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REVIEW

Attaching-effacing Bacteria in Animals

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Summary

Enteric bacteria with a demonstrable or potential ability to form attaching-effacing lesions, so-called attaching-effacing (AE) bacteria, have been found in the intestinal tracts of a wide variety of warm-blooded animal species, including man. In some host species, for example cattle, pigs, rabbits and human beings, attaching-effacing Escherichia coli (AEEC) have an established role as enteropathogens. In other host species, AE bacteria are of less certain significance. With continuing advances in the detection and typing of AE strains, the importance of these bacteria for many hosts is likely to become clearer. The pathogenic effects of AE bacteria result from adhesion to the intestinal mucosa by a variety of mechanisms, culminating in the formation of the characteristic intimate adhesion of the AE lesion. The ability to induce AE lesions is mediated by the co-ordinated expression of some 40 bacterial genes organized within a so-called pathogenicity island, known as the "Locus for Enterocyte Effacement". It is also believed that the production of bacterial toxins, principally Vero toxins, is a significant virulence factor for some AEEC strains. Recent areas of research into AE bacteria include: the use of Citrobacter rodentium to model human AEEC disease; quorum-sensing mechanisms used by AEEC to modulate virulence gene expression; and the potential role of adhesion in the persistent colonization of the intestine by AE bacteria. This review of AE bacteria covers their molecular biology, their occurrence in various animal species, and the diagnosis, pathology and clinical aspects of animal diseases with which they are associated. Reference is made to human pathogens where appropriate. The focus is mainly on natural colonization and disease, but complementary experimental data are also included.

Keywords: attaching-effacing bacteria; bacterial infection; Escherichia coli; review article

Definitions and History Pertaining to Attaching-effacing Bacteria

Attaching-effacing (AE) bacteria are so-called because they are capable of forming AE lesions on the intestinal mucosa in vivo and on certain tissues and cell cultures in vitro. The mucosal lesion is associated with stunting and fusion of villi in severe cases (Fig. 1a). Bacteria can be seen on the mucosal surface (Fig. 1b), and may often be specifically identified by immunolabelling (Fig. 1c). With transmission electron microscopy (Fig.1d), the AE lesion is seen to be characterized by intimate adhesion of the bacterium to the epithelial cell membrane, with an intervening gap of approximately 10 nm, accompanied by effacement of enterocyte microvilli. Beneath the adherent bacterium a cytoskeletal rearrangement, including the accumulation of filamentous actin (F-actin), is seen. The bacteria often rest upon a pedestal-like structure, which may extend for up to 10 μm away from the epithelial cell surface (Kaper et al., 1998). The first description and illustration of the AE lesion, although not referred to as such at that time, was from neonatal piglets inoculated with a human strain of Escherichia coli (Staley et al., 1969). Takeuchi et al. (1978) described in detail a similar lesion from rabbits inoculated with RDEC-
1, a strain of *E. coli* associated with diarrhoea in rabbits. The term “attaching-effacing” was first used by Moon *et al.* (1983) in describing the same type of lesion induced experimentally in the intestines of pigs and rabbits.

Most reported AE bacteria are *E. coli*, known as “attaching-effacing *E. coli*” (AEEC), but *Citrobacter rodentium*, which infects mice (Luperchio *et al.*, 2000), is also capable of producing AE lesions.

AEEC strains associated with human gastrointestinal disease are classified as either enteropathogenic or enterohaemorrhagic *E. coli* (EPEC or EHEC, respectively), depending on their inability or ability to produce one or more Vero toxins (VTs; also known as Shiga toxins or Shiga-like toxins).

Fig. 1a–d. Ileum of calf infected with *E. coli* O26. (a) The mucosa has a flat appearance due to villous stunting and fusion. The surface epithelium has an irregular appearance. Haematoxylin and eosin (HE). Bar, 100 μm. (b) Adherent bacteria (arrows) are present on the surface of enterocytes. HE. Bar, 15 μm. (c) Adherent bacteria, labelled with an O26 antiserum, are present on the irregular epithelial surface. Immunoperoxidase. Bar, 20 μm. (d) Adherent bacteria are intimately attached to the surface of enterocytes and microvilli are effaced. Condensed actin filaments are seen at cell apex (arrow). TEM. Bar, 500 nm.
Vero toxins are encoded by temperate bacteriophages and are potent ribosomal inhibitors, targeting blood vessels and other tissues, depending upon the distribution of toxin receptors in the host species (O’Loughlin and Robins-Browne, 2001; Paton and Paton, 1998). EPEC strains produce AE lesions in the small and large intestine (Ulshen and Rollo, 1980; Rothbaum et al., 1982); they do not produce VT and are now mainly associated with infant diarrhoea in developing countries (Nataro and Kaper, 1998). EHEC produce VT and are associated with haemorrhagic colitis and the haemolytic-uraemic syndrome (HUS) (Nataro and Kaper, 1998). E. coli O157:H7 is the prototype EHEC; it forms AE lesions on animal intestinal mucosa (Tzipori et al., 1986), and in-vitro studies (Phillips et al., 2000) suggested that human intestinal mucosa was similarly affected. Such lesions may assist colonization of the human large intestine. The designations EPEC and EHEC were developed in the context of observed clinical disorders; their definition and their usage in relation to individual bacterial strains and to virulence factors has varied, both over time and between workers. For example, the designation “EHEC” in a communication may or may not infer an established association between the strain in question and the clinical entities of haemorrhagic diarrhoea and HUS. Furthermore, some disease-associated EHEC strains lack an AE capability (Willshaw et al., 1992).

When discussing veterinary AEEC the term “EPEC” is widely employed (e.g., bovine EPEC [Goffaux et al., 2001]), often with a letter denoting...
the natural host species. Thus, for example, rabbit (R) EPEC is referred to as REPEC (Adams et al., 1997) and pig EPEC as PEPEC (An et al., 2000). The term “EHEC” is used less commonly in the context of veterinary AEEC, with a limited overlap between the disease patterns associated with human EHEC and with veterinary VT-producing AEEC. The terms EPEC-like and EHEC-like may be used to demarcate veterinary pathotypes from similar human strains.

In the present review the term “AEEC” is reserved for those strains with a proven capability to form AE lesions. The term “putative AEEC” is used for bacterial strains that encode genes associated with the production of AE lesions but for which evidence of the ability to produce such lesions is lacking.

The Attaching-effacing Lesion

Much of the work on elucidating the mechanisms that play a role in the formation of the AE lesion has been performed on human EPEC, and later contrasted with EHEC studies. EPEC O127:H6, an established experimental type strain, has a 35.6 kilo base-pair (kbp) chromosomal insertion that is necessary and sufficient for expression of the AE phenotype in vitro (McDaniel and Kaper, 1997). This “pathogenicity island” encodes 41 predicted open reading frames (ORF), with a distinct cytosine and guanidine nucleotide percentage (38.3%) as compared with that of the rest of the E. coli genome (50.8%), suggesting an origin outside the species (Elliott et al., 1998). It has been designated the “Locus of Enterocyte Effacement” (LEE). The LEE was invariably present in the genome of diverse AE pathogens including EPEC, EHEC, a rabbit EPEC and C. rodentium, but was absent from related non-AE bacteria (McDaniel et al., 1995). The LEE of EHEC O157:H7 is larger (43 kbp) than that of EPEC O126:H6 but has the same overall genetic structure (Perna et al., 1998).

For both EPEC and EHEC there are five polycistronic LEE operons; LEE 1 to 4 (encoding secreted proteins and a type III secretion apparatus) and the Tir (LEE 5) operon. This last operon contains the eae (enterocyte attaching and effacing) and tir (translocated intimin receptor) genes encoding the intimin adhesin and its receptor (Tir; see Stages 2 and 3 below), respectively. An ORF within LEE 1, termed the LEE-encoded regulator (Ler), “upregulates” expression of LEE 2, 3 and 4 (Mellies et al., 1999). The EPEC and EHEC LEE, including Ler, is under the influence of global regulators, including the Integration Host Factor (IHF) (Friedberg et al., 1999) and a quorum sensing mechanism mediated by a bacterial autoinducer whose production is dependent upon the luxS gene (Sperandio et al., 1999, 2003). It has been hypothesized that quorum sensing functions to upregulate virulence determinants, such as the LEE, in an intestinal environment, by means of signals from the intestinal flora (Sperandio et al., 2001). Recent evidence also suggests a role for host catecholamine hormones (e.g., adrenaline) in the upregulation of virulence of the LEE (Sperandio et al., 2003). A three-stage model for the AE process has been proposed (Donnenberg et al., 1997); however, a fourth (invasion) stage may be included (Tesh and O’Brien, 1992). Fig. 2 illustrates the three-stage model.

Stage 1: Initial Non-intimate Attachment

An early non-intimate attachment (Fig. 2a) appears to be necessary for initial signalling leading to the development of the AE lesion, but the mediators of this attachment are poorly understood. Studies have shown a potentiating effect of adhesion factors (such as the EPEC Adherence Factor [EAF] plasmid and the REPEC-associated AF/R1 fimbrial adhesin) upon AE lesion formation in vitro (Knutton et al., 1987; Francis et al., 1991) and on AEEC virulence in vivo (Levine et al., 1985; Wolf et al., 1988). The EAF plasmid contains a gene cluster (bfp) encoding bundle-forming pili (BFP) (Stone et al., 1995), which mediate microcolony formation in culture and localized adhesion to HEp-2 cells (Giron et al., 1991). However, the role of BFP in vivo is still unclear, as adhesion of EPEC to human duodenal and jejunal organ cultures appeared to be independent of EAF or BFP (Knutton et al., 1991; Hicks et al., 1998). EspA filaments (see Stage 2 below and Fig. 2a) may act as an adhesin, with evidence for this in EPEC (Knutton et al., 1998; Daniell et al., 2001b) and EHEC O157:H7 (Tatsuno et al., 2000). Components of the LEE, including intimin and Tir, which are directly involved in intimate attachment, appeared to be important for primary adhesion and microcolony formation by EHEC O157:H7 in vitro (McKee et al., 1995; Devinney et al., 1999; Tatsuno et al., 2000), and for detectable adhesion of a REPEC strain in vivo (Marches et al., 2000), but intimin-deficient mutants nonetheless adhere diffusely in vitro (Tatsuno et al., 2000).

Stage 2: Signal Transduction Leading to Cytoskeletal Reorganization and Microvillus Effacement

This is illustrated in Fig. 2b. AEEC strains use a type-III secretory apparatus, which allows
the translocation of bacterial proteins into the cytosol of the host eukaryotic cell (Hueck, 1998). This is encoded on the LEE by sep (secretion of \textit{E. coli} proteins) and esc (\textit{E. coli} secretion) genes (Elliott \textit{et al.}, 1998). The LEE encodes several secreted proteins, termed EPEC-secreted proteins (Esp), three of which (A, B and D) are essential for normal AE lesion formation. EspA forms filaments on the bacterial surface, in an EspD-dependent process (Knutton \textit{et al.}, 1998), and these filaments

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**Fig. 2a–c.** Composite, schematic diagram illustrating proposed mechanisms in the formation of the attaching-effacing lesion. (a) Non-intimate adhesion and protein translocation. EspA, -B and -D are exported via a type III secretion system and form a bi-functional organelle permitting adhesion of the AE bacterium to the host cell and translocation of Tir into the host cell. Esp, \textit{E. coli} secreted protein; Tir, translocated intimin receptor. (b) Signal transduction. The host cell cytoskeleton is dissolved locally, leading to effacement of microvilli. Tir is inserted in the host cell membrane. Tir, translocated intimin receptor. (c) Intimate adhesion. Tir focuses filamentous (F) actin, forming a pedestal, and binds intimin in the bacterial outer membrane. Tir, translocated intimin receptor.
adhere to host cells (Shaw et al., 2002). EspD also participates in forming pores in the host cell membrane (Daniell et al., 2001a). EspB is translocated to the host cell in an EspA-dependent manner (Ebel et al., 1998; Knutton et al., 1998), and is distributed in the plasma membrane and the cytosol of the host cell (Wolff et al., 1998).

In a current model it is proposed that a bacterial transmembrane structure, encoded by escC, links with a hollow EspA filament to form a bi-functional organelle which has adhesive and protein-translocating roles (Daniell et al., 2001b). EspB and EspD may create a pore-forming structure in the eukaryotic cell membrane. Together, these elements might form a molecular “syringe” for the introduction of bacterial macromolecules into the host cytosol (Frankel et al., 1998b).

AEEC translocate a LEE-encoded receptor for intimin, termed the translocated intimin receptor (Tir), into the host cell (Rosenshine et al., 1992; Kenny et al., 1997; Deibel et al., 1998; DeVinney et al., 1999). Tir undergoes an apparent increase in molecular mass within the host cell, which for EPEC O127:H6 is from 78 to 85 kDa and is based in part on serine residue phosphorylation (Warawa and Kenny, 2001). Phosphorylation of tyrosine residues occurs on Tir molecules of some AE strains (for example: EPEC O127:H6 [Kenny et al., 1997]; a VT-producing bovine-derived E. coli O26:H-[Deibel et al., 1998]; and C. rodentium [Deng et al., 2003]), but not others (notably EHEC O157:H7 [Ismaili et al., 1995a]). Phosphorylation of proteins at the site of the lesion appears to be dependent upon EspB secretion (Ismaili et al., 1998).

At the site of intimate attachment, considerable eukaryotic cytoskeletal reorganization occurs, with depolymerization of actin, the formation of F-actin and the accumulation of α-actinin, myosin light chain, talin and ezrin (Donnenberg et al., 1997). These cytoskeletal changes are associated with the effacement of microvilli, the formation of pedestals topped by attached bacteria, and the disruption of the intestinal barrier function of enterocytes (Simonovic et al., 2001). The mechanisms by which cytoskeletal elements are dissolved and reaggregated are unclear. Current models implicate translocated bacterial proteins such as EspB and Tir, which are co-localized at the site of cytoskeletal reorganization, together with activation of host cell protein kinase activity (Frankel et al., 1998b). EPEC Tir appears to interact with the cytoskeletal elements in at least two different ways: it binds α-actinin directly (Goosney et al., 2000) but also acts via the Nck protein, neural Wiskott–Aldrich syndrome proteins (N-WASP) and the actin-related protein complex (Arp2/3c) to focus (nucleate) actin, promoting its polymerization (Kalman et al., 1999; Gruenheid et al., 2001). This latter process is dependent upon phosphorylation of tyrosine residue 474 in the EPEC Tir molecule (Kenny, 1999), but appears to be significantly different in EHEC O157:H7 lesions, where a serine residue occupies position 474 in Tir and tyrosine phosphorylation does not occur (Ismaili et al., 1995a).

