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Review article

## Rabbit EPEC: a model for the study of enteropathogenic *Escherichia coli*

Alain Milon<sup>\*</sup>, Eric Oswald, Jean De Rycke

Unité associée Inra/ENV de microbiologie moléculaire, École nationale vétérinaire, 23, chemin des Capelles, 31076 Toulouse cedex 3, France

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**Abstract** – Colibacillosis has become, in rational rabbit breeding units of western Europe, one of the most economically and pathologically important issues since the beginning of the 1980s. Data on the virulence mechanisms and the phenotypic characters of the *E. coli* strains that are responsible for lethal diarrhoea epizootics have been gathered throughout the years. These strains are representative of a pathovar called enteropathogenic *E. coli* (EPEC) in diarrhoeagenic strains of human origin. EPEC are mainly characterized by their ability to induce a typical lesion called attachment/effacement, whose determinism lies in a pathogenicity island: the locus of enterocyte effacement. The understanding of the pathogenesis mechanisms of this type of bacteria should lead to new tools helping to control the disease in rabbit farming. © Inra/Elsevier, Paris.

***E. coli* / pathogenesis / pathovar / pathogenic island / rabbit**

**Résumé** – EPEC du lapin : un modèle d'études des *Escherichia coli* enteropathogènes. La colibacillose est devenue, depuis le début des années 1980, une dominante pathologique de l'élevage du lapin en Europe de l'Ouest et une cause importante de pertes économiques pour la filière. De nombreuses connaissances ont été acquises sur les caractères phénotypiques et les mécanismes de virulence des souches d'*E. coli* responsables d'épizooties de diarrhées fatales. Ces souches sont représentatives d'un pathovar connu chez les colibacilles d'origine humaine sous la dénomination d'*Escherichia coli* entéropathogènes (EPEC). Les EPEC sont caractérisées essentiellement par leur aptitude à induire des lésions dites d'attachement/effacement, dont le déterminisme réside dans un îlot de virulence chromosomique : le *locus of enterocyte effacement*. La compréhension des mécanismes de pathogénie de ces souches conduira probablement à de nouveaux outils de contrôle de la maladie en élevage. © Inra/Elsevier, Paris.

***E. coli* / pathogénie / pathovar / îlot de virulence / lapin**

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\* Correspondence and reprints  
Tel.: (33) 5 61 19 38 84; fax (33) 5 61 19 39 75; e-mail: a.milón@envt.fr

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## 1. INTRODUCTION

Rabbit (*Oryctolagus cuniculus*) meat production has been progressively structured in western Europe since the mid 1970s. In rational breeding units, it has become evident that digestive disorders in weaned animals are a highly dominant problem, inducing major commercial losses due to weight impairment, epizootic diarrhoeas, mortalities and veterinary costs. Upon searching for infectious causes that may be responsible for such disorders, several parasites and bacteria can be selected as potential diarrhoea-inducers. Digestive *Eimeria* spp., which were very common in the rabbit, have been controlled since the beginning of the 1980s by supplementation of the diet with anticoccidial drugs. The frequent rise of *Escherichia coli* populations in the faeces of animals during diarrhoeic episodes questions the role of this bacteria, which is, as in most species, a normal component of the digestive flora of rabbit. Several research teams have tried to answer the question: is the rise in *E. coli* due to modifications in the gut environment associated with diarrhoea (therefore, *E. coli* might be a simple indicator of these modifications and not a primary cause of the illness) or is *E. coli* a real inducer of diarrhoea in weaned rabbits? Based on the fulfilment of Koch's postulates (i.e. by experimental infection with individual *E. coli* isolates from diarrhoeic rabbits), both hypotheses appear to be true.

In most diarrhoeic episodes, the normal *E. coli* strains from rabbit digestive flora show high populations; however, these stains could not induce diarrhoea when given orally to healthy weaned rabbits. On the contrary, some strains or groups of strains are highly pathogenic for the rabbit, and bear a significant epidemiological repartition in western European breeding units. It is now established that these strains have pathogenic mechanisms that are analogous to those of the *E. coli* pathovar described in some human *E. coli* diarrhoeas: the enteropathogenic *E. coli* (EPEC).

## 2. THE EPEC PATHOVAR

Diarrhoea-inducing *E. coli* strains isolated from humans are currently divided into five pathovars on the basis of their pathogenic mechanisms. In the mid 1980s, four of these pathovars were consensually accepted [39, 73]: ETEC (enterotoxigenic *E. coli*), EIEC (enteroinvasive *E. coli*), EHEC (enterohaemorrhagic *E. coli*) and EPEC. Then, a fifth pathovar was individualized: the EAaggEC (enteroaggregative *E. coli*) [129]. *Table 1* summarizes the main characters of these different pathovars. Readers may refer to reviews that have been devoted to EPEC [68, 36].

The term EPEC was first proposed in 1955 by Neter (cited by Levine [73]) to refer

**Table I.** Diarrhoea-inducing *E. coli* pathovars in humans [73, 94, 115].

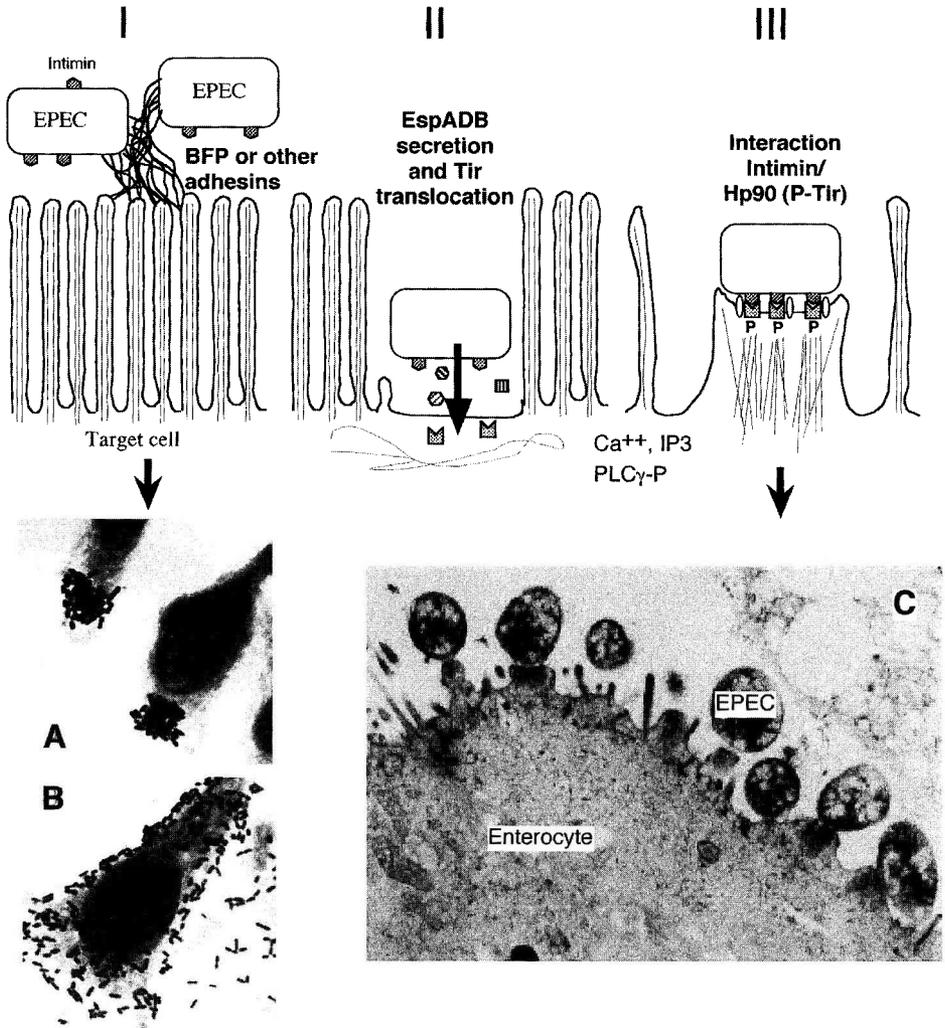
Pathotype	Strains characters
EPEC (enteropathogenic <i>E. coli</i> )	<ul style="list-style-type: none"> <li>– typical serogroups and serovars (see text)</li> <li>– adhesion to enterocyte by specific adhesins</li> <li>– in vitro adhesion to cell lines: localized (class I) or diffuse (class II)</li> <li>– attachment/effacement of enterocytes microvilli and <i>eae</i> (locus of enterocyte effacement (LEE)) loci</li> <li>– no invasive character of ‘<i>Shigella</i>’ type</li> <li>– no production of enterotoxins</li> <li>– no production of Verocytotoxins</li> </ul>
ETEC (enterotoxigenic <i>E. coli</i> )	<ul style="list-style-type: none"> <li>– typical serovars: O6:H16, O8:H9, O15:H11, O25:H42, O78:H12, O120:H7, O20:H–, etc.</li> <li>– adhesion to enterocytes by mean of specific fimbriae (CFA/I to IV)</li> <li>– production of heat-stable (ST) and/or heat-labile (LT) enterotoxins</li> </ul>
EIEC (enteroinvasive <i>E. coli</i> )	<ul style="list-style-type: none"> <li>– virulence plasmid (ca. 140 MDa)</li> <li>– invasion and proliferation in epithelial cells in vivo and in vitro</li> <li>– Sereny test positive (inoc. to guinea-pig conjunctiva)</li> <li>– non-motile, lactose neg., lysine decarboxylase neg.</li> <li>– typical serogroups: O28ac, O112, O124, O136, O143, O144, O173.</li> </ul>
EHEC (enterohemorrhagic <i>E. coli</i> )	<ul style="list-style-type: none"> <li>– high production of Verocytotoxins (Shiga-like toxins)</li> <li>– attachment/effacement of enterocytes microvilli and <i>eae</i> (LEE) loci</li> <li>– production of enterohaemolysin</li> <li>– typical serovars: see Mainil [77]</li> <li>– virulence plasmids (ca. 60 Mda)</li> </ul>
EAggEC (entero-aggregative <i>E. coli</i> ) or EA-AggEC (entero-adherent-aggregative <i>E. coli</i> )	<ul style="list-style-type: none"> <li>– typical in vitro ‘stack brick’ adherence to cell lines</li> <li>– production of a cytotoxin (ST entero-toxin-like: enteroaggregative stable toxin (EAST toxin))</li> </ul>

