Vet. Res. 30 (1999) 259–284
© Inra/Elsevier, Paris

Review article

Enterotoxigenic *Escherichia coli* (ETEC) in farm animals

Béla Nagy*, Péter Zs. Fekete

Veterinary Medical Research Institute of the Hungarian Academy of Sciences,
H–1143 Budapest, Hungária krt. 21, Hungary

(Received 4 November 1998; accepted 22 January 1999)

Abstract – Animal diseases due to enterotoxigenic *Escherichia coli* (ETEC) typically appear as severe watery diarrhoea during the first few days of life (also a few days after weaning in pigs). ETEC adhere to the small intestinal microvilli without inducing morphological lesions and produce enterotoxins acting locally on enterocytes. This action results in the hypersecretion (of water and electrolytes) and reduced absorption. Adhesins and toxins are the two prominent virulence attributes of ETEC and the level of knowledge of these factors determines the chances for successful prevention and therapy of the disease. For animal ETEC the most common adhesins are the fimbriae (pili) on the surface: F4(K88), F5(K99), F6(987P), F41, F42, F165, F17 and F18. Enterotoxins (extracellular proteins or peptides) of animal ETEC are classified as heat-labile (LT) and heat-stable (ST) enterotoxins with further subdivisions (LTh-I, LTp-I, LTIIa, LTIIb, STaH, STaP, STb) according to antigenic and biological differences. Fimbriae and LT enterotoxins are made up of large molecular weight proteins which facilitate their utilisation in vaccines and their detection using immunodiagnostic systems. The adhesive fimbriae and enterotoxins of animal ETEC are plasmid determined (except F41 and F17). Virulence gene probes (DNA hybridisation, PCR) are specific and sensitive diagnostic and epidemiologic tools for the detection of ETEC. Based on genetic typing, the ETEC, in spite of limited serogroups, seem to represent a population of *E. coli* with a diverse genetic background.
© Inra/Elsevier, Paris.

enterotoxin / fimbria / pilus / ETEC

Résumé – ***Escherichia coli* entérotoxigène (ETEC) chez les animaux de la ferme.** L'infection des animaux par *Escherichia coli* entérotoxigène (ETEC) se manifeste typiquement par une diarrhée liquide aiguë au cours des premiers jours de vie ainsi que quelques jours après le sevrage chez le porc. Les ETEC adhèrent aux microvillosités de l'intestin grêle sans provoquer de lésions morphologiques et produisent des entérotoxines qui agissent localement sur les entérocytes. Cette action entraîne une hypersécrétion d'eau et d'électrolytes ainsi qu'une absorption réduite. Les adhésines et les toxines sont deux attributs importants de la virulence des ETEC et le niveau de connaissances concernant ces facteurs détermine les chances d'une prévention et d'une thérapie réussie de la mala-

* Correspondence and reprints
Tel.: (36) 1 252-2455; fax: (36) 1 252-1069; e-mail: bnagy@novell.vmri.hu

die. Pour les ETEC des animaux, les adhésines les plus répandues sont les fimbriae (pili) de types : F4(K88), F5(K99), F6(987P), F41, F42, F165, F17 et F18. Les entérotoxines (protéines extracellulaires ou peptides) des ETEC sont classées en fonction de leur stabilité à la chaleur [entérotoxines instables à la chaleur (LT) et stables à la chaleur (ST)] et se subdivisent selon les différences antigéniques et biologiques en : LTh-I, LTp-I, LTIIa, LTIIb, StaP, STb. Les fimbriae et les entérotoxines LT sont constituées de protéines de masse moléculaire élevée ce qui facilite leur utilisation comme vaccin et leur détection par des tests immunologiques. Les fimbriae et les entérotoxines des ETEC d'animaux sont codés par des plasmides (sauf pour F41 et la majorité des F17). Des sondes nucléiques ainsi que la PCR sont des outils spécifiques et sensibles pour le diagnostic et les études épidémiologiques. Ils sont utilisés pour la détection des ETEC qui, malgré un nombre limité de groupes sérologiques, semblent constituer une population d'*E. coli* avec une grande diversité génétique. © Inra/Elsevier, Paris.

entérotoxine / fimbria / pilus / ETEC

Plan

1. Introduction 261
2. General statements 261
   2.1. Aetiology and pathogenesis 261
   2.2. Classification of adhesins and toxins 261
   2.3. Adhesins 262
   2.4. Enterotoxins 262
   2.5. Mechanism of action of LT and ST enterotoxins 263
   2.6. Diagnosis of ETEC infections 267
   2.7. Therapy of ETEC infections 267
   2.8. Prevention or metaphylaxis by vaccination 268
3. ETEC infections in main species of farm animals 268
   3.1. Enterotoxic colibacillosis of calves and small ruminants 268
      3.1.1. Diagnosis 269
      3.1.2. Vaccines 269
      3.1.3. Treatment of diarrhoea due to ETEC in calves and small ruminants 270
      3.1.4. ETEC infections in goat kids and lambs 270
   3.2. ETEC infections of pigs 270
      3.2.1. Aetiology, pathogenesis and epidemiology of neonatal diarrhoea 270
      3.2.2. Postweaning ETEC infection of pigs 271
      3.2.3. Aetiology, pathogenesis and epidemiology of PW diarrhoea 272
      3.2.4. Adhesins of PWD ETEC 272
      3.2.5. Adhesin receptors of PWD ETEC 273
      3.2.6. Enterotoxins of PWD ETEC 273
      3.2.7. Diagnosis of porcine ETEC infections 274
      3.2.8. Treatment and prevention of neonatal and PW diarrhoea 275
         3.2.8.1. Prevention 275
         3.2.8.2. Vaccinations against neonatal diarrhoea 276
         3.2.8.3. Vaccinations against PWD 277
         3.2.8.4. Final comments on immune prevention 277
   3.3. ETEC infection of other farm animals 277
4. Clonality of ETEC 277
5. Conclusion 278

1. INTRODUCTION

Enterotoxic *Escherichia coli* (ETEC) refers to *E. coli* bacteria which adhere to the microvilli of small intestinal epithelial cells without inducing morphological lesions and producing enterotoxins that act locally on enterocytes. Enteric diseases due to strains of ETEC are the most commonly occurring form of colibacillosis in pigs and calves. ETEC also occurs widely in man and is less common in dogs and cats. So far, little information has been published about the diseases caused by ETEC in birds or reptiles. The subject of ETEC in farm animals has always attracted much interest because it can be related to human diseases in many aspects. Therefore, there are huge amounts of literature on the subject and it is almost impossible to discuss all important aspects in the necessary detail.

This paper will focus on the clinical, epidemiological and pathogenetic aspects of ETEC infection in farm animals. It will not deal with molecular mechanisms such as genetic regulation or secretion mechanisms which are discussed in other papers of this issue. Although such a review may be somewhat subjective and is almost necessarily incomplete, it is still hoped that it will give an updated outline of the main issues as well as useful hints for future research and expected developments.

2. GENERAL STATEMENTS

2.1. Aetiology and pathogenesis

The common features of ETEC infections in different species are that bacteria adhere to the small intestinal epithelial cells (overwhelmingly in newborn or very young animals), thereby colonising the gut. They also secrete proteins or peptides (enterotoxins) which stimulate the small intestine for increased water and electrolyte secretion and/or decreased fluid absorption. The ability of adhesion of ETEC to intestinal epithelial cells is mainly due to the production of thin (3–7 nm) proteinaceous surface appendages (fimbriae or pili) which can be morphologically, biologically and antigenically different on various strains. Some of them morphologically resemble the common fimbriae ('Type 1' fimbriae or pili) of *E. coli* [26]. With the help of these adhesins (fimbriae), the bacteria are able to attach themselves to the microvilli of small intestinal epithelial cells, thereby more intensively transferring the enterotoxins to the target cells.