Stage 3: Intimate Attachment

This is illustrated in Fig. 2c. Intimin, encoded by the enterocyte attaching and effacing (eae) gene, is a surface-exposed bacterial outer membrane protein (Jerse and Kaper, 1991; Louie et al., 1993). Tir binds intimin (Rosenshine et al., 1996; DeVinney et al., 1999; Hartland et al., 1999) and both proteins are necessary for the development of the mature AE lesion (Donnenberg et al., 1993; Rosenshine et al., 1996; Kenny et al., 1997). Tir binds to the C-terminus of the intimin molecule (Liu et al., 1999; Luo et al., 2000), the amino-acid sequence of which varies between AE organisms. Four principal subtypes of intimin (α, β, γ and ε) have been established, based on antigenic, amino-acid and eae sequence differences at the C-terminus domain (Adu-Bobie et al., 1998; Oswald et al., 2000). However, up to seven further subtypes have been proposed, based upon eae sequence variation (Zhang et al., 2002; Jones et al., 2003).

In the C-terminus domain of intimin there is also a Tir-independent c-lectin-like binding site, which is necessary for expression of the AE phenotype (Frankel et al., 1998a; Hartland et al., 1999). Intimin subtype may confer tissue tropism upon AEEC, as reflected in the different intestinal regions affected by EPEC and EHEC (Tzipori et al., 1995). As the intimin receptor Tir is not a host protein, tissue tropism may be determined by the c-lectin-like binding site on intimin; however, binding of c-lectins by intimin in the conventional manner seems improbable (Luo et al., 2000). Intimin type seems unlikely to be the sole determinant of the distribution of AE lesions, as human EPEC strains O127:H6 and O55:H6, both of which encode α-intimin and BFP, differ in their tropism on human intestine in organ culture (Fitzhenry et al., 2002). Frankel et al. (1996a) suggested that intimin may bind cells in a non-Tir-dependent manner by association with β-integrins.

Deng et al. (2003) showed that the ability of C. rodentium to form AE lesions and to induce pathological changes and disease was independent of its actin-focusing and pedestal-forming capability.
at the AE lesion. This demonstrates that stage three of the lesion is much less dependent upon stage two than previously assumed.

**Stage 4: Invasion**

The tendency for invasion of host cells by EPEC is promoted by EAF and eae (Donnenberg et al., 1989; Francis et al., 1991). The invasiveness of EPEC *in vitro* may rival that exhibited by enteroinvasive *E. coli* (Donnenberg et al., 1989), *Salmonella enterica* serotype Typhimurium (Geyid et al., 1996) and *Shigella flexneri* (McKee and O’Brien, 1995). However, marked variation in the extent of invasion has been observed between EPEC strains, and with different combinations of strain and host cell type (Geyid et al., 1996; Dibb-Fuller et al., 2001). EHEC strains may invade *in vitro* but generally to a lesser extent than EPEC (Ismaili et al., 1998). Intracytoplasmic bacteria (both in vacuoles and free within the cytoplasm) have been observed in AE lesions due to EPEC and EPEC-like organisms in human patients (Fagundes-Neto et al., 1995), rabbits (Takeuchi et al., 1978; Peeters et al., 1984a), pigs (Helie et al., 1991), dogs (Wada et al., 1996a) and cotton-top tamarins (Mansfield et al., 2001b). The numbers of such bacteria appear to be small relative to the surface population (Takeuchi et al., 1978; Peeters et al., 1984a; Helie et al., 1991). In some cases, bacteria were seen in the lamina propria (Helie et al., 1991; Wada et al., 1996a).

**Host Cell Specificity In Vitro**

Although the alimentary mucosa is the target site for all known AE pathogens, AE bacteria will produce lesions *in vitro* not only with enterocyte-derived cells (Knutton et al., 1989) but also with other epithelial-type cells (e.g., the HeLa human cervical carcinoma line [Fagundes-Neto et al., 1995]) and with non-epithelial cells (e.g., the HEL 229 human embryonic fibroblast line [Knutton et al., 1989]). However, there is some specificity in the bacterium–host cell pairing. For example, a REPEC O103 strain formed AE lesions on a rabbit cell line but not on HeLa cells (Nougayrede et al., 1999); moreover, the proportion of bovine LEE-positive VT-positive *E. coli* strains that produced AE lesions on a bovine cell line was higher than that producing such lesions on the human HEp-2 cell line (Wieler et al., 1998). Genetic modification of an AE organism leading to the expression of a non-intimate adhesin appropriate to the host cell type may considerably enhance the organism’s AE capability on the homologous cell line (Deng et al., 2003).

**Detection and Diagnosis of AE Lesions**

The definitive method for determining the presence of AE lesions is observation of the characteristic ultrastructural appearance of the lesion by transmission electron microscopy (TEM). For routine detection of AE lesions *in vitro*, the associated characteristic cytoskeletal reorganization has proved to be a useful target for fluorescence microscopy. Knutton et al. (1989) used the specific affinity of the fungal toxin phalloidin for the F-actin beneath the attached bacteria to develop the fluorescence actin staining (FAS) test. In this test, phalloidin labelled with a fluorescent dye is used; “co-localization” of adherent bacteria and bright fluorescence by phase-contrast and epifluorescent microscopy is a sensitive, specific and technically straightforward indicator of AE lesions on cultured cell monolayers (Donnenberg and Nataro, 1995). Use of a fluorescence-labelled α-actinin antibody has also proved successful in the specific detection of AE lesions *in vitro* (Ismaili et al., 1995b). FAS has also been performed with apparent success on frozen histopathological sections viewed by epifluorescent microscopy (Vallance et al., 2002) or confocal laser scanning microscopy (Abe et al., 1998).

Descriptions of the histopathological appearance of intestines colonized by AE bacteria are consistent across species. These species include cattle (Hall et al., 1985; Moxley and Francis, 1986; Pospischil et al., 1987; Schoonderwoerd et al., 1988; Janke et al., 1989, 1990; Pearson et al., 1989; Iijima et al., 1990), pigs (Helie et al., 1991; Neef et al., 1994; Higgins et al., 1997), rabbits (Polotsky et al., 1977; Peeters et al., 1984a), human beings (Ulshen and Rollo, 1980; Rothbaum et al., 1982), monkeys (Mansfield et al., 2001b) and poultry (Fukui et al., 1995). In general, bacteria adhere to enterocytes in an extensive or multifocal pattern and typically have a distinct, coccolid appearance (Janke et al., 1989). Colonized cells appear degenerate and many are hyperchromatic, rounded-up or pyknotic. Mucosal erosions and detachment of enterocytes are commonly seen. At low magnification this gives a ragged, irregular, “scalloped” or “cobblestone” appearance to the mucosal surface (Moon et al., 1983; Hall et al., 1985; Janke et al., 1989; Pearson et al., 1989; Higgins et al., 1997; Mansfield et al., 2001b). Bacteria may be seen inside enterocytes, which are heavily colonized on their apical surface (Helie et al., 1991). An inflammatory infiltrate of variable intensity, typically neutrophilic but sometimes mixed, is often seen in the lamina propria.
AE lesions have been reported in all regions of the gastrointestinal tract from the stomach to the rectum. In the small intestine, blunting, atrophy and fusion of villi accompany the changes described above (Moon et al., 1983; Peeters et al., 1984a; Moxley and Francis, 1986; Schoonderwoerd et al., 1988; Pearson et al., 1989, 1999; Iijima et al., 1990; Helie et al., 1991; Fagundes-Neto et al., 1995; Higgins et al., 1997; Gunning et al., 2001). Crypt hyperplasia is also reported in the small intestine (Ulshen and Rollo, 1980; Pospischil et al., 1987). In the large intestine, the ragged appearance of the colonized mucosa is prominent, and attached AE bacteria are particularly dense on the surface epithelium but may extend some way into the crypts (Janke et al., 1989, 1990; Fukui et al., 1995). In mice, hyperplasia of the colonic crypts is a prominent feature of infection with C. rodentium (Lupercio and Schauer, 2001), and a similar but less marked change is reported in other species, for example tamarin monkeys (Mansfield et al., 2001b).

Loss of the enterocyte brush border may be discernible by light microscopy in well-preserved specimens (Janke et al., 1989), an important distinction from infection with enterotoxigenic E. coli (ETEC) organisms, which adhere to the small intestinal mucosa via fimbriae, and which do not disrupt the brush border (Acres, 1985). However, the fine detail of an attachment suspected to be AE in nature cannot be discerned fully by light microscopy. Some reports assert that a diagnosis of AE lesions can often be made on the basis of microscopic examination of tissues is not undertaken. There is an association between disease and the subtype of intimin present in faecal isolates of putative AEEC from cattle (see below). This suggests that, for some host species, the typing of LEE elements from clinical isolates may help to determine their pathogenicity.

**Host Species and Clinical Diseases Associated with AE Bacteria**

**Cattle**

EPEC-like strains have been associated with AE lesions in the large and small intestines of diarrhoeic calves (Janke et al., 1989, 1990; Hollander et al., 1999b); in only one case, however, was a positive identification of the organism (E. coli O80:H-) made in situ (Pearson et al., 1989; Wales et al., 2000). VT-producing AEEC O5:H- and O111:H- were associated with naturally occurring haemorrhagic enteritis (dysentery) and AE lesions in calves (Hall et al., 1985, 1988a; Schoonderwoerd et al., 1988). These strains were shown to produce AE lesions in experimentally infected calves (Chanter et al., 1984; Schoonderwoerd et al., 1988). Other VT-producing E. coli strains were reported in association with AE lesions in diarrhoeic calves in which dysentery was present in only a proportion of cases; the serovars included O5:H-, O5, O23:H-, O26:H-, O26:H11, O26, O111:H-, O111:H11 and O111 (Moxley and Francis, 1986; Pospischil et al., 1987; Janke et al., 1989, 1990; Iijima et al., 1990; Gunning et al., 2001). In older animals,
haemorrhagic diarrohoea with AE lesions was reported in an 8-month-old heifer infected with E. coli O26 (Pearson et al., 1999) and in an adult cow infected with E. coli O15 (Wada et al., 1994). The report of the former case describes the detection of VT and other EHEC genes in an O26:H11 strain isolated from an in-contact animal with similar clinical signs. In cases of AEEC-associated diarrhoea there is commonly concurrent infection with one or more of the following: coronavirus, bovine viral diarrhoea virus, rotavirus, Cryptosporidium, Salmonella and ETEC (Hall et al., 1985, 1988b; Moxley and Francis, 1986; Pospischil et al., 1987; Janke et al., 1989, 1990; Vorster et al., 1994; Holland et al., 1999b; Gunning et al., 2001).

Gross findings in cases of AEEC-associated bovine disease vary from no significant mucosal lesions (Moxley and Francis, 1986; Pearson et al., 1989) to fibrino-haemorrhagic enteritis (Schoonderwoerd et al., 1988). Haemorrhage, evident in the mucosa or intestinal contents (or both) is a frequent finding (Hall et al., 1985; Janke et al., 1989; Pearson et al., 1989; Wada et al., 1994). However, haemorrhage is not invariably present with VT-producing AEEC enteritis (Janke et al., 1990); conversely, haemorrhage may be seen when the AEEC strains do not produce VT (Pearson et al., 1989).

Naturally occurring AE lesions have been reported most commonly in the large intestine (Hall et al., 1985, 1988b; Moxley and Francis, 1986; Mainil et al., 1987; Janke et al., 1989, 1990; Iijima et al., 1990; Wada et al., 1994, 1997; Holland et al., 1999b; Pearson et al., 1999) or large and small intestines, with the small intestinal lesions often confined to the ileum (Pospischil et al., 1987; Hall et al., 1988b; Schoonderwoerd et al., 1988; Janke et al., 1989, 1990; Pearson et al., 1989; Holland et al., 1999b; Gunning et al., 2001). Cases in which AE lesions are confined to the small intestine are less common (Janke et al., 1989, 1990). Serotype does not appear to be closely associated with the distribution of lesions; thus, AE lesions associated with E. coli O26:H11 were found by Mainil et al. (1987) and Pearson et al. (1999) to occur only in the large intestine, but by Gunning et al. (2001) to occur in both the large and small intestines. The relative contribution of host and strain to the observed distribution of lesions is uncertain, and in some cases the sensitivity of detection may have been a significant factor.

Putative AEEC are isolated frequently from cattle. Orden et al. (2002) reported a prevalence of EPEC-like AEEC of 8.2% amongst cattle of all ages, and Aktan et al. (2004) found eae-positive E. coli in 3% of animals entering abattoirs in the UK. In other studies eae-positive E. coli were isolated from 5 to 20% of diarrhoeic calves and 21 to 40% of healthy calves (China et al., 1998; Orden et al., 1998; Holland et al., 1999b). Whilst these calf studies showed an apparent lack of any relationship between the prevalence of putative AEEC and the presence of diarrhoea, a longitudinal study of calves between one and 12 weeks of age showed a positive relationship (China et al., 1998). There is evidence of a particular association between calf diarrhoea and the presence of the β subtype of intimin (China et al., 1998, 1999; Orden et al., 2003). The reported proportions of bovine putative AEEC isolates that produce VT vary between 20 and 50% (Orden et al., 1998, 2002; Holland et al., 1999b). Serogroups particularly associated with bovine eae-positive E. coli are O4, O5, O14, O26, O111, O118 and O123, with O26 having been reported most consistently (Mainil et al., 1993; Orden et al., 1998, 2003; Holland et al., 1999b). Strains of E. coli O26:H11 and O26 possessing EPEC-like features, i.e., hybridizing with eae probes but lacking VT, have been isolated from diarrhoeic calves (Fischer et al., 1994; Saridakis et al., 1997; Orden et al., 2003). It appears, therefore, that bovine pathogenic O26 AEEC strains include not only those that produce VT (described above in association with AE lesions in calves) but also EPEC-like strains that do not. There is some evidence of geographical variation in putative bovine pathogenic AEEC. In particular, the predominant eae-positive, VT-producing isolates reported from diarrhoeic calves in Germany and Belgium are of serotype O118:H16 (Wieler et al., 1998). Furthermore, analysis of E. coli O118:H16 and O118:H- strains has shown an association between bovine-derived AEEC and human-pathogenic EHEC isolates (Wieler et al., 2000).

Oral inoculation studies in young calves with putative bovine pathogenic AEEC of serotypes O5:H- O8:H9, O26:H11, O111:H- and O118:H16 produced AE lesions in the large intestine (Hall et al., 1985; Mainil et al., 1987; Schoonderwoerd et al., 1988) or in both the small and large intestines (Moxley and Francis, 1986; Wray et al., 1989; Stordeur et al., 2000). Typically, there was accompanying diarrhoea, but in two reports (Mainil et al., 1987; Schoonderwoerd et al., 1988) diarrhoea was not observed despite the presence of intestinal AE lesions.

In summary, bovine AEEC causing natural diarrhoeal disease includes VT-producing (EHEC-like) and non-VT (EPEC-like) strains. Calves are affected predominantly, but older animals, including adults, may also show AE enteritis. Putative
AEEC strains are common in healthy and diarrhoeic animals. Certain serogroups (O5, O26 and O111) are prominent amongst both proven and putative bovine-pathogenic AE ECC strains. Certain bovine VT-producing AE ECC strains (for example O26:H11 and O118:H16) share serotypes with human EHEC; cross-species pathogenicity, however, remains largely a matter of speculation.

