

## Virulence plasmids of enterotoxigenic *Escherichia coli* isolates from piglets

J.G. Mainil<sup>a</sup>, G. Daube<sup>a</sup>, E. Jacquemin<sup>a</sup>, P. Pohl<sup>b</sup>, A. Kaeckenbeeck<sup>a</sup>

*a. Chaire de Bactériologie et de Pathologie des Maladies Bactériennes, Faculté de Médecine Vétérinaire, Université de Liège, Campus du Sart Tilman, Bât B43a, Liège, B4000, Belgium*

*b. Centre d'Etudes et de Recherches Vétérinaires et Agrochimiques, Groeselenberg, 99, Bruxelles, B1180, Belgium*

### Abstract

Virulence plasmids of 68 ETEC isolates from piglets belonging to different pathotypes and six ETEC isolates from calves with pathotypes typical of porcine ETEC were identified with seven virulence probes for the heat-stable (STa and STb) and heat-labile (LT) enterotoxins, for the F4, F5, F6, and F41 fimbrial adhesin subunit, and also with five Rep probes for the RepFIA and RepFIB basic replicons, and the RepFIC family of basic replicons. With the exception of the F41 probe, the other virulence probes hybridized with at least one plasmid band of a size range from 65 to more than 100 Mda. Common associations of virulence factor-encoding genes on plasmid bands were: STb/LT, STa/F5, STa/F6, STa/STb. Other associations, STa/F4, STa/F4/F6, and STa/STb/LT/F6, were rarer. On the other hand the F4 adhesin-encoding genes were isolated on one plasmid band in all but three F4+ isolates. All but one of the 92 virulence plasmids which were studied have Rep probe hybridization profiles and replicon types typical of the uni- or multireplicon plasmids belonging to the various incompatibility groups of the F incompatibility complex.

**Keywords:** *Escherichia coli*; Pig-bacteria; Plasmids; Replicon typing

### 1. Introduction

*Escherichia coli* is a versatile bacterial species with various virulent strains which produce specific virulence factors or possess virulence-associated properties (reviews by Pohl, 1993; Lior, 1994). In pigs, several categories of pathogenic *E. coli* have been described (reviews by Holland, 1990; Fairbrother, 1993; Alexander, 1994; Bertschinger and Gyles, 1994; Fairbrother and Ngeleka, 1994; Hampson, 1994).

Enterotoxigenic strains, or ETEC, are associated with neonatal, preweaning and postweaning diarrhea (Fairbrother, 1993; Alexander, 1994; Hampson, 1994). They belong to several pathotypes according to the combination of fimbrial adhesins and enterotoxins they produce or whose encoding genes they harbour. Porcine ETEC isolates can be classified into three subgroups according to the production of the heat-stable enterotoxins (STa and/or STb) or to the hybridization results of gene probes specific for these ST toxins: class 1 isolates produce the type b (STb), class 2 isolates the type a (STa), and the class 3 isolates both enterotoxins (STa and STb) (Whipp et al., 1981; Harnett and Gyles, 1983; Mainil et al., 1995).

The enterotoxins and most of the fimbrial adhesins of ETEC from cattle, man, and pig are coded by genes located on plasmids belonging to the F incompatibility complex by incompatibility and/or replicon typing (Harnett and Gyles, 1985; Smith et al., 1985; Broes et al., 1988; Mainil et al., 1992; Gyles, 1994; Smyth et al., 1994).

The goals of this study were: (i) to confirm the plasmid location of the various virulence factor-encoding genes of porcine ETEC; (ii) to study their association on the virulence plasmids; and (iii) to identify the virulence plasmid replicon types.

## 2. Materials and methods

### 2.1. *E. coli* isolates

A total of 68 ETEC isolates from piglets belonging to different pathotypes (Table 1) were chosen on the basis of their different pathotypes for virulence plasmid identification and replicon typing. The isolates originate from Belgium (Mainil et al., 1995) but also from USA, Canada and UK. The virulence plasmids of the Canadian isolates have already been partially characterized (Broes et al., 1988). Six American ETEC isolates from cattle with pathotypes of porcine ETEC were added to this number (Isaacson et al., 1978; Mainil et al., 1990).