2.2. Classification of adhesins and toxins

The virulence characteristics of ETEC are strongly dependent on the production of adhesins (fimbriae) and enterotoxins. It would, however, be an oversimplification if we do not consider other microbial attributes such as the production of cell wall or capsular lipopolysaccharide (LPS) antigens or if we disregard the general microbial housekeeping mechanisms that make ETEC strains not only particularly able to survive but also able to flourish in the intestine. A further possible virulence trait of some ETEC may be the epithelial cell invasion [28], although the pathogenetic significance of that in vitro detectable trait has yet to be determined.

In addition to adhesive and enterotoxic virulence factors, pathogenesis also involves host factors among which the most important ones are adhesin and/or enterotoxin receptors. Species specificity – which is a general characteristic of ETEC infections – is largely due to the presence of specific receptors in only one or in a limited spectrum of animal species.

Several of these virulence factors and their receptors are known and will be discussed in detail below, but some of them are still unknown. Future research on this area is clearly needed and could bring further understanding of pathogenesis and thereby would contribute to more successful strate-

gies in the prevention and treatment of enteric enterotoxic colibacillosis due to ETEC.

2.3. Adhesins

According to our present understandings, the pathogenesis of enterotoxic colibacillosis starts with the adhesin–ligand interaction on the small intestinal microvilli, resulting in a strong but morphologically non-destructive attachment of bacteria to the microvilli.

In the case of animal ETEC strains, the most common adhesive fimbriae can be differentiated as surface antigens such as K88 or K99, 987P or F41 or F107 and 2134P in pigs and calves, also designated as F4, F5, F6, F41, or F18ab and F18ac, respectively [90, 96] (*table I*).

Morphologically these fimbriae are straight, bent or kinky proteinaceous appendages originating from the outer membrane of the bacterial cells. They have various molecular weights (from 15 to 25 kDa). In general, fimbriae are composed of 'major' and 'minor' subunit structures governed and assembled under the direction of structural and accessory genes, respectively. For adhesive fimbriae, the adhesive function is often represented by molecules at the tip of the filaments. The ability of fimbriae (pili) to agglutinate red blood cells of different species was recognised very early [28] and it has been used for classification along with the effect of 0.5 % D-mannose: MS = mannose-sensitive (adhesion blocked by mannose) or MR = mannose-resistant adhesion. Among fimbriae of animal ETEC bacteria we can recognise the following categories: MS haemagglutinating fimbriae (Type 1), MR haemagglutinating fimbriae (K88, K99, F41) and MR non-haemagglutinating fimbriae (987P, F18ab, F18ac) (*table I.*).

2.4. Enterotoxins

Enterotoxins are extracellular proteins or peptides (exotoxins) which are able to exert their actions on the intestinal epithelium. ETEC strains are characterised by the production of one or both of the following enterotoxin categories [104], all of which are plasmid regulated:

Table I. Designation and characterisation of fimbria (pili) of enterotoxigenic *E. coli* (ETEC) of farm animals.

Fimbria	Variants	Diameter (mean nm)	Subunit size (kDa)	Mannose sensitivity	Associate O-type	Localisation of genes
F4 (K88)	ab ac ad	2.1	27.6	R	O8, O141, O149	plasmid
F5 (K99)		5	16.5	R	O8, O20, O101	plasmid
F6 (987P)		7	17.2	NH	O9, O20	plasmid
F17 (Fy)		3.4	20			chromosome
F18	ab (F107) ac (2134P) (8813)			R	O139, O141, O147, O157	plasmid
F41		3.2	29	R	O101	chromosome
F42			32	R	?	plasmid
F165		4–6	17.5, 19	R	O115	chromosome

R: mannose resistant; S: mannose sensitive; NH: non-hemagglutinating.

- large molecular weight (88 kDa) heat-labile enterotoxins (LT);
- small molecular weight (11–48 amino acid containing) heat-stable peptide toxins (ST) resisting to 100 °C for at least 15 min.

LT enterotoxins are produced predominantly by human and porcine ETEC, while ST enterotoxins are produced by ETEC of human, porcine and bovine origin.

LT toxins have good antigenicity while ST toxins do not. LT toxins can be divided into two antigenically and biologically distinct but structurally similar groups: LTI and LTII. Within LTI, the LTh-I (human) and LTp-I (porcine) strains can be distinguished, and within LTII, two antigenic variants (LTIIa and LTIIb) can be distinguished [87].

ST toxins have two classes: STa and STb (also referred to as STI and STII, respectively). STa toxins have variants which are STaH and STaP [indicating human (H) or porcine (P) type of the STa enterotoxins]. STa toxins are further characterised by methanol solubility and by the ability to induce small intestinal fluid secretion in baby mice and to a lesser extent in weaned pigs. STb is not soluble in methanol and does not react in baby mice; however, it can induce small intestinal fluid secretion in newborn and weaned pigs (*table II*).

2.5. Mechanism of action of LT and ST enterotoxins

The common feature in the mechanisms of action of LT and ST enterotoxins is that they do not produce pathological lesions or morphological alterations on the mucosa. They only produce functional changes such as an increased secretion of H_2O, Na^+ and Cl^- and a concomitant decrease of fluid absorption. As a result, fluid and $NaHCO_3$ is lost from the body, leading to exsiccosis (dehydration) and acidosis. The actions of ST are less clear (see below in this section).

The mechanisms of action and structure of LT enterotoxin are well known and are very similar to the heat-labile toxin of *Vibrio cholerae* (CT). The major difference between LT and CT is that LT is exported much less onto the surface of the bacteria, and a significant amount of this *E. coli* toxin accumulates in the periplasmic space. This difference can be readily explained if we accept the hypothesis that LT of ETEC could have originated from *V. cholerae* where it needs about 20 different proteins to be expressed [47]. Such an army of usher proteins may not be readily available in *E. coli*. CT and LT consist of a single A domain and five B subunits containing 240 and 103 amino acids, respectively. The B subunits bind predominantly to the GM1 ganglioside acting as receptors on the cell

Table II. Designation and characterisation of enterotoxins of enterotoxigenic *E. coli* (ETEC) of farm animals.

Enterotoxin	Variant	Molecular size	Action	Receptor
LT-I LT-II	LTh-I, LTp-I LTIIa, LTIIb	1A (28 kDa) 5B (11.5 kDa)	hypersecretion: stimulation of adenylate-cyclase system	LTI: GM1 LTIIa: GD1b LTIIb: GD1a
STa	STaH, STaP	18 or 19 aa. peptide (2 kDa)	hypersecretion: stimulation of guanylate-cyclase system	pGCc
STb	–	48 aa. peptide (5 kDa)	malabsorption: opening G-protein linked Ca^{++} plasma membrane channel	G protein linked Ca^{++} channel