Sheep and Goats

The first illustration of AE lesions in sheep was in the small and large intestines of two lambs, which were part of an experiment with Cryptosporidium in which the final inoculum had been originally derived from the intestinal contents of a calf (Angus et al., 1982). Consequently, it was not clear whether these AE bacteria were of bovine or ovine origin. Two cases of natural infection by AE pathogens were reported in diseased neonatal lambs (Janke et al., 1989). The colon was affected in both lambs and the ileum in one. E. coli isolates from the lambs were not typed. AE lesions produced by untyped bacteria were also observed on the ileal and large intestinal mucosa of symptomless neonatal lambs (Wales et al., 2005a). In addition, multifocal colonization of the large intestinal mucosa of older, weaned sheep by bacteria forming AE lesions was reported by Cookson et al. (2002a) and Woodward et al. (2003). Animals in these last two reports had been inoculated with the human pathogen EHEC O157:H7, but the AE lesions were shown to be formed by E. coli O115:H- in the first case, and by E. coli O26 together with other AE organisms in the second; the animals remained clinically normal.

Natural enteric disease in goats associated with AE lesions is the subject of three reports. Duhamel et al. (1992) reported a 2-month-old goat with diarrhoea of 3 weeks’ duration. There were extensive AE lesions in the large intestine. These were attributed to a VT-producing E. coli O103:H2 recovered from the large intestine. Coccidia were also seen in the intestinal tissues. An outbreak of fatal, acute diarrhoea associated with AE ECC in one-week-old kids was reported by Drolet et al. (1994b). Investigation of an individual case revealed focally extensive AE lesions in the large intestine and an untypable eae-positive E. coli was recovered from the intestines. Recently, Barlow et al. (2005), found AE lesions caused by E. coli O145 in the ileum and colon of a 2-year-old goat with mild diarrhoea and severe dehydration, from a herd experiencing several cases of anorexia, weakness and death in milking females. In addition, Wales et al. (2005b) reported AE lesions caused by one or more unidentified organisms in the large intestine of clinically normal neonatal kids that had been inoculated with E. coli O157:H7. Experimentally, colostrum-deprived kid goats inoculated with a putative calf VT-producing AE ECC developed colonic AE lesions (Tominaga et al., 1989).

Examination of lamb and kid faeces for putative AE ECC by screening for eae (Orden et al., 2000; Cid et al., 2001; de la Fuente et al., 2002) showed a lower prevalence in diarrhoeic animals (7 to 21%) than in healthy animals (33 to 50%). However, in one study of 1013 animals (de la Fuente et al., 2002), E. coli O26 accounted for nearly 43% of eae-positive isolates from diarrhoeic lambs but only 2.5% from healthy lambs. This suggests that, whilst LEE genes are widespread, only certain LEE-positive strains, some of which may have characteristic serotypes, have the pathogenic phenotype in any particular host species. The serovars of eae-positive E. coli isolated from sheep are diverse and dissimilar to those from goats (Cid et al., 2001; de la Fuente et al., 2002; Aktan et al., 2004).

Thus, AE ECC strains appear to affect young sheep and goats clinically, but also to form subclinical lesions in neonatal and older animals. Survey evidence suggests that certain AE ECC serovars, particularly O26, may commonly have a role in lamb diarrhoea, at least in some geographical regions.

Pigs

Diarrhoea associated with naturally occurring AE lesions has been reported in pigs from the neonatal to the post-weaning period (Janke et al., 1989; Wada et al., 1996b; Higgins et al., 1997; Holland et al., 2000), and there is some evidence that diet may play a part in the occurrence of such lesions (Neef et al., 1994). Diarrhoea may be haemorrhagic (Janke et al., 1989), but gross findings typically are unremarkable (Janke et al., 1989; Higgins et al., 1997) and isolates rarely produce VT (Janke et al., 1989; Zhu et al., 1994; Higgins et al., 1997). AE lesions may be present in the small or large intestines, or in both (Janke et al., 1989; Higgins et al., 1997; Holland et al., 2000). In the field, dual infection of the small and large intestines of weaned pigs with ETEC O149 and AE ECC O45 has been reported (Wada et al., 1996b). Serogroup O45 is implicated commonly in AE ECC postweaning diarrhoea (Helie et al., 1991; Zhu et al., 1994), but within this serogroup strains that lack eae and possess attributes of ETEC (K88 adhesin, enterotoxin) may also be found (Zhu et al., 1994).
Several other serogroups, including O26, O75, O116 and O−, have been reported in association with AEEC in pigs (Neef et al., 1994; Higgins et al., 1997; Holland et al., 2000). Porcine diarrhoea-associated E. coli strains of various serogroups that produce the FAS reaction and AE lesions in vitro, but which are negative in conventional tests for eae and intimin, have been reported (Penteado et al., 2001). It is possible that these possess a hitherto unknown variant of intimin. Pathogenic E. coli strains producing a variant of VT also colonize the pig intestine and cause oedema disease, but attachment of these strains to the mucosa occurs via F18 fimbriae, not AE mechanisms (Gyles, 1998).

Experimentally, gnotobiotic or caesarean-derived colostrum-deprived piglets have proved to be convenient and useful subjects for studying AE lesions. Piglets are usually inoculated at 1–2 days of age, and develop AE lesions in the distal ileum and the large intestine when infected with EPEC, EHEC, bovine EHEC-like and rabbit EPEC strains (Moon et al., 1983; Tzipori et al., 1985, 1989; Hall et al., 1988a). The susceptibility of piglets to AE lesions appears to decline with increasing age (Moon et al., 1983).

In summary, AE pathogens in pigs typically are EPEC-like and cause diarrhoea in animals up to the postweaning period. Both the presence of haemorrhage and the distribution of the pathogen in the intestine are variable. Young piglets are susceptible to AE lesion production by a range of AEEC strains from other host species.

**Rabbits**

AEEC is an especially prominent enteric pathogen in rabbits. Epizootics of colibacillosis are a major cause of disease in commercial rabbit farms (Peeters et al., 1984d; Blanco et al., 1996; Milon et al., 1999), and are characterized by infection with AEEC strains that adhere to the intestinal mucosa (Peeters et al., 1984c), forming typical AE lesions (Prescott, 1978; Blanco et al., 1997). Rabbit AEEC strains rarely produce enterotoxins or VT (Pohl et al., 1993; Blanco et al., 1996), and accordingly are classified as rabbit EPEC (REPEC). In contrast to other host species, the presence of the eae gene in rabbit intestinal E. coli is closely associated with diarrhoeal disease (Blanco et al., 1996). REPEC strains have been widely studied in view of their commercial importance, their similarity in many respects to human EPEC, and the suitability of the rabbit for experimental studies.

Field isolates of REPEC can be subdivided into strains affecting suckling rabbits and those affecting weanlings. The former are associated with yellowish, watery diarrhoea in preweaned rabbits typically aged 7–12 days (Peeters et al., 1984a,c). Experimentally, Peeters et al. (1984a,d) found that diarrhoea in neonatal rabbits commenced 1–3 days post-inoculation and mortality was high; AE lesions were found throughout the large and small intestines from 24 h after inoculation, and there was accompanying mucosal ulceration and haemorrhage. Strains in suckling rabbits, which appear to be restricted to serotype O109:H2, cause minimal lesions in weaned rabbits (Peeters et al., 1984d).

By contrast, weanling rabbits are typically affected at 4–6 weeks of age (Peeters et al., 1984d) by strains belonging to a range of serotypes, the most common of which include O15:H-, O26:H11, O103:H2 and O109:H2 (Peeters et al., 1984d, 1988; Blanco et al., 1996). Experimentally, strains produced diarrhoea of variable consistency in a proportion of inoculated rabbits (Peeters et al., 1984d; Heczko et al., 2000); they differed in virulence (Peeters et al., 1988) and appeared to be avirulent in suckling rabbits, despite forming AE lesions in vivo (Peeters et al., 1984a). Diarrhoea started typically at approximately 6 days post-inoculation (Cantey and Blake, 1977; Peeters et al., 1984d) and the gross findings included liquid intestinal contents, thickening or oedema of the intestinal wall, and swollen mesenteric lymph nodes (Cantey and Blake, 1977; Peeters et al., 1984d). Initial attachment of the bacteria, within 24 h of inoculation, was non-intimate and was restricted to the follicle-associated epithelium of Peyer’s patches in the ileum (Cantey and Inman, 1981; Peeters et al., 1984b; von Moll and Cantey, 1997; Heczko et al., 2000). For one strain (RDEC-1), non-intimate attachment was further shown to be restricted to the microfold (M) epithelial cells covering the specialized dome villi over the ileal Peyer’s patches (Inman and Cantey, 1983). Subsequent intimate (AE) attachment, which was observed from 3 days after inoculation, occurred in the ileum and large intestine (Cantey and Inman, 1981; Heczko et al., 2000), and was accompanied by inflammation and ulceration (Peeters et al., 1984d; Heczko et al., 2000). This progressive sequence of attachment may explain the differences between suckling and weanling strains in terms of the speed of onset of diarrhoea. Furthermore, the resistance of suckling rabbits to weanling-associated strains may be due to the fact that Peyer’s patches do not develop before 2 weeks of age (Heczko et al., 2000). In addition, weanling
rabbit-associated strains do not adhere to neonatal rabbit intestinal villi in vitro (Peeters et al., 1984d).

Strains of AEEC appear to differ between geographical regions. For example, weanling-associated REPEC O15 is reported commonly from Belgium, the Netherlands and North America, but less commonly from France and Spain (Blanco et al., 1996). REPEC O15:H- includes the prototypical strain RDEC-1 (Cantey and Blake, 1977; Cantey et al., 1981) that encodes a plasmid-born rabbit-specific fimbrial adhesin, termed AF/R1, which mediates non-intimate adhesion to ileal M cells and is an established virulence factor (Berendson et al., 1983; Wolf et al., 1988). RDEC-1 also elaborates a soluble factor which affects the electrical properties of rabbit ileal mucosa in vivo and may therefore have diarrhoeagenic properties (Raimondi et al., 2001). Another REPEC O15:H- strain (U83/39) resembled RDEC-1 in respect of the disease produced and the lesion distribution (Peeters et al., 1984b, 1985), but it encoded a different plasmid-born adhesin on the ral operon; this adhesin, which is also a virulence factor, is homologous with the K88 (F4) fimbria of bovine and porcine ETEC (Adams et al., 1997).

E. coli O103, and particularly the O103-K:H2 serotype, is the predominant REPEC reported from France and Spain (Camguilhem and Milon, 1989; Blanco et al., 1996, 1997). Virulence is closely associated with the rhamnose non-fermenting biotype (Camguilhem and Milon, 1989) that encodes chromosomal genes for a fimbrial-type adhesin termed AF/R2, which is related to both the ETEC K88 adhesin and the ral-encoded adhesin of REPEC O15 (Pillien et al., 1996; Fiederling et al., 1997). AF/R2 also appears to be encoded by some other REPEC serotypes (Penteado et al., 2002). Experimentally, REPEC O103 strains caused watery or haemorrhagic diarrhoea in weaned rabbits (Camguilhem and Milon, 1989) and elaborated fimbriae transiently in vivo (Heczko et al., 2000).

The diversity of REPEC is underlined by the observation that chromosomal LEE insertion sites vary between isolates (Penteado et al., 2002). Furthermore, adhesion of one REPEC O103-K:H2 strain to the rabbit intestine was dependent upon the presence of a 117 kb plasmid (Licois et al., 1991). This plasmid (pREC-1) also apparently conferred an AE capacity upon laboratory strains of E. coli, including K12, which is LEE-negative (Perna et al., 2001). This suggests that the LEE, or elements thereof, may in some cases be plasmid-born (Licois et al., 1991), an hypothesis supported by the observation of plasmid-like sequences adjacent to the LEE of C. rodentium (Deng et al., 2001).

The roles of LEE genes in AE lesion formation and of AE lesions in the virulence of REPEC have been studied with LEE mutants of virulent REPEC O103:K:H2. Mutants deficient in intimin, Tir, EspA and EspB proved unable to form AE lesions in vitro and to be avirulent in weaned rabbits (Abe et al., 1998; Marches et al., 2000); the Tir and intimin mutants, however, had persistence characteristics similar to those of the wild-type strain. Use of the ligated intestinal loop and oral inoculation techniques in rabbits has proved valuable in studies of AEEC affecting other host species, particularly man (Moon et al., 1983; Fagundes-Neto et al., 1995). Oral inoculation of infant rabbits with EHEC O157:H7 produced colonization of the small and large intestines, AE lesions and non-haemorrhagic diarrhoea (Potter et al., 1985; Pai et al., 1986; Sherman et al., 1988).

A slow, progressive, irreversible and lethal cytopathogenic effect (CPE) was observed in HeLa cells infected with single strains of REPEC (serogroups O103, O26, O132, O-rough), RDEC-1, and human EPEC (de Rycke et al., 1997). The lesion cannot be induced with bacterial culture supernates or cell lysates, i.e., it is associated with intact bacterial cells. It is characterized by the rearrangement of the host actin cytoskeleton into stress fibres, accompanied by vinculin rod formation in the cytoplasm. With REPEC O103, the CPE is dependent on the presence of EspA, EspB and EspD but independent of the presence of intimin or Tir (Nougayrede et al., 1999; Marches et al., 2000); it is also associated with arrest of the HeLa cell cycle (Nougayrede et al., 2001).

In summary, the typical AE pathogens of rabbits are EPEC-like in nature. REPEC strains are diverse in terms of serotype, target age groups, patterns of intestinal colonization, and virulence. Primary, non-intimate adhesion is critical in REPEC virulence and is accomplished apparently by a variety of adhesins, often targeted at the follicle-associated epithelium of the ileal Peyer’s patches. REPEC appears to share this primary target with the important human pathogen EHEC O157:H7 (Phillips and Frankel, 2000; Phillips et al., 2000), although mechanisms of adhesion may differ.

Mice

The only known natural AE pathogen for mice, unlike other host species, is C. rodentium rather than E. coli. It is the aetiological agent of transmissible murine colonic hyperplasia (TMCH), reviewed by
Luperchio and Schauer (2001). TMCH-associated Citrobacter isolates, originally designated atypical Citrobacter freundii, were subsequently renamed Citrobacter freundii biotype 4280 (Barthold et al., 1976) and Citrobacter genospecies 9 (Brenner et al., 1993). Comparison of isolates then led to their further redesignation as C. rodentium, a new species which, uniquely amongst the Citrobacter isolates examined, possessed LEE genes (Schauer et al., 1995). A murine pathogen in Japan, which caused a disease (“infectious megaentron”) similar to TMCH, was classified as an atypical E. coli and termed mouse-pathogenic E. coli (MPEC). However, MPEC was subsequently shown by comparative biochemistry and genetics to be indistinguishable from C. rodentium (Luperchio et al., 2000; Luperchio and Schauer, 2001).

