WEANED RABBIT COLIBACILLOSIS: A MODEL FOR STUDY OF ENTEROPATHOGENIC *ESCHERICHIA COLI* (EPEC).

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Abstract - Colibacillosis has raised in rational rabbit breeding units as one of the most economically and pathologically important issue since the beginning of the eighties. Data on the virulence mechanisms and the phenotypic characters of the *E.coli* strains that are responsible of epizootics of lethal diarrheas have been gathered through the years. These strains are representative of a "pathovar" that is known in strains of human origin as "enteropathogenic *E. coli*" (EPEC). The understanding of the pathogenesis mechanisms of this type of bacteria will probably lead to new tools in the control of the disease in the field.

INTRODUCTION

Rabbit (*Oryctolagus cuniculus*) meat production has been progressively structured in western Europe since the mid-seventies. In rational breeding units, it became evident that digestive disorders in weaned animals was a highly dominant problem, inducing major commercial losses due to weight impairement, epizootic diarrheas, mortalities and veterinary costs. In search of infectious causes that may be responsible of such disorders, several parasites and bacterias could be selected as potential diarrhea-inducers. Digestive *Eimeria spp,* that were very common in rabbit, were controlled since the beginning of eighties by supplementation of the diet with anti-coccidial drugs. The frequent raise of *Escherichia coli* populations in feces of animals during diarrheic episodes questionned about the role of this bacteria, which is also, as in most species, a normal component of the digestive flora of rabbit. Then, several teams tried to answer the question: is the *E.coli* rise due to modifications in the gut environment associated with diarrhea (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 diarrhea in weaned rabbit ? Based on the fulfillment of Koch's postulates (i.e. by experimental infection with individual *E. coli* isolates from diarrheic rabbits), both hypothesis appeared to be true. In most diarrheic episodes, the normal *E. coli* strains from rabbit digestive flora raised to high populations; however, these stains could not induce diarrhea when orally given to healthy weaned rabbits. On the contrary, some strains or groups of strains were highly pathogenic for rabbit, and bear a significant epidemiological repartition in the western european breeding units. Several lines of evidence indicate that these strains have pathogenic mechanisms that are analogous to those of an *E. coli* "pathovar" described in some human *E.coli* diarrheas: the EnteroPathogenic *E. coli* (EPEC).

THE EPEC PATHOVAR

Diarrhea-inducing *E.coli* strains isolated from humans are currently shared into five pathovars on the basis of their pathogenic mechanisms. In the mid-eighties, four of these pathovars were consensually accepted (Edelman and Levine, 1983; Levine, 1987): ETEC (Enterotoxigenic *E. coli*), EIEC (EnteroInvasive *E. coli*), EHEC (EnteroHemorrhagic *E. coli*) and EPEC. Then, a fifth pathovar was individualized: the EAggEC (EnteroAggregative *E. coli*) (VIAL *et al.*, 1988). Table 1 summarizes the main characters of these different pathovars. Readers may refer to a recent review that has been devoted to EPEC (LAW, 1994).

The term EPEC was fist proposed in 1955 by NETER (cited by LEVINE, 1987) to refer to a series of *E.coli* strains that were epidemiologically incriminated in severe and epidemic outbreaks of infantile diarrheas 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 (SANSONETTI, 1985; ØRSKOV and ØRSKOV, 1992). Serotyping remained the only diagnostic tool for EPEC until the early 1970s. At that time, the frequence of epidemic episodes caused by EPEC decreased in

developped countries (Cohen 1991), but EPEC are still a very important cause of diarrhea in infants in the developping ones as well as agents of travellers diarrheas (GOMES *et coll.*, 1989, 1991; SENERWA *et coll*, 1989a et b; ETCHEVERRIA *et coll.*, 1991; KIM, 1989; BHAN *et coll.*, 1989; CHATKAEOMORAKOT *et coll.*, 1987).