to a series of *E. coli* strains that were epidemiologically incriminated in severe and epidemic outbreaks of infantile diarrhoeas throughout the world. These strains were first identified by their O serogroup, and later by their O:H serovar. The classical ‘true’ EPEC serovars are O55:H6 or :H7, O86:H2 or :H34, O111:H2, :H12 or :H21, O114:H2, O119:H6, O125ac:H6 or :H21, O126:H2 or :H27, O127:H6, :H9 or :H27, O128:H2, :H7 or :H12 and O142:H2 [115, 94]. Serotyping was the only diagnostic tool for EPEC until the early 1970s. At that time, the frequency of epidemic episodes caused by EPEC decreased in developed countries [31], but EPEC is still a very important

cause of diarrhoea in infants in developing countries as well as agents of travellers’ diarrhoeas [41, 50, 51, 60, 104, 114, 119, 120, 135].

## 2.1. Pathogenesis mechanisms of EPEC

Models for the pathogenesis of EPEC have been proposed successively by Knutton et al. [64], Donnenberg and Kaper [33] and Donnenberg et al. [36]. EPEC are characterized by a rather specific way of interaction with target cells (i.e. enterocytes in vivo, or cell lines in vitro) (*figure 1*). This interaction may be divided into different



**Figure 1.** Three-stage model of enteropathogenic *E. coli* (EPEC) adherence to epithelial cells and formation of attaching/effacing lesion (modified from [36]). I: Class-I EPEC adhere loosely to each other and to target enterocyte apical membrane by class IV fimbriae such as bundle forming pili. On HeLa cells, the adhesion phenotype is called 'localized adhesion' (A). Rabbit EPEC use other fimbrial adhesins, such as AF/R1, Ral, or AF/R2 (with a diffuse adhesion pattern: B). II: Type III secretion-dependant (*esc/sep*) triggers secretion of EspA, -D and -B. Esp A and -D allow translocation of EspB and Tir (and perhaps other proteins). Tir is phosphorylated by the eukaryotic cell and inserted into its membrane. III: Intimin interacts with P-Tir/Hp90 (and perhaps other eukaryotic receptors), which nucleates actin,  $\alpha$ -actinin and other cytoskeletal proteins. Signal transduction results in A/E lesion (as shown by transmission electron microscopy for a REPEC O103: C) and other signals, such as Ca<sup>++</sup> and inositol triphosphate flux and phosphorylation of phospholipase C.

steps that include: i) a loose attachment of the bacteria to the cell, which may be mediated by several specific adhesins; ii) a close contact of the bacteria with the target cell that needs expression of a set of procaryotic proteins among which is intimin (Eae) and its translocated receptor Tir; and iii) a signal, probably delivered through secreted proteins driven to the cell by a type III secretion system (*esc/sep*) and an 'injection apparatus' formed by the Esp proteins, which translocates at least the Tir protein. All the known proteins involved in the second and third steps are determined by genes carried on a pathogenicity island inserted into the chromosome, which is called the locus of enterocyte effacement (LEE). Phenotypically and in vivo, the result of the interaction is a microscopic lesion known as 'attachment/effacement' (A/E) of the microvilli of the enterocytes, followed by the appearance of a pedestal-like structure upon which bacteria are closely attached. The fate of the target cell is less well known. It is presumed, however, that a set of bacteria may penetrate the target cell by endocytosis and/or the result of the transduced signal(s) may explain the death of the cell or at least an efflux of water and ions implicated in the pathogenesis of diarrhoea.

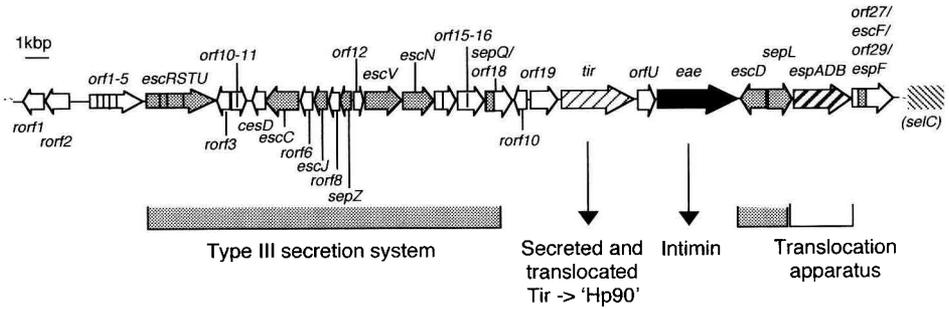
## 2.2. EPEC adhesins

The first step of interaction between EPEC and the target cell (either in vivo or in vitro, on cultured cell lines such as Hep-2 or HeLa cells) is a loose adhesion mediated by specific proteic structures called adhesins. These adhesins usually act as lectins that recognize carbohydrate moieties of glycolipids or glycoproteins at the surface of the eukaryotic cell. The interaction fits the bacteria onto the epithelium. *In fine*, the adhesins are thought to allow colonization of the enteric biotope by the bacteria, which thwart several non-specific defence mechanisms of the host (such as peristaltism and barrier effects induced by the resident flora). Mechanisms of EPEC adhesion from most

classical serovars have been studied in vitro on cultured epithelial cell lines, such as Hep-2 or HeLa cells. Scaletsky et al. [117] first reported, in 1984, two distinctive patterns of EPEC adherence to HeLa cells: localized adhesion (LA), characterized by bacterial microcolonies attached to discrete zones of the cell surface, and diffuse adhesion (DA) where bacteria attached in a scattered pattern to the entire cell surface. Because of the strong association of LA and classical EPEC serovars from diarrhoea outbreaks, strains giving a LA phenotype were later considered as class I EPEC. Strains showing DA or no adhesion to cell lines were less often incriminated in outbreaks of diarrhoea and were termed class II EPEC [73, 91]. The LA phenotype is known to be generated by plasmid-borne type IV fimbriae called 'bundle forming pili' (BFP) [48, 49], which is rather unique to the E2348/69 human EPEC reference strain and other typical EPEC of human origin.

## 2.3. The attachment/effacement lesion and the LEE locus

Recently, new data have been gathered on the mechanisms of the attachment/effacement lesion ([59], for a review, see [36]). A summary of these data is presented in *figure 1*. From a bacterial genetic point, the ability to induce A/E lesions depends upon a group of chromosomal genes forming a pathogenicity island called the locus of enterocyte effacement (LEE) [84]. The LEE and the A/E ability are also present in EHEC stains, some strains of *Hafnia alvei* and *Citrobacter freundii* biotype 4280 (*C. rodentium*) [45, 118]. In the EPEC O127:H6 E2348/69 strain, the LEE is 35 kbp long and is inserted downstream from the tRNA locus *selC* (*figure 2*) [84]. The complete sequence of the LEE from this strain has recently been published [40]. As usual for pathogenicity islands (for a review, see [69]), the G+C content of the LEE (38.4 %) is far below that of the *E. coli* chromosome (50.8 %),



**Figure 2.** Genetic structure of the locus of enterocyte effacement from the EPEC strain E248/69 (O127:H6) (modified from [40]). White open reading frames have no defined function at the moment. Cross-hatched genes are part of the type III secretion machinery (*esc/sep*). Hatched genes encode secreted proteins (Tir and Esp) and the black gene encodes intimin (*eae*). The LEE is inserted into the chromosome at minute 82, near the selenocysteine tRNA locus *selC*.