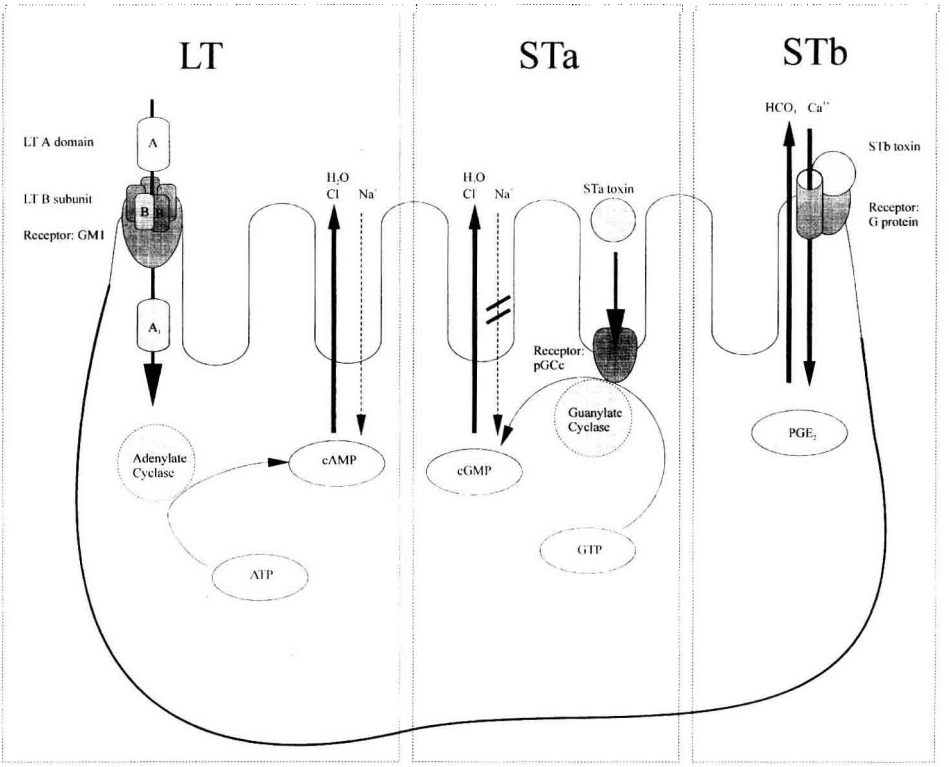

Figure 1. A schematic representation of mechanisms of action of heat-labile (LT) and heat-stable (STa and STb) enterotoxins of enterotoxigenic *Escherichia coli* (ETEC) of farm animals.

surfaces [87] (*figure 1*). Once the B subunits have fixed the toxin molecule to the cell surface, the A1 fragments will translocate into the cell where they activate the adenylate-cyclase system resulting in increased fluid and electrolyte secretion and decreased absorption. This system activates the catalytic unit of cAMP-dependent protein kinase, which phosphorylates membrane proteins leading to transepithelial ion transport disorders. The effect of LT is irreversible [87]. Besides the above classical mode of action there are alternative potential mechanisms of secretion induced by LT, which involve prostaglandins, the enteric nervous system and cytokine activation [86].

LTIIa does not stimulate fluid secretion in ligated rabbit loop (in contrast to LTI) and is generally much less efficiently produced. The A subunit sequence of LTIIa has a 57 % homology with LTI and a much lower homology with the B subunit (*figure 2*). LTIIa and LTIIb also differ in their membrane receptors.

ST enterotoxins were discovered by Smith and Halls [105] as produced by porcine ETEC. Further studies revealed that two kinds of completely unrelated ST toxins are produced by porcine ETEC: STa (also named STI) and STb (also named STII). The biological activities and mechanisms of action of ST enterotoxins can be assayed

in suckling mice or in ligated intestinal loops of pigs and calves. STa is a 2-kDa peptide, containing 11–18 amino acids, and it is secreted into the culture medium or into the intestinal fluid by the bacteria. Receptors for STa are heterogeneous (*figure 1*). On the cell surface there are glycoproteins and a particulate transmembrane form of guanylate cyclase-c (pGC-c) [38, 39]. The effects of STa are reversible. However, STa receptors seem to be occupied after binding. Therefore, this association is not a typical reversible binding system [28]. The biological activity of STa is exerted through stimulation of the guanylate-cyclase system, leading to intracellular accumulation of cGMP and reduced absorption of water and electrolytes (Na^+ coupled Cl^-) on villus tips, and simultaneously to an elevated secretion of Cl^- and H_2O in crypt cells [35]. STa toxins produced by human and animal strains of ETEC seem to differ in some amino acids [72] and are labelled as STaH and STaP. Human ETEC may produce both STaH or STaP, while ETEC from calves and pigs produce STaP [97]. STa toxin genes are located on plasmids and are part of a transposon (Tn1681) flanked by inverted repeats of IS1 [109] (*figure 2*).

The mechanism of action and the molecular characteristics of STb are much less known and it seems that poor absorption of fluids and electrolytes plays an important role in pathogenesis. STb does not elevate intracellular cyclic nucleotide levels but seems to stimulate a non-chloride anion secretion by intestinal epithelial cells [121], although the mechanism of action is still not quite clear (*figure 1*). One way of action could be through the activation of prostaglandin E2 [48], while Dreyfus et al. [23] suggest that STb acts by opening a G protein-linked calcium channel. Elevated intracellular Ca^{++} can activate prostaglandin endoperoxidase synthetase and may lead to

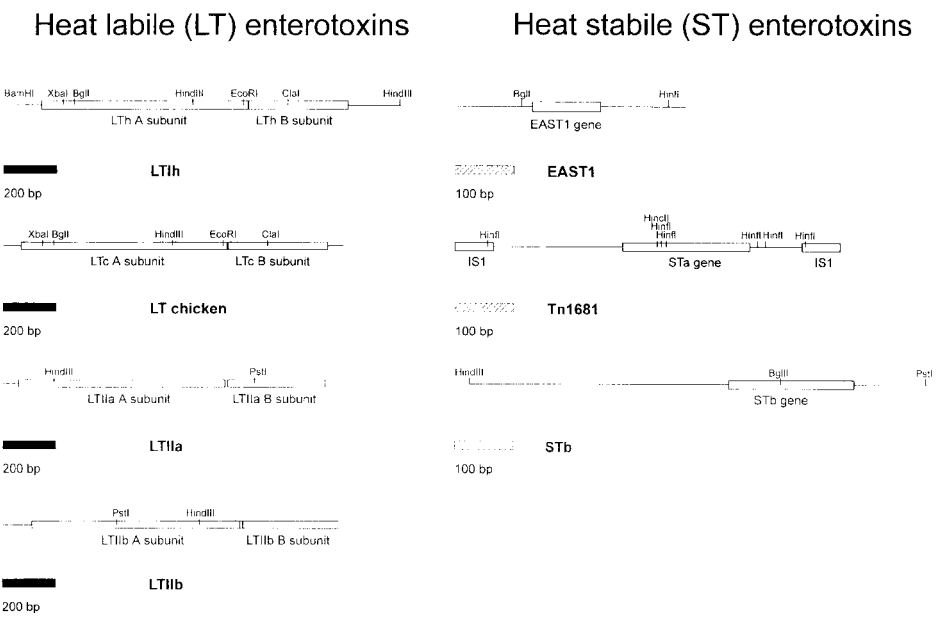

Figure 2. Organisation of toxin genes of enterotoxigenic *Escherichia coli*.

formation of prostaglandins. STb also binds to lipids of the intestinal mucus which could further complicate the mode of entry and action of this peculiar toxin [24]. At present there are some doubts about the pathogenetic significance of STb, based on experiments using newborn pigs [14]. In these experiments STb did not contribute to the severity of diarrhoea in newborn pigs. It should, however, be kept in mind that STb is mainly present in ETEC-isolated weaned pigs with diarrhoea. There are sporadic observations about the isolation of ETEC producing STb from cases of diarrhoea in men, calves and chickens [3]. The production of STb is regulated by plasmids which may also regulate LT and STa. The STb gene is also part of a transposon (Tn4521) which may explain a relatively high frequency of detection of these genes in ETEC and in non-ETEC strains from animals [62] and man [64] (*figure 2*). In contrast to STa, STb is sensitive to proteases. Protection of STb from digestion by the addition of protease inhibitors to STb, makes it possible to detect its biological function in other animal species [120].