TMCH affects suckling mice and some adult inbred strains; infection of outbred adults is usually subclinical. The factors underlying the susceptibility of mouse strains to overt disease are unclear (Vallance et al., 2003). Clinically affected animals have soft faeces and mortality in suckling mice is high (Barthold et al., 1978). The main gross lesion is a thickening of the intestine commencing with the distal colon and extending in some cases to affect the rest of the colon and sometimes the caecum and ileum (Luperchio and Schauer, 2001). On histopathological examination of experimentally infected mice, heavy colonization of the luminal colonic mucosa and superficial portions of crypts by C. rodentium is seen from 4 days post-inoculation. There is accompanying marked hyperplasia of crypts, peaking 2–3 weeks after inoculation (Barthold et al., 1978; Johnson and Barthold, 1979; Luperchio and Schauer, 2001). Bacterial attachment is AE in nature (Johnson and Barthold, 1979; Luperchio and Schauer, 2001). Multifocal mucosal erosions, ulceration and suppurative inflammation, progressing to mononuclear cell infiltration in survivors, may accompany the hyperplastic changes (Barthold et al., 1978; Luperchio and Schauer, 2001), but the variation in inflammatory responses is poorly understood. In animals that recover, the intestinal lesion regresses completely by 7 weeks post-inoculation of adults but more slowly in young mice (Barthold et al., 1978; Johnson and Barthold, 1979). Acquired immunity, mediated via CD4+ T lymphocytes and possibly B lymphocytes, limited the pathological changes and mortality associated with infection by C. rodentium (Simmons et al., 2003). However, in a highly susceptible strain of mice, the presence of T and B lymphocytes was associated with more severe intestinal changes (Vallance et al., 2003).

The LEE of C. rodentium is closely similar in structure and sequence to that of EPEC, EHEC and RDEC-1 (Rabbit EPEC), but the chromosomal location into which it is inserted differs, being adjacent to plasmid-like sequences (Deng et al., 2001). It therefore appears to have been acquired independently of E. coli LEEs and may have been plasmid-borne. LEE genes of C. rodentium, including eae, tir, espB and espD, are colonization factors in mice (Schauer and Falkow, 1993; Newman et al., 1999; Deng et al., 2003; Mundy et al., 2003); eae and tir are also virulence factors for murine infection (Deng et al., 2003). Outside of the LEE, disruption of a C. rodentium gene cluster which is homologous to EPEC hfp attenuates mouse colonization profoundly, indicating that pilus-mediated non-intimate adhesion may be a significant mechanism for TMCH in vivo (Mundy et al., 2003). This indicates that similar non-intimate adhesins may be used by large intestinal (C. rodentium) as well as small intestinal (EPEC) AE pathogens.

C. rodentium and TMCH offer a promising combination for experimental investigations into AE pathogens of other species, and of human beings in particular. The bacterium has been modified successfully in studies based on the deletion of AE-associated genes and their substitution by genes from other AE pathogens (Frankel et al., 1996b; 1998a; Hartland et al., 2000). This, and the established role of mice in pathogen studies, which may include genetic and immunological manipulation of the host (Ghaem-Maghami et al., 2001; Vallance et al., 2002), has led to the use of C. rodentium to further the understanding of AE bacteria and to develop strategies to combat them. A recent experimental report of AE lesions formed by E. coli O157:H7 in mice (Nagano et al., 2005) indicates that murine models based on direct inoculation of AEEC from other host species may also prove to be useful.

Other Mammals

Domestic carnivores. The literature contains reports of 15 cases of canine enteritis associated with AEEC and verified by histopathology and electron microscopy (Broes et al., 1988; Drolet et al., 1994a; Wada et al., 1996a; Holland et al., 2000). The 15 animals were young, usually less than 3 months of age and none exceeded 5 months. Diarrhoea, which was invariably present, was often chronic (Broes et al., 1988; Wada et al., 1996a; Holland et al., 2000) and non-haemorrhagic (Drolet et al., 1994a). Typically the dogs were seriously ill and concurrently infected with one or more other agents
(distemper virus, parvovirus, coccidia, Giardia and Cryptosporidium). There were often associated additional losses of puppies which were not subjected to pathological examination. Gross findings, apart from soft or watery intestinal contents that were sometimes blood-tinged, were unremarkable or not described. AE lesions were reported in the small intestine of all cases, and in the large intestine of seven of the nine cases in which it was examined. In one unusual case, lesions were described in the small and large intestines and the gastric pylorus (Wada et al., 1996a), in association with evidence of enteric distemper virus. Various serovars of eae-positive E. coli, including O49:H10 (Broes et al., 1988; Drolet et al., 1994a), O118:H- (Wada et al., 1996a), O118, O119 and O115 (Drolet et al., 1994a), as well as O- and untypable strains, were isolated from the cases. In some instances, E. coli O49 (Broes et al., 1988) and O118 (Wada et al., 1996a) were demonstrated in lesions by immunolabelling. Most isolates were examined for enterotoxin and VT, with negative results. Six of eight isolates examined for a BFP sequence by gene probe were positive (Drolet et al., 1994a).

Twenty-three further cases of presumptive AE lesions in dogs with enteritis were diagnosed on the basis of light microscopy by Janke et al. (1989) or Turk et al. (1998). In the first of these reports, of three cases aged 7–8 weeks two had tarry faeces and died acutely, without macroscopical changes in the intestinal mucosa (Janke et al., 1989). Small-intestinal AE lesions were seen in all three cases, and large-intestinal AE lesions in two. In the second report (20 cases), the animals were diarrhoeic and were aged between 3 days and 5 years, with an average of 56 days (Turk et al., 1998). Findings (AE lesions) from the large intestine only were reported, and there was no evidence of VT in E. coli isolates.

A study in dogs (Sancak et al., 2004) demonstrated a significant association between putative AEEC in faeces and diarrhoea (acute or chronic). In the same study a similar association between VTEC and diarrhoea was found. In cases of acute diarrhoea positive for potential enteric pathogens, putative AEEC was the only such agent detected in 20 of 23 cases. In cases of chronic diarrhoea, other infectious and non-infectious factors were frequently present in addition to putative AEEC. Many serogroups and untypable strains were isolated. In an experimental study (Hart et al., 1990), AE lesions were readily induced in vitro on canine jejunal and ileal organ cultures inoculated with a human disease-derived EPEC O111:H-.

When putative canine strains of EPEC (Drolet et al., 1994a) were examined in vitro, it was found that all of nine eae-positive isolates adhered to piglet ileum and produced AE lesions, but only one adhered and formed FAS-positive lesions on HEp-2 cells (Beaudry et al., 1996). Putative AEEC isolated from dogs and cats encompassed a range of serogroups (Holland et al., 1999a). Such eae-positive isolates also frequently encoded the BFP structural gene (bfpA), had a variety of chromosomal insertion sites for the LEE, and produced AE lesions in ligated ileal loops of rabbits (Goffaux et al., 2000). A novel combination of virulence factors (eae plus the ETEC heat-stable enterotoxin) was detected in E. coli isolates from four of 52 dogs (Holland et al., 1999a).

Pospischil et al. (1987) reported fatal enteritis with AE lesions (verified by TEM) in a 2-month-old kitten and an adult cat. AE lesions were present in the ileum and colon of both animals, with accompanying villous atrophy, crypt hyperplasia and acute inflammation. Coronavirus was seen concurrently by electron microscopy in intestinal epithelium from the kitten.

In summary, canine and feline AEEC strains are of diverse serotypes; they are proven pathogens, which are EPEC-like (lacking VT) and can cause spontaneous, sometimes haemorrhagic, enteric disease, often in association with other enteropathogens. Both adults and juveniles are potentially susceptible. BFP sequences, associated with typical human EPEC, are common, but their expression has not been determined. There is some epidemiological evidence of an association between canine diarrhoea and VT-producing organisms, and isolates from some dogs appear to have a mixed AEEC and ETEC genotype.

Horses. The role of E. coli in equine diarrhoea is uncertain. A survey of 304 diarrhoeic and 32 healthy foals failed to show an association between diarrhoea and E. coli encoding a range of virulence factors; LEE genes were not used as markers of potential virulence (Browning et al., 1991). There is no reported clinical evidence that AE lesions are associated with equine enteric disease. However, the potential susceptibility of equine intestine to AE lesions was demonstrated in ileal organ cultures inoculated with a human disease-derived EPEC O111:H- strain (Batt et al., 1989). In addition, of eight eae-positive E. coli strains isolated from 63 diarrhoeic and 30 healthy foals, seven came from the diarrhoeic foals (Holland et al., 1996). None of these putative AEEC strains encoded VT, and three were of the O2 serogroup.
**Primates.** Typical AE lesions were seen in the colon of five captive cotton-top tamarins with acute diarrhoea (Mansfield et al., 2001b). An E. coli O26:NM (non-motile) isolate exhibited localized adhesion on HEp-2 cells; it encoded eae and bfpA but did not possess genes for VT. In a follow-up study of the tamarin colony there was a significant association between faecal isolates encoding eae and colitis (as demonstrated by biopsy). The precise role of AEEC in the colitis that is commonly seen in captive cotton-top tamarins remains unclear, as additional factors (diet, environment, and possibly other pathogens) also appear to play a role (Mansfield et al., 2001b).

Similar findings were reported in simian immunodeficiency virus (SIV)-infected rhesus macaque monkeys with diarrhoea, and an associated EPEC-like O156:NM strain was recovered (Mansfield et al., 2001a). In the same report a review of 96 rhesus macaques with SIV-associated immune deficiency syndrome revealed that AE lesions were present in 27 cases, invariably in the colon and, in severe cases, in the distal small intestine also. All 27 cases had diarrhoea, typically chronic and non-haemorrhagic. AEEC was the most frequent enteropathogen identified, and was the sole enteropathogen in seven cases.

**Birds**

AEEC strains were reported in association with clinical disease in broiler chickens (Fukui et al., 1995). The disease was associated with depression, ruffled feathers and death, but not with diarrhoea. The affected birds, which were 1–2 months of age, came from two separate farms and had typical AE lesions throughout the intestine, particularly in the ileocaecal area. One or more concurrent infections (Marek’s disease, infectious bursal disease, coccidiosis, cryptosporidiosis) were present in each of nine birds examined. The AEEC O103:H- isolate lacked VT and enterotoxins but produced AE lesions experimentally in day-old chicks.

In turkeys, AEEC strains were implicated in the pathogenesis of poult enteritis-mortality syndrome (PEMS), characterized by diarrhoea, depression and dehydration (Guy et al., 2000; Pakpinyo et al., 2002). AE lesions or AEEC strains (lacking genes for VT or BFP) were detected in 10 of 12 affected flocks (Pakpinyo et al., 2002). Inoculation of young turkeys with putative turkey EPEC produced severe disease when accompanied by turkey coronavirus (TCV), but only symptomless intestinal AE lesions when inoculated alone (Guy et al., 2000; Pakpinyo et al., 2002). TCV inoculated alone produced mild disease. Several serogroups, including O111, were represented amongst the turkey EPEC strains implicated in PEMS (Pakpinyo et al., 2002).

AE disease in a 6-month-old pigeon co-infected with intestinal adenovirus was reported by Wada et al. (1995). Pennycott et al. (1998) reported a fatal disease of finches in Scotland, associated with eae-positive E. coli O86:K61, which produced cytolethal distending toxin, but intestinal examination for AE lesions was precluded by autolysis. Further investigation of avian O86:K61 strains showed that the majority encoded eae, and four of five strains produced AE lesions in vitro, but neither of two strains produced detectable AE lesions in inoculated chicks (la Ragione et al., 2002). E. coli O86 does not appear to be a common avian serogroup outside the finch family (Pennycott et al., 1998).

Microbiological surveys demonstrated substantial variation between different avian species in the nature of intestinal putative AEEC. Approximately 10% of healthy pigeons carried E. coli encoding Stx2f, a specific subtype of VT (Dell’Omo et al., 1998; Schmidt et al., 2000; Morabito et al., 2001); almost all strains also carried the eae gene (Dell’Omo et al., 1998; Morabito et al., 2001). The β subtype of intimin and the O45 serogroup appeared to be particularly common amongst VT-producing eae-positive E. coli from pigeons (Morabito et al., 2001; Kobayashi et al., 2002). As pigeon surveys have concentrated upon characterizing VT-positive isolates, the prevalence of eae-positive E. coli not encoding VT is, however, uncertain. By contrast, 15% of healthy broiler chickens carried eae-positive E. coli encoding β-intimin but not VT, and 40% of healthy gulls carried eae-positive, VT-negative E. coli encoding non-β-intimin (Kobayashi et al., 2002). Schremmer et al. (1999) reported that psittacine birds yielded eae-positive E. coli, predominantly from diarrhoeic cases (six of seven isolates); three of the isolates were of serotype O110:H6, and all carried BFP genes, unlike pigeon, broiler and gull isolates (Kobayashi et al., 2002). Thus, the information available, albeit incomplete, suggests that AEEC strains from different avian host species often differ in respect of serovar, intimin subtype, the presence and subtype of VT, and the presence of other virulence factors such as BFP. It is not known whether carriage of these bacterial strains is related to enteric disease in the various host species. In one study, young pigeons that carried VT-producing organisms were significantly lighter in weight than those that did not (Pohl et al., 1994; Morabito et al., 2001). This, together with evidence of AEEC strains in dead finches (Pennycott et al., 1998) and diarrhoeic
Psittaciformes (Schremmer et al., 1999), suggests that AEEC may have clinical effects in birds.

Persistent *E. coli* O157:H7 infection of chicken caeca was induced by oral inoculation of one-day-old chicks with a high dose (10^9 colony-forming units). AE lesions and caecal oedema were induced, but clinical signs were not observed (Beery et al., 1985). Similarly, AE lesions were seen, but only in the caeca, in symptomless chicks inoculated orally with AE strains of various serotypes from calves, chicks, pigs and human beings (Sueyoshi and Nakazawa, 1994).

In summary, birds carry a variety of AE strains, many encoding VT; like mammals, birds appear to be susceptible to AE lesions, but few confirmed cases of AE disease have been reported. Diarrhoea would seem not to be an invariable feature of avian AE disease. Avian AE may often cause clinical disease only in association with other enteropathogens or with immunosuppressive disease. The pathogenic role of avian AE requires further study.

### Attaching-effacing Lesions as a Possible Method of Bacterial Persistence

Whilst some strains of AEEC and *C. rodentium* clearly have a role as enteric pathogens, there may be an additional effect of the LEE, namely the promotion of persistence of AE organisms in individual animals and in animal populations. The presence of *eae*, typically in conjunction with approximately 40 other LEE genes, in a substantial proportion of diverse *E. coli* strains from healthy animals is described above for various host species. Only some of these putative AEEC strains are demonstrably pathogenic, suggesting that the maintenance of the LEE in the wider *E. coli* population confers a survival advantage. One AEEC strain, the human pathogen *EHEC* O157:H7, is normally non-pathogenic but persists in a proportion of cattle and sheep (Cray and Moon, 1995; Kudva et al., 1995; Cornick et al., 2000; Wray et al., 2000; Conedera et al., 2001; Cookson et al., 2002a), pigs (Booher et al., 2002) and possibly other non-human species. The LEE-mediated capacity of *E. coli* O157:H7 to adhere to epithelial cells may play a role in its persistence in animals (Gyles, 1998). In fact, isogenic *eae* mutants are less persistent than wild-type *E. coli* O157:H7 in cattle and sheep (Cornick et al., 2002; Woodward et al., 2005).