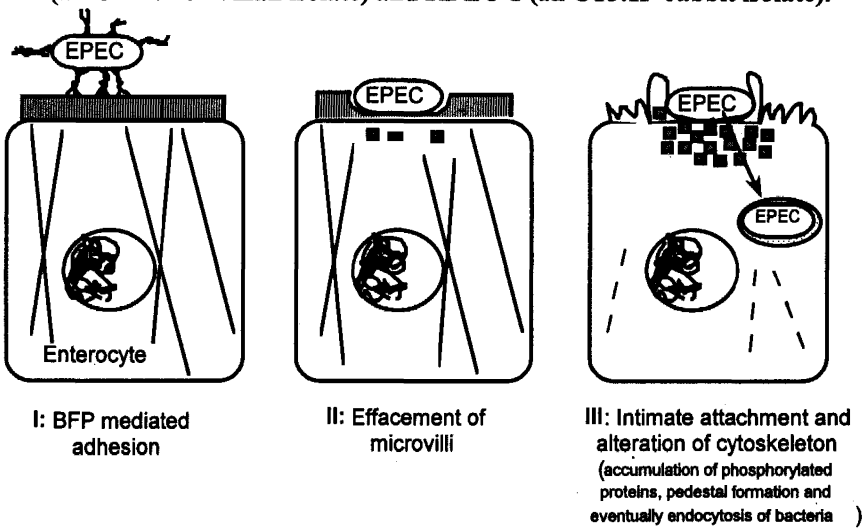
Table 1 : Diarrhea-inducing *E.coli* pathovars in humans (SANSONETTI, 1985; LEVINE, 1987; BOEDECKER and SHERMAN, 1986, ØRSKOV and ØRSKOV , 1992).

Pathotype	Strains characters
EPEC (enteropathogenic *E.coli*)	- Typical serogroups and serovars (see text) - Adhesion to enterocyte by specific adhesins - *In vitro* adhesion to cell lines: localized (class I) or diffuse (class II) - Attachement-effacement of enterocytes microvilli and *eae* (LEE) loci - No invasive character of "*Shigella*" type - No production of enterotoxins - No production of Shiga-like (Vero-) cy-totoxins
ETEC (enterotoxigenic *E.coli*)	- Typical serovars: O6:H16, O8:H9, O15:H11, O25:H42, O78:H12, O120:H7, O20:H-,... - Adhesion to enterocytes by mean of specific fimbriae (CFA/I à IV) - Production of ST and/or LT enterotoxins
EIEC (enteroinvasive *E.coli*)	- Virulence plasmid (*ca* 140 MDa) - Invasion and proliférâtion in epithelial cells *in vivo* and *in vitro* - Sereny test positive (inoc. to guinea pig conjunctiva) - non motile, lactose neg., LDC neg. - Typical serogroups: O28ac, O112, O124, O136, O143, O144, O173.
EHEC (enterohemorrhagic *E.coli*)	- High production of Shiga-like (Vero-) cytotoxins - Attachement-effacement of enterocytes microvilli and *eae* (LEE) loci - Typical serovars: O157:H7, O26:H11, O103:H2, O172:H?,... - Virulence plasmids (*ca* 60 Mda)
EAggEC (enteroaggregative *E. coli*) or EA-AggEC (enteroadherent-aggregative *E. coli*)	- Typical *in vitro* "stack brick" adherence to cell lines - Production of a cytotonin (ST entero-toxin-like)

Pathogenesis mechanisms of EPEC -

Models for the pathogenesis of EPEC have been proposed successively by KNUTTON *et al.* (1987) and DONNENBERG and KAPER (1992). 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 in different steps that include: (i) a loose attachment of the bacteria to the cell, which may be mediated by several specific proteic structures called adhesins; (ii) a close contact of the bacteria with the target cell that needs expression of a procaryotic protein called intimin and (iii) a signal transduction, probably delivered through secreted proteins driven to the cell by a type III secretion system. 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 localized. The fate of the target cell is less well known. However it is presumed that a set of bacteria may penetrate into the target cell by endocytosis and/or the result of the transduced signal(s) may explain death of the cell or at least an efflux of water and ions implicated in the pathogenesis of diarrhea.

Figure 1 : Three-stage model of EPEC pathogenesis (Donnenberg and Kaper, 1992). The model is mainly based on data gathered from studies of the strains E2348/69 (an O127:H6 human isolate) and RDEC-1 (an O15:H- rabbit isolate).