### 2.2. Gene probes

The gene probes for the enterotoxins (STaP, STb, and LT) and for the fimbrial adhesin subunits (F4, F5, F6, and F41) were derived as already described (Mainil et al., 1990, 1995). The DNA probes for the RepFIA, RepFIB, RepFIC, and RepFIIA basic replicons of plasmids from the F incompatibility complex, and for the RepII basic replicon (rep probes) were derived according to Couturier et al. (1988). The FIA and FIB probes are specific of their replicons of origin. The FIC, FIIA and II probes originate from basic replicons of the so-called RepFIC family: the FIC probe detects all described variants whereas the FIIA and II probes hybridize with two subgroups of this family of basic replicons allowing partial characterization (Couturier et al., 1988).

### 2.3. Plasmid DNA hybridization

Plasmid DNA was extracted from the *E. coli* isolates according to the method described by Kado and Liu (1981) with some modifications (Broes et al., 1988). The plasmids were separated by agarose gel electrophoresis and hybridized within the gels with the virulence and with the Rep probes according to Maas et al. (1985) with modifications (Broes et al., 1988). The ETEC strains 431 (STaP+F5+F41+) (Moon et al., 1977), 987 (STaP+STb+F6+) (Nagy et al., 1977), and Abbotstown (STb+LT+F4+) (Sojka, 1972) were used as controls for the virulence probes. The control plasmids for the Rep probes were the plasmids FTacpro (FIA+FIB+FIC+FIIA+), P307 (FIB+FIC+FIIA+ 1+), Rldrdl9 (FIC+FIIA+), and R64drdl1 (FIC+II+) (Couturier et al., 1988). Plasmid size markers were the six plasmids from *Erwinia uredovora* 391: 173.3, 120, 70, 43.3, 18.7 and 5.2 Mda (Thiry, 1984) and the four plasmids of strain 39R681: 98, 42, 23.9 and 4.6 Mda (received from Dr. Jack Levy of the St. Pierre Hospital, Brussels, Belgium).

## 3. Results

### 3.1. Hybridization of plasmid DNA with virulence probes

Plasmid bands were detected by hybridization in agarose gels with the gene probes for the STaP, STb, and LT enterotoxins and for the F4, F5, and F6 adhesins. On the opposite, the F41 probe detected no plasmid band in any of these isolates. In three isolates (1535, 1540, 1878) no plasmid band had homology to the F6 probe, in another isolate (GIS25) to the F4 probe, and in a fifth isolate (19KP85) to the STaP and F6 probe were observed.

The virulence plasmid bands of the 74 ETEC isolates ranged from 65 Mda to more than 100 Mda, except for the STaP+ plasmid band of isolate 1676. The virulence plasmid pathotypes were identical for most of the ETEC isolates within the same bacterial pathotype. The six isolates from cattle (1533, 1535, 1540, 1876, 1877, and 1878) had the same virulence plasmid profiles as the porcine ETEC belonging to the same pathotype (Table 1). Specific association of genes coding for virulence factors on the plasmids were the following: STaP and F5, STaP and F6, STaP and STb, STb and LT. On the opposite the genes encoding for the F4 adhesins were isolated on particular plasmids in most F4+ ETEC.

One virulence plasmid band was detected in all STb+LT+, STb+, STaP+F5+, STaP+F41+, and STaP+STb+ ETEC and in most STaP+F5+F41+ and STaP+F6+ ETEC isolates. Two virulence plasmid bands were present in all STb+LT+F4+ and in most STaP+STb+F6+ isolates. One to three plasmid bands were observed in the nine STaP+STb+LT+F4+F6+ ETEC isolates.

A few ETEC isolates gave peculiar plasmid DNA hybridization results compared to the majority of isolates (Table 1), either by lack of hybridization of one or two gene probes (isolates 1535, 1540, 1878, GIS25, 19KP85) or by additional hybridization of one gene probe by a second plasmid band (isolates 603 and 1676).