When commensal AE organisms colonize the ovine intestinal tract, AE lesion formation sometimes occurs. In seven of 39 experimental lambs, incidental AE lesions were detected in the intestines of clinically normal animals colonized by naturally acquired *E. coli* O115 and O26 and at least one other AE organism (Wales et al., 2005a). Similar observations were made in three of six kid goats (Wales et al., 2005b), and further evidence of AE lesions in the absence of symptoms was reported in turkeys (Guy et al., 2000) and calves (Mainil et al., 1987; Schoonwoerd et al., 1988).

Together, these findings suggest that the results of interactions of AE bacteria with their hosts may range from overt pathogenicity to LEE-promoted commensal persistence. If it becomes possible to intervene in LEE–host interactions, there may be significant benefits in terms of protection against enteric disease and reduction in the persistence of zoonotic AE pathogens in animals.

### Conclusions

AE bacteria are widespread in the intestinal flora of animals and include many pathogenic strains. In addition to the capacity for intimate adhesion to the intestinal mucosa, AE pathogens may possess a variety of other virulence factors, including VT and non-intimate adhesins. The AE capacity appears to have been acquired independently by many AE strains, via insertion of the LEE pathogenicity island at different locations in the bacterial chromosome. AE lesions are typically associated with diarrhoea and may be seen in the small or large intestine (or both), or even in the stomach. The large intestine is usually affected, but colonization of the small intestine is more variable. Haemorrhagic diarrhoea does not appear to be closely associated with VT-producing AEEC. Factors that may affect the presence or severity of AE lesions include: age, concurrent infection with other enteropathogens, and diet. AEEC strains are sometimes regarded as minor pathogens in species such as cattle, but the ubiquity of *E. coli* in intestinal samples may mask potentially significant AE pathogens unless routine screening for relevant virulence markers (e.g., *eae* and certain serogroups) is undertaken. The relationship between veterinary and human pathogenic AEEC strains that share a serotype, for example O26:H11, remains uncertain. The possible role of the LEE as an intestinal colonization factor is under scrutiny in the light of the carriage by animals of proven and putative human AE pathogens.
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References

Abe, A., Heczko, U., Hegele, R. G. and Finlay, B. B. (1998). Two enteropathogenic Escherichia coli type III secreted proteins, EspA and EspB, are virulence factors. Journal of Experimental Medicine, 188, 1907–1916.

Acres, S. D. (1985). Enterotoxicogenic Escherichia coli infections in newborn calves: a review. Journal of Dairy Science, 68, 229–256.

Adams, L. M., Simmons, C. P., Rezmann, L., Strugnell, R. A. and Robins-Browne, R. M. (1997). Identification and characterization of a K88- and CS31A-like operon of a rabbit enteropathogenic Escherichia coli strain which encodes fimbriae involved in the colonization of rabbit intestine. Infection and Immunity, 65, 5222–5230.

Adu-Bobie, J., Frankel, G., Bain, C., Goncalves, A. G., Abe, A., Heczko, U., Hegele, R. G. and Finlay, B. B. with Fig. 2.

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An, H., Fairbrother, J. M., Desautels, C., Mabrouk, T., Aktan, I., Sprigings, K. A., La Ragione, R. M., Woodward, M. J. and Harel, J. (2000). Presence of the LEE (locus of enteroocyte effacement) in pig attaching and effacing Escherichia coli and characterization of eae, espA, espB and espD genes of PEPEC (pig EPEC) strain 1390. Microbial Pathogenesis, 28, 291–300.

Angus, K. W., Tzipori, S. and Gray, E. W. (1982). Intestinal lesions in specific-pathogen-free lambs associated with a cryptosporidium from calves with diarrhoea. Veterinary Pathology, 19, 67–78.

Barlow, A. M., Wales, A. D., Burch, A. A., LaRagione, R. M., Woodward, M. J. and Pearson, G. R. (2005). Attaching and effacing lesions in the intestines of an adult goat associated with natural infection with Escherichia coli O145. Veterinary Record, in press.

Barthold, S. W., Coleman, G. L., Bhatt, P. N., Osbaldeston, G. W. and Jonas, A. M. (1976). The etiology of transmissible murine colonic hyperplasia. Laboratory Animal Science, 26, 889–894.

Barthold, S. W., Coleman, G. L., Jacoby, R. O., Livstone, E. M. and Jonas, A. M. (1978). Transmissible murine colonic hyperplasia. Veterinary Pathology, 15, 223–236.

Batt, R. M., Embaye, H., Hunt, J. and Hart, C. A. (1989). Ultrastructural damage to equine intestinal epithelium induced by enteropathogenic Escherichia coli. Equine Veterinary Journal, 21, 373–375.

Beaudry, M., Zhu, C., Fairbrother, J. M. and Harel, J. (1996). Genotypic and phenotypic characterization of Escherichia coli isolates from dogs manifesting attaching and effacing lesions. Journal of Clinical Microbiology, 34, 144–148.

Beery, J. T., Doyle, M. P. and Schoeni, J. L. (1985). Colonization of chicken ceca by Escherichia coli associated with hemorrhagic colitis. Applied and Environmental Microbiology, 49, 310–315.

Berendson, R., Cheney, C. P., Schad, P. A. and Boedeker, E. C. (1983). Species-specific binding of purified pili (AF/R1) from the Escherichia coli RDEC-1 to rabbit intestinal mucosa. Gastroenterology, 85, 837–845.

Blanco, J. E., Blanco, M., Loustau, J., Mora, A., Balaguer, L., Cuervo, I., Balsalobre, C. and Munoz, F. (1997). Prevalence and characteristics of enteropathogenic Escherichia coli with the eae gene in diarrheic rabbits. Microbiology and Immunology, 41, 77–82.

Blanco, J. E., Blanco, M., Blanco, J., Mora, A., Balaguer, L., Mourino, M., Juarez, A. and Jansen, W. H. (1996). O serogroups, biotypes, and eae genes in Escherichia coli strains isolated from diarrheic and healthy rabbits. Journal of Clinical Microbiology, 34, 3101–3107.

Booher, S. L., Cornick, N. A. and Moon, H. W. (2002). Persistence of Escherichia coli O157:H7 in experimentally infected swine. Veterinary Microbiology, 89, 69–81.

Brenner, D. J., Grimont, P. A., Steigerwalt, A. G., Fanning, G. R., Ageron, E. and Riddle, C. F. (1993). Classification of citrobacteria by DNA hybridization: designation of Citrobacter farmeri sp. nov., Citrobacter youngae sp. nov., Citrobacter braakii sp. nov., Citrobacter werkmanii sp. nov., Citrobacter sedlakii sp. nov., and three unnamed Citrobacter genomospecies. International Journal of Systematic Bacteriology, 43, 645–658.

Bros, A., Drolet, R., Jacques, M., Fairbrother, J. M. and Johnson, W. M. (1988). Natural infection with an attaching and effacing Escherichia coli in a diarrheic puppy. Canadian Journal of Veterinary Research, 52, 280–282.

Browning, G. F., Chalmers, R. M., Snodgrass, D. R., Batt, R. M., Hart, C. A., Ormarod, S. E., Leadon, D., Stoneham, S. J. and Rossdale, P. D. (1991). The prevalence of enteric pathogens in diarrhoeic thoroughbred foals in Britain and Ireland. Equine Veterinary Journal, 23, 405–409.

Camguilhem, R. and Milon, A. (1989). Biotypes and O serogroups of Escherichia coli involved in intestinal infections of weaned rabbits: clues to diagnosis of pathogenic strains. Journal of Clinical Microbiology, 27, 743–747.
Cantey, J. R. and Blake, R. K. (1977). Diarrhea due to *Escherichia coli* in the rabbit: a novel mechanism. *Journal of Infectious Diseases*, **135**, 454–462.

Cantey, J. R. and Inman, L. R. (1981). Diarrhea due to *Escherichia coli* strain RDEC-1 in the rabbit: the Peyer’s patch as the initial site of attachment and colonization. *Journal of Infectious Diseases*, **143**, 440–446.

Cantey, J. R., Lushbaugh, W. B. and Inman, L. R. (1981). Attachment of bacteria to intestinal epithelial cells in diarrhea caused by *Escherichia coli* strain RDEC-1 in the rabbit: stages and role of capsule. *Journal of Infectious Diseases*, **143**, 219–230.

Chanter, N., Morgan, J. H., Bridger, J. C., Hall, G. A. and Reynolds, D. J. (1984). Dysentery in gnotobiotic calves caused by atypical *Escherichia coli*. *Veterinary Record*, **114**, 71.

China, B., Jacquemin, E., Devrin, A. C., Pirson, V. and Mainil, J. (1998). Prevalence and molecular typing of attaching and effacing *Escherichia coli* among calf populations in Belgium. *Veterinary Microbiology*, **63**, 249–259.

Cid, D., Ruiz-Santa-Quiteria, J. A., Marin, I., Sanz, R., Orden, J. A., Amils, R. and de la Fuente, R. (2001). Association between intimin (*eae*) and EspB gene subtypes in attaching and effacing *Escherichia coli* strains isolated from diarrhoeic lambs and goat kids. *Research in Microbiology*, **150**, 323–332.

China, B., Pirson, V. and Mainil, J. (1998). Prevalence and molecular typing of attaching and effacing *Escherichia coli* among calf populations in Belgium. *Veterinary Microbiology*, **63**, 249–259.

Cid, D., Ruiz-Santa-Quiteria, J. A., Marin, I., Sanz, R., Orden, J. A., Amils, R. and de la Fuente, R. (2001). Association between intimin (*eae*) and EspB gene subtypes in attaching and effacing *Escherichia coli* strains isolated from diarrhoeic lambs and goat kids. *Research in Microbiology*, **150**, 323–332.

Conedera, G., Chapman, P. A., Matanson, S., Tisato, E., Dalvit, P. and Zuin, A. (2001). A field survey of *Escherichia coli* O157 ecology on a cattle farm in Italy. *International Journal of Food Microbiology*, **66**, 85–93.

Cookson, A. L., Hayes, C. M., Pearson, G. R., Roe, J. M., Wales, A. D. and Woodward, M. J. (2002b). Isolation from a sheep of an attaching-effacing *E. coli* O115:H7 with a novel combination of virulence factors. *Journal of Medical Microbiology*, **51**, 1041–1049.

Cookson, A. L., Wales, A. D., Roe, J. M., Hayes, C. M., Pearson, G. R. and Woodward, M. J. (2002a). Variation in the persistence of *Escherichia coli* O157:H7 in experimentally inoculated six-week-old conventional lambs. *Journal of Medical Microbiology*, **51**, 1032–1040.

Cornick, N. A., Booher, S. L., Casey, T. A. and Moon, H. W. (2000). Persistent colonization of sheep by *Escherichia coli* O115:H7 and other *E. coli* pathotypes. *Applied and Environmental Microbiology*, **66**, 4926–4934.

Cornick, N. A., Booher, S. L. and Moon, H. W. (2002). Intimin facilitates colonization by *Escherichia coli* O157:H7 in adult ruminants. *Infection and Immunity*, **70**, 2704–2707.

Cray, W. C. and Moon, H. W. (1995). Experimental infection of calves and adult cattle with *Escherichia coli* O157:H7. *Applied and Environmental Microbiology*, **61**, 1586–1590.

Daniell, S. J., Delahay, R. M., Shaw, R. K., Hartland, E. L., Pallen, M. J., Booy, F., Ebel, F., Knutton, S. and Frankel, G. (2001a). Coiled-coil domain of enteropathogenic *Escherichia coli* type III secreted protein EspD is involved in EspA filament-mediated cell attachment and hemolysis. *Infection and Immunity*, **69**, 4055–4064.

Daniell, S. J., Takahashi, N., Wilson, R., Friedberg, D., Rosenshine, I., Booy, F. P., Shaw, R. K., Knutton, S., Frankel, G. and Aizawa, S. (2001b). The filamentous type III secretion translocon of enteropathogenic *Escherichia coli*. *Cellular Microbiology*, **3**, 865–871.

Dean-Nystrom, E. A., Bosworth, B. T., O’Brien, A. D. and Moon, H. W. (1999). Bovine infection with *Escherichia coli* O157:H7. In: *E. coli O157 in Farm Animals*, C. S. Stewart and H. J. Flint, Eds, CAB International, Wallingford, UK, pp. 51–58.

Deibel, C., Kramer, S., Chakraborty, T. and Ebel, F. (1998). EspE, a novel secreted protein of attaching and effacing bacteria, is directly translocated into infected host cells, where it appears as a tyrosine-phosphorylated 90 kDa protein. *Molecular Microbiology*, **28**, 463–474.

De la Fuente, R., Garcia, S., Orden, J. A., Ruiz-Santa-Quiteria, J. A., Diez, R. and Cid, D. (2002). Prevalence and characteristics of attaching and effacing strains of *Escherichia coli* isolated from diarrheic and healthy sheep and goats. *American Journal of Veterinary Research*, **63**, 262–266.

Dell’Omo, G., Morabito, S., Quondam, R., Agrimi, U., Ciuchini, F., Macri, A. and Caprioli, A. (1998). Feral pigeons as a source of verocytotoxin-producing *Escherichia coli*. *Veterinary Record*, **142**, 309–310.

Deng, W., Li, Y., Vallance, B. A. and Finlay, B. B. (2001). Locus of enterocyte effacement from *Citrobacter rodentium* sequence analysis and evidence for horizontal transfer among attaching and effacing pathogens. *Infection and Immunity*, **69**, 6323–6335.

Deng, W., Vallance, B. A., Li, Y., Puente, J. L. and Finlay, B. B. (2003). *Citrobacter rodentium* translocated intimin receptor (Tir) is an essential virulence factor needed for actin condensation, intestinal colonization and colonic hyperplasia in mice. *Molecular Microbiology*, **48**, 95–115.

De Rycke, J., Comtet, E., Chalareng, C., Boury, M., Tasca, C. and Milon, A. (1997). Enteropathogenic *Escherichia coli* O103 from rabbit elicits actin stress fibers and focal adhesions in HeLa epithelial cells, cytopathic effects that are linked to an analog of the locus of enterocyte effacement. *Infection and Immunity*, **65**, 2555–2563.

DeVinney, R., Stein, M., Reinscheid, D., Abe, A., Ruschkowski, S. and Finlay, B. B. (1999). Enterohemorrhagic *Escherichia coli* O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated. *Infection and Immunity*, **67**, 2389–2398.
Dibb-Fuller, M. P., Best, A., Stagg, D. A., Cooley, W. A. and Woodward, M. J. (2001). An in-vitro model for studying the interaction of Escherichia coli O157:H7 and other enteropathogens with bovine primary cell cultures. Journal of Medical Microbiology, 50, 759–769.