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 bacterias onto the epithelium. *In fine*, adhesins are thought to allow colonization of the enteric biotope by the bacterias, that thwart several non specific defense mechanisms of the host (such as peristaltism, and barrier effects induced by the resident flora). Mechanisms of adhesion of EPEC from most classical serovars have been studied *in vitro* on cultured epithelial cell lines, such as Hep-2 or HeLa cells. SCALETSKY *et al.* fist 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 bacterias attached in a scattered pattern to the entire cell surface. Because of the strong association of LA and classical EPEC serovars from diarrhea 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 diarrhea and were termed class II EPEC (NATARO *et al.*, 1985; LEVINE 1987). The LA phenotype is now known to be generated by plasmid-born fimbriae called "Bundle Forming Pili" (BFP) (GIRON *et al.*, 1991).

The attachment-effacement lesion and the LEE locus

Recently, new data have been gathered on the mechanisms of the attachment-effacement lesion (FOUBISTER *et al.*, 1994b, KENNY and FINLAY, 1995, MCDANIEL *et al.*, 1995, JARVIS *et al.*, 1995). A sum-up on these data is presented in Figures 2 and 3. On a bacterial genetic point, the ability to induce A/E lesion is under dependance of a group of chromosomal genes forming a "pathogenicity island" inserted near the rare tRNA locus *selC* (figure 2) (for a review, see LEE, 1996). This 35†kbp DNA region has been called LEE, for Locus of Enterocyte Effacement (McDANIEL *et al.*, 1995), and contains the *eaeA* locus (JERSE *et al.*, 1990), that encodes a 94 kDa outer membrane protein called intimin. This protein is thought to mediate close contact between the EPEC and the target cell. It may serve further as a "secondary" adhesin, binding to a putative membrane receptor, and the complex intimin/receptor as an organizer for aggregation of phosphorylated cytoskeletal proteins beyond the intimin receptor (Rosenshine *et al.*, 1992) (figure 3). A second chromosomal gene called *eaeB*, located *ca* 5 kb downstream from *eaeA* in the LEE locus, has been shown necessary for A/E lesion induction by EPEC (DONNENBERG *et al.*, 1993). EaeB is a 37 kDa protein that is released from EPEC through a type III secretion apparatus that is also encoded by genes (*sepABCD*) from this locus (KENNY and FINLAY, 1995; JARVIS *et al.*, 1995, McDANIEL *et al.*, 1995). EaeB is involved in signal transduction associated with tle A/E lesion, which includes tyrosine phosphorylation of a 90†kDa host protein (Hp90) (ROSENSHINE *et al.*, 1992). Phosphorylated Hp90 is a membrane protein that creates a binding site for intimin. It also acts in nucleation of cytoskeletal elements along the EPEC-cell contact area (Figure 3) (ROSENSHINE *et al.*, 1996). Tyrosine phosphorylation also activates host phospholipase C (FOUBISTER *et*

al., 1994a), which leads to elevation of intracellular free calcium levels (BALDWIN *et al.*,1991) and to activation of serine or threonine kinases and subsequent phosporylation of different host proteins (BALDWIN *et al.*, 1990, MANJARREZ-HERNANDEZ *et al*, 1992). Phosphorylation of membrane proteins may be responsible of ion secretion and subsequent diarrhea, while cytoskeletal changes may result in disruption of microvilli and A/E lesions (LAW, 1994).

Figure 2 : EPEC genetic systems involved in pathogenesis of E2348/69. The EAF plasmid carries genes involved in synthesis of Bundle Forming Pili (*bfp*) and a locus called *perA* which acts as a positive regulator on *eaeA* (intimin) gene. A chromosomal 35kbp pathogenicity islet (LEE for locus of enterocyte effacement) is inserted near the *selC* (selenocysteine) tRNA locus. This region contains the *eaeA* gene, which encodes the 94 kDa intimin, the *sep* genes which are involved in a type III secretion apparatus synthesis and the gene *eaeB* which encodes a 37 kDa protein excreted *via* the *sep* system and involved in signal transduction to target eukariotic cell. Other proteins (40, 39 and 25 kDa) appear to share this secretion pathway, but their roles are unknown. (FOUBISTER *et al.*, 1994, KENNY and FINLAY, 1995, McDANIEL *et al.*, 1995, JARVIS *et al.*, 1995)