and it contains sequences homologous to IS600 at its right extremity [37]. Several lines of evidence indicate that the LEE has been horizontally transferred on several occasions within *E. coli* clones (therefore giving rise to several lineages of EPEC and EHEC), which differ by the insertion site, length of the locus and/or alleles of some genes [14, 101, 121, 131]. For instance, in the EHEC EDL933 strain, the LEE is inserted downstream of *selC*, but presents 13 open reading frames (ORFs) belonging to a putative P4 family prophage that are not present in the EPEC E2348/69 strain [101]. In E2348/69, the LEE contains 41 ORFs which may be divided into three functional regions (figure 2) [40]. The central region contains the *eae* locus (for *E. coli* attachment/effacement) [54], which encodes a 94-kDa outer membrane protein called intimin. This protein mediates close contact between the bacteria and the target cell, upon interaction with its translocated receptor Tir (for translocated intimin receptor), whose gene is located upstream [59]. Tir was initially identified as a 90-kDa tyrosine-phosphorylated protein from the target cell membrane and was called the Hp90 [110]. The C-terminal 280-amino acid domain of intimin supports the interaction site with the receptor [46], and shows high polymorphism

which leads to the recognition of at least five subgroups within EPEC and EHEC, irrespective of the strain origins and pathotypes ([4, 5], A. Caprioli, pers. comm.). Preliminary results demonstrate that Tir also has a related polymorphism, which may indicate a role in the tropism of the strains for the intimin/tir couple. Some discrepancies exist, however, in related strains. For instance, rabbit EPEC O103 and human EHEC O103 have identical Tir and different intimins (our unpublished data). This suggests that Tir/Hp90 is perhaps not the only receptor of intimin, or acts as a co-receptor with the help of eukaryotic molecules such as  $\beta 1$ -integrin [47]. Tir (78 kDa) is tyrosine-phosphorylated inside the target cell, bound to the cell membrane where it forms the previously described Hp90 ('host protein 90 kDa'). Then, the complex intimin/Hp90 triggers or subverts signal transduction pathways and acts as an organizer for cytoskeletal proteins nucleation and pseudopod formation beyond the intimin receptor [57, 111–113] (figure 1). This leads to the disarrangement of microvilli and the formation of the pedestal structure beyond the site of bacterial attachment. The demonstration of actin accretion in that site is used in the so-called FAS test (fluorescent actin staining test) [65], which is widely used in studies on the

interaction of EPEC and/or EHEC and cell lines *in vitro*. Tyrosine phosphorylation also activates host phospholipase C [44], which leads to the elevation of intracellular free calcium levels [8] and to the activation of serine or threonine kinases and subsequent phosphorylation of different host proteins [7, 81]. Phosphorylation of membrane proteins may be responsible for ion secretion and subsequent diarrhoea, while cytoskeletal changes may result in the disruption of microvilli and A/E lesions [68].

A second set of genes, *espADB* (for EPEC-secreted proteins), located at the right hand of the LEE locus, has been shown necessary for A/E lesion induction by EPEC [35, 43, 56, 58, 67]. EspA (25 kDa), EspB (37 kDa, formerly named EaeB) and EspD (39 kDa) are secreted by strains upon interaction with target cells. Recent evidence indicates that EspA forms a surface organelle during the early stage of A/E lesion formation, leading to a physical bridge between bacteria and the infected eukaryotic cell and allowing the translocation of EspB [66, 134]. EspA, EspB and EspD as well as Tir proteins are secreted via a type III secretion apparatus ([53, 56, 84], for a review, see [70]) which is encoded mainly by the left hand of the LEE: the *sep/esc* machinery comprises at least 12 genes and 11 more Orfs within the LEE are putatively part of the system too [40]. Initially called *sep* (for secretion of EPEC proteins), these genes have recently been proposed to be renamed as *esc* (for *E. coli* secretion), on the basis of their homologies with other bacterial type III secretion systems, including that of *Yersinia* (Ysc).

Little is known about the host immune responses to EPEC. It has been shown, however, that EPEC lysates could modulate mucosal immune responses. Inhibition of interleukin-2 (IL-2), IL-4, IL-5 and gamma-interferon production by mitogen-activated human blood mononuclear cells was first demonstrated [61]. The same results were found for human gastrointestinal lymphocytes [62] and murine splenic or mucosal

lymphocytes [80], suggesting that protein(s) from EPEC, as well as from EHEC, rabbit diarrhoeal *E. coli*-1 (RDEC-1) or *C. rodentium* [80] could suppress specific local immune responses. EPEC have been shown, however, to have proinflammatory activities through the activation of IL-8 synthesis by epithelial cells [116].

### 3. RABBIT EPEC (REPEC) AND OTHER ANIMAL EPEC

EPEC have been isolated from several animal species, such as pigs, dogs and cats, based on their A/E lesion induction ability and/or on *eae* gene detection [3, 6, 9, 38, 79, 125, 136, 137]. In swine, EPEC are reported to be involved in cases of post-weaning diarrhoeas [63, 137]. Gnotobiotic newborn piglets have also been used as a model to test virulence of some EPEC or EHEC isolates [34, 126–128]. In dogs, there is some evidence for a diarrhoeagenic role of EPEC [38, 125]. Only one paper in the literature describes a chicken isolate with A/E ability [122]. Older reports on A/E lesions inducing strains from animals question whether or not these isolates really belonged to the EPEC group. A/E lesions are also induced by EHEC. In bovine, for instance, most strains with EPEC characters also carry the *slt* genes that encode for verotoxins (VT) and belong to the EHEC pathotype [77, 78]. In our own experiments, strains with EPEC characteristics that do not produce VT may be isolated from calf faeces, whether or not these animals experienced diarrhoea (unpublished results). The rabbit is the only animal species that naturally develops severe diarrhoeas at a high rate due to EPEC.

#### 3.1. The rabbit diarrhoeal *E. coli* 1 (RDEC-1) strain

A link between EPEC strains of human origin and rabbit diarrhoeagenic *E. coli* was discovered initially by Cantey and Blake [21]. The study of an outbreak of diarrhoea

in a colony of laboratory rabbits led them to isolate the RDEC-1 (rabbit diarrhoeal *E. coli*-1) strain. This O15:H- strain could reproduce diarrhoea upon oral administration to weaned rabbits, did not produce known enterotoxin, was not invasive as judged by the Sereny test [21] and induced A/E lesions of the enterocytes [123]. At the time of the study, the A/E lesion was considered as a new mechanism and research conducted on RDEC-1 significantly participated in the definition of the mechanisms of virulence in the EPEC pathovar.

The RDEC-1 strain reproduces diarrhoea upon oral administration of  $10^2$ – $10^{10}$  colony-forming units to weaned rabbits [21]. It colonizes within a few hours ileal Peyers patches [23, 52, 130], then spreads to the ileal mucosa ( $10^3$ – $10^6$  CFU/g) and caecal/colon mucosa ( $10^5$ – $10^7$  CFU/g), where the colonization is more uniform. The strain persists at high populations in the distal parts of the rabbit digestive tract for at least 15 days [22]. Usually, the diarrhoea appears 6–8 days after infection. The ability to colonize is correlated to the aptitude of the strain to adhere *in vitro* to enterocyte brush borders isolated from rabbits over 3 weeks [26, 27]. The RDEC-1 strain appears rather specific to the rabbit, because it does not colonize rats or guinea pigs, and does not adhere to human enterocytes [28]. It induces A/E lesions in newborn colostrum-deprived piglets, however, without generating clinical signs [90].

The adhesion to enterocytes and the colonization aptitude of RDEC-1 were quickly correlated to the presence of a plasmid-encoded fimbrial adhesin called AF/R1 (adhesive factor/rabbit 1) [10, 29, 52, 133]. Purified AF/R1 fimbriae have a major subunit with an apparent m.w. of 19 kDa. The *in vitro* expression of fimbriae is modulated by culture medium and temperature. The *afr* operon is carried by a plasmid of m.w. ca. 86 MDa. It seems that the cellular receptor for AF/R1 is a membrane heteropolymeric glycoprotein of m.w. ca 500 kDa that interacts with the cytoskeleton [105]. Mutants of AF/R1 have been obtained by repeated sub-

cultures at 42 °C [24] or by transposition [133]. The mutants show an altered ability to adhere to Peyer's patch M cells and to colonize the digestive tract. However, a residual virulence remains, and mutants still have the ability to induce A/E lesions, and diarrhoea in some animals [24]. The *afr* operon has been cloned and the gene for the major subunit (AfrA) has been sequenced [132]. It revealed that AF/R1 belongs to the family of the type 1 fimbriae (class I adhesins): AfrA has 43 and 42 % respective homologies with the major subunits of type 1 fimbriae (FimA) and Pap fimbriae (PapA) from uropathogenic *E. coli* [132].

As with the classical EPEC of human origin, the RDEC-1 strain bears an *eae* locus [71, 103]. More recently, it has been shown to possess the entire pathogenicity island (LEE locus) that has been identified in the reference EPEC E2348/69, thus including genes analogous to *esp* and *sep/esc* [1, 55]. It seems therefore likely that RDEC-1 induces diarrhoea by using the mechanisms described above and common to the EPEC pathovar: in the RDEC-1 case, the AF/R1 adhesin may promote the initial colonization of the Peyer's patches and the distal parts of the digestive tract as well, allowing secondary close contacts with enterocytes and the development of A/E lesions, under the dependence of the LEE locus.

