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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-

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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).

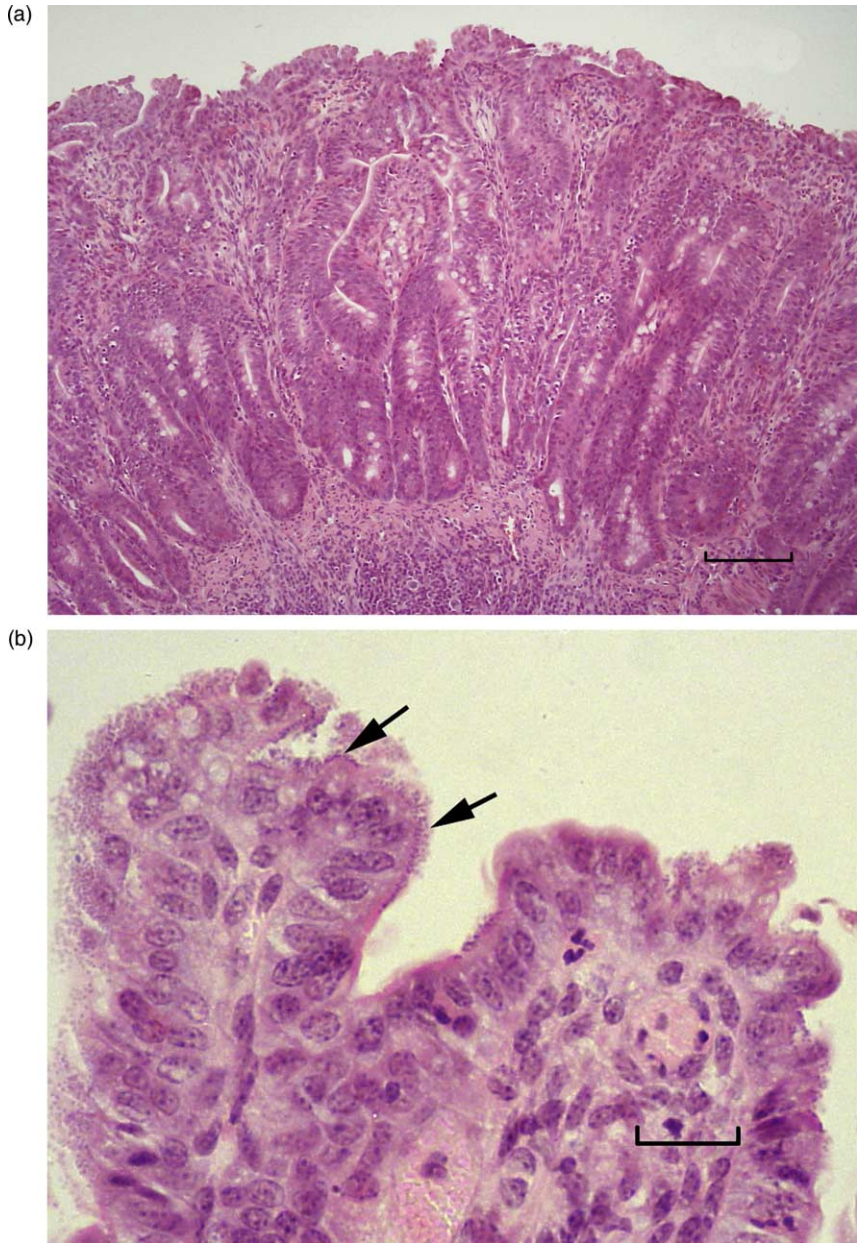

Fig. 1a–d. Ileum of calf infected with *E. coli* O26. (a) The mucosa has a flat appearance due to villous stunting and fusion. The surface epithelium has an irregular appearance. Haematoxylin and eosin (HE). Bar, 100 µm. (b) Adherent bacteria (arrows) are present on the surface of enterocytes. HE. Bar, 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.