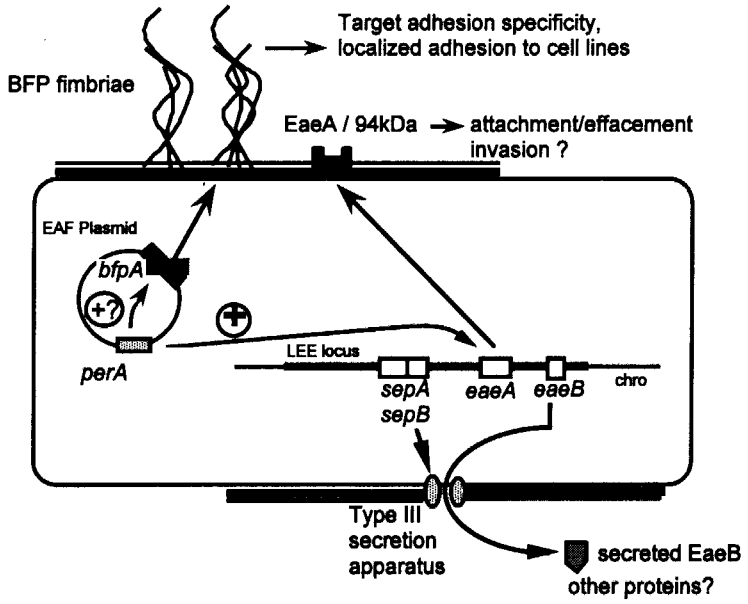
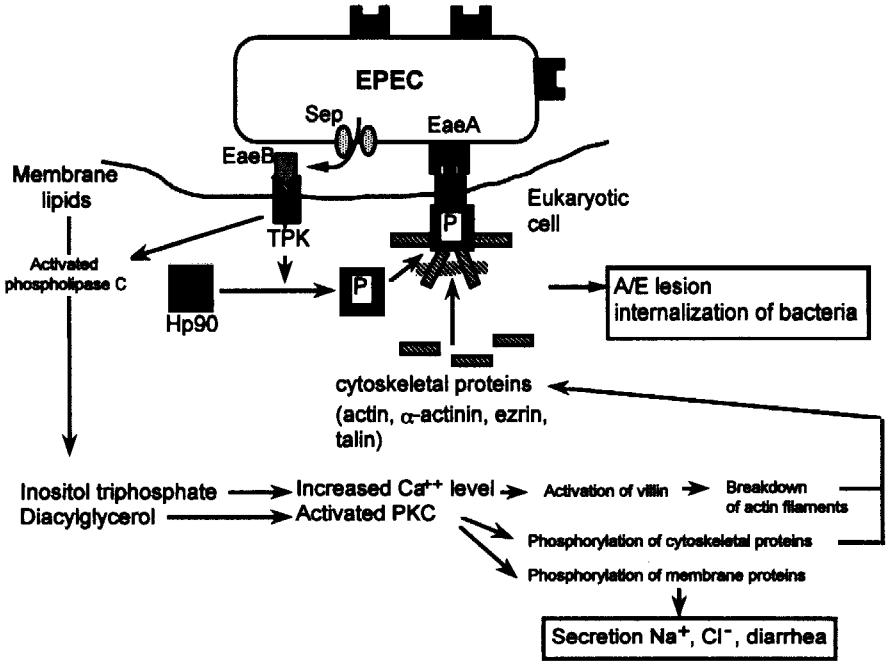


Figure 3 : Possible mechanisms for development of A/E lesions (modified from LAW, 1994)



RABBIT EPEC-LIKE

The RDEC-1 strain

A link between EPEC strains of human origin and rabbit diarrheagenic *E. coli* has been discovered initially by CANTEY and BLAKE (1977). The study of an outbreak of diarrheas in a colony of laboratory rabbits led them to isolate the RDEC-1 (Rabbit Diarrheal *E. coli* -1) strain. This O15:H- strain could reproduce diarrhea upon oral administration to weaned rabbits, did not produce known enterotoxin, was not invasive as judged by the Sereny test (CANTEY and BLAKE, 1977) and induced A/E lesions of the enterocytes (TAKEUCHI *et al.*, 1978). At that time, the A/E lesion was considered as a new mechanism and studies conducted on RDEC-1 participated significantly to the definition of the mechanisms of virulence in the EPEC pathovar.