**Table 1:** Pathotypes and replicon types of the virulence plasmids of porcine ETEC isolates'

Pathotype	No. Isolates	Plasmid pathotype	Rep probe hybridization
<b>Class 1 ETEC isolates.</b>			
STb+LT+F4+	6	STb+LT+ and F4+	FIB+FIC+IIA+I1 + FIC+IIA+
STb+LT+	11	STb+LT+	FIB+FIC+FIIA+I1 +
	1	STb+LT+	FIA+FIB+FIC+FIIA+I1+
STb+	5	STb+	FIA+FIB+FIC+FIIA+
	5	STb+	FIB+FIC+FIIA+
	1	STb+	FIC+FIIA+
	1 <sup>b</sup>	STb+	FIC+I1+
<b>Class 2 ETEC isolates.</b>			
STaP+F5+F41 <sup>c</sup>	7	STaP+F5+	FIA+FIB+FIC+FIIA+
	1	STaP+F5+	FIB+FIC+FIIA+
	1	STaP+F5+ and STaP+	FIA+FIB+FIC+FIIA+ - <sup>d</sup>
STaP+F5+	1	STaP+F5+	FIB+FIC+FIIA+
	2	STaP+F5+	ND <sup>e</sup>
STaP+F41+	1	STaP+	ND
STaP+F6+	3	STaP+F6+	FIB+FIC+FIIA+
	1	STaP+F6+	FIB+FIC+I1 +
	1	STaP+F6+	FIC+FIIA+
	2	STaP+F6+	FIC+FIIA+I1 +
	1	STaP+F6+	FIC+I1+
	1	STaP+F6+ and STaP+	FIC+FIIA+I1 + FIC+FIIA+
<b>Class 3 ETEC isolates.</b>			
STaP+STb+LT+F4+F6+	2	STaP+STb+LT+F6+ and F4+	FIB+FIC+FIIA+ FIC+FIIA+
STaP+STb+LT+F4+F6+	1	STaP+STb+LT+F6+ and F4+	FIB+FIC+FIIA+I1 + FIC+FIIA+
STaP+STb+LT+F4+F6+	2	STaP+STb+LT+F6+ and STaP+F4+	FIB+FIC+FIIA+ FIC+FIIA+
STaP+STb+LT+F4+F6+	1	STb+LT+ and STaP+F6+ and F4+	FIB+FIC+FIIA+I1 + FIC+FIIA+ FIC+FIIA+
STaP+STb+LT+F4+F6+	1	STb+LT+ and STaP+F4+F6+	FIB+FIC+FIIA+ FIC+FIIA+
STaP+STb+LT+F4+F6+	1 <sup>f</sup>	STaP+STb+LT+F6+	FIB+FIC+FIIA+
STaP+STb+LT+F4+F6+	1 <sup>g</sup>	STb+LT+ and F4+	ND ND
STaP+STb+F6+	6	STaP+STb+ and STaP+F6+	FIC+FIIA+ FIC+FIIA+I1 +
	1	STaP+STb+ and STaP+F6+	ND ND
	1 <sup>b</sup>	STb+ and STaP+F6+	FIC+I1+ FIC+I1+
	2 <sup>h</sup>	STaP+STb+	FIC+FIIA+
STaP+STb+	1	STaP+STb+	
	2	STaP+STb+	FIC+FIIA+

a. Isolates 1533, 1535, 1540, 1876, 1877 and 1878 are of cattle origin (Isaacson et al., 1978; Mainil et al., 1990).

b. This STb+ isolate (1797) derives from an STa+STb+F6+ isolate (P16; Moon, pc) with STb+ and STaP+F6+ plasmids (see class 3 isolates).

c. F41-encoding genes are not plasmid-located.

d. This STaP+ plasmid hybridized with RepQ probe (Mainil et al., 1992).

e. ND: not done

f. This isolate has lost homology with the F4 probe.

g. This isolate has lost homology with the STaP and F6 probes.

h. These isolates have lost homology with the F6 probe.

### 3.2. Replicon typing of virulence plasmids

Of the three Rep probes derived from basic replicons of the RepFIC family, the FIC probe hybridized with all but one of the 92 virulence plasmid bands (99%) which were studied whereas the FIIA probe tested positive with 86 plasmid bands (93%) and the I1 probe with 34 plasmid bands (37%). Twenty-nine plasmid bands (32%) tested positive with these three Rep probes (all but one STb+LT+, half of the STaP+F6+ and one STaP+STb+LT+F6+); 57 plasmid bands (62%) tested positive with the FIC and FIIA probes (all or most of the STb+, STaP+F5+, STaP+STb+, STaP+STb+LT+F6+, F4+, several STaP+F6+ and a few others); and five plasmid bands (5%) tested positive with the FIC and I1 probes (two STb+ and three STaP+F6+).