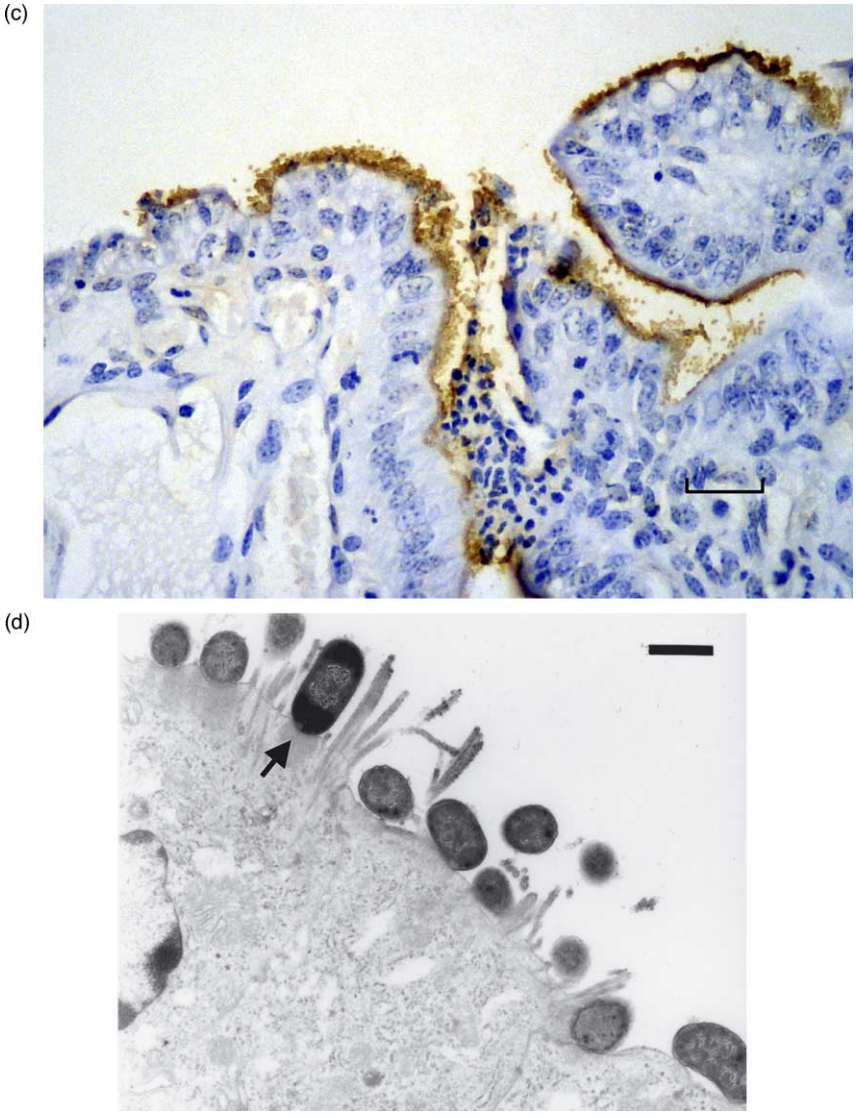

Fig. 1a–d (*continued*)

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, there being 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 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.*, 1996), 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