The RDEC-1 strain reproduces diarrhea upon oral administration of 102 to 1010 colony forming units (i.e. viable bacterias) to weaned rabbits (CANTEY and BLAKE, 1977). It colonizes within few hours ileal Peyers patches (CANTEY and INMAN, 1981; INMAN and CANTEY, 1984), then spreads to 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 is able to persist at high populations in the distal parts of rabbit digestive tract at least during 15 days (CANTEY and HOSTERMAN, 1979). Usually, the diarrhea appears at peak of colonization, 3 to 4 days after infection. The colonization ability is corelated to the aptitude of the strain to adhere in vitro to enterocytes brush borders isolated from rabbits over 3 weeks (CHENEY *et al.*, 1979; CHENEY and BOEDECKER, 1984). The RDEC-1 strains appears rather specific of rabbit, as it does not colonize rats or guinea pigs, and does not adhere to human enterocytes (CHENEY *et al.*, 1980). However, it induces A/E lesions in newborn colostrum-deprived piglets without generating clinical signs (MOON *et al.*, 1984).

The adhesion to enterocytes and the colonization aptitude were quickly corelated to the presence of a plasmid-encoded fimbrial adhesin called AF/R1 (Adhesive Factor/ Rabbit 1) (CHENEY *et al.*, 1983; INMAN *et al.*, 1984; BERENDSON *et al.*, 1983; WOLF *et al.*, 1988). 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 cytoskeletton (RAFIEE *et al.* 1990). Mutants of AF/R1 have been obtained by repeated subcultures at 42°C (CANTEY *et al*, 1989) or by transposition (WOLF *et al.*, 1988). The mutants show altered ability to adhere to Payer's patches 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 diarrhea in some animals (CANTEY *et al*, 1989). The *afr* operon has been cloned and the gene for the major subunit (AfrA) has been sequenced (WOLF and BOEDECKER, 1990). It revealed that AF/R1 belongs to the family of the type 1 fimbriae (class I adhesins): AfrA has 43 p.100 and 42 p.100 of homologies respectively with the major subunits of type 1 fimbriae (FimA) and Pap fimbriae (PapA) from uropathogenic *E. coli* (WOLF and BOEDECKER, 1990).

Like classical EPEC of human origin, the RDEC-1 strain bears an *eaeA* locus (POHL *et al.*, 1993; LEROY *et al*, 1994). 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 *eaeB* and *sep* (McDaniel *et al*, 1995). It seems therefore likely that RDEC-1 induces diarrhea by using 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 Payer's patches, and, further, of the distal parts of the digestive tract, allowing secondary close contacts with enterocytes and the development of A/E lesions, under dependance of the LEE locus.

Epidemiologically significant groups of rabbit EPEC-like in Western Europe

During eighties, several european teams tried to solve the question about the existence of really pathogenic field strains of *E. coli* in rabbit. Strains isolated from diarrheic rabbits in breeding units were serotyped and/or biotyped and their experimental ability to reproduce diarrhea upon oral administration to rabbits was tested. Epidemiological data were gathered mainly from Belgium, the Netherlands, France and Spain, including serovar and biovar data, experimental pathogenesis and characterization of intestinal lesions, and sometimes genetic identification of relevant virulence loci (PEETERS *et al.*, 1984a, 1984b, 1984c, 1985, 1988; OKERMAN and DEVRIESE, 1985; Licois *et al.*, 1982, 1991; CAMGUILHEM *et al.*, 1986; CAMGUILHEM and MILON, 1989; POHL *et al.*, 1993; LEROY *et al.*, 1994; LEROY-SETRIN *et al.*, 1995; BLANCO *et al.*, 1994). As a whole, the results indicated that several groups of *E. coli* inducing A/E lesions were highly

pathogenic for rabbit. In Benelux, these include 0109:K-:H2 strains that were found in suckling rabbits and induced severe and lethal yellowish diarrhea 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 diarrheas with high mortality 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 diarrheic weaned rabbits. These strains, as well as some O26:H11 french isolates, induced severe and lethal diarrheas upon oral inoculations with as few as 10^4 CFU. All studies indicated that some strains of other serovars and/or biotypes (such as O128 or O132) could induce mild diarrheas and/or weight losses (and may be isolated either from diarrheic or from healthy animals). All highly pathogenic groups were shown to induce *in vivo* A/E lesions (PEETERS *et al.*, 1984c, 1985; LICOIS *et al.*, 1991; ROBINS-BROWNE *et al.*, 1994b) and hybridized with an *eaeA* DNA probe (POHL *et al.*, 1993; LEROY *et al.*, 1994; ROBINS-BROWNE *et al.*, 1994a), that refered them to EPEC or EHEC pathovars. However, phenotypic or genetic research on their ability to produce Shiga-like toxins has always been negative, suggesting that they belonged to an EPEC-like group (MARENDA *et al.*, 1992; MARIANI-KURKDJIAN *et al.*, 1993; POHL *et al.*, 1993; LEROY *et al.*, 1994; ROBINS-BROWNE *et al.*, 1994a). We have now recent evidences that rabbit strains of the O103 group present on their chromosome an equivalent of the entire LEE locus, which confirms their closeness to EPEC (J. DE RYCKE, E. COMTET, M. BOURY and A. MILON, unpublished data). Interestingly, by use of discriminant genetic techniques, LEROY-SETRIN *et al.*, (1995) 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, following the animal diffusion channels.