### 3.2. Epidemiologically significant groups of rabbit EPEC-like strains in western Europe

During the 1980s, several European teams tried to solve the question about the existence of pathogenic field strains of *E. coli* in the rabbit. Strains isolated from diarrhoeic rabbits in breeding units were serotyped and/or biotyped and their ability to experimentally induce diarrhoea upon oral administration to rabbits was tested. Epidemiological data were gathered mainly from Belgium, the Netherlands, France and Spain, and included serovar and biovar data, experimental pathogenesis and characterization

of intestinal lesions, and sometimes genetic identification of relevant virulence loci [11–13, 18, 19, 70, 71, 74, 76, 93, 96–100, 103, 106]. As a whole, the results indicated that several groups of *E. coli* inducing A/E lesions were highly pathogenic for rabbits. In Benelux, these include the O109:K–:H2 strains that were found in suckling rabbits and which induced severe and lethal yellowish diarrhoea in these animals (but not in weaned ones). In weaned animals, highly incident O15:H– strains and less frequent O103:K–:H2 strains were able to induce diarrhoeas with a high mortality rate and often haemorrhagic lesions of the digestive tract. These types of strains were not isolated from healthy rabbits. In France and Spain, a high predominance of O103:K–:H2 rhamnose-negative *E. coli* was found in sets of strains isolated from diarrhoeic weaned rabbits. These strains, as well as some O26:H11 French isolates, induced severe and lethal diarrhoeas after oral inoculations with as few as  $10^4$  CFU. All studies indicated that some strains of other serovars and/or biovars (such as O128 or O132) could induce mild diarrhoeas and/or weight losses (and may be isolated either from diarrhoeic or from healthy animals). All highly pathogenic groups were shown to induce A/E lesions *in vivo* [76, 96, 99, 109] and hybridized with an *eae* DNA probe [12, 13, 71, 103, 110], which referred them to EPEC or EHEC pathovars. However, phenotypic or genetic research on their ability to produce verocytotoxins has always been negative, suggesting that they belong to an EPEC-like group [71, 82, 83, 103, 110]. Rabbit strains of the O103 group present an equivalent of the entire LEE locus on their chromosome, which confirms their closeness to EPEC [32]. Interestingly enough, by the use of discriminant genetic techniques, Leroy-Sétrin et al. [72] showed a high predominance of a few clones in a set of O103 rhamnose-negative French isolates. This suggests that the highly virulent strains may diffuse throughout the rabbit breeding network, using the animal diffusion channels.

### 3.3. Peculiarities of rabbit EPEC-like strains

Besides their ability to induce A/E lesions and to possess genes that are necessary for that purpose, rabbit EPEC-like strains present specificities that link them to their natural host. As already mentioned for the RDEC-1 strain, their ability to induce diarrhoea in different hosts has been tested and their virulence seems restricted to the rabbit. Robins-Browne et al. [109] showed that, despite their adhesion *in vitro* to enterocytes of different species, their colonizing ability (in mice) and their induction of A/E lesions (in guinea-pigs), none of the four rabbit EPEC-like strains (including RDEC-1, another O15:H–, an O103:H2 and an O109:H2) was virulent for the mouse or the guinea-pig. This strongly suggests that the colonization ability and A/E lesion induction are necessary for pathogenicity of rabbit EPEC-like strains. It seems likely that adhesins such as AF/R1 intervene in the gut colonization process. Little is known on the adhesins of rabbit EPEC-like isolates except for RDEC-1, another O15:H– strain called 83/39 [2] and O103 strains. The adhesin AF/R1 (from the RDEC-1 strain) seems to be rather unique, since it has been scarcely detected in field isolates (including O15:H– ones) [103, 109], except in some pathogenic O26:H11 [130]. Adams et al. [2] have recently described a new adhesin, called Ral (REPEC adherence locus) in a field O15:H– isolate. This adhesin belongs to the K88 (F4)/CS31A group and is similar to the AF/R2 adhesin of O103 strains (see below), but its operon is located on a plasmid. We have extensively studied the adhesive properties of O103 strains and showed that they adhere to cellular substrates by means of a fimbrial adhesin that we called AF/R2 [42, 88, 102]. This fimbrial adhesin is clearly different from AF/R1; it has a major subunit of an apparent m.w. of 32 kDa, and mediates adhesion of the bacteria to rabbit intestinal villi regardless of the age of animals, and to HeLa cells in a diffuse manner (*figure 1*). A mutant that does not express

AF/R2 has impaired colonization properties and a significantly reduced pathogenicity in weaned rabbits [102]. We have cloned the whole operon *af/r2*, which is carried by the chromosome, and sequenced the gene that encodes its major subunit [42]. The deduced protein has significant homologies with class II adhesin major subunits, such as F4 (K88 fimbriae) FaeG or the afimbrial Cs31a subunit. If AF/R2 is clearly involved in pathogenesis of the O103 strains, it must be stressed that it may also be expressed by some other strains, such as O128 isolates, that are far less virulent than O103 [88, 89]. A few highly pathogenic O103 strains do not express AF/R2 (unpublished data). The intervention of other specific adhesins cannot be ruled out and has to be searched for, including in the O103 group.

The LEE locus is involved in the pathogenesis of O103 strains. We selected a set of transposed mutants from the reference O103 strain B10 on the basis of their impaired ability to induce a specific cytopathic effect (CPE) after interaction with HeLa cells [32]. This CPE is characterized by long-term accumulation of actin stress fibres and focal adhesions in cells after a short contact with the REPEC O103 [32]. In the CPE mutants, the transposon was inserted into the LEE locus [32]. When tested in weaned rabbits, one of these mutants, the transposon located in *sep/esc* region, had completely lost its virulence [25]. On the basis of analysis of non-polar mutants obtained by allelic exchange into *eae*, *tir*, *espA*, *espB* or *espD* genes, we further demonstrated that CPE development needs all Esp proteins but is independent of the intimin/Tir system [92]. This may suggest that other virulence protein(s) should use the secretion/translocation apparatus and trigger the CPE phenotype. All mutants had lost their ability to induce A/E lesions and completely lost their virulence in experimental infections (submitted). On the above basis, we propose to develop specific mutants as vaccines against colibacillosis in rabbit (see below).

#### 4. DIAGNOSIS, THERAPY, PREVENTION OF WEANED RABBIT COLIBACILLOSIS

Diarrhoeic disease in weaned rabbit, associated or not with haemorrhages of caecal and colic serosae, must lead to the search for REPEC strains in caecal content or in animal faeces. In O103 colibacillosis, the sensitivity of the animals seems to be maximal a few weeks after weaning [75]. In some cases, however, O103 colibacillosis is detected in suckling rabbits as well as in does [87]. As previously explained, the isolation of *E. coli* from the digestive tract is not sufficient for diagnosis of colibacillosis. *E. coli* proliferation may be secondary to zootechnical errors that may induce multifactorial diarrhoeas. One also has to be reminded that EPEC may be carried by adults, or suckling animals, without any trouble. For this reason, at least two measures must be met for positive diagnosis: i) a high level of *E. coli* CFUs/g or mL of material (over  $10^8$ ), which implies that an enumeration must be carried out in an appropriate selective medium; ii) the possession by isolated strain(s) of REPEC characters. In western Europe, it means that the serogroup is to be determined (O15, O103, or, less importantly, O26, O128, O132 and O109) and that biovar may greatly help (rhamnose-negative reaction for O103, O26 [16], and/or Peeters biovars determination [100]). Further detection of genes specific for rabbit EPEC pathogenesis (either in the isolates, or in the future, directly in the samples) is (or will be) helpful for a guaranteed diagnosis, but these tests are not currently available for use in classical medical laboratories.

The use of antibiotics to treat or prevent EPEC colibacillosis is not easy, owing to the peculiarities of the digestive physiology and of the normal flora of the rabbit, on the one hand, and to the frequently acquired multiple resistance of *E. coli* strains, on the other. It must be kept in mind that wide spectrum antibiotics, such as  $\beta$ -lactames (ampicillin, amoxicillin, cephalosporins,

etc.), lincomycin and clindamycin are highly toxic for the rabbit, essentially because they induce tremendous disequilibrium of the digestive flora (usually associated with death). Narrow spectrum antibiotics, such as polypeptides (colistin, or polymyxin B) or quinolones (flumequine, enrofloxacin) may help to decrease mortality during epizootics [30, 95]. However, both field and experimental data show that antibiotics used in supplemented feed or by individual administrations cannot really solve the problems linked with EPEC strains in the rabbit. Furthermore, EPEC strains show high frequency conjugative plasmid-borne resistances that may be selected by in vivo use of relevant antibiotics and which may spread easily in the enteric bacterial populations [11, 19, 108].

Other means of therapy and prevention have been tested in the rabbit EPEC colibacillosis. Reynaud et al. [108] described a lytic bacteriophage specific for the O103 *E. coli* strain. However, attempts to use this phage for therapy of experimental colibacillosis failed [108].

In our laboratory, we tried to prevent weaned rabbit colibacillosis by vaccination. Owing to the epidemiological predominance of O103 strains in France, we focused the study on this type of REPEC. Early studies revealed that parenteral administration of formalin- or heat-killed bacteria did not protect weaned animals against an oral challenge by the reference O103 B10 strain [15]. We then tried oral vaccination and showed that per os administration just after weaning of high and repeated doses of formalin killed O103 B10 strain could very efficiently protect the animals against an experimental challenge [17, 20, 85, 86]. Vaccination protocols give good results under field conditions, in breeding units with O103 colibacillosis problems ([20], Milon and Boury, unpublished data). The heaviness of schedules, doses and protocols and the rigour that is necessary when applying inactivated vaccines do not, however, allow their use. That is the reason why we turned to trials with

live strains, that could be administered only once at weaning, in moderate doses, with the advantage of in vivo multiplication in the digestive tract of vaccinated animals. By using this technique, we tested two non-pathogenic rabbit isolates which shared either the O103 LPS or the AF/R2 adhesin with virulent O103 strains [89]. One of these strain, an O128 AF/R2 positive isolate called C6, gave very good results by preventing colonization of the gut by the B10 challenge strain. However, the C6 strain could induce slight but significant growth retardation in rabbits, suggesting that it was not completely devoid of pathogenicity. The possible intervention of colicins produced by C6 and active against O103 strains in the effect of 'resistance to colonization' and the possible use of this strain in ecological therapy of O103 colibacillosis was then questioned [124]. The results of crossed experimental administrations of C6 and B10 showed that when the strains are given to rabbits at the same time, or when the O103 strain B10 is given before the C6 strain, the competition is always won by the pathogenic O103 B10 strain, suggesting that the C6 could not displace a virulent strain and be used in ecological therapy [124]. Overall, the results on vaccine trials suggest that oral vaccination with live strains will be a good method to deal with rabbit colibacillosis. The identification of the main virulence mechanisms of EPEC and that of corresponding genes allows us now to construct stable non-virulent mutants of pathogenic strains that may scientifically solve this problem.