Finally, a recently discovered kind of ST toxin (EAST1) should be mentioned, which has been detected in human enteroaggregative *E. coli* (EAggEC) by Savarino et al. [101]. It is interesting to note that this toxin also interacts with the STa receptor (GC-c) but according to database information the structural gene for EAST1 has no significant homology with the STa gene [109]. Furthermore, it seems that the EAST1 was also acquired by some ETEC and enteropathogenic *E. coli* (EPEC) and by all enterohaemorrhagic *E. coli* (EHEC) strains at some points in their clonal development [32]. These EAST1 toxins are secreted by a type IV secretion system [34]. An intriguing question remains as to whether EAST1-producing strains are common amongst classical ETEC strains?

Secretion of virulence determinants of ETEC: there are three general secretion pathways recognised in gram-negative bacteria that export virulence factors (I–III). Another group of bacterial proteins (IV) mediate their own transport and are therefore called autotransporter systems [34]. Such an autotransporter class of enterotoxins (EAST1) has recently been described in EaggEC, as above [30, 101]. It is also known that the secretion of STa and STb involves an energy and secA-dependent (Type II) conversion of the performed toxins to the extracellular ones [60, 128]. However, several steps of enterotoxin production as related to secretion systems have yet to be clarified. It should be noted that the maturation of virulence proteins is also part of the different secretion and expression mechanisms, i.e. formation of disulphide bonds within the periplasm (for cholera toxin and for LT of *E. coli*).

Regulation of adhesins and toxins: changes in the gene expression can be the result of a random genetic event (stochastic process), but expression of virulence factors is usually linked more to environmental signals, such as temperature, ion concentration, osmolarity, carbon source, Fe^{++}, pH, O_2, etc. These signals can also be sensed by ETEC bacteria in order to more appropriately accommodate the in vitro and in vivo environment (stereotypic response). Under in vivo conditions some of the above factors can induce a whole cascade of virulence functions, turning on different genes while turning off others at different steps of the infectious process (for instance: invasion genes are turned on early in the infection but are repressed once bacteria are within the host cell) [34]. For ETEC, much less is known about regulation. Virulence factors are influenced by the above signals through 'regulator elements'. Some of these control the expression of many unrelated genes and are therefore called 'global regulators'. In general, virulence factor regulators can be grouped into a few 'families' (based on their conserved sequences and mechanisms). Virulence genes of enteric pathogen strains of *E. coli* are mainly genes 'foreign' to *E. coli* and they can be controlled by several regulators. These regulators are therefore a possible exciting area of research for

ETEC in terms of pathogenesis (in vivo functions) and diagnosis. We can hope to find the right culture conditions for the efficient in vitro production of adhesins and enterotoxins only with more knowledge on regulation. Consequently, improvement of methods to detect these factors in vivo and in vitro could enhance the detection rates of ETEC (see sections 3.1.1 and 3.2.7).

2.6. Diagnosis of ETEC infections

Diagnosis of ETEC infections requires the detection of virulence factors (adhesins, enterotoxins) using in vitro tests (slide or latex agglutination or ELISA) in most cases [113]. Adhesive fimbriae can, however, be most efficiently detected in vivo, by an immunofluorescent method using absorbed polyclonal or monoclonal antifimbrial antibodies [55]. In contrast to fimbriae, enterotoxins produced in vivo are much more difficult to detect. Therefore, at the early ages of ETEC studies in vitro-produced toxins could only be tested by biological assays: ligated small intestinal segments (for all enterotoxins) or baby mouse assay (for STa), followed by cell cultures (for LT), and later on by ELISA assays (for LT and ST) [20]. Now, with the advent of molecular methods in the diagnostic laboratories, the cumbersome biological assays can be replaced by so-called gene probes: DNA hybridisation and PCR (recently in a complex form) for detecting the genes of different virulence characters [37, 66, 115]. The question can be raised, however, whether our chances to discover new toxic and other virulence attributes will not be limited if we disregard biological assays in the long run.

2.7. Therapy of ETEC infections

The classical antibiotic therapy of diarrhoeal diseases of animals requires oral application of broad-spectrum antibiotics which would be slowly or not absorbed from the intestine, such as neomycin, colistin, aminosidine, kanamycin and polymyxin. At present more and more antibiotics are produced for parenteral application against bacterial diarrhoea (ampicillin, quinolones and fluoroquinolones). During the last few years several very effective antibiotics (i.e. chloramphenicol, nitrofurans) had to be removed from veterinary use in Europe – and some others are on their way out – because of long-range hazards on human health or because of antibiotic resistances developing among human pathogens. In the area of antibiotic therapy of ETEC infections, theory and practice seem to function in a somewhat 'peaceful contradiction'. Namely, exact diagnosis requires isolation and identification of the ETEC strains causing the disease (necessitating the determination of virulence factors), a procedure which usually takes a few days under conditions in a routine diagnostic laboratory. Determination of antibiotic resistance would theoretically follow only after the virulence factors are known. The pressing need in veterinary practice requires much quicker actions and therefore the use of antibiotics almost always precedes the results of the resistance pattern. As a result, in most cases the right choice of antibiotics remains the practitioner's responsibility, for which today – in spite of all the latest technical developments – the laboratory only gives a retrospective diagnosis.

It is necessary to replace the loss of fluid and electrolytes, independently of the results of detailed bacteriological investigations partly because of the above difficulties and partly because of the common pathomechanism of ETEC infections. For these reasons, several 'ready-to-use' formulae are available for oral applications in different animal species to cure and to prevent dehydration and to restore electrolyte balance. Glucose is an important component of many of these formulae; it helps to revert intestinal epithelial cellular functions from secretion back to absorption.

2.8. Prevention or metaphylaxis by vaccination

ETEC infections can, and should be prevented by several hygienic and management techniques which are outside the scope of this paper. Among these, the most important factor, in the case of newborn animals, remains the early and sufficient colostral supply.

The protective value of colostrum against diarrhoeal diseases of the offspring caused by ETEC can be increased essentially by maternal immunisation. For that purpose several vaccines are used mainly for parenteral application (which can be adjuvanted by oral immunisation). These vaccines contain the so-called protective antigens (virulence factors – fimbrial adhesins with or without LT enterotoxins). Vaccinations should usually take place in late pregnancy and can be repeated as 'reminder' vaccinations before each subsequent farrowing. As a result, colostral antibodies would block virulence factors and propagation of bacteria in the intestine. Similar effects can be expected in the case of passive immunisation, i.e. the oral application of polyclonal or monoclonal antibodies [104]. Immune colostrum or specific antibodies can also be applied metaphylactically, however, with much less success.

Amongst the above-described mechanisms of action, the success of colostral vaccines depends largely upon matching the right protective antigens with the pathogens present in a given animal population. Our knowledge about the possible existing virulence factors is, however, still limited and further improvements in this area are to be expected. Whatever vaccine is applied against ETEC, the old truth remains for all: even the most effective vaccines are only good to correct minor failures in management practices but they cannot be expected to be 'the overall cure' against high challenge doses and a large variety of infectious agents having the capacity to induce diarrhoea.

3. ETEC INFECTIONS IN MAIN SPECIES OF FARM ANIMALS

Based upon the above general knowledge about the pathogenetic traits and mechanisms of ETEC, the following section will focus on special characteristics of ETEC infections that relate to calves, sheep and goats as well as to pigs.