Peculiarities of rabbit EPEC-like

Besides their ability to induce A/E lesions and to possess genes that are necessary for that purpose, rabbit EPEC-like present specificities that link them to their natural host. As already mentionned for the RDEC-1 strain, their ability to induce diarrhea in different hosts has been tested and their virulence seems quite restricted to rabbit. Robins-Browne *et al.*(1994b) showed that, despite their adhesion in vitro to enterocytes of different species, their colonization ability (in mice) and their induction of A/E lesions (in guinea-pigs), none of 4 rabbit EPEC-like strains (including RDEC-1, another O15:H-, an O103:H2 and an O109:H2) was virulent for mouse or guinea-pig. This suggests strongly that colonization ability and A/E lesions induction are necessary for pathogenicity of rabbit EPEC-like. It seems likely that adhesins like AF/R1 intervene in the gut colonization process. Little is known on the adhesins of rabbit EPEC-like isolates except for RDEC-1 and O103 strains. The adhesin AF/R1 (from the RDEC-1 strain) seems to be rather unique, since it is not detected in field isolates (including O15:H- ones) (POHL *et al.*, 1993; ROBINS-BROWNE *et al.* 1994b). We have studied extensively the adhesive properties of O103 strains and showed that they adhere to cellular substrates by mean of a fimbrial adhesin that we called AF/R2 (MILON *et al.*, 1990; PILLIEN *et al.*, 1996). This fimbrial adhesin is clearly different from AF/R1; it has a major subunit of apparent m.w. 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. A mutant that does not express AF/R2 has impaired colonization properties and a significantly reduced pathogenicity in weaned rabbit. We have recently cloned the whole operon *af/r2* and sequenced the gene that encodes its major subunit. The deduced protein has significant homologies with class II adhesins major subunits, such as F4 (K88 fimbriae) FaeG or the afimbrial Cs31a subunit ClpG (F. FIEDERLING, M. BOURY, C. PETIT and A. MILON, submited). If AF/R2 is clearly involved in pathogenesis of 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 (MILON *et al.*, 1990, 1992). On another hand, 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. We have also some evidences that the LEE locus is involved in the pathogenesis of O103 strains. We selected recently a transposed mutant of the reference O103 strain B10 on the basis of its impaired ability to induce a specific cytopathic effect after interaction with HeLa cells. In this mutant, the transposon is inserted into the LEE locus. When tested in weaned rabbits, this mutant had completely lost its virulence (CHALARENG *et al.*, 1994).

DIAGNOSIS, THERAPY, PREVENTION OF WEANED RABBIT COLIBACILLOSIS

Diarrheic disease in weaned rabbit, associated or not with haemorrhages of caecal and colic serosae, must lead to the search for EPEC-like strains in caecal content or in feces of animals. In O103 colibacillosis, the sensitivity of animals seems maximal the very few weeks after weaning (LICOIS *et al.*, 1990). However, in few

cases, O103 colibacillosis has been detected in suckling rabbits as well as in does (MILON *et al.*, 1990b). As already explained herein, 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 diarrheas. One has also to remind that EPEC may be carried by adults, or suckling animals, without any trouble, depending on their usual target. In this respect, at least two characters must be found for positive diagnosis: (i) a high level of *E. coli* CFUs/g or ml of material (over 10^8), which implies that an enumeration is done in appropriate selective medium; (ii) the possession by isolated strain(s) of EPEC-like characters. In western Europe, it means that serogroup is to be determined (O15, O103, or, less importantly, O26, O128, O132 and O109) and that biovar may greatly help [rhamnose reaction for O103, O26 (CAMGUILHEM and MILON, 1989), and/or Peeters biovars determination (PEETERS *et al.*, 1988)]. Further detection of genes specific for rabbit EPEC pathogenesis (either in the isolates, or, in future, directly in samples) is (or will be) helpful for a certitude diagnosis, but these tests are out of the possibilities of a classical medical laboratory for the moment.