## 5. CONCLUSION

The apparent uniformity of mechanisms and genes of pathogenicity of the highly virulent *E. coli* strains that are responsible for digestive colibacillosis in rabbits leads us to the conclusion that these strains are representative of the enteropathogenic (EPEC) pathovar. Progress in the knowledge of these types of pathogenic strains gives reasonable

hopes of coping with colibacillosis in the near future. This includes increased diagnostic possibilities as well as new vaccinal approaches, which will permit overall sanitary and medical prevention against – at least – the main epidemiologically relevant strains.

## REFERENCES

- [1] Abe A., Kenny B., Stein M., Finlay B.B., Characterization of two virulence proteins secreted by Rabbit Enteropathogenic *Escherichia coli*, EspA and EspB, whose maximal expression is sensitive to host body temperature, *Infect. Immun.* 65 (1997) 3547–3555.
- [2] Adams L.M., Simmons C.P., Rezmann L., Strugnell R.A., Robins-Browne R.M., 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, *Infect. Immun.* 65 (1997) 5222–5230.
- [3] Adesiyun A.A., Campbell M., Kaminjolo J.S., Prevalence of bacterial enteropathogens in pet dogs in Trinidad, *Zentralbl. Veterinarmed. Reihe B* 44 (1997) 19–27.
- [4] Adu-Bobie J., Frankel G., Bain C., Goncalves A.G., Trabulsi L.R., Douce G., Knutton S., Dougan G., Detection of intimins alpha, beta, gamma, and delta, four intimin derivatives expressed by attaching and effacing microbial pathogens, *J. Clin. Microbiol.* 36 (1998) 662–668.
- [5] Agin T.S., Wolf M.K., Identification of a family of intimins common to *Escherichia coli* causing attaching-effacing lesions in rabbits, humans, and swine, *Infect. Immun.* 65 (1997) 320–326.
- [6] An H., Fairbrother J.M., Dubreuil J.D., Harel J., Cloning and characterization of the *eae* gene from a dog attaching and effacing *Escherichia coli* strain 4221, *FEMS Microbiol. Lett.* 148 (1997) 239–245.
- [7] Baldwin T.J., Brooks S.F., Knutton S., Manjarrez-Hernandez H.A., Aitken A., Williams P.H., Protein phosphorylation by protein kinase C in HEp-2 cells infected with enteropathogenic *Escherichia coli*, *Infect. Immun.* 58 (1990) 761–765.
- [8] Baldwin T.J., Ward W., Aitken A., Knutton S., Williams P.H., Elevation of intracellular free calcium levels in HEp-2 cells infected with enteropathogenic *Escherichia coli*, *Infect. Immun.* 59 (1991) 1599–1604.
- [9] Beaudry M., Zhu C., Fairbrother J.M., Harel J., Genotypic and phenotypic characterization of *Escherichia coli* isolates from dogs manifesting attaching and effacing lesions, *J. Clin. Microbiol.* 34 (1996) 144–148.
- [10] Berendson R., Cheney C.P., Schad P.A., Boedecker E.C., Species-specific binding of purified pili (AF/R1) from the *Escherichia coli* RDEC-1 to rabbit intestinal mucosa, *Gastroenterology* 85 (1983) 837–845.
- [11] Blanco J.E., Blanco M., Blanco J., Rioja L., Duchá J., Serotypes, toxins and antibiotic resistance of *Escherichia coli* strains isolated from diarrhoeic and healthy rabbits in Spain, *Vet. Microbiol.* 38 (1994) 193–201.
- [12] Blanco J.E., Blanco M., Blanco J., Mora A., Balaguer L., Mourino M., Juárez A., Jansen W.H., O serogroups, biotypes and *eae* genes in *Escherichia coli* strains isolated from diarrheic and healthy rabbits, *J. Clin. Microbiol.* 34 (1996) 3101–3107.
- [13] Blanco J.E., Blanco M., Blanco J., Mora A., Balaguer L., Cuervo L., Balsalobre C., Munoa F., Prevalence and characteristics of enteropathogenic *Escherichia coli* with *eae* gene in diarrheic rabbits, *Microbiol. Immunol.* 41 (1997) 77–82.
- [14] Boerlin P., Chen S., Colbourne J.K., Johnson R., De Grandis S., Gyles C., Evolution of enterohemorrhagic *Escherichia coli* hemolysin plasmids and the locus for enterocyte effacement in shiga toxin-producing *E. coli*, *Infect. Immun.* 66 (1998) 2553–2561.
- [15] Camguilhem R., Essai de vaccination des lapins par voie intradermique contre l'entérite colibacillaire à *E. coli* O103, *Proceedings of IVth Journées de la Recherche Cunicole, Inra-ITAVI, Paris, 1986, communication no. 35.*
- [16] Camguilhem R., Milon A., Biotypes and O serogroups of *Escherichia coli* involved in intestinal infections of weaned rabbits: clues to diagnosis of pathogenic strains, *J. Clin. Microbiol.* 27 (1989) 743–747.
- [17] Camguilhem R., Milon A., Protection of weaned rabbits against experimental *Escherichia coli* O103 intestinal infection by oral formalin-killed vaccine, *Vet. Microbiol.* 21 (1990) 353–362.
- [18] Camguilhem R., Lebas F., Labie C., Reproduction expérimentale chez le lapin en engraissement d'une diarrhée provoquée par une souche d'*Escherichia coli* de séro-groupe O103, *Ann. Rech. Vét.* 17 (1986) 409–424.
- [19] Camguilhem R., Murcau G., Nicolas J.A., Brocas J., Tournou J., Groupage sérologique O et antibiosensibilité des souches d'*Escherichia coli* isolées en France sur les lapins diarrhéiques après le sevrage, *Rev. Méd. Vét.* 137 (1986) 205–212.
- [20] Camguilhem R., Milon A., Esslinger J., Gregory J.N., Maire C., Chmitelín F., Vaccination contre la colibacillrose O103: vaccin inactivé utilisé par voie orale en situation d'infection permanente, *Proceedings of Vth Journées de la Recherche Cunicole, Inra-ITAVI, Paris, 1990, communication no. 19.*