3.1. Enterotoxic colibacillosis of calves and small ruminants

The overwhelming majority of ETEC responsible for diarrhoea in newborn calves are characterised by adhesins K99 (F5) and F41, and by STaP enterotoxins. They usually belong to the O8, O9, O20 and O101 serogroups and often produce an acidic polysaccharide type of K(A) antigen (K25, K28, K30, K35), making the colonies of such strains more compact and less transparent. It seems that such capsular polysaccharide antigens enhance colonisation induced by K99 [44, 54]. K99 and other fimbrial adhesins mediate attachment of the ETEC to the small intestinal (mainly ileal) microvilli, thereby resisting removal and facilitating colonisation. Thus, bacteria are able to efficiently transmit the STa that they typically produce, which in turn induces extensive excretion and loss of water and electrolytes, rapidly leading to dehydration. Other, less frequently occurring adhesins are the so-called F17 (earlier known as FY and Att25) [63]. Adhesions mediated by these surface proteins are dependent on the presence of glycoprotein receptors which are abundantly present in newborn calves and lambs, and which, in the case of K99, gradually decrease with age [99]. It seems that K99 and F41 are frequently produced simultaneously by bacteria of the same ETEC strain. There are different receptors for K99 (sheep and horse haemagglutinin) and for F41 (guinea pig and human-A haemagglutinin). K99 and F41 also differ in their genetic regulation (K99 is regulated

by a plasmid while F41 is regulated by a chromosome). Both K99 and F41 as well as F17 can, however, adhere to the porcine small intestinal brush border and can induce porcine enterotoxic colibacillosis. Association of F17 (FY/Att25) with enterotoxins is not quite clear. Original descriptions of antigens reported enterotoxic activities [63, 92]. In Hungary (in a limited study) the three FY+ (= F17+) isolates that were K99– and F41– did not produce STaP, although they had been isolated from diarrhoeal calves [74]. Studies in recent years revealed that F17 fimbrial adhesins are somewhat heterogeneous and they form a so-called F17 family of fimbriae (F17a, F17b, F17c, F17d and G fimbriae) based on their receptor specificities [61]. It should be mentioned that another (non-fimbrial) surface protein (CS31A) has also been associated with calf diarrhoea [42] but it is also detected on septicaemic *E. coli* from calves, in contrast to K99 or F41. Interestingly, CS31A is genetically related to K88 fimbria (known as a typical porcine adhesin) [42]. Pathogenetic significance of the F17 fimbrial family, and of other (non-fimbrial) adhesins (such as CS31A) in enteric and in extraintestinal infections is reviewed in another paper of this issue [61].

Finally, a special kind of the ETEC strain isolated from a diarrhoeal water buffalo calf should be mentioned in this section [103]. This ETEC produces a variant of LT (designated LTII), which has also been detected in calves [93] and in humans in some Asian countries. This indicates that these strains may have adhesins that find receptors in bovine and human small intestines. Not much information is, however, available on their adhesins yet.

3.1.1. Diagnosis

According to our present knowledge, the diagnosis of ETEC infection in calves is greatly facilitated by the high frequency of K99 antigens on bovine ETEC. The presence of K99 can, however, be covered by the K(A) antigens. Besides, the production of K99 may also be repressed by the presence of glucose, while for other strains glucose may even enhance K99 production [41]. Therefore, special media such as Minimal Casein Agar with Isovitalex® added (MINCA-Is) are required [43] for the detection of K99 in vitro. Alternatively, the immunostaining of small intestinal segments from calves that died as a result of diarrhoea proved to be more efficient [55, 75]. Monoclonal based latex reagents [113, 114] and DNA probes (hybridisation and PCR) that detect the above fimbrial genes are available for more efficient diagnosis [66].

3.1.2. Vaccines

Vaccines against enterotoxic colibacillosis of calves or small ruminants should also contain both K99 and F41 [1, 17, 73]. Vaccines should also contain the F17(FY/Att25) antigens as well considering the cross protection between these and F17 in countries where F17(FY/Att25) fimbriae are prevalent [15, 63]. As ETEC infections of calves and small ruminants frequently occur simultaneously with rotavirus infection, most of the vaccines used today contain bovine rotavirus antigens as well [6, 59]. So far, no information is available about a possible shift in fimbrial characteristics of ETEC in herds or areas where K99- and/or F41-containing vaccines are used. There is evidence, however, suggesting that the strongly reduced incidence of K99 and F17 may be explained by the use of vaccines containing these antigens [16]. During the last decade, no new adhesins or toxins of calf or ruminant ETEC strains were discovered, although it seems almost impossible that the adhesin (and toxin) spectrum in these animal species is that limited all over the world.

In the future, further questions should be raised about age resistance and genetic resistance to F41 and F17 fimbrial adhesion (and to enterotoxins) in calves. So far, no solid

information is available concerning these questions in ruminants.

3.1.3. Treatment of diarrhoea due to ETEC in calves and small ruminants

As the main effect of ETEC diarrhoea is exsiccosis, metabolic acidosis and hyponatraemia, effective therapeutic regimes should include oral or intravenous rehydration using one of the several commercially available formulae. Antibiotics such as oral polymixin and quinolones or fluoroquinolones have been so far successfully applied. Immune treatment or prophylaxis by feeding K99 monoclonal antibodies or powdered egg yolk of K99-immunised hens may also be applied besides the usual oral or parenteral antibiotic therapy, as described above [29, 51, 104].

3.1.4. ETEC infections in goat kids and lambs

As mentioned several times above, lambs have a very similar clinical diarrhoeal disease and similar strains of ETEC as calves. However, this seems much less certain in goat kids. In general, it is true for both animal species that we have much more limited information about their ETEC infections as compared to those of calves. For instance, the adhesins F17(FY/Att25) and CS31A detected on calf diarrhoea strains have not been described so far for *E. coli* bacteria from lamb or goat diarrhoea, but such isolates can be prevalent among septicaemic strains of lambs and goat kids [61]. Information about ETEC infection in goats is even more limited. According to our earlier studies [77], infection by K99+ ETEC may also cause diarrhoea in young goat kids in some herds but cryptosporidiosis and rotavirus infections seem to be the main aetiological agents. This observation is supported by the experimental infection of goat kids with K99+ ETEC strains and by successful prevention of diarrhoea due to such strains by the K99 vaccine [18]. In contrast to ETEC, verotoxic *E. coli* (VTEC) strains have been isolated more frequently from 1- to 2-month-old goat kids with diarrhoea and they seem to be the major diarrhoeal agent of this age group [27]. More information is needed, however, about ETEC (and in general about enteric *E. coli*) infection of goat kids and lambs.

3.2. ETEC infection of pigs

It is well known that enteric enterotoxic colibacillosis produces significant losses in two different age groups of pigs: first among newborn pigs and later at the postweaning age. For the economy of words and space only aetiology, pathogenesis and epidemiology will be discussed separately for the two age groups. Diagnosis, treatment and prevention have enough in common to be described under one heading.

3.2.1. Aetiology, pathogenesis and epidemiology of neonatal diarrhoea

E. coli strains causing enterotoxic colibacillosis in suckling piglets are characterised by one or the other of the K88 adhesins (in variants K88ab, K88ac and K88ad) also known as the (F4), by K99 (F5) or 987P (F6) adhesins and occasionally by the F41 [118], F165 [31] or F42 adhesins [110]. ETEC strains possessing K88 (especially K88ac) are the most common cause of diarrhoea and they usually produce LT in addition to STaP or STb. K88+ ETEC are also characterised by haemolysin production in vitro. ETEC strains carrying K99 and/or F41 or 987P produce only STaP and are non-haemolytic. While K88+ ETEC may represent about 40–60 % of the *E. coli* strains causing diarrhoea in piglets, the above non-K88 strains make up between 20 and 30 % [74, 127]. The typical O serogroups for neonatal porcine ETEC infections are O8, O9, O20, O101, O141, O147

and O157 representing both K88+ and non-K88 ETEC. In our experience, the two groups (K88+ and non-K88) of ETEC have a somewhat different clinical picture: K88 strains cause more severe diarrhoea at a younger age (1–5 days) while non-K88 strains give rise to milder diarrhoea with a later onset (approx. 4–14 days of age). It should also be noted that the rotavirus infection often complicates neonatal colibacillosis of pigs, especially in non-K88 ETEC infections at the second week of age. In Hungary, rotavirus was found in approximately 10 % of the pigs that died as a result of diarrhoea, and in approximately 25 % of live diarrhoeal piglets [74].