Donnenberg, M. S., Donohue-Rolfe, A. and Keusch, G. T. (1989). Epithelial cell invasion: an overlooked property of enteropathogenic Escherichia coli (EPEC) associated with the EPEC adherence factor. Journal of Infectious Diseases, 160, 452–459.

Donnenberg, M. S., Kaper, J. B. and Finlay, B. B. (1997). Interactions between enteropathogenic Escherichia coli and host epithelial cells. Trends in Microbiology, 5, 109–114.

Donnenberg, M. S. and Nataro, J. P. (1995). Methods for studying adhesion of diarrheagenic Escherichia coli. Methods in Enzymology, 253, 324–336.

Duhamel, G. E., Moxley, R. A., Maddox, C. W. and Erickson, E. D. (1992). Enteric infection of a goat with enterohemorrhagic Escherichia coli (O111:H2). Journal of Veterinary Diagnostic Investigation, 4, 197–200.

Ebel, F., Podzadl, T., Rohde, M., Kresse, A. U., Kramer, S., Deibel, C., Guzman, C. A. and Chakraborty, T. (1998). Initial binding of Shiga toxin-producing Escherichia coli to host cells and subsequent induction of actin rearrangements depend on filamentous EspA-containing surface appendages. Molecular Microbiology, 30, 147–161.

Elliott, S. J., Wainwright, L. A., McDaniel, T. K., Jarvis, K. G., Deng, Y. K., Lai, L. C., McNamara, B. P., Donnenberg, M. S. and Kaper, J. B. (1998). The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic Escherichia coli E2348/69. Molecular Microbiology, 28, 1–4.

Fagundes-Neto, U., Freymuller, E., Gatti, M. S., Schmitz, L. G. and Scaletsky, I. (1995). Enteropathogenic Escherichia coli O111:H2 penetrates the small bowel epithelium in an infant with acute diarrhoea. Acta Paediatrica, 84, 453–455.

Fiederling, F., Boury, M., Petit, C. and Milon, M. (1997). Adhesive Factor/Rabbit 2, a new fimbrial adhesin and a virulence factor from Escherichia coli O103, a serogroup enteropathogenic for rabbits. Infection and Immunity, 65, 847–851.

Fischer, J., Maddox, C., Moxley, R., Kinden, D. and Miller, M. (1994). Pathogenicity of a bovine attaching effacing Escherichia coli isolate lacking Shiga-like toxins. American Journal of Veterinary Research, 55, 991–999.

Fitzhenry, R. J., Reece, S., Trabulsi, L. R., Heuschkel, R., Murch, S., Thomson, A., Frankel, G. and Phillips, A. D. (2002). Tissue tropism of enteropathogenic Escherichia coli strains belonging to the O55 serogroup. Infection and Immunity, 70, 4362–4368.

Francis, C. L., Jerse, A. E., Kaper, J. B. and Falkow, S. (1991). Characterization of interactions of enteropathogenic Escherichia coli O127:H6 with mammalian cells in vitro. Journal of Infectious Diseases, 164, 693–703.

Frankel, G., Lider, O., Hershkoviz, R., Mould, A. P., Kachalsky, S. G., Candy, D. C. A., Cahalon, L., Humphries, M. J. and Dougan, G. (1996a). The cell-binding domain of intimin from enteropathogenic Escherichia coli binds to beta 1 integrins. Journal of Biological Chemistry, 271, 20359–20364.

Frankel, G., Phillips, A. D., Novakova, M., Batchelor, M., Hicks, S. and Dougan, G. (1998a). Generation of Escherichia coli intimin derivatives with differing biological activities using site-directed mutagenesis of the intimin C-terminus domain. Molecular Microbiology, 29, 559–570.

Frankel, G., Phillips, A. D., Novakova, M., Field, H., Candy, D. C. A., Schauer, D. B., Douce, G. and Dougan, G. (1996a). Intimin from enteropathogenic Escherichia coli restores murine virulence to a Citrobacter rodentium eaeA mutant: induction of an immunoglobulin A response to intimin and EspB. Infection and Immunity, 64, 5315–5325.

Frankel, G., Phillips, A. D., Rosenshine, I., Dougan, G., Kaper, J. B. and Knutton, S. (1998b). Enteropathogenic and enterohaemorrhagic Escherichia coli: more subversive elements. Molecular Microbiology, 30, 911–921.

Friedberg, D., Umanski, T., Fang, Y. A. and Rosenshine, I. (1999). Hierarchy in the expression of the locus of enterocyte effacement genes of enteropathogenic Escherichia coli. Molecular Microbiology, 34, 941–952.

Fukui, H., Sueyoshi, M., Haritani, M., Nakazawa, M., Naitoh, S., Tani, H. and Uda, Y. (1995). Natural infection with attaching and effacing Escherichia coli (O 103:H-) in chicks. Avian Diseases, 39, 912–918.

Geyid, A., Fletcher, J., Gashe, B. A. and Ljungh, A. (1996). Invasion of tissue culture cells by diarrhoeagenic strains of Escherichia coli which lack the enteroinvasive gene. FEMS Immunology and Medical Microbiology, 14, 15–24.

Ghaem-Maghami, M., Simmons, C. P., Daniell, S., Pizza, M., Lewis, D., Frankel, G. and Dougan, G. (2001). Intimin-specific immune responses prevent bacterial colonization by the attaching-effacing pathogen Citrobacter rodentium. Infection and Immunity, 69, 5597–5605.
Giron, J. A., Ho, A. S. and Schoolnik, G. K. (1991). An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. *Science*, **254**, 710–713.

Goffaux, F., China, B., Janssen, L. and Mainil, J. (2000). Genotypic characterization of enteropathogenic *Escherichia coli* (EPEC) isolated in Belgium from dogs and cats. *Research in Microbiology*, **151**, 865–871.

Goffaux, F., China, B. and Mainil, J. (2001). Organisation and in vitro expression of esp genes of the LEE (locus of enterocyte effacement) of bovine enteropathogenic and enterohemorrhagic *Escherichia coli*. *Veterinary Microbiology*, **83**, 275–286.

Goosney, D. L., DeVinney, R., Pfuetzner, R. A., Frey, E. A., Strynadka, N. C. and Finlay, B. B. (2000). Enteropathogenic E-coli translocated intimin receptor, Tir, interacts directly with alpha-actinin. *Current Biology*, **10**, 735–738.

Gruenheid, S., DeVinney, R., Bladt, F., Goosney, D., Goffaux, F., China, B. and Mainil, J. (2001). Enteropathogenic *E. coli* Tir binds Nck to initiate actin pedestal formation in host cells. *Nature Cell Biology*, **3**, 856–859.

Gunning, R. F., Wales, A. D., Pearson, G. R., Done, E., Cookson, A. L. and Woodward, M. J. (2001). Attaching and effacing lesions in the intestines of two calves associated with natural infection with *Escherichia coli* O26:1111. *Veterinary Record*, **148**, 780–782.

Guy, J. S., Smith, L. G., Breslin, J. J., Vaillancourt, J. P. and Barnes, H. J. (2000). High mortality and growth depression experimentally produced in young turkeys by dual infection with enteropathogenic *Escherichia coli* and turkey coronavirus. *Avian Diseases*, **44**, 105–113.

Gyles, C. L. (1998). Vaccines and shiga toxin-producing *Escherichia coli* in animals. In: *Escherichia coli O157:H7 and other Shiga Toxic-Producing E. coli Strains*, J. B. Kaper and A. D. O’Brien, Eds, ASM Press, Washington, D.C., pp. 434–444.

Hall, G. A., Chanter, N. and Bland, A. P. (1988a). Comparison in gnotobiotic pigs of lesions caused by verotoxigenic and non-verotoxigenic *Escherichia coli*. *Veterinary Pathology*, **25**, 205–210.

Hall, G. A., Reynolds, D. J., Chanter, N., Morgan, J. H., Parsons, K. R., Debney, T. G., Bland, A. P. and Bridger, J. C. (1985). Dysentery caused by *Escherichia coli* (ST02-9) in calves: natural and experimental disease. *Veterinary Pathology*, **22**, 156–163.

Hall, G. A., Reynolds, D. J., Parsons, K. R., Bland, A. P. and Morgan, J. H. (1988b). Pathology of calves with diarrhoea in southern Britain. *Research in Veterinary Science*, **45**, 240–250.

Hart, C. A., Embaye, H., Getty, B., Saunders, J. R. and Batt, R. M. (1990). Ultrastructural lesions to the canine intestinal epithelium caused by enteropathogenic *Escherichia coli*. *Journal of Small Animal Practice*, **31**, 591–594.

Hartland, E. L., Batchelor, M., Delahay, R. M., Hale, C., Matthews, S., Dougan, G., Knutton, S., Connerton, I. and Frankel, G. (1999). Binding of intimin from enteropathogenic *Escherichia coli* to Tir and to host cells. *Molecular Microbiology*, **32**, 151–158.

Hartland, E. L., Huter, V., Higgins, L. M., Goncalves, N. S., Dougan, G., Phillips, A. D., MacDonald, T. T. and Frankel, G. (2000). Expression of intimin gamma from enterohemorrhagic *Escherichia coli* in *Citrobacter rodentium*. *Infection and Immunity*, **68**, 4637–4646.

Hezko, U., Abe, A. and Finlay, B. B. (2000). In vivo interactions of rabbit enteropathogenic *Escherichia coli* O103 with its host: an electron microscopic and histopathologic study. *Microbes and Infection*, **2**, 5–16.

Helie, P., Morin, M., Jacques, M. and Fairbrother, J. M. (1991). Experimental infection of newborn pigs with an attaching and effacing *Escherichia coli* O45 K”E65” strain. *Infection and Immunity*, **59**, 814–821.

Hicks, S., Frankel, G., Kaper, J. B., Dougan, G. and Phillips, A. D. (1998). Role of intimin and bundle-forming pili in enteropathogenic *Escherichia coli* adhesion to pediatric intestinal tissue in vitro. *Infection and Immunity*, **66**, 1570–1578.

Higgins, R. J., Pearson, G. R. and Wray, C. (1997). Attaching and effacing *E. coli*. Microscopic and ultrastructural observation of intestinal infections in pigs. *Advances in Experimental Medicine and Biology*, **412**, 59–62.

Holland, M. S., Kennedy, F. A. and Holland, R. E. (2000). Companion animals as reservoirs of *eaeA*: *Escherichia coli*. *Journal of Veterinary Diagnostic Investigation*, **12**, 78–80.

Holland, R. E., Schmidt, A., Sriranganathan, N., Grimes, S. D., Wilson, R. A., Brown, C. M. and Walker, R. D. (1996). Characterization of *Escherichia coli* isolated from foals. *Veterinary Microbiology*, **48**, 243–255.

Holland, R. E., Walker, R. D., Sriranganathan, N., Wilson, R. A. and Ruhl, D. C. (1999a). Characterization of *Escherichia coli* isolated from healthy dogs. *Veterinary Microbiology*, **70**, 261–268.

Holland, R. E., Wilson, R. A., Holland, M. S., YuzbasiyanGurkan, V., Mullaney, T. P. and White, D. G. (1999b). Characterization of *eae* (+) *Escherichia coli* isolated from healthy and diarrheic calves. *Veterinary Microbiology*, **66**, 251–263.

Hueck, C. J. (1998). Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiology and Molecular Biology Reviews*, **62**, 379–433.

Iijima, T., Sueyoshi, M., Yamamoto, T., Yoshioha, K. and Nakazawa, M. (1990). Diarrhea due to “attaching and effacing *Escherichia coli* (O 26)” infection in a calf. *Nippon Juigaku Zasshi (Japanese Journal of Veterinary Science)*, **52**, 1347–1350.

Inman, L. R. and Cantey, J. R. (1983). Specific adherence of *Escherichia coli* (strain RDEC-1) to membranous (M) cells of the Peyer’s patch in *Escherichia coli* diarrhea in the rabbit. *Journal of Clinical Investigation*, **71**, 1–8.

Ismaili, A., McWhirter, E., Handselman, M. Y. C., Brunswick, J. L. and Sherman, P. M. (1998). Divergent signal
transduction responses to infection with attaching and effacing *Escherichia coli*. *Infection and Immunity*, 66, 1688–1696.

Ismaili, A., Philpott, D. J., Dytoc, M. T. and Sherman, P. M. (1995a). Signal transduction responses following adhesion of verocytotoxin-producing *Escherichia coli*. *Infection and Immunity*, 63, 3316–3326.

Ismaili, A., Philpott, D. J., Dytoc, M. T., Soni, R., Ratnam, S. and Sherman, P. M. (1995b). Alpha-actinin accumulation in epithelial cells infected with attaching and effacing gastrointestinal pathogens. *Journal of Infectious Diseases*, 172, 1393–1396.

Janke, B. H., Francis, D. H., Collins, J. E., Libal, M. C., Zeman, D. H. and Johnson, D. J. (1989). Attaching and effacing *Escherichia coli* infections in calves, pigs, lambs, and dogs. *Journal of Veterinary Diagnostic Investigation*, 1, 6–11.

Janke, B. H., Francis, D. H., Collins, J. E., Libal, M. C., Zeman, D. H., Johnson, D. D. and Neiger, R. D. (1990). Attaching and effacing *Escherichia coli* infection as a cause of diarrhea in young calves. *Journal of the American Veterinary Medical Association*, 196, 897–901.

Jerse, A. E. and Kaper, J. B. (1991). The eae gene of enteropathogenic *Escherichia coli* encodes a 94-kilodalton membrane protein, the expression of which is influenced by the EAF plasmid. *Infection and Immunity*, 59, 4302–4309.

Johnson, E. and Barthold, S. W. (1979). The ultrastructure of transmissible murine colonic hyperplasia. *American Journal of Pathology*, 97, 291–313.

Jores, J., Zehmke, K., Eichberg, J., Rumer, L. and Wieler, L. H. (2003). Description of a novel intimin variant (type zeta) in the bovine O84:NM verotoxin-producing *Escherichia coli* strain 537/89 and the diagnostic value of intimin typing. *Experimental Biology and Medicine*, 228, 370–376.

Kalman, D., Weiner, O. D., Goosney, D. L., Sedat, J. W., Finlay, B. B., Abo, A. and Bishop, J. M. (1999). Enteropathogenic *E. coli* acts through WASP and Arp2/3 complex to form actin pedestals. *Nature Cell Biology*, 1, 389–391.

Kaper, J. B., Elliott, S., Sperandio, V., Perna, N. T., Mayhew, G. F. and Blattner, F. R. (1998). Attaching-and-effacing intestinal histopathology and the locus of enterocyte effacement. In: *Escherichia coli O157:H7 and Other Shiga Toxin-producing E. coli Strains*, A. D. O’Brien and J. B. Kaper, Eds, ASM Press, Washington, D.C., pp. 163–182.

Kenny, B. (1999). Phosphorylation of tyrosine 474 of the enteropathogenic *Escherichia coli* (EPEC) Tir receptor molecule is essential for actin nucleating activity and is preceded by additional host modifications. *Molecular Microbiology*, 31, 1229–1241.

Kenny, B., Devinney, R., Stein, M., Reinscheid, D. J., Frey, E. A. and Finlay, B. B. (1997). Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell*, 91, 511–520.