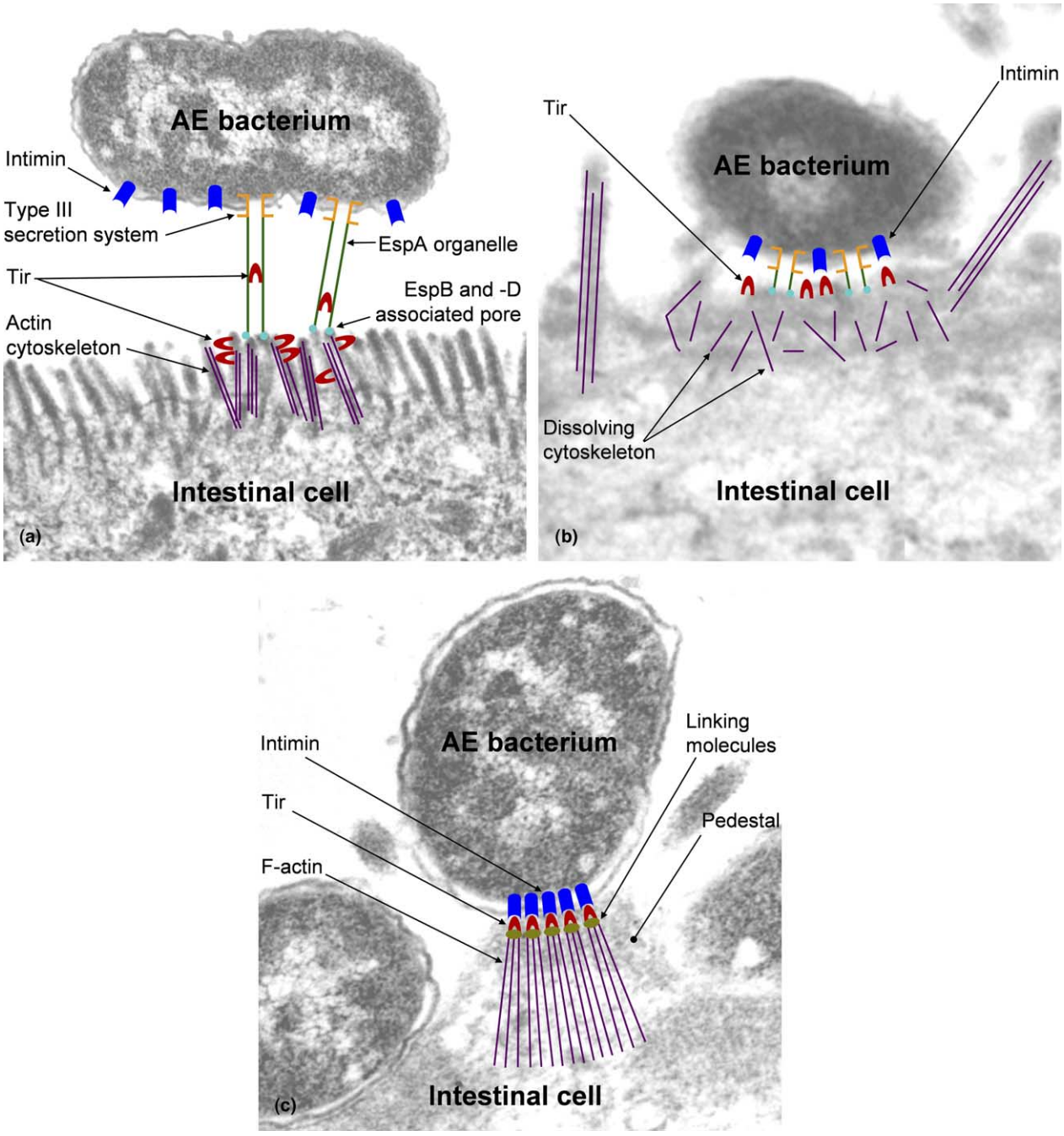

Fig. 2a–c. Composite, schematic diagram illustrating proposed mechanisms in the formation of the attaching-effacing lesion. (a) Non-intimate adhesion and protein translocation. EspA, -B and -D are exported via a type III secretion system and form a bi-functional organelle permitting adhesion of the AE bacterium to the host cell and translocation of Tir into the host cell. Esp, *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.

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 *E. coli* proteins) and *esc* (*E. coli* secretion) genes (Elliott *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 *et al.*, 1998), and these filaments

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; Jores *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 formed 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, coccoid 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* (Luperchio 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 conventional histopathology alone (Janke *et al.*, 1989, 1990). However, enteric disease is often associated with more than one enteropathogen (Moxley and Francis, 1986; Hall *et al.*, 1988b; Janke *et al.*, 1989, 1990; Zhu *et al.*, 1994; Holland *et al.*, 1999b), and this may complicate the histopathological appearance of tissues. Hall *et al.* (1988b) found that light microscopy was unreliable for distinguishing between AE and non-intimate bacterial attachment in the large intestines of diarrhoeic calves. TEM remains the definitive diagnostic technique for AE lesions *in vivo*. If an antiserum specific for the suspected AE pathogen is available, immunohistochemical techniques may be used to identify the lesional organisms (Moxley and Francis, 1986; Wales *et al.*, 2001).

AE lesions may be small and sparse when naturally present in some clinically normal animals (Cookson *et al.*, 2002b; Wales *et al.*, 2005a), or after inoculation with an AEEC strain of low virulence for the experimental species (Dean-Nystrom *et al.*, 1999; Wales *et al.*, 2001). Under such circumstances, verification of the nature of the lesion may require closely targeted TEM techniques, necessitating the retrieval of tissues from wax blocks (Wales *et al.*, 2001) or glass slides (Drolet *et al.*, 1994a) for embedding in resin.

When suitable histopathological material is not available, a suspected AE pathogen may nonetheless be implicated in enteric disease by its isolation from the faeces or intestinal tract (Fischer *et al.*, 1994). However, studies seeking putative AE pathogens by screening faeces for LEE-associated genes have found such genes to be commonly present in the flora of both healthy and diseased animals (Holland *et al.*, 1996, 1999b; Orden *et al.*, 2000; de la Fuente *et al.*, 2002; Penteado *et al.*, 2002). Furthermore, isolates encoding intimin frequently lack a demonstrable AE phenotype *in vitro* (Sandhu *et al.*, 1999; la Ragione *et al.*, 2002) or *in vivo* (la Ragione *et al.*, 2002). Therefore, the presence of genetic markers of AE bacteria should be interpreted with caution in the diagnosis of possible AE-associated disease when histopathological 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; Holland *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 diarrhoea 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 AEEC strains. Certain bovine VT-producing AEEC 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 AEEC 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 AEEC developed colonic AE lesions (Tominaga *et al.*, 1989).

Examination of lamb and kid faeces for putative AEEC 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, AEEC 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 AEEC 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 AEEC O45 has been reported (Wada *et al.*, 1996b). Serogroup O45 is implicated commonly in AEEC 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-borne 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-borne 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-borne (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* genomospecies 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 in 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 *bfp* 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.*, 2003) 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 exceeding 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), O45, 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 expresion 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 acquired 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 AEEC strains of various serotypes from calves, chicks, pigs and human beings (Sueyoshi and Nakazawa, 1994).

In summary, birds carry a variety of AEEC strains, many encoding VT; like mammals, birds appear to be susceptible to AE lesions, but few confirmed cases of AEEC disease have been reported. Diarrhoea would seem not to be an invariable feature of avian AEEC disease. Avian AEEC may often cause clinical disease only in association with other enteropathogens or with immunosuppressive disease. The pathogenic role of avian AEEC 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.*, 2003).

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; Schoonderwoerd *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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