- [21] Cantey J.R., Blake R.K., Diarrhea due to *Escherichia coli* in the rabbit: a novel mechanism, *J. Infect. Dis.* 135 (1977) 454–462.
- [22] Cantey J.R., Hosterman D.S., Characterization of colonization of the rabbit gastrointestinal tract by *Escherichia coli* RDEC-1, *Infect. Immun.* 26 (1979) 1099–1103.
- [23] Cantey J.R., Inman L.R., Diarrhea due to strain RDEC-1 in the rabbit: the Peyer's patch as initial site of attachment and colonization, *J. Infect. Dis.* 143 (1981) 440–446.
- [24] Cantey J.R., Inman L.R., Blake R.K., Production of diarrhea in the rabbit by a mutant of *Escherichia coli* (RDEC-1) that does not express adherence (AF/R1) pili, *J. Infect. Dis.* 160 (1989) 136–141.
- [25] Chalareng C., Pillien F., Boury M., Tasca C., De Rycke J., Milon A., Mutants d'adhésion et d'effet cytopathique : nouveaux candidats à la vaccination contre la colibacillose O103 du lapereau sevré ?, *Proceedings of VI Journées de la recherche Cunicole, Inra-ITAVI-ASFC, 1994*, pp. 17–24.
- [26] Cheney C.P., Boedecker E.C., Rabbit mucosal receptors for an enteropathogenic *Escherichia coli* strain: appearance of bacterial receptor activity at weaning, *Gastroenterology* 87 (1984) 821–826.
- [27] Cheney C.P., Boedecker E.C., Formal S.B., Quantitation of adherence of an enteropathogenic *Escherichia coli* to isolated rabbit intestinal brush borders, *Infect. Immun.* 26 (1979) 736–743.
- [28] Cheney C.P., Schad P.A., Formal S.B., Boedecker E.C., Species specificity of in vitro *Escherichia coli* adherence to host intestinal cell membranes and its correlation with in vivo colonization and infectivity, *Infect. Immun.* 28 (1980) 1019–1027.
- [29] Cheney C.P., Formal S.B., Schad P.A., Boedecker E.C., Genetic transfer of a mucosal adherence factor (RI) from an enteropathogenic *Escherichia coli* strain into a *Shigella flexneri* strain and the phenotypic suppression of this adherence factor, *J. Infect. Dis.* 147 (1983) 711–723.
- [30] Chow T.T., Peeters J.E., Geeroms R., Effet inhibiteur de la polymyxine B sur l'excrétion fécale d'*Escherichia coli* entéropathogène (sérototype O109/biotype 1+) après infection expérimentale de lapereaux sevrés, *Proceedings of VIth Journées de la Recherche Cunicole, Inra-ITAVI-ASFC, Paris, 1994*, pp.35–38.
- [31] Cohen M.B., Etiology and mechanisms of acute infectious diarrhea in infants in the United States, *J. Pediatr.* 118 (1991) S34–S39.
- [32] De Rycke J., Comtet E., Chalareng C., Boury M., Tasca C., Milon A., Rabbit enteropathogenic *Escherichia coli* O103 elicits actin stress fibers and focal adhesions in HeLa cells, a cytopathic effect that is linked to an analogue of Locus of Enterocyte Effacement, *Infect. Immun.* 65 (1997) 2555–2563.
- [33] Donnenberg M.S., Kaper J.B., Enteropathogenic *Escherichia coli*, *Infect. Immun.* 60 (1992) 3953–3961.
- [34] Donnenberg M.S., Tzipori S., McKee M.L., O'Brien A.D., Alroy J., Kaper J.B., The role of *eae* gene of enterohemorrhagic *Escherichia coli* in intimate attachment in vitro and in a porcine model, *J. Clin. Invest.* 92 (1993) 1418–1424.
- [35] Donnenberg M.S., Yu J., Kaper J.B., A second chromosomal gene necessary for intimate attachment of enteropathogenic *Escherichia coli* to epithelial cells, *J. Bacteriol.* 175 (1993) 4670–4680.
- [36] Donnenberg M.S., Kaper J.B., Finlay B.B., Interactions between enteropathogenic *Escherichia coli* and host epithelial cells, *Trends Microbiol.* 5 (1997) 109–114.
- [37] Donnenberg M.S., Lai L.C., Taylor K.A., The locus of enterocyte effacement pathogenicity island of enteropathogenic *Escherichia coli* encodes secretion functions and remnants of transposons at its extreme right end, *Gene* 184 (1997) 107–114.
- [38] Drolet R., Fairbrother J.M., Harel J., Helie P., Attaching and effacing and enterotoxigenic *Escherichia coli* associated with enteric colibacillosis in the dog, *Can. J. Vet. Res.* 58 (1994) 87–92.
- [39] Edelman R., Levine M.M., Summary of a workshop on enteropathogenic *Escherichia coli*, *J. Infect. Dis.* 147 (1983) 1108–1118.
- [40] Elliott S.J., Wainwright L.A., McDaniel T.K., Jarvis K.G., Deng YK., Lai L.-C., McNamara B.P., Donnenberg M.S., Kaper J.B., The complete sequence of the locus of enterocyte effacement (LEE) from enteropathogenic *Escherichia coli* E2348/69, *Mol. Microbiol.* 28 (1998) 1–4.
- [41] Etcheverria P., Ørskov F., Ørskov I., Knutton S., Scheutz F., Brown J.E., Loxombon U., Attaching and effacing enteropathogenic *Escherichia coli* as cause of infantile diarrhea in Bangkok, *J. Infect. Dis.* 164 (1991) 550–554.
- [42] Fiederling F., Boury M., Petit C., Milon A., Adhesive Factor/ Rabbit 2, a new fimbrial adhesin and a virulence factor from *Escherichia coli* O103, enteropathogenic for rabbit, *Infect. Immun.* 65 (1997) 847–851.
- [43] Foubister V., Rosenshine I., Donnenberg M.S., Finlay B.B., The *eaeB* gene of enteropathogenic *Escherichia coli* (EPEC) is necessary for signal transduction in epithelial cells, *Infect. Immun.* 62 (1994) 3038–3040.
- [44] Foubister V., Rosenshine I., Finlay B.B., A diarrheal pathogen, enteropathogenic *Escherichia coli* (EPEC), triggers a flux of inositol phosphates in infected epithelial cells, *J. Exp. Med.* 179 (1994) 993–998.
- [45] Frankel G., Candy D.C., Everest P., Dougan G., Characterization of the C-terminal domains

- of intimin-like proteins of enteropathogenic and enterohemorrhagic *Escherichia coli*, *Citrobacter freundii*, and *Hafnia alvei*, *Infect. Immun.* 62 (1994) 1835–1842.
- [46] Frankel G., Candy D.C.A., Fabiani E., Adu-Bobbie J., Gil S., Novakova M., Phillips A.D., Dougan G., Molecular characterization of a carboxy-terminal eukaryotic-cell binding domain of intimin from enteropathogenic *Escherichia coli*, *Infect. Immun.* 63 (1995) 4323–4328.
- [47] Frankel G., Lider O., Hershkoviz R., Mould A.P., Kachalsky S.G., Candy D.C.A., Cahalon L., Humphries M.J., Dougan G., The cell-binding domain of intimin from enteropathogenic *Escherichia coli* binds to beta1 integrins, *J. Biol. Chem.* 271 (1996) 20359–20364.
- [48] Giron J.A., Ho A.S.Y., Schoolnik G.K., An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*, *Science* 254 (1991) 710–713.
- [49] Giron J.A., Qadri F., Azim T., Jarvis K.J., Kaper J.B., Albert M.J., Monoclonal antibodies specific for the Bundle-Forming Pilus of enteropathogenic *Escherichia coli*, *Infect. Immun.* 63 (1995) 4949–4952.
- [50] Gomes T.A.T., Vieira M.A.M., Wachsmuth I.K., Blake P.A., Trabulsi L.R., Serotype-specific prevalence of *Escherichia coli* strains with EPEC adherence factor genes in infants with and without diarrhea in Sao Paulo, Brazil, *J. Infect. Dis.* 160 (1989) 131–135.
- [51] Gomes T.A.T., Rassi V., MacDonald K.L., Silva Ramos S.R., Trabulsi L.R., Vieira M.A.M., Guth B.E.C., Candeias J.A.N., Ivey C., Toledo M.R., Blake P.A., Enteropathogens associated with acute diarrheal disease in urban infants in Sao Paulo, Brazil, *J. Infect. Dis.* 164 (1991) 331–337.
- [52] Inman L.R., Cantey J.R., Peyer's patch lymphoid follicle epithelial adherence of a rabbit enteropathogenic *Escherichia coli* (strain RDEC-1), Role of plasmid-mediated pili in initial adherence, *J. Clin. Invest.* 74 (1984) 90–95.
- [53] Jarvis K.G., Giron J.A., Jerse A.E., McDaniel T.K., Donnenberg M.S., Kaper J.B., Enteropathogenic *Escherichia coli* contains a putative type III secretion system necessary for the export of proteins involved in attaching and effacing lesion formation, *Proc. Natl. Acad. Sci. USA* 92 (1995) 7996–8000.
- [54] Jerse A.E., Yu J., Tall B.D., Kaper J.B., A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells, *Proc. Natl. Acad. Sci. USA* 87 (1990) 7839–7843.
- [55] Karaolis D.K., McDaniel T.K., Kaper J.B., Boedeker E.C., Cloning of the RDEC-1 locus of enterocyte effacement (LEE) and functional analysis of the phenotype on Hep-2 cells, *Adv. Exp. Med. Biol.* 412 (1997) 241–245.
- [56] Kenny B., Finlay B.B., Protein secretion by enteropathogenic *Escherichia coli* is essential for transducing signals into epithelial cells, *Proc. Natl. Acad. Sci. USA* 92 (1995) 7991–7995.
- [57] Kenny B., Finlay B.B., Intimin-dependent binding of enteropathogenic *Escherichia coli* to host cells triggers novel signaling events, including tyrosine phosphorylation of phospholipase C-g1, *Infect. Immun.* 65 (1997) 2528–2536.
- [58] Kenny B., Lai L.C., Finlay B.B., Donnenberg M.S., EspA, a protein secreted by enteropathogenic *Escherichia coli*, is required to induce signals in epithelial cells, *Mol. Microbiol.* 20 (1996) 313–323.
- [59] Kenny B., DeVinney R., Stein M., Reinscheid D.J., Frey E.A., Finlay B.B., Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells, *Cell* 91 (1997) 511–520.
- [60] Kim K-H., Suh I-S., Kim J.M., Kim C.W., Cho Y-J., Etiology of childhood diarrhea in Korea, *J. Clin. Microbiol.* 27 (1989) 1192–1196.
- [61] Klapproth J.M., Donnenberg M.S., Abraham J.M., Mobley H.L., James S.P., Products of enteropathogenic *Escherichia coli* inhibit lymphocyte activation and lymphokine production, *Infect. Immun.* 63 (1995) 2248–2254.
- [62] Klapproth J.M., Donnenberg M.S., Abraham J.M., James S.P., Products of enteropathogenic *E. coli* inhibit lymphokine production by gastrointestinal lymphocytes, *Am. J. Physiol.* 271 (1996) G841–G848.
- [63] Knutton S., Attaching and effacing *Escherichia coli*, in: Gyles C.L.(Ed.) *Escherichia coli* in Domestic Animals and Human, CAB International, Guilford, UK, 1994, pp. 567–591.
- [64] Knutton S., Lloyd D.R., McNeish A.S., Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa, *Infect. Immun.* 55 (1987) 69–77.
- [65] Knutton S., Baldwin T., Williams P.H., McNeish A.S., Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*, *Infect. Immun.* 57 (1989) 1290–1298.
- [66] Knutton S., Rosenshine I., Pallen M.J., Nisan I., Neves B.C., Bain C., Wolff C., Dougan G., Frankel G., A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells, *EMBO J.* 17 (1998) 2166–2176.
- [67] Lai L.C., Wainwright L.A., Stone K.D., Donnenberg M.S., A third secreted protein that is encoded by the enteropathogenic *Escherichia coli* pathogenicity island is required for transduction of signals and for attaching and effacing activities in host cells, *Infect. Immun.* 65 (1997) 2211–2217.