Knutton, S., Baldini, M. M., Kaper, J. B. and McNeish, A. S. (1987). Role of plasmid-encoded adherence factors in adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells. *Infection and Immunity*, 55, 78–85.

Knutton, S., Baldwin, T., Williams, P. H. and McNeish, A. S. (1989). Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. *Infection and Immunity*, 57, 1290–1298.

Knutton, S., Phillips, A. D., Smith, H. R., Gross, R. J., Shaw, R., Watson, P. and Price, E. (1991). Screening for enteropathogenic *Escherichia coli* in infants with diarrhea by the fluorescent-actin staining test. *Infection and Immunity*, 59, 365–371.

Knutton, S., Rosenshine, I., Pallen, M. J., Nisan, I., Neves, B. C., Bain, C., Wolf, C., Dougan, G. and Frankel, G. (1998). A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *EMBO Journal*, 17, 2166–2176.

Kobayashi, H., Pohjanvirta, T. and Pelkonen, S. (2002). Prevalence and characteristics of intimin- and Shiga toxin-producing *Escherichia coli* from gulls, pigeons and broilers in Finland. *Journal of Veterinary Medical Science*, 64, 1071–1073.

Kudva, I. T., Hatfield, P. G. and Hovde, C. J. (1995). Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. *Applied and Environmental Microbiology*, 61, 1563–1570.

La Ragione, R. M., McLaren, I. M., Foster, G., Cooley, W. A. and Woodward, M. J. (2002). Phenotypic and genotypic characterization of avian *Escherichia coli* O86:K61 isolates possessing a gamma-like intimin. *Applied and Environmental Microbiology*, 68, 4932–4942.

Levine, M. M., Nataro, J. P., Karch, H., Baldini, M. M., Kaper, J. B., Black, R. E., Clements, M. L. and O’Brien, A. D. (1985). The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. *Journal of Infectious Diseases*, 152, 550–559.

Licois, D., Reynaud, A., Federighi, M., Gaillardmartinie, B., Guillot, J. F. and Joly, B. (1991). Scanning and transmission electron-microscopic study of adherence of *Escherichia coli* O103 enteropathogenic and or enterohaemorrhagic strain GV in enteric infection in rabbits. *Infection and Immunity*, 59, 3796–3800.

Liu, H., Magoun, L., Luperchio, S., Schauer, D. B. and Leong, J. M. (1999). The Tir-binding region of enterohaemorrhagic *Escherichia coli* intimin is sufficient to trigger actin condensation after bacterial-induced host cell signalling. *Molecular Microbiology*, 34, 67–81.

Louie, M., de Azavedo, J. C., Handelsman, M. Y., Clark, C. G., Ally, B., Dytoc, M., Sherman, P. and Brunton, J. (1993). Expression and characterization of the eaeA gene product of *Escherichia coli* serotype O157:H7. *Infection and Immunity*, 61, 4085–4092.
Luo, Y., Frey, E. A., Pfu¨etzner, R. A., Creagh, A. L., Knoechel, D. G., Haynes, C. A., Finlay, B. B. and Srynadka, N. G. (2000). Crystal structure of enteropathogenic Escherichia coli intimin-receptor complex. Nature, 405, 1073–1077.

Luperchio, S. A., Newman, J. V., Dangler, C. A., Schrenzel, M. D., Brenner, D. J., Steigerwalt, A. G. and Schauer, D. B. (2000). Citrobacter rodentium, the causative agent of transmissible murine colonic hyperplasia, exhibits clonality: synonymy of C. rodentium and mouse-pathogenic Escherichia coli. Journal of Clinical Microbiology, 38, 4343–4350.

Luperchio, S. A. and Schauer, D. B. (2001). Molecular pathogenesis of Citrobacter rodentium and transmissible murine colonic hyperplasia. Microbes and Infection, 3, 335–340.

Mainil, J. G., Duchesnes, C. J., Whipp, S. C., Marques, L. R., O’Brien, A. D., Casey, T. A. and Moon, H. W. (1987). Shiga-like toxin production and attaching effacing activity of Escherichia coli associated with calf diarrhea. American Journal of Veterinary Research, 48, 743–748.

Mainil, J. G., Jacquemin, E. R., Kaeckenbeek, A. E. and Pohl, P. H. (1993). Association between the effacing (eae) gene and the Shiga-like toxin-encoding genes in Escherichia coli isolates from cattle. American Journal of Veterinary Research, 54, 1064–1068.

Mansfield, K. G., Lin, K. C., Newman, J., Schauer, D., MacKey, J., Lackner, A. A. and Carville, A. (2001a). Identification of enteropathogenic Escherichia coli in simian immunodeficiency virus-infected infant and adult rhesus macaques. Journal of Clinical Microbiology, 39, 971–976.

Mansfield, K. G., Lin, K. C., Xia, D. L., Newman, J. V., Schauer, D. B., MacKey, J., Lackner, A. A. and Carville, A. (2001b). Enteropathogenic Escherichia coli and ulcerative colitis in cotton-top tamarins (Saguinus oedipus). Journal of Infectious Diseases, 184, 803–807.

Marches, O., Nougayrede, J. P., Boulier, S., Mainil, J., Charlier, G., Raymond, I., Pohl, P., Boury, M., De Rycke, J., Milon, A. and Oswald, E. (2000). Role of Tir and intimin in the virulence of rabbit enteropathogenic Escherichia coli serotype O103:H2. Infection and Immunity, 68, 2171–2182.

McDaniel, T. K., Jarvis, K. G., Donnenberg, M. S. and Kaper, J. B. (1995). A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. Proceedings of the National Academy of Sciences of the United States of America, 92, 1664–1668.

McDaniel, T. K. and Kaper, J. B. (1997). A cloned pathogenicity island from enteropathogenic Escherichia coli confers the attaching and effacing phenotype on E. coli K-12. Molecular Microbiology, 23, 399–407.

McKee, M. L., Melton-Celsa, A. R., Moxley, R. A., Francis, D. H. and O’Brien, A. D. (1995). Enterohemorrhagic Escherichia coli O157:H7 requires intimin to colonize the gnotobiotic pig intestine and to adhere to HEp-2 cells. Infection and Immunity, 63, 3739–3744.

McKee, M. L. and O’Brien, A. D. (1995). Investigation of enterohemorrhagic Escherichia coli O157:H7 adherence characteristics and invasion potential reveals a new attachment pattern shared by intestinal E. coli. Infection and Immunity, 63, 2070–2074.

Mellies, J. L., Elliott, S. J., Sperandio, V., Donnenberg, M. S. and Kaper, J. B. (1999). The Per regulon of enteropathogenic Escherichia coli: identification of a regulatory cascade and a novel transcriptional activator, the locus of enterocyte effacement (LEE)-encoded regulator (Ler). Molecular Microbiology, 33, 296–306.

Milon, A., Oswald, E. and de Rycke, J. (1999). Rabbit EPEC: a model for the study of enteropathogenic Escherichia coli. Veterinary Research, 30, 203–219.

Moon, H. W., Whipp, S. C., Argenzio, R. A., Levine, M. M. and Giannella, R. A. (1983). Attaching and effacing activities of rabbit and human enteropathogenic Escherichia coli in pig and rabbit intestines. Infection and Immunity, 41, 1340–1351.

Morabito, S., Dell’Omo, G., Agrimi, U., Schmidt, H., Karch, H., Cheasty, T. and Caprioli, A. (2001). Detection and characterization of Shiga toxin-producing Escherichia coli in feral pigeons. Veterinary Microbiology, 82, 275–283.

Moxley, R. A. and Francis, D. H. (1986). Natural and experimental infection with an attaching and effacing strain of Escherichia coli in calves. Infection and Immunity, 53, 339–346.

Mundy, R., Pickard, D., Wilson, R. K., Simmons, C. P., Dougan, G. and Frankel, G. (2003). Identification of a novel type IV pilus gene cluster required for gastrointestinal colonization of Citrobacter rodentium. Molecular Microbiology, 48, 795–809.

Nagano, K., Taguchi, K., Hara, T., Yokoyama, S., Kawada, K. and Mori, H. (2003). Adhesion and colonization of enterohemorrhagic Escherichia coli O157:H7 in cecum of mice. Microbiology and Immunology, 47, 125–132.

Nataro, J. P. and Kaper, J. B. (1998). Diarrheagenic Escherichia coli. Clinical Microbiology Reviews, 11, 142–201.

Neef, N. A., McOrist, S., Lyons, R. J., Bland, A. P. and Miller, B. G. (1994). Development of large intestinal attaching and effacing lesions in pigs in association with the feeding of a particular diet. Infection and Immunity, 62, 4325–4332.

Newman, J. V., Zabel, B. A., Jha, S. S. and Schauer, D. B. (1999). Citrobacter rodentium espB is necessary for signal transduction and for infection of laboratory mice. Infection and Immunity, 67, 6019–6025.

Nougayrede, J. P., Boury, M., Tasca, C., Marches, O., Milon, A., Oswald, E. and De Rycke, J. (2001). Type III secretion-dependent cell cycle block caused in HeLa cells by enteropathogenic Escherichia coli O103. Infection and Immunity, 69, 6785–6795.

Nougayrede, J. P., Marches, O., Boury, M., Mainil, J., Charlier, G., Pohl, P., de Rycke, J., Milon, A. and Oswald, E. (1999). The long-term cytoskeletal...
O’Loughlin, E. V. and Robins-Browne, R. M. (2001). Effect of Shiga toxin and Shiga-like toxins on eukaryotic cells. *Microbes and Infection*, 3, 493–507.

Orden, J. A., Cid, D., Ruiz-Santa-Quiteria, J. A., García, S., Martinez, S. and de la Fuente, R. (2002). Verotoxin-producing *Escherichia coli* (VTEC), enteropathogenic *E. coli* (EPEC) and necrotogenic *E. coli* (NTEC) isolated from healthy cattle in Spain. *Journal of Applied Microbiology*, 93, 29–35.

Orden, J. A., Ruiz-Santa-Quiteria, J. A., Cid, D., García, S., Sanz, R. and de la Fuente, R. (1998). Verotoxin-producing *Escherichia coli* (VTEC) and eae-positive non-VTEC in 1–30-days-old diarrhoeic dairy calves. *Veterinary Microbiology*, 63, 239–248.

Orden, J. A., Ruiz-Santa-Quiteria, J. A., García, S., Cid, D. and de la Fuente, R. (2000). Quinolone resistance in *Escherichia coli* strains isolated from diarrhoeic lambs in Spain. *Veterinary Record*, 147, 576–578.

Orden, J. A., Yuste, M., Cid, D., Piacesi, T., Martinez, S., Ruiz-Santa-Quiteria, J. A. and Dela Fuente, R. (2003). Typing of the *eae* and espB genes of attaching and effacing *Escherichia coli* isolates from ruminants. *Veterinary Microbiology*, 96, 203–215.

Oswald, E., Schmidt, H., Morabito, S., Karch, H., Marches, O. and Caprioli, A. (2000). Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli* characterization of a new intimin variant. *Infection and Immunity*, 68, 64–71.

Pai, C. H., Kelly, J. K. and Meyers, G. L. (1986). Experimental infection of infant rabbits with verotoxin-producing *Escherichia coli*. *Infection and Immunity*, 51, 16–23.

Pakpinyo, S., Ley, D. H., Barnes, H. J., Vaillancourt, J. P. and Guy, J. S. (2002). Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. *Infection and Immunity*, 68, 360–369.

Paton, J. C. and Paton, A. W. (1998). Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clinical Microbiology Reviews*, 11, 450–479.

Pearson, G. R., Bazeley, K. J., Jones, J. R., Gunning, R. F., Green, M. J., Cookson, A. and Woodward, M. J. (1999). Attaching and effacing lesions in the large intestine of an eight-month-old heifer associated with *Escherichia coli* O26 infection in a group of animals with dysentery. *Veterinary Record*, 145, 370–373.

Pearson, G. R., Watson, C. A., Hall, G. A. and Wray, C. (1989). Natural infection with an attaching and effacing *Escherichia coli* in the small and large intestines of a calf with diarrhoea. *Veterinary Record*, 124, 297–299.

Peeters, J. E., Charlier, G. J. and Halen, P. H. (1984a). Pathogenicity of attaching effacing enteropathogenic *Escherichia coli* isolated from diarrhoeic suckling and weanling rabbits for newborn rabbits. *Infection and Immunity*, 46, 690–696.

Peeters, J. E., Charlier, G. J. and Raeymaekers, R. (1985). Scanning and transmission electron microscopy of attaching effacing *Escherichia coli* in weanling rabbits. *Veterinary Pathology*, 22, 54–59.

Peeters, J. E., Geeroms, R. and Gloreux, B. (1984b). Experimental *Escherichia coli* enteropathy in weanling rabbits: clinical manifestations and pathological findings. *Journal of Comparative Pathology*, 94, 521–528.

Peeters, J. E., Geeroms, R. and Orskov, F. (1988). Biotype, serotype, and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated from diarrhoeic commercial rabbits. *Infection and Immunity*, 56, 1442–1448.

Peeters, J. E., Pohl, P. and Charlier, G. (1984c). Infectious agents associated with diarrhoea in commercial rabbits: a field study. *Annales de Recherches Vétérinaires*, 15, 335–340.

Pennycook, T. W., Ross, H. M., McLaren, I. M., Park, A., Hopkins, G. F. and Foster, G. (1998). Causes of death of wild birds of the family Fringillidae in Britain. *Veterinary Record*, 143, 155–158.

Penteado, A. S., Aitdar, L., de Castro, A. F. P., Yamada, A., Andrade, J. R. C., Blanco, J., Blanco, M. and Blanco, J. E. (2001). *eae*-Negative attaching and effacing *Escherichia coli* from piglets with diarrhea. *Research in Microbiology*, 152, 75–81.

Penteado, A. S., Ugrinovich, L. A., Blanco, J., Blanco, M., Blanco, J. E., Mora, A., Andrade, J. R. C., Correa, S. S. and de Castro, A. F. P. (2002). Serobiotypes and virulence genes of *Escherichia coli* strains isolated from diarrhoeic and healthy rabbits in Brazil. *Veterinary Microbiology*, 89, 41–51.

Perna, N. T., Mayhew, G. F., Posfai, G., Elliott, S., Donnenberg, M. S., Kaper, J. B. and Blattner, F. R. (1998). Molecular evolution of a pathogenicity island from enterohemorrhagic *Escherichia coli* O157:H7. *Infection and Immunity*, 66, 3810–3817.

Perna, N. T., Plunkett, G., Burland, V., Mau, B., Glaser, J. D., Rose, D. J., Mayhew, G. F., Evans, P. S., Gregor, J., Kirkpatrick, H. A., Posfai, G., Hackett, J., Klink, S., Boutin, A., Shao, Y., Miller, L., Grotbeck, E. J., Davis, N. W., Link, A., Dimalanta, E. T., Potamousis, K. D., Apodaca, J., Anantharaman, T. S., Lin, J. Y., Yen, G., Schwartz, D. C., Welch, R. A. and Blattner, F. R. (2001). Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature*, 409, 529–533.