Thirty-seven plasmid bands (40%) were positive with the FIB probe (all or most STb+LT+, STaP+STb+LT+F6+, several STb+ and a few others); whereas 15 others (16%) tested positive with both FIA and FIB probes (most STaP+F5+, several STb+ and one STb+LT+).

Plasmids with identical rep types can belong to various pathotypes and plasmids with identical pathotypes to various rep types (Tables 2 and 3).

With the exceptions of the STaP+, STb+ and STaP+F6+ plasmid bands, the great majority of virulence plasmids within one pathotype gave the same replicon typing results whatever the pathotype of the isolate of origin was (Table 2).

**Table 2:** Replicon types of the 92 virulence plasmids according to the pathotype of the ETEC isolate of origin<sup>a</sup>

Plasmid pathotype	Isolate pathotype	Replicon type	No. plasmids	
<b>Enterotoxin plasmids</b>				
STaP+	STaP+F5+F41 +	-	1 <sup>b</sup>	
	STaP+F6+	FIC+FIIA+	1	
STb+	STb+	FIB+FIC+FIIA+	1	
		FIA+FIB+FIC+FIIA+	5	
		FIB+FIC+FIIA+	5	
		FIC+FIIA+	1	
		FIC+I1 +	1	
STaP+STb+	STaP+STb+F6+	FIC+I1 +	1	
	STaP+STb+	FIB+FIC+FIIA+	1	
STb+LT+	STaP+STb+F6+	FIC+FIIA+	2	
		FIC+FIIA+	8	
	STb+LT+	FIB+FIC+FIIA+I1+	11	
		FIA+FIB+FIC+FIIA+I1 +	1	
		STb+LT+F4+	FIB+FIC+FIIA+I1+	6
	STaP+STb+LT+F4+F6+	FIB+FIC+FIIA+	1	
		FIB+FIC+FIIA+I1+	1	
<b>Adhesin plasmids.</b>				
F4+	STb+LT+F4+	FIC+FIIA+	6	
	STaP+STb+LT+F4+F6+	FIC+FIIA+	4	
<b>Enterotoxin and adhesin plasmids.</b>				
STaP+F5+	STaP+F5+	FIB+FIC+FIIA+	1	
	STaP+F5+F41 +	FIA+FIB+FIC+FIIA+	8	
		FIB+FIC+FIIA+	1	
STaP+F4+	STaP+STb+LT+F4+F6+	FIC+FIIA+	2	
STaP+F6+	STaP+F6+	FIB+FIC+FIIA+	2	
		FIB+FIC+I1 +	1	
		FIC+FIIA+	1	
		FIC+FIIA+I1+	3	
		FIC+I1 +	1	
		STaP+STb+F6+	FIC+FIIA+I1+	6
			FIC+I1 +	1
		STaP+STb+LT+F4+F6+	FIC+FIIA+	1
	STaP+F4+F6+	STaP+STb+LT+F4+F6+	FIC+FIIA+	1
	STaP+STb+LT+F6+	STaP+STb+LT+F4+F6+	FIB+FIC+FIIA+	5
		FIB+FIC+FIIA+I1+	1	

- a. Seven virulence plasmids were not studied by replicon typing.  
 b. This STaP+ plasmid hybridized with RepQ probe (Mainil et al., 1992).