The availability of adhesin receptors on the small intestinal epithelium is a very important factor in the pathogenesis of diarrhoea due to ETEC. It has been shown that the receptors for K88 are glycoproteins and the lack of production is a recessive trait. Thus, homozygous piglets are resistant to K88-mediated adhesions, to colonisation and disease [102]. Receptor functions seem to be dependent on the 'b' and 'c' components, and in genetically resistant piglets the receptors are usually absent for both of these components [11, 49]. In vitro adhesion tests have revealed a polymorphism of intestinal receptors for K88 and indicated that there are five to six adhesion patterns (A–F) among piglets according to the adhesion of K88ab, K88ac and K88ad variants [11, 12, 95]. Unfortunately, this phenomenon of genetically determined resistance could not gain wide practical application. It may, however, complicate epidemiological pictures, by partially producing non-diarrhoeal homozygous recessive (ss) litters, and by partially leaving heterozygous (Ss) piglets (which are born to resistant sows and sensitive boars), without colostral immunity (such sows would not have acquired the infection and could not produce specific antibodies in their colostrum). The practical application of this knowledge is even more complicated by the fact that the correlation of the adhesion of K88 variants to the small intestinal brush borders with susceptibility to colonisation and diarrhoea may be lacking. This can be explained by the most recent findings of Francis et al. [36], suggesting that the intestinal brush border mucin-type glycoprotein (IMTGP) is a biologically more relevant receptor for K88ab and K88ac as compared to the so far widely accepted enterocyte brush border glycoprotein.

So far, no information is available about the genetic determination of receptors for K99, F41 or 987P in pigs, but there are mice which are genetically resistant to colonisation by K99 [25]. Such information is, however, available for F18 fimbrial receptors of weaned pigs (see section 3.2.5).

The production of receptors also influences age-related resistance to the disease. This is, however, manifested in different ways for different adhesins. Receptors for K88 are abundant in newborn pigs and will decrease with age but remain relatively stable throughout the weaning and postweaning periods. Receptors for K99 gradually decrease with age [99]. In contrast, production of receptors for 987P in fact increase with age [22]. This invariably leads to a lower adhesion and colonisation because the receptors shed into the lumen and block bacterial adhesion before contacting intestinal epithelial cells. The ageing nature of receptors for F41 are unknown but data indicate that they may be produced all through the weaning age [82].

3.2.2. Postweaning ETEC infection of pigs

Postweaning diarrhoea (PWD) is usually the most constant disease problem of large-scale farms, especially of those that wean around 3–4 weeks of age. PWD starts a few days after lacteal protection completely ceases, and pigs are placed in an environment which is completely new from technical, social and microbiological points of view. It is widely accepted that specific serotypes and pathotypes of ETEC are

responsible for the major part of PWD. It is also without debate that the disease is a highly complex one. ETEC only plays a part (although an essential one). It is frequently seen in almost all large-scale piggeries but it is one of the most difficult diseases to experimentally reproduce. Diarrhoea and reduction in weight are only part of the losses. Retarded growth, which usually follows diarrhoeal episodes in weaned pigs, makes the losses even worse.

3.2.3. Aetiology, pathogenesis and epidemiology of PW diarrhoea

The main cause of postweaning diarrhoea is the weaning itself. Only on this basis can we understand the aetiology and pathogenesis more realistically and be more humble about our capacities to bring real (economically feasible) improvement in this enigma. ETEC strains involved are most frequently of the O serogroup: O8, O141, O138, O147, O149 and O157, of which O149:K88 seems to be the predominant serotype in most countries [45]. So far, all the typical PWD strains of ETEC are haemolytic, although haemolysin does not play an essential part in virulence of porcine ETEC [107].

3.2.4. Adhesins of PWD ETEC

The main adhesive virulence factors of ETEC PWD strains are K88 (mainly K88ac) fimbria. Furthermore, K99 and 987P and F41 have also been described in some PWD strains [78, 82, 85], but they seem to be rarely involved in diarrhoea at that age. Recently, a new fimbrial adhesin has been recognised under the F18 designation (*figure 3*).

Some additional information on the recently discovered and classified F18 fimbriae should be given here, because they have been described under different names, and misunderstandings are frequent in the use of the earlier names and new designations. During the last few years, three new colonisation factors or adhesive fimbriae have been described for groups of *E. coli* involved in PWD or oedema disease: F107 on oedema strains [8], 2134P on ETEC strains [81], and '8813' also on ETEC strains [100]. Additionally, fimbriae of two ETEC strains of serogroup O141 have also been described [58], although no data have been given on their adhesive or pathogenetic significances. As a first attempt to clarify the relationships between these factors, pili 2134P were compared to fimbriae F107 by means of polyclonal and monoclonal antibodies. It was provisionally concluded that these two adhesins were morphologically similar and shared a common antigenic determinant in addition to a type-specific one [80]. These findings were confirmed [125] and it was suggested that the symbol 'a' should be used for the common determinant and the symbols 'b' and 'c' for the specific determinants of F107 and 2134P, respectively. Furthermore, Rippinger et al. [96] investigated the morphological, immunological, genetic and receptor-binding relatedness of fimbriae F107 and 2134P, together with the colonisation factor '8813'. Based on earlier suggestions made by Ida and Frits Ørskov (International *Escherichia coli* Centre, Copenhagen, 1992) for a new F18 fimbria, it was shown that two serological variants were determined and should be designated as follows: F18ab (for F107) and F18ac (for 2134P and 8813) [96]. The genetic relatedness of the above family of F18 fimbriae was described by Imberechts et al. [52], supporting the above grouping, and adding the fimbriae of Kennan's O141 strains to the group of F18ac. In a recent study, it was pointed out that F18ab and F18ac fimbriae are biologically distinct: F18ab fimbriae are poorly expressed both in vitro and in vivo. They are frequently linked with the production of SLT-IIv (VTEC strains), while F18ac are more efficiently expressed both in vitro and in vivo and they are more characteristic of ETEC strains [84].

It should also be mentioned that some ETEC strains may produce multiple

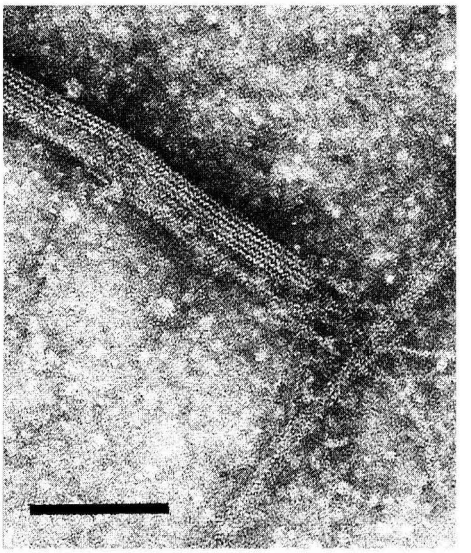

Figure 3. Electron micrograph of negatively stained, aggregated F18 fimbriae showing a characteristic zig-zag pattern. Bar = 100 nm.

adhesins such as K88, F18ac or K88, F41 or even K88, F18ac and F41 [82]. It remains to be shown if such strains have a pathogenetic advantage over strains with one kind of an adhesin. It may also be questioned under what conditions there are receptors for these rarely occurring adhesins (K99, 987P, F41) available at the right amount on the small intestinal mucosae.

3.2.5. Adhesin receptors of PWD ETEC

Information about receptors for the post-weaning adhesins has also facilitated our understanding of earlier findings on the epidemiology and pathogenesis of PWD. Receptors for K88 are produced, although to a somewhat reduced extent, all through the weaning age, while receptors for the variants of F18 (F18ab and F18ac) are increasingly produced up to the weaning age [81, 84] and the fimbriae F18ac seem to have more receptors around the ileal Peyer's patches [81]. The lack of receptors for F18ab and F18ac in newborn pigs offers an explanation as to why these VTEC and ETEC strains (and why the oedema disease itself) are only prevalent in weaned pigs.

Genetic determinants for the production of intestinal receptors of fimbria F18ab have also been investigated by oral inoculation of weaned pigs and by in vitro adhesion tests [9], and it seems that phenotypes susceptible or resistant to F18 adhesion can be differentiated. Pigs with at least one copy of a dominant allele for receptors are susceptible to colonisation and in vitro adhesion (similarly to the K88 receptors). Additional genetic marker studies localised the receptor gene on the porcine chromosome 6, closely linked to the gene encoding halothane sensitivity [119]. It seems that the lack of receptors will coincide with halothane (stress) sensitivity, making it difficult to select and raise pigs without small intestinal receptors for F18 fimbria.

Small intestinal receptors for K88 and for F18 seem to be different, based on comparative in vitro studies [84] and on the different localisations of their regulation on the porcine chromosome [40, 119].

Although it seems to be an attractive idea to breed pigs resistant to ETEC adhesion, it may have more drawbacks than advantages. It is not only difficult to select subdominant alleles of two different, independently inherited traits (lack of receptors for K88 and F18) but we should also consider that the *E. coli* bacteria are genetically much more flexible than their host. This would ultimately lead to the emergence and proliferation of new ETEC pathotypes. Furthermore, we should take into account the possible co-selection of unwanted traits (such as halothane-sensitivity).

3.2.6. Enterotoxins of PWD ETEC

ETEC isolates of PWD are characterised by production of either LT, STa or STb or combinations (i.e. STa is almost always accompanied by STb and/or LT). Some strains (ETEC/VTEC) inducing PWD pro-

duce both ST enterotoxin and SLT-IIv verotoxin [79, 108]. LT and STa enterotoxins induce a secretory diarrhoea without epithelial damage. STb is the exception for which the mechanism of action is still not clear and which also involves reduced absorption and shortening of small intestinal villi [98]. It should also be noted that the small intestine becomes transiently more susceptible to ST enterotoxins immediately after weaning [67, 111]. Although STa is known to affect small intestinal fluid accumulation in weaned pigs much less than in newborn pigs, this cannot be explained by the lower activity of membrane-bound particulate GC-c or by intracellular GC [57].

It is interesting to note that ETEC strains producing STa are haemolytic only in weaned pigs but not in newborn pigs. Another interesting phenomenon is the connection between fimbrial and toxin genes. Porcine ETEC strains producing LT almost always produce K88 (and haemolysin) as well. Those that produce STa and/or STb mainly produce F18ac, while the VTEC strains (especially those of serogroup O139) have a strong tendency to produce F18ab [80, 82, 84]. It would be logical to assume that the ST toxin and F18 adhesin genes are on the same plasmid. However, it was proved not to be the case [89].

Knowing the complexity of the aetiology of PWD the question remains as to why so few serogroups and why so relatively few virulence determinants are known to be involved in PWD throughout the world [46, 124].

3.2.7. Diagnosis of porcine ETEC infections

Diarrhoea in pigs at the suckling age and within the first 2 weeks after weaning is almost always accompanied by some type of non-commensal *E. coli* infection. Today, we already know of several types of porcine ETEC (although it seems that other pathotypes can also complicate and partly induce diarrhoea in newborn and especially in weaned pigs). Furthermore, it should be remembered that on the herd level diarrhoeal episodes are infrequently monocausal. The presence of one or more types of ETEC (for example) can often be accompanied by rotaviruses, caliciviruses, coccidia or by the coronavirus of porcine epidemic diarrhoea (PED) in both age groups but especially in weaned pigs [45, 83]. In this chapter only the diagnosis of infections due to known and established types of ETEC will be discussed, which are in most cases the dominant element of sporadic diarrhoeal diseases on the herd level.

Diagnosis of ETEC infection is based on the detection of known virulence factors (and of the serogroup) of the suspected ETEC. This would not necessarily require culturing of bacteria (see below), but the need to determine antibiotic resistance patterns simultaneously makes culture and test of bacterial attributes in vitro an accepted routine for diagnostic laboratories. For cultures, usually small intestinal or faecal samples are available, from which it is advisable to inoculate specific media (besides classical media) required for preferential growth of some adhesins (such as MINCA-Is for K99, or Difco Blood agar Base with sheep blood for 987P) [43, 76]. To test if the isolates are ETEC, the fimbrial antigens K88, K99, F41 and 987P can be detected by slide agglutination using specific absorbed sera or by latex agglutination for which there are monoclonal antibody-based kits available [113, 114]. Adhesive fimbria produced in vivo can be more efficiently detected by testing small intestinal smears of diarrhoeal pigs using fluorescence antibody assays. As there may be ETEC strains without known (or detectable) adhesive virulence factors, it is advisable to perform tests for enterotoxins as well. LT and STa toxins can be identified by ELISA or by latex agglutination; unfortunately no such tests are available for STb. DNA probes (hybridisation and PCR) are also in use for in vitro detection of almost all known virulence genes of porcine ETEC [66, 78]. Their use,

however, seems to be too expensive and time consuming under routine diagnostic laboratory conditions. Therefore, further research has been, and is still being carried out on techniques, such as the complex PCR, that will facilitate their use by diagnostic laboratories [37].

The diagnosis of PWD diarrhoea requires careful consideration of the predisposing factors and bacteriological results. There is almost always a need for differential diagnostic investigations (such as virus detection) as well. Therefore, in the case of PWD, it is strongly advised not to be content with a possible bacteriological result detecting some types of ETEC, but it is also necessary to consider other physiological, environmental, dietary and viral factors that may sometimes be as important as the given ETEC bacteria themselves [45]. Culturing and/or immunofluorescent in vivo identification of ETEC strains from the ilea of diarrhoeal pigs is the most effective and simplest way of making a bacteriological diagnosis (as described for diarrhoea of newborns). The bacteriological analysis of faecal samples for ETEC is more difficult because the bacteria present in the faeces may not reflect the microbial status of the small intestine. There are a variety of in vitro techniques that detect virulence factors (adhesins and toxins) of ETEC including immunological and biological assays, molecular probes (DNA hybridisation and PCR) as mentioned above for newborn diarrhoea. Differential diagnosis should frequently include the detection of rota- and coronaviruses as well as spirochaetes and Salmonella [45, 83].

3.2.8. Treatment and prevention of neonatal and PW diarrhoea

Pigs suspected to have ETEC-induced diarrhoea can be treated by oral and/or parenteral antibiotics as well as by fluids and electrolytes, for which a wide range of therapeutic products are available (as mentioned above). In severe outbreaks, antibiotics can be given (orally or parenterally) in the form of routine treatment to all pigs soon after birth (or after weaning). Depending on the kind and the usage of antibiotics, treatments should be continued for 3–5 days, and may even be applied twice daily. Oral electrolyte solutions (containing at least potassium, dextrose and sodium chloride) are helpful in diarrhoeal cases of both age groups, provided that they are accurately applied. In most cases such fluids are offered for drinking. It would be impossible to list all the available products of different companies within the frame of this review.

Medication and preventive treatment of PWD is based on oral antibiotics (preferentially through water) and on fluid/electrolyte replacement. When choosing feed as a drug vehicle (as part of a preventive regime), it should be remembered that weaned pigs eat less after weaning, and feed intake may greatly vary between pigs within the same group. Therefore, diarrhoeal pigs need individual treatment. As antibiotic resistance is very common among PWD ETEC isolates, it is necessary to have resistance tests performed very frequently, and to modify preventive and curative treatments accordingly. Although the prophylactic use of antibiotics is not an ideal solution for PWD either, several piggeries have to rely on that kind of prevention as well.

3.2.8.1. Prevention

There are several important aspects of diarrhoeal disease prevention due to ETEC in pigs. Among them, management techniques ('all in all out', clean and dry places, 'feed back of diarrhoeal faeces', minimising mastitis, metritis, agalactia syndrome (MMA) in sows, etc.) and 'good farming practice' are the most decisive in the long run. This is especially true for techniques serving for the prevention of postweaning diarrhoea (feeding regimes, early and isolated weaning, etc.). Prevention by breeding resistant pigs, although theoretically possible, does not seem to be feasible for practice today, as mentioned above. Immune

prevention, primarily vaccination, is more realistic and more in the scope of this paper and will therefore be dealt with in some detail.

One of the several areas of non-antibiotic and non-immune prevention of PWD is the reduction of managemental factors predisposing to the disease. These factors include weaning age and weight, weaning diet, overstocking and contaminated environment from earlier stocks [45]. Supplementation of water with acids [112] or of feed with probiotics has been discussed and advised but with no convincing evidence for long-term efficiency [21].

As a way of passive immune prevention, the addition of egg yolk containing specific ETEC antibodies to postweaning pigs has been suggested to be of good prophylactic use [53, 123, 129].

3.2.8.2. Vaccinations against neonatal diarrhoea

Vaccinations against neonatal diarrhoea due to ETEC have been very successful especially since the most prevalent adhesins (K88, K99, 987P) and toxin (LT) became standard components of the vaccines [68]. It seems that LT could not only act as a protective antigen, but also as an oral adjuvant [2].

Such vaccines are almost always used to provide maternal immunity through immune colostrum to the offspring. This requires parenteral (or oral) application of the above antigens well before farrowing. As a result, passively acquired antibodies through colostrum will protect piglets for about a week against most types of ETEC under normal farming conditions, provided that piglets ingest immune colostrum early enough and in an adequate quantity during the first 12 h of life (before the sharp decline in their absorptive capacity for colostral immunoglobulins). There have been several ways to improve the efficiency of maternal parenteral vaccines against ETEC. One of them is by applying the protective antigens in the form of an 'immune stimulating complex' (ISCOM). ISCOM consists of a matrix (of cage-like structures) with the unique components QuilA and lipids in equimolar ratios (for adjuvant activity), with the antigens incorporated into the complex by hydrophobic interaction [70]. Subunits of fimbriae are hydrophobic and would therefore be potentially incorporated into ISCOM by hydrophobic interaction. In this way much less antigen is needed for the same level of immunity, thereby making vaccine production more effective [79].

Some companies advise to use 'in-feed' vaccines (containing killed or live bacteria) for sows or to combine them with parenteral vaccines. The results of Moon et al. [69] suggest that effective presentation of the protective antigens would require the use of live oral vaccines for such purposes. Such oral vaccines, if licensed, could efficiently stimulate the mucosa-associated lymphoid system (GALT) so that secretory antibodies (especially SIgA) – which are protected from digestion – could be produced and provide the firmest protection. Strong lactogenic immunity mediated in this way lasts for about the first 10–14 days of life. It should be noted that first farrowing gilts are less able to produce high levels of antibodies whatever the route of immunisation. The combination of 'in-feed' and parenteral vaccines can be recommended for first and second pregnant gilts as well [69]. It should be remembered, however, that licensing of live oral bacterial vaccines for use in veterinary medicine, especially those produced by genetic engineering, is difficult in most countries. Killed oral vaccines are, however, of limited value. Live oral vaccines still represent a more controlled and more effective way of specific immune prevention of neonatal diarrhoea as compared to the so-called 'feed back' (feeding of diarrhoeal faecal material to pregnant sows, as practised on some farms). We could also use recombinant Salmonella-vector vaccines expressing the necessary adhesive epitopes [5, 71]. Finally, it is hoped that more

progress in the area of genetically engineered plants (containing the required antigens produced for feeding) will be made in the future.

3.2.8.3. Vaccinations against PWD

Vaccinations against PWD have not shown much progress lately, although the theoretical basis is clear and the need is unquestionable. In-feed vaccines containing heat-treated ETEC bacteria have not been consistently effective and most have been removed from the market. Parenteral vaccination of piglets before weaning is advised by some companies but their efficacy against PWD has not been convincingly demonstrated. At present the most promising experiments are in the area of live oral vaccines applied before weaning. Bertschinger et al. [7] demonstrated the efficacy of such a vaccine when a low-energy diet was also given. Further experiments of this group provided evidence about the protection of pigs against PWD and oedema disease by a live oral vaccine containing F18 fimbria. A combined (live oral plus killed parenteral) vaccine against PWD also seems to be successful in preventing losses [4].

3.2.8.4. Final comments on immune prevention

Protection of pigs from pathogenic *E. coli* is a constant challenge for farmers and veterinarians alike. This is partly because *E. coli* is a highly flexible organism (acquiring new virulence characters or masking the ones that may be disadvantageous for survival) [65], partly because there are several kinds of infections (due to viruses and protozoa as described above) and conditions that may predispose the host to colonisation by ETEC, thereby enhancing the chances for *E. coli* to utilise its pathogenic potential. This is especially true if the porcine reproductive and respiratory syndrome (PRRS) virus is introduced into a hitherto uninfected herd, in which neonatal and/or postweaning colibacillosis will be triggered. In such herds, preventive measures should include vaccinations and management techniques not only against ETEC but also against PRRS to suppress this viral infection on a herd basis (Nagy B. et al., unpublished observations).

3.3. ETEC infection of other farm animals

Although there are several descriptions of diarrhoeal diseases of farm animals (other than the above-mentioned species) characterised by higher than usual *E. coli* numbers in the small intestine and/or in the faeces, at present there is little evidence about enterotoxins and/or adhesins of *E. coli* strains isolated from farm animals other than pigs or ruminants.

Strains of *E. coli* inducing diarrhoea in suckling or in weaned rabbits are characteristic representatives of EPEC [91].

ETEC infection among horses was described only in the late 1980s [50] without details about adhesins and toxins or epidemiology. Experimental infections using an *E. coli* K99+ strain derived form a diarrhoeal foal did not lead to reproduction of the disease. By using the same model, however, a strain of K88+ ETEC of porcine origin did induce diarrhoea. In vitro studies indicated that foals had some intestinal receptors for K88 but not for K99 [117].

ETEC strains producing LT and/or ST (belonging to mostly unidentifiable O groups) were isolated from diarrhoeal chicks on several farms in the Philippines [56]. The LT of these avian strains proved to be identical with the LT of human ETEC strains [116]. *E. coli* in cats and dogs is the subject of an accompanying paper [10].

4. CLONALITY OF ETEC

During the last two decades, plenty of new information has become available about gene flow, genetic drift and changes in

genomic organisation influencing the variation and population structure of *E. coli*. Furthermore, exciting questions about the emergence and spread of pathogenic *E. coli* such as ETEC and enterohaemorrhagic *E. coli* (EHEC) seem to have been answered. A bacterial clone – sensu stricto – is a closed system of ancestors of a single bacterial cell that accumulates differences only through genetic processes within a single cell (point mutations, inversions, duplications, deletions, transpositions). In a more practical sense, however, the term 'clone' is used for a well-defined group of bacteria within a species with many similarities from one ancestor and not shared by other groups [122]. Thus, genotypes are the criteria for belonging to a clone, which may represent attributes that have resulted not only by divergence through mutations and subsequent periodic selection, but also by recombination between bacterial cell lines. The most likely way that pathogenic types of *E. coli* can evolve is by recombination, especially in its additive form (genetic elements from other bacterial species will integrate into the *E. coli* genome). Examples of non-enteric virulence genes recently transferred into the *E. coli* genome are haemolysin-encoding genes [33] or the genes encoding macrolide