Phillips, A. D. and Frankel, G. (2000). Intimin-mediated tissue specificity in enteropathogenic *Escherichia coli* interaction with human intestinal organ cultures. *Journal of Infectious Diseases*, 181, 1496–1500.

Phillips, A. D., Navabpour, S., Hicks, S., Dougan, G., Wallis, T. and Frankel, G. (2000).
Enterohaemorrhagic *Escherichia coli* O157:H7 target Peyer’s patches in humans and cause attaching/effacing lesions in both human and bovine intestine. *Gut*, 47, 377–381.

Pillien, F., Chalareng, C., Boury, M., Tasca, C., de Rycke, J. and Milon, A. (1996). Role of Adhesive Factor-Rabbit 2 in experimental enteropathogenic *Escherichia coli* O103 diarrhea of weaned rabbits. *Veterinary Microbiology*, 50, 105–115.

Pohl, P., Marin, M., Imberechts, H., Attaching-effacing, D. N. A. and Mainil, J. G. (1994). (eaeA) are significantly more frequent in verotoxigenic *Escherichia coli* isolates from diarrhoeic than from healthy calves. *Annales de Medecine Veterinaire*, 138, 597–599.

Pohl, P. H., Peeters, J. E., Jacquemin, E. R., Lintemans, P. F. and Mainil, J. G. (1993). Identification of eae sequences in enteropathogenic *Escherichia coli* strains from rabbits. *Infection and Immunity*, 61, 2203–2206.

Polotsky, Y. E., Dragunskaya, E. M., Seliverstova, V. G., Avdeeva, T. A., Chakhutinskaya, M. G., Ketyi, I., Vertenyl, A., Falovich, B., Emody, L., Malovics, I., Safonova, N. V., Snigirevskaia, E. S. and Karyagina, E. I. (1977). Pathogenic effect of enterotoxigenic *Escherichia coli* and *Escherichia coli* causing infantile diarrhoea. *Acta Microbiologica Academiae Scientiarum Hungaricae*, 24, 221–236.

Pospischil, A., Mainil, J. G., Baljer, G. and Moon, H. W. (1987). Attaching and effacing bacteria in the intestines of calves and cats with diarrhea. *Veterinary Pathology*, 24, 330–334.

Potter, M. E., Kaufmann, A. F., Thomason, B. M., Blake, P. A. and Farmer, J. D. (1985). Diarrhea due to *Escherichia coli* O157:H7 in the infant rabbit. *Journal of Infectious Diseases*, 152, 1341–1343.

Prescott, J. F. (1978). *Escherichia coli* and diarrhoea in the rabbit. *Veterinary Pathology*, 15, 237–248.

Raimondi, F., Kaper, J. B., Boedeker, E. C., Wolf, M. K., Guandalini, S. and Fasano, A. (2001). Enteropathogenic *Escherichia coli* strain RDEC-1 produces a novel electrogenic factor active on rabbit ileum in vitro.*Journal of Pediatric Gastroenterology and Nutrition*, 32, 122–126.

Rosenshine, I., Donnenberg, M. S., Kaper, J. B. and Finlay, B. B. (1999). Enteropathogenic *Escherichia coli* strain of *Citrobacter rodentium* sp. nov. *Journal of Clinical Microbiology*, 33, 2064–2068.

Schauer, D. B., Zabel, B. A., Pedraza, I. F., O’Hara, C. M., Steigerwalt, A. G. and Brenner, D. J. (1995). Genetic and biochemical characterization of *Citrobacter rodentium* sp. nov. *Journal of Clinical Microbiology*, 33, 2064–2068.

Schmidt, H., Scheef, J., Morabito, S., Caprioli, A., Wieler, L. H. and Karch, H. (2000). A new Shiga toxin 2 variant (Stx2f) from *Escherichia coli* isolated from pigeons. *Applied and Environmental Microbiology*, 66, 1205–1208.

Schoonderwoerd, M., Clarke, R. C., Van Dreumel, A. A. and Rawluk, S. A. (1988). Colitis in calves: natural and experimental infection with a verotoxin-producing strain of *Escherichia coli* O111:NM. *Canadian Journal of Veterinary Research*, 52, 484–487.

Schremmer, C., Lohr, J. E., Westphlhuber, U., Kosters, J., Ravelshofer, K., Steinruick, H. and Wieler, L. H. (1999). Enteropathogenic *Escherichia coli* in *Psittaci* forms. *Avian Pathology*, 28, 349–354.

Shaw, R. K., Daniell, S., Frankel, G. and Knutton, S. (2002). Enteropathogenic *Escherichia coli* translocate Tir and form an intimin-Tir intimate attachment to red blood cell membranes. *Microbiology*, 148, 1355–1365.

Sherman, P., Soni, R. and Karmali, M. (1988). Attaching and effacing adherence of Vero cytotoxin-producing *Escherichia coli* to rabbit intestinal epithelium in vivo. *Infection and Immunity*, 56, 756–761.

Simmons, G. P., Clare, S., Ghaem-Maghami, M., Uren, T. K., Rankin, J., Huet, A., Goldin, R., Lewis, D. J., MacDonald, T. T., Strugnell, R. A., Frankel, G. and Dougan, G. (2003). Central role for B lymphocytes and CD4+/T cells in immunity to infection by the attaching and effacing pathogen *Citrobacter rodentium*. *Infection and Immunity*, 71, 5077–5086.

Simonovic, I., Arpin, M., Koutsouris, A., Falk-Krzesinski, H. J. and Hecht, G. (2001). Enteropathogenic *Escherichia coli* activates ezrin, which participates in disruption of tight junction barrier function. *Infection and Immunity*, 69, 5679–5688.

Sperandio, V., Mellies, J. L., Nguyen, W., Shin, S. and Kaper, J. B. (1999). Quorum sensing controls...
expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic Escherichia coli. Proceedings of the National Academy of Sciences of the United States of America, 96, 15196–15201.

Sperandio, V., Torres, A. G., Giron, J. A. and Kaper, J. B. (2001). Quorum sensing is a global regulatory mechanism in enterohemorrhagic Escherichia coli O157:H7. Journal of Bacteriology, 183, 5187–5197.

Sperandio, V., Torres, A. G., Jarvis, B., Nataro, J. P. and Kaper, J. B. (2005). Bacteria–host communication: the language of hormones. Proceedings of the National Academy of Sciences of the United States of America, 100, 8951–8956.

Staley, T. E., Jones, E. W. and Corley, L. D. (1969). Attachment and penetration of Escherichia coli into intestinal epithelium of the ileum in newborn pigs. American Journal of Pathology, 65, 371–392.

Stone, K. D., Zhang, H. Z., Carlson, L. K. and Donnenberg, M. S. (1996). A cluster of fourteen genes from enteropathogenic Escherichia coli is sufficient for the biogenesis of a type IV pilus. Molecular Microbiology, 20, 325–337.

Stordeur, P., China, B., Charlier, G., Roels, S. and Mainil, J. (2000). Clinical signs, reproduction of attaching-effacing lesions, and enterocyte invasion after oral inoculation of an O118 enterohaemorrhagic Escherichia coli in neonatal calves. Microbes and Infection, 2, 17–24.

Sueyoshi, M. and Nakazawa, M. (1994). Experimental infection of young chicks with attaching and effacing Escherichia coli. Infection and Immunity, 62, 4066–4071.

Takeuchi, A., Inman, L. R., O’Hanley, P. D., Cantey, J. R. and Lushbaugh, W. B. (1978). Scanning and transmission electron microscopic study of Escherichia coli O15 (RDEC-1) enteric infection in rabbits. Infection and Immunity, 19, 686–694.

Tatsuno, I., Kimura, H., Okutani, A., Kanamaru, K., Abe, H., Nagai, S., Makino, K., Shinagawa, H., Yoshida, M., Sato, K., Nakamoto, J., Tobe, T. and Sasakawa, C. (2000). Isolation and characterization of mini-Tn5Km2 insertion mutants of enterohemorrhagic Escherichia coli O157:H7 deficient in adherence to Caco-2 cells. Infection and Immunity, 68, 5943–5952.

Tesh, V. L. and O’Brien, A. D. (1992). Adherence and colonization mechanisms of enteropathogenic and enterohemorrhagic Escherichia coli. Microbial Pathogenesis, 12, 245–254.

Tominaga, K., Nakazawa, M., Haritani, M. and Hirata, K. (1989). Bio-chemical characteristics and pathogenicity of attaching and effacing Escherichia coli (AEEC) isolated from calves with diarrhoea. Journal of the Japanese Veterinary Medical Association, 42, 775–779.

Turk, J., Maddox, C., Fales, W., Ostlund, E., Miller, M., Johnson, G., Pace, L., Turnquist, S. and Kreeger, J. (1998). Examination for heat-labile, heat-stable, and Shiga-like toxins and for the eaeA gene in Escherichia coli isolates obtained from dogs dying with diarrhea: 122 cases (1992–1996). Journal of the American Veterinary Medical Association, 212, 1733–1736.

Tzipori, S., Gibson, R. and Montanaro, J. (1989). Nature and distribution of mucosal lesions associated with enteropathogenic and enterohemorrhagic Escherichia coli in piglets and the role of plasmid-mediated factors. Infection and Immunity, 57, 1142–1150.

Tzipori, S., Gunzer, F., Donnenberg, M. S., de Montigny, L., Kaper, J. B. and Donohue-Rolfe, A. (1995). The role of the eaeA gene in diarrhea and neurological complications in a gnotobiotic piglet model of enterohemorrhagic Escherichia coli infection. Infection and Immunity, 63, 3621–3627.

Tzipori, S., Robins-Browne, R. M., Gonis, G., Hayes, J., Withers, M. and McCartney, E. (1985). Enteropathogenic Escherichia coli enteritis: evaluation of the gnotobiotic piglet as a model of human infection. Gut, 26, 570–578.

Tzipori, S., Wachsmuth, I. K., Chapman, C., Birden, R., Brittingham, J., Jackson, C. and Hogg, J. (1986). The pathogenesis of hemorrhagic colitis caused by Escherichia coli O157:H7 in gnotobiotic piglets. Journal of Infectious Diseases, 154, 712–716.

Ulshen, M. H. and Rollo, J. L. (1980). Pathogenesis of Escherichia coli gastroenteritis in man—another mechanism. New England Journal of Medicine, 302, 99–101.

Vallance, B. A., Deng, W., Jacobson, K. and Finlay, B. B. (2003). Host susceptibility to the attaching and effacing bacterial pathogen Citrobacter rodentium. Infection and Immunity, 71, 3443–3453.

Vallance, B. A., Deng, W., Knodler, L. A. and Finlay, B. B. (2002). Mice lacking T and B lymphocytes develop transient colitis and crypt hyperplasia yet suffer impaired bacterial clearance during Citrobacter rodentium infection. Infection and Immunity, 70, 2070–2081.

von Moll, L. K. and Cantey, J. R. (1997). Peyer’s patch adherence of enteropathogenic Escherichia coli strains in rabbits. Infection and Immunity, 65, 3788–3793.

Vorster, J. H., Henton, M. M., Bastianello, S. S. and Van der Lugt, J. J. (1994). Attaching and effacing Escherichia coli strains as a cause of diarrhoea and mortality in calves in South Africa. Journal of the South African Veterinary Association, 65, 3.

Wada, Y., Kondo, H., Nakaoka, Y. and Kubo, M. (1996a). Gastric attaching and effacing Escherichia coli lesions in a puppy with naturally occurring enteric colibacillosis and concurrent canine distemper virus infection. Veterinary Pathology, 33, 717–720.

Wada, Y., Kondo, H., Nakazawa, M. and Kubo, M. (1995). Natural infection with attaching and effacing Escherichia coli and adenovirus in the intestine of a pigeon with diarrhea. Journal of Veterinary Medical Science, 57, 531–533.

Wada, Y., Kondo, H., Sueyoshi, M., Kubo, M. and Adachi, Y. (1997). A novel developmental process of intestinal epithelial lesions in a calf infected with attaching and effacing Escherichia coli. Journal of Veterinary Medical Science, 59, 401–403.
Wieler, L. H., Busse, B., Steinruck, H., Beutin, L., Weber, A., Karch, H. and Baljer, G. (2000). Enterohemorrhagic Escherichia coli (EHEC) strains of serogroup O118 display three distinctive clonal groups of EHEC pathogens. Journal of Clinical Microbiology, 38, 2162–2169.

Wieler, L. H., Schwanitz, A., Vieler, E., Busse, B., Steinruck, H., Kaper, J. B. and Baljer, G. (1998). Virulence properties of Shiga toxin-producing Escherichia coli (STEC) strains of serogroup O118, a major group of STEC pathogens in calves. Journal of Clinical Microbiology, 36, 1604–1607.

Willshaw, G. A., Scotland, S. M., Smith, H. R. and Rowe, B. (1992). Properties of Vero cytotoxin-producing Escherichia coli of human origin of O serogroups other than O157. Journal of Infectious Diseases, 166, 797–802.

Wolf, M. K., Andrews, G. P., Fritz, D. L., Sjogren, R. W. and Boedeker, E. C. (1988). Characterization of the plasmid from Escherichia coli RDEC-1 that mediates expression of adhesin AF/R1 and evidence that AF/R1 pili promote but are not essential for enteropathogenic disease. Infection and Immunity, 56, 1846–1857.

Wolff, C., Nisan, I., Hanski, E., Frankel, G. and Rosenshine, I. (1998). Protein translocation into host epithelial cells by infecting enteropathogenic Escherichia coli. Molecular Microbiology, 28, 143–155.

Woodward, M. J., Best, A., Spriggins, K. A., Pearson, G. R., Skuse, A. M., Wales, A., Hayes, C. M., Roe, J. M., Low, C. J. and la Ragione, R. M. (2003). Non-toxigenic Escherichia coli O157:H7 strain NCTC12900 causes attaching-effacing lesions and aedependent persistence in weaned sheep. International Journal of Medical Microbiology, 293, 299–308.

Wray, C., McLaren, I. and Pearson, G. R. (1989). Occurrence of “attaching and effacing” lesions in the small intestine of calves experimentally infected with bovine isolates of verocytotoxic E. coli. Veterinary Record, 125, 365–368.

Wray, C., McLaren, I. M., Randall, L. P. and Pearson, G. R. (2000). Natural and experimental infection of normal cattle with Escherichia coli O157. Veterinary Record, 147, 65–68.

Zhang, W. L., Kohler, B., Oswald, E., Beutin, L., Karch, H., Morabito, S., Caprioli, A., Suerbaum, S. and Schmidt, H. (2002). Genetic diversity of intimin genes of attaching and effacing Escherichia coli strains. Journal of Clinical Microbiology, 40, 4486–4492.

Zhu, C., Harel, J., Jacques, M., Desautels, C., Donnenberg, M. S., Beaudry, M. and Fairbrother, J. M. (1994). Virulence properties and attaching-effacing activity of Escherichia coli O45 from swine postweaning diarrhea. Infection and Immunity, 62, 4153–4159.