**Table 3:** Plasmid and isolate pathotypes according to the replicon types

Plasmid retype <sup>a</sup>	Plasmid pathotype	Isolate pathotype	No. plasmids/ No. studied
<b>Unireplicon virulence plasmids.</b>			
FIC+FIIA+	STaP+	STaP+F6+	1/2
	STb+	STb+	1/12
	STaP+STb+	STaP+STb+	2/3
		STaP+STb+F6+	8/8
	F4+	STb+LT+F4+	6/6
		STaP+STb+LT+F4+F6+	4/4
	STaP+F4+	STaP+STb+LT+F4+F6+	2/2
	STaP+F6+	STaP+F6+	1/8
		STaP+STb+LT+F4+F6+	1/1
	STaP+F4+F6+	STaP+STb+LT+F4+F6+	1/1
FIC+FIIA+I1+	STaP+F6+	STaP+F6+	3/8
		STaP+STb+F6+	6/7
FIC+I1 +	STb+	STb+	1/12
		STaP+STb+F6+	1/1
	STaP+F6+	STaP+F6+	1/8
		STaP+STb+F6+	1/7
<b>Bireplicon virulence plasmids</b>			
FIB+FIC+FIIA+	STaP+	STaP+F6+	1/2
	STb+	STb+	5/12
	STaP+STb+	STaP+STb+	1/3
	STb+LT+	STaP+STb+LT+F4+F6+	1/2
	STaP+F5+	STaP+F5+	1/1
		STaP+F5+F41+	1/9
	STaP+F6+	STaP+F6+	2/8
STaP+STb+LT+F6+	STaP+STb+LT+F4+F6+	5/6	
FIB+FIC+FIIA+I1+	STb+LT+	STb+LT+	11/12
		STb+LT+F4+	6/6
		STaP+STb+LT+F4+F6+	1/2
	STaP+STb+LT+F6+	STaP+STb+LT+F4+F6+	1/6
FIB+FIC+I1 +	STaP+F6+	STaP+F6+	1/8
<b>Trireplicon virulence plasmids.</b>			
FIA+FIB+FIC+FIIA+	STb+	STb+	5/12
	STaP+F5+	STaP+F5+F41+	8/9
FIA+FIB+FIC+FIIA+I1 +	STb+LT+	STb+LT+	1/12

1. Seven virulence plasmids were not studied by replicon typing and one STaP+ plasmid hybridized with the RepQ probe only (Mainil et al., 1992).

#### 4. Discussion

Many virulence factors of *E. coli* pathogenic to man and animals are coded by genes located on plasmids belonging to the F incompatibility complex. The virulence plasmids of human and bovine ETEC (size, combination of virulence genes, incompatibility properties, replicon typing) are relatively easy to analyze because these ETEC isolates belong to only a few pathotypes. At the opposite porcine ETEC can belong to many different pathotypes and few data on their various virulence plasmids have been published (reviews by Smith et al., 1985; Gyles, 1994; Smyth et al., 1994).

The porcine ETEC isolates of this work harbour one to six high-molecular weight plasmid bands. DNA sequences homologous to the structural genes coding for the STaP, STb, and LT enterotoxins and for the F4, F5, and F6, but not the F41 fimbrial adhesin subunit are located on plasmid bands in all but five isolates that were positive by colony hybridization. These results are in agreement with already published data, including the presence of F41 fimbriae-encoding genes on the chromosome (reviews by Smith et al., 1985; Gyles, 1994;

Smyth et al., 1994). The absence of hybridization with the STaP, F4, or F6 probes by the plasmid bands of five isolates could also be the consequence of the localization of these genes on the chromosome, but is most probably the consequence of the loss of the genes or of the virulence plasmids carrying those genes as these five isolates were subsequently negative when tested by colony hybridization. This kind of loss is not a rare observation either during in vitro storage or even during in vivo experiments (Mainil et al., 1987; Casey and Moon, 1990).

In the majority of isolates only one plasmid band is positive with each of the virulence gene probes. Exceptions are the STaP+STb+F6+ isolates, the STaP+STb+LT+F4+ F6+ isolates, and a very few isolates with other pathotypes in which copies of the STaP enterotoxin encoding gene are detected on two different plasmid bands. This duplication may be the results of a transposition event on another plasmid band of the STaP enterotoxin encoding transposon Tnl681 (So et al., 1979).

With the exceptions of most F4 probe-positive plasmid bands and of the single probe-positive isolates of course, almost all probe positive plasmid bands hybridize with at least two gene probes. Common associations of virulence factor-encoding genes on plasmid bands are: STb/LT, STaP/F5, STaP/F6, STaP/STb. Other associations, STaP/F4, STaP/F4/ F6, and STaP/STb/LT/F6, are rarer. These virulence factor-encoding genes are most probably associated on a single plasmid molecule ranging in size from 65 to more than 100 Mda. Presence of two or more plasmid bands of identical or similar size cannot be excluded absolutely, but these associations correspond to the associations phenotypically or genotypically already described in porcine and bovine ETEC (reviews by Smith et al., 1985; Gyles, 1994; Smyth et al., 1994). Exceptions may be the plasmid bands of STaP+STb+LT+F4+F6+ ETEC which are heterogeneous with regard to the virulence plasmid bands detected (Table 1). The STaP+STb+LT+F6+ and STaP+F4+F6+ plasmid bands of these isolates may well be composed of different plasmids which could not be separated under the conditions of electrophoresis used.

All but one virulence plasmid bands of porcine ETEC have replicon types typical of the plasmids belonging to the various incompatibility groups of the F incompatibility complex (Couturier et al., 1988). The virulence plasmid bands of porcine ETEC are uni- or multireplicon plasmids carrying at least one basic replicon of the RepFIC family. Moreover many plasmids, especially the STb+, STb+LT+, STaP+F5+, are multireplicon plasmids carrying two (RepFIB-RepFIC) or even three (RepFIA-RepFIB-RepFIC) basic replicons. Plasmids with identical rep types can belong to various pathotypes and plasmids with identical pathotypes to various rep types. They do not differ from the various virulence-associated plasmids of members of the family Enterobacteriaceae (reviews by Smith et al., 1985 and Couturier et al., 1988; Pohl et al., 1987; Silva et al., 1988; Oswald et al., 1989; Tinger and Curtiss III, 1990; Mainil et al., 1992). In addition IncF group plasmid multireplicon types are common on non-virulence associated plasmids of members of the family Enterobacteriaceae (Bergquist et al., 1986; Chaslus-Dancla et al., 1991; Mainil et al., 1992). For these reasons, the replicon type does not represent a universal epidemiologic tool although it may be of help in limited surveys, f.i. to trace the spread of a particular plasmid among bacteria.

On the other hand the more heterogeneous results for the STaP+ and STb+ plasmid bands may reflect the variety of origin (loss of other virulence genes or plasmid, as f.i. for the STb+ isolate 1797 which derives from the STaP+STb+F6+ isolate P16 after loss of the STaP+F6+ plasmid band; Moon, personal communication) and for the STaP+ and STaP+F6+ plasmid bands the presence of the STaP enterotoxin-encoding genes on transposable structure (So et al., 1979).

If some ETEC pathotypes may derive from others after gene and/or plasmid loss, other ETEC pathotypes may originate on the opposite from others after gene and/or plasmid acquisition. F.i. some of the STaP+STb+LT+F4+F6+ isolates may originate from STb+LT+F4+ isolates of the 0149:K91 serotype after acquisition of one STaP+F6+ plasmid of a similar size to the STb+LT+ plasmids (IPVS). Similarly, the STaP+STb+F6+ isolates may originate from STaP+F6+, STaP+STb+ or STb+ (for isolate P16) isolates, after acquisition of the second virulence plasmid. These observations highlight the importance of the plasmids in determining the virulence properties of enterotoxigenic *E. coli* just as phages determine the production of verotoxins by the verotoxigenic *E. coli* (O'Brien and Holmes, 1987).

Plasmid transfer attempts and comparisons of phenotypic and genotypic properties of bacterial isolates may bring answers in the future to the questions and hypotheses raised by the studies reported here.

## Acknowledgements

The authors thank Pr S. Lariviere (Faculté de Médecine Vétérinaire, Université de Montreal, St Hyacinthe, Quebec, Canada), Dr Moon (Iowa State University, Ames, Iowa, USA), and Dr Cox (Faculteit van de Geneeskunde, Rijksuniversitair Centrum, Universiteit Antwerpen, Antwerpen, België) for providing some of the porcine ETEC isolates studied. Dr Martine Couturier from the Molecular Biology Department of the University of Brussels (ULB) is also thanked for fruitful discussion. This work was supported by a grant from the "Fonds National de la Recherche Scientifique (FNRS, Crédits aux Chercheurs)".

## References

- Alexander, T.J.L., 1994. Neonatal Diarrhoea in Pigs. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans. CAB International, Guildford, UK, pp. 151-170.
- Bergquist, P.L., Saadi, S., Maas, W.K., 1986. Distribution of basic replicons having homology with RepFIA, RTepFIB and RepFIC among incF group plasmids. *Plasmid* 15, 19-34.
- Bertschinger, H.U., Gyles, C.L., 1994. Oedema Disease of Pigs. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans. CAB International, Guildford, UK, pp. 193-220.
- Broes, A., Fairbrother, J.M., Mainil, J.G., Harel, J., Lariviere, S., 1988. Phenotypic and genotypic characterization of enterotoxigenic *Escherichia coli* serotypes O8:KX105 and O8:K2829 strains isolated from piglets with diarrhea. *J. Clin. Microbiol.* 26, 2402-2409.
- Casey, T.A., Moon, H.W., 1990. Genetic characterization and virulence of enterotoxigenic *Escherichia coli* mutants which have lost virulence genes in vivo. *Infect. Immun.* 58, 4156-4-158.
- Chaslus-Dancla, E., Pohl, P., Meurisse, M., Marin, M., Lafont, J.P, 1991. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamycin and apramycin. *Antimicrob. Agents Chemother.* 35, 590-593.
- Couturier, M., Bex, F, Bergquist, P.L., Maas, W.K., 1988. Identification and classification of bacterial plasmids. *Microbiol. Rev.* 52, 305-317.
- Fairbrother, P., 1993. Les colibacilloses du porc. *Ann. Med. Vet.* 137, 369-375.
- Fairbrother, J.M., Ngeleka, M., 1994. Extraintestinal *Escherichia coli* infections in pigs. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans, CAB International, Guildford, UK, pp. 221-236.
- Gyles, C.L., 1994. *Escherichia coli* enterotoxins. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans, CAB International, Guildford, UK, pp. 337-364.
- Hampson, D.J., 1994. Postweaning *Escherichia coli* diarrhoea in pigs. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans, CAB International, Guildford, UK, pp. 171-192.
- Harnett, N.M., Gyles, C.L., 1983. Enterotoxigenicity of bovine and porcine *Escherichia coli* of O groups 8, 9, 20, 64, 101, and X46. *Am. J. Vet. Res.* 44, 1210-1214.
- Harnett, N.M., Gyles, C.L., 1985. Linkage of genes for heat-stable enterotoxin, drug resistance, K99 antigen, and colicin production in bovine and porcine strains of enterotoxigenic *Escherichia coli*. *Am. J. Vet. Res.* 46, 428-433.
- Holland, R.E., 1990. Some infectious causes of diarrhea in young farm animals. *Clin. Microbiol. Rev.* 3, 345-375.
- Isaacson, R.E., Schneider, R.A., Moon, H.W., 1978. *Escherichia coli* in the small intestines of calves with and without diarrhea: identification of virulence factors. In: Acres, S.D. (Ed.), *Proceedings of the Second International Symposium on Neonatal Diarrhea*, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, pp. 17-28.

- Kado, C.L., Liu, S.T., 1981. Rapid procedure for detection and isolation of large and small plasmids. *J. Bacteriol.* 145, 1365-1373.
- Lior, H., 1994. Classification of *Escherichia coli*. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans. CAB International, Guildford, UK, pp. 31-72.
- Maas, R., Silva, A.M., Gomes, T.A.T., Trabulsi, L.R., Maas, W.K., 1985. Detection of genes for heat-stable enterotoxin I in *Escherichia coli* strains isolated in Brazil. *Infect. Immun.* 49, 46-51.
- Mainil, J.G., Bex, F., Dreze, P., Pohl, P., Kaeckenbeeck, A., Couturier, M., 1992. Replicon typing of virulence plasmids in enterotoxigenic *Escherichia coli* isolates from cattle. *Infect. Immun.* 60, 3376-3380.
- Mainil, J.G., Bex, F., Jacquemin, E., Pohl, P., Couturier, M., Kaeckenbeeck, A., 1990. Prevalence of four enterotoxin (STaP, STaH, STb, and LT) and four adhesin subunit (K99, K88, 987P, and F41) genes among *Escherichia coli* isolates from cattle. *Am. J. Vet. Res.* 51, 187-190.
- Mainil, J.G., Daube, G., Jacquemin, E., Kaeckenbeeck, A., Pohl, P., 1995. Typage des *Escherichia coli* isolés d'intestins de porcelets en Belgique au moyen de sondes génétiques: souches entérotoxigènes, vérotoxigènes ou entéropathogènes. *Ann. Méd. Vét.* 139, 5-13.
- Mainil, J.G., Sadowski, P.L., Tarsio, M., Moon, H.W., 1987. In vivo emergence of enterotoxigenic *Escherichia coli* variants lacking genes for K99 fimbriae and heat-stable enterotoxin. *Infect. Immun.* 55, 3111-3116.
- Moon, H.W., Nagy, B., Isaacson, R.E., Orskov, I., 1977. Occurrence of K99 antigen on *Escherichia coli* isolated from pigs and colonization of pig ileum by K99+ enterotoxigenic *Escherichia coli* from calves and pigs. *Infect. Immun.* 15, 614-620.
- Nagy, B., Moon, H.W., Isaacson, R.E., 1977. Colonization of porcine intestine by enterotoxigenic *Escherichia coli*: selection of piliated forms in vivo, adhesion of piliated forms to epithelial cells in vitro, and incidence of a pilus antigen among porcine enteropathogenic *Escherichia coli*. *Infect. Immun.* 16, 344-352.
- O'Brien, A.D., Holmes, R.K., 1987. Shiga and Shiga-like toxins. *Microbiol. Rev.* 51, 206-220.
- Oswald, E., De Rycke, J., Guillot, J.F., Boivin, R., 1989. Cytotoxic effect of multinucleation in HeLa cell cultures associated with the presence of Vir plasmid in *Escherichia coli*. *FEMS Microbiol. Letters* 58, 95-100.
- Pohl, P., 1993. Les souches pathogènes d'*Escherichia coli*: histoire et classification. *Ann. Méd. Vét.* 137, 325-333.
- Pohl, P., Lintermans, P., Bex, F., Desmyter, A., Dreze, P., Fonteyne, P.A., Couturier, M., 1987. Propriétés phénotypiques et génotypiques de quatre plasmides de virulence de *Salmonella typhimurium*. *Ann. Inst. Pasteur (Paris)* 138, 523-528.
- Silva, R.M., Saadi, S., Maas, W.K., 1988. A basic replicon of virulence-associated plasmids of *Shigella* sp. And enteroinvasive *Escherichia coli* is homologous with a basic replicon in plasmids of incF groups. *Infect. Immun.* 56, 836-842.
- Smith, H.R., Scotland, S.M., Rowe, B., 1985. Genetics of *Escherichia coli* Virulence. In: Sussman, M. (Ed.), *The virulence of Escherichia coli*. The Society for General Microbiology, Academic Press, London, UK, pp. 227-269.
- Smyth, C.J., Marron, M., Smith, S.G.J., 1994. Fimbriae of *Escherichia coli*. In: Gyles, C.L. (Ed.), *Escherichia coli* in domestic animals and humans. CAB International, Guildford, UK, pp. 399-436.
- So, M., Heffron, F., McCarthy, B.J., 1979. The *Escherichia coli* gene encoding heat-stable toxin is a bacterial transposon flanked by inverted repeats of IS1. *Nature* 277, 453-456.
- Sojka, W.J., 1972. Colibacillose intestinale des porcelets. *Ann. Med. Vet.* 116, 377-446.
- Thiry, G., 1984. Plasmids of the epiphytic bacterium *Erwinia uredovora*. *J. Gen. Microbiol.* 130, 1623-1631.

Tinger, S.A., Curtiss III, R., 1990. Isolation of the replication and partitioning regions of the *Salmonellatyphimurium* virulence plasmid and stabilization of heterologous replicons. *J. Bacteriol.* 130, 1623-1631.

Whipp, S.C., Moon, H.W., Argenzio, R.A., 1981. Comparison of enterotoxic activities of heat-stable enterotoxins from class 1 and class 2 *Escherichia coli* of swine origin. *Infect. Immun.* 31, 245-251.