- [68] Law D., Adhesion and its role in the virulence of enteropathogenic *Escherichia coli*, Clin. Microbiol. Rev. 7 (1994) 152–173.
- [69] Lee C.A., Pathogenicity islands and the evolution of bacterial pathogens, Infect. Agents Dis. 5 (1996) 1–7.
- [70] Lee C.A., Type III secretion systems: machines to deliver bacterial proteins into eukariotic cells?, Trends Microbiol. 5 (1997) 148–156.
- [71] Leroy S.M., Lesage M.C., Chaslus-Dancla E., Lafont J.P., Presence of *eaeA* in pathogenic and non-pathogenic *Escherichia coli* strains isolated from weaned rabbits, J. Med. Microbiol. 40 (1994) 90–94.
- [72] Leroy-Sétrin S., Lesage M.C., Chaslus-Dancla E., Lafont J.P., Clonal diffusion of EPEC-like *Escherichia coli* from rabbits as detected by ribotyping and random amplified polymorphic DNA assays, Epidemiol. Infect. 114 (1995) 113–121.
- [73] Levine M.M., *Escherichia coli* that cause diarrhea: enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic, and enteroadherent, J. Infect. Dis. 155 (1987) 377–389.
- [74] Licois D., Coudert P., Guillot J.F., Renault L., Diarrhée expérimentale du lapin : étude de la pathologie due à des coccidies intestinales (*E. intestinalis*) et à des *Escherichia coli*, Proceedings of 111rd Journées de la Recherche Cunicole, Inra-ITAVI, Paris, 1982, communication no. 27.
- [75] Licois D., Guillot J.F., Mouline C., Sensibilité du lapin à une souche pathogène d'*Escherichia coli* O103 : effet de l'âge des animaux, Proceedings of Vth Journées de la Recherche Cunicole, Inra-ITAVI, Paris, 1990, communication no. 24.
- [76] Licois D., Reynaud A., Fédérighi M., Gaillard-Martinie B., Guillot J.F., Joly B., Scanning and transmission electron microscopic study of adherence of *Escherichia coli* O103 enteropathogenic and/or enterohemorrhagic strain GV in enteric infection in rabbits, Infect. Immun. 59 (1991) 3796–3800.
- [77] Mainil J.G., Shiga/Verocytotoxins and Shiga/Verotoxigenic *Escherichia coli* in animals, Vet. Res. 30 (1999) 235–257.
- [78] Mainil J.G., Duchesnes C.J., Whipp S.C., Marques L.R., O'Brien A.D., Casey T.A., Moon H.W., Shiga-like toxin production and attaching effacing activity of *Escherichia coli* associated with calf diarrhea, Am. J. Vet. Res. 48 (1987) 743–748.
- [79] Mainil J., Bez S., Jacquemin E., Kaackenbeeck A., Les souches pathogènes d'*Escherichia coli*. Détection des souches entérotoxigènes (ETEC), entéropathogènes (EPEC), vérotoxigènes (VTEC), entérohémorragiques (EHEC) et nécrotoxigènes (NTEC), Ann. Méd. Vét. 142 (1998) 39–46.
- [80] Malstrom C., James S., Inhibition of murine splenic and mucosal lymphocyte function by enteric bacterial products, Infect. Immun. 66 (1998) 3120–3127.
- [81] Manjarrez-Hernandez H.A., Baldwin T.J., Aitken A., Knutton S., Williams P.H., Intestinal epithelial cell protein phosphorylation in enteropathogenic *Escherichia coli* diarrhoea, Lancet 339 (1992) 521–523.
- [82] Marena M., Milon A., Bauerfeind R., Boury M., Toxines Shiga-like et *Escherichia coli* entérotoxigènes (EPEC-like) du lapin sevré, Rev. Méd. Vét. 143 (1992) 333–340.
- [83] Mariani-Kurkdjian P., Denamur E., Milon A., Picard B., Cave H., Lambert-Zechovsky N., Loirat C., Goulet P., Sansonetti P., Elion J., Identification of a clone of *E. coli* O103:H2 as a potential agent of Hemolytic and Uremic Syndrome in France, J. Clin. Microbiol. 31 (1993) 296–301.
- [84] McDaniel T.K., Jarvis K.G., Donnenberg M.S., Kaper J.B., A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens, Proc. Natl. Acad. Sci. USA 92 (1995) 1664–1668.
- [85] Milon A., Camguilhem R., Essais de protection des lapereaux sevrés contre l'entérite à *Escherichia coli* O103 : vaccinations des mères avec un vaccin inactivé, Rev. Méd. Vét. 140 (1989) 389–395.
- [86] Milon A., Camguilhem R., Esslinger J., Vaccination des lapins contre l'entérite à *Escherichia coli* O103 : nouveaux résultats, Rev. Méd. Vét. 140 (1989) 835–839.
- [87] Milon A., Camguilhem R., Chmitel F., Maire C., Gregory J.N., Esslinger J., Colibacillose O103 : à propos d'une épi-zootie d'élevage atteignant les mères, les nouveau-nés et les lapereaux sevrés, Proceedings of Vth Journées de la Recherche Cunicole, Inra-ITAVI, Paris, 1990, communication no. 20.
- [88] Milon A., Esslinger J., Camguilhem R., Adhesion of *Escherichia coli* strains isolated from diarrheic weaned rabbits to intestinal villi and HeLa cells, Infect. Immun. 58 (1990) 2690–2695.
- [89] Milon A., Esslinger J., Camguilhem R., Oral vaccination of weaned rabbits against Enteropathogenic *Escherichia coli*-like *E. coli* O103 infection: use of heterologous strains harboring lipopolysaccharide or adhesin of pathogenic strains, Infect. Immun. 60 (1992) 2702–2709.
- [90] Moon H.W., Whipp S.C., Argenzio R.A., Levine M.M., Gianella R.A., Attaching and effacing activities of rabbit and human enteropathogenic *Escherichia coli* in pig and rabbit intestines, Infect. Immun. 39 (1983) 1340–1351.
- [91] Nataro J.P., Scaletsky I.C.A., Kaper J.B., Levine M.M., Trabulsi L.R., Plasmid-mediated factors

- conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*, Infect. Immun. 48 (1985) 378–383.
- [92] Nougayrede J.P., Marches O., Boury M., Mainil J., Charlier G., Pohl P., De Rycke J., Milon A., Oswald E., The long-term cytoskeletal rearrangement induced by rabbit enteropathogenic *Escherichia coli* is Esp-dependent but intimin-independent, Mol. Microbiol. (1998) in press.
- [93] Okerman L., Devriese L.A., Biotypes of enteropathogenic *Escherichia coli* strains from rabbits, J. Clin. Microbiol. 22 (1985) 955–958.
- [94] Ørskov F., Ørskov I., *Escherichia coli* serotyping and disease in man and animals, Can. J. Microbiol. 38 (1992) 699–704.
- [95] Peeters J.E., Geeroms R., Traitement et éradication de la colibacillose (sérotype O15:K:H-) sur le terrain, Proceedings of Vth Journées de la Recherche Cunicole, Inra-ITAVI, Paris, 1990, communication no. 21.
- [96] Peeters J.E., Charlier G.J., Halen P.H., Pathogenicity of attaching effacing enteropathogenic *Escherichia coli* isolated from diarrheic suckling and weanling rabbits for newborn rabbits, Infect. Immun. 46 (1984) 690–696.
- [97] Peeters J.E., Geeroms R., Glorieux B., Experimental *Escherichia coli* enteropathy in weanling rabbits: clinical manifestations and pathological findings, J. Comp. Pathol. 94 (1984) 521–528.
- [98] Peeters J.E., Pohl P., Okerman L., Devriese L.A., Pathogenic properties of *Escherichia coli* strains isolated from diarrheic commercial rabbits, J. Clin. Microbiol. 20 (1984) 34–39.
- [99] Peeters J.E., Charlier G.T., Raeymaekers R., Scanning and transmission electron microscopy of attaching and effacing *Escherichia coli* in weanling rabbits, Vet. Pathol. 22 (1985) 54–59.
- [100] Peeters J.E., Geeroms R., Ørskov F., Biotype, serotype and pathogenicity of attaching and effacing enteropathogenic *Escherichia coli* strains isolated from diarrheic commercial rabbits, Infect. Immun. 56 (1988) 1442–1448.
- [101] Perna N.T., Mayhew G.F., Posfai G., Elliott S., Donnenberg M.S., Kaper J.B., Blattner F.R., Molecular evolution of a pathogenicity island from enterohemorrhagic *Escherichia coli* O157:H7, Infect. Immun. 66 (1998) 3810–3817.
- [102] Pillien F., Chalareng C., Boury M., Tasca C., De Rycke J., Milon A., Role of adhesive factor/rabbit 2 in experimental enteropathogenic *Escherichia coli* O103 diarrheic of weaned rabbit, Vet. Microbiol. 50 (1996) 105–115.
- [103] Pohl P., Peeters J.E., Jacquemin E.R., Lintermans P.F., Mainil J.G., Identification of *eae* sequences in enteropathogenic *Escherichia coli* strains from rabbits, Infect. Immun. 61 (1993) 608–613.
- [104] Porat N., Levy A., Fraser D., Deckelbaum R.J., Dagan R., Prevalence of intestinal infections caused by diarrheagenic *Escherichia coli* in Bedouin infants and young children in southern Israel, Pediatr. Infect. Dis. J. 17 (1998) 482–488.
- [105] Rafiee P., Byrd J.C., Leffler H., Cassels F., Boedecker E., Kim Y.S., Characterization of the rabbit intestinal receptor for the rabbit diarrheagenic *E. coli*, RDEC-1 (abstract), Gastroenterology 98 (1990) A197.
- [106] Renault L., Roux J., Le Bourhis E., Coudert P., Licois D., Guillot J.F., Description d'un sérotype (O103) d'*Escherichia coli* entéropathogène chez le lapin au sevrage, Bull. Acad. Vét. Fr. 56 (1983) 387–400.
- [107] Reynaud A., Fédérighi M., Licois D., Guillot J.F. and Joly B., R plasmid in *Escherichia coli* O103 coding for the colonization of the rabbit intestinal tract, Infect. Immun. 59 (1991) 1888–1892.
- [108] Reynaud A., Cloastre L., Bernard J., Laveran H., Ackermann H.W., Licois D., Joly B., Characteristics and diffusion in the rabbit of a phage for *Escherichia coli* O103. Attempts to use this phage for therapy, Vet. Microbiol. 30 (1992) 203–212.
- [109] Robins-Browne R.M., Tokhi A.M., Adams L.M., Bennett-Wood V., Host specificity of enteropathogenic *Escherichia coli* from rabbits: lack of correlation between adherence in vitro and pathogenicity for laboratory animals, Infect. Immun. 62 (1994) 3329–3336.
- [110] Robins-Browne R.M., Tokhi A.M., Adams L.M., Bennett-Wood V., Moisisidis A.V., Krejany E.O., O'Gorman L.E., Adherence characteristics of attaching and effacing strains of *Escherichia coli* from rabbits, Infect. Immun. 62 (1994) 1584–1592.
- [111] Rosenshine I., Donenberg M.S., Kaper J.B., Finlay B.B., Signal transduction between enteropathogenic *Escherichia coli* and epithelial cells; EPEC induces tyrosine phosphorylation of host cell proteins to initiate cytoskeletal rearrangements and bacterial uptake, EMBO J. 11 (1992) 3551–3560.
- [112] Rosenshine I., Ruschkowski S., Finlay B.B., Expression of attaching/effacing activity by enteropathogenic *Escherichia coli* depends on growth phase, temperature, and protein synthesis upon contact with epithelial cells, Infect. Immun. 64 (1996) 966–973.
- [113] Rosenshine I., Ruschkowski S., Stein M., Reinscheid D.J., Mills S.D., Finlay B.B., A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation, EMBO J. 15 (1996) 2613–2624.
- [114] Samonis G., Maraki S., Christidou A., Georgiadakis A., Tselentis Y., Bacterial pathogens associated with diarrhoea on the island of Crete, Eur. J. Epidemiol. 13 (1997) 831–836.
- [115] Sansonetti P.J., *Escherichia coli* entéropathogènes : données récentes sur la virulence, Bull. Inst. Pasteur 83 (1985) 5–18.