The use of antibiotics to treat or prevent EPEC colibacillosis is not easy, due to the peculiarities of the digestive physiology and of the normal flora of rabbit, on one hand, and to the frequent acquired multiple resistance of *E. coli* strains, on another hand. It must be kept in mind that wide spectrum antibiotics, such as ß-lactames (ampicillin, amoxicillin, cephalosporins,..), lincomycin, clindamycin are highly toxic for rabbit, essentially because they induce tremendous desequilibrium of the digestive flora (usually associated with death). Narrow spectrum antibiotics, as polypeptides (colistin, or polymyxin B) or quinolones (flumequine, enrofloxacin) may help to decrease mortality during an epizootics (PEETERS and GEEROMS, 1990; CHOW *et al.*, 1994). 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 rabbit. Furthermore, EPEC strains show at high frequency conjugative plasmid-born resistances that may be selected by *in vivo* use of relevant antibiotics and may spread easily in the enteric bacterial populations (CAMGUILHEM *et al.*, 1986b; REYNAUD *et al.*, 1991; .BLANCO *et al*, 1994).

Other means of therapy and prevention have been tested in rabbit EPEC colibacillosis. REYNAUD *et al.* (1992) described a lytic bacteriophage specific for O103 *E. coli* strain. However, attemps to use this phage for therapy of experimental colibacillosis failed (REYNAUD *et al.*, 1992).

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 EPEC. Early studies revealed that parenteral administration of formalin- or heat-killed bacteria did not protect weaned animals against oral challenge by the reference O103 strain B10 (CAMGUILHEM, 1986c). We moved to oral vaccination and showed that *per os* administration just after weaning of high and repeated doses of formalin killed O103 strain B10 could protect very efficiently the animals against experimental challenge (CAMGUILHEM and MILON, 1990; MILON and CAMGUILHEM, 1989; MILON *et al.*, 1989). Vaccination protocols gave good results under field conditions, in breeding units with O103 colibacillosis problems (CAMGUILHEM *et al.*, 1990; MILON and BOURY, unpublished data). However, the heaviness of schedules, doses and protocols and the rigour that is necessary to the application of inactivated vaccines do not allow their use in practice. That is the reason why we turned to trials with live strains, that could be administrered only once at weaning, in moderate doses, with the advantage of *in vivo* multiplication in the digestive tract of vaccinated animals. In that respect, we tested two non pathogenic rabbit isolates which shared with virulent O103 strains either the O103 LPS or the AF/R2 adhesin (MILON *et al.*, 1992). One of these strain, an O128 AF/R2 positive isolate called C6, gave very good results, by preventing colonization of the gut by the challenge strain B10. 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 questionned (TRAN CONG *et al.*, 1992). The results of crossed experimental administrations of C6 and B10 showed that when the strain are given to rabbits at the same time, or when the strain B10 is given before the strain C6, the competition is always win by the pathogenic O103 strain B10, suggesting that C6 could not displace a virulent strain and be used in ecological therapy (TRAN CONG *et al.*, 1992). Globally, 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 solve this problem on a scientific basis.

CONCLUSION

The apparent uniformity of mechanisms and genes of pathogenicity of the highly virulent *E.coli* strains that are responsible of digestive colibacillosis in rabbit lead to the conclusion that these strains are representative of the enteropathogenic (EPEC) pathovar. Progress of knowledge on this type of pathogenic strains give reasonable hopes for the near future on several points coping with colibacillosis control in the field. This includes increased diagnosis possibilities as well as new vaccinal approaches, which will permit a global sanitary and medical prevention against -at least- the main epidemiologically relevant strains.

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Colibacillose chez le lapereau sevré : un modèle pour l'étude des *Escherichia coli* enteropathogènes (EPEC) - La colibacillose est devenue, depuis le début des années 1980, une dominante pathologique de l'élevage du lapin 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). La compréhension des mécanismes de pathogénie de ces souches conduira probablement à de nouveaux outils de contrôle de la maladie en élevage.