- [116] Savcovic S.D., Koutsouris A., Hecht G., Activation of NF- $\kappa$ B in intestinal epithelial cells by enteropathogenic *Escherichia coli*, *Am. J. Physiol.* 273 (1997) C1160–C1167.
- [117] Scaletsky I.C.A., Silva M.L.M., Trabulsi L.R., Distinctive patterns of adherence of enteropathogenic *Escherichia coli* to HeLa cells, *Infect. Immun.* 45 (1984) 534–536
- [118] Schauer D.B., Falkow S., The *eae* gene of *Citrobacter freundii* biotype 4280 is necessary for colonization in transmissible murine colonic hyperplasia, *Infect. Immun.* 61 (1993) 4654–4661
- [119] Senerwa D., Olsvik Ø., Mutanda L.N., Gathuma J.M., Wachsmuth K., Colonization of neonates in a nursery ward with Enteropathogenic *Escherichia coli* and correlation to the clinical histories of the children, *J. Clin. Microbiol.* 27 (1989) 2539–2543.
- [120] Senerwa D., Olsvik Ø., Mutanda L.N., Lindqvist K.J., Gathuma J.M., Fossum K., Wachsmuth K., Enteropathogenic *Escherichia coli* serotype O111:HNT isolated from preterm neonates in Nairobi, Kenya, *J. Clin. Microbiol.* 27 (1989) 1307–1311.
- [121] Sperandio V., Kaper J.B., Bortolini M.R., Neves B.C., Keller R., Trabulsi L.R., Characterization of the locus of enterocyte effacement (LEE) in different enteropathogenic *Escherichia coli* (EPEC) and Shiga-toxin producing *Escherichia coli* (STEC) serotypes, *FEMS Microbiol. Lett.* 164 (1998) 133–139.
- [122] Sueyoshi M., Fukui H., Tanaka S., Nakazawa M., Ito K., A new adherent form of an attaching and effacing *Escherichia coli* (*eae*A+, *bfp*-) to the intestinal epithelial cells of chicks, *J. Vet. Med. Sci.* 58 (1996) 1145–1147.
- [123] Takeuchi A., Inman L.R., O'Hanley P.D., Canteley J.R., Lushbaugh W.B., Scanning and transmission electron microscopic study of *Escherichia coli* O15 (RDEC-1) enteric infection in rabbits, *Infect. Immun.* 19 (1978) 686–694.
- [124] Tran Cong T., Milon A., Boury M., Tasca C., Colicines et effets de barrière écologique dans l'entérocologie à *Escherichia coli* O103 du lapin sévré, *Rev. Méd. Vét.* 143 (1992) 655–665.
- [125] Turk J., Maddox C., Fales W., Ostlund E., Miller M., Johnson G., Pace L., Turnquist S., Kreeger J., Examination for heat-labile, heat-stable, and Shiga-like toxins and for the *eae*A gene in *Escherichia coli* isolates obtained from dogs dying with diarrhea: 122 cases (1992–1996), *J. Am. Vet. Med. Assoc.* 212 (1998) 1735–1736.
- [126] Tzipori S., Gibson R., Montanaro J., Nature and distribution of mucosal lesions associated with enteropathogenic and enterohemorrhagic *Escherichia coli* in piglets and the role of plasmid mediated factors, *Infect. Immun.* 57 (1989) 1142–1150.
- [127] Tzipori S., Robins-Browne R.M., Gonis G., Hayes J., Withers M., McCartney E., Enteropathogenic *Escherichia coli* enteritis: evaluation of the gnotobiotic piglet as a model of human infection, *Gut* 26 (1985) 570–578.
- [128] Tzipori S., Gunzer F., Donnenberg M.S., De Montigny L., Kaper J.B., Donohue-Rolfe A., The role of *eae*A gene in diarrhea and neurological complications in a gnotobiotic piglet model of enterohemorrhagic *Escherichia coli* infection, *Infect. Immun.* 63 (1995) 3621–3627.
- [129] Vial P.A., Robins-Browne R., Lior H., Prado V., Kaper J.B., Nataro J.P., Maneval D., Elsayed A., Levine M.M., Characterization of enteroadherent-aggregative *Escherichia coli*, a putative agent of diarrheal disease, *J. Infect. Dis.* 158 (1988) 70–79.
- [130] Von Moll L.K., Canteley J.R., Peyer's patch adherence of enteropathogenic *Escherichia coli* strains in rabbits, *Infect. Immun.* 65 (1997) 3788–3793.
- [131] Wieler L.H., McDaniel T.K., Whittam T.S., Kaper J.B., Insertion site of the locus of enterocyte effacement in enteropathogenic and enterohemorrhagic *Escherichia coli* differs in relation to the clonal phylogeny of the strains, *FEMS Microbiol. Lett.* 156 (1997) 49–53
- [132] Wolf M.K., Boedecker E.C., Cloning of the genes for AF/R1 pili from rabbit enteroadherent *Escherichia coli* RDEC-1 and DNA sequence of the major structural subunit, *Infect. Immun.* 58 (1990) 1124–1128.
- [133] Wolf M.K., Andrews G.P., Fritz D.L., Sjogren R.W.Jr, Boedecker E.C., Characterization of the plasmid from *Escherichia coli* RDEC-1 that mediates expression of adhesion AF/R1 and evidence that AF/R1 pili promote but are not essential for enteropathogenic disease, *Infect. Immun.* 56 (1988) 1846–1857.
- [134] Wolff, C., Nisan I., Hanski E., Frankel G., Rosenshine I., Protein translocation into host epithelial cells by infecting enteropathogenic *Escherichia coli*, *Mol. Microbiol.* 28 (1998) 143–155.
- [135] Yamashiro T., Nakasone N., Higa N., Iwanaga M., Insiengmay S., Phounane T., Munnalath K., Sithivong N., Sisavath L., Phanthauamath B., Chomlasak K., Sisulak P., Vongsanith P., Etiological study of diarrheal patients in Vientiane, Lao People's Democratic Republic, *J. Clin. Microbiol.* 36 (1998) 2195–2199.
- [136] Zhu C., Harel J., Dumas F., Fairbrother J.M., Identification of EaeA protein in the outer membrane of attaching and effacing *Escherichia coli* O45 from pigs, *FEMS Microbiol. Lett.* 129 (1995) 237–242.
- [137] Zhu C., Harel J., Jacques M., Desautels C., Donnenberg M.S., Beaudry M., Fairbrother J.M., Virulence properties and attaching-effacing activity of *Escherichia coli* O45 from swine postweaning diarrhea, *Infect. Immun.* 62 (1994) 4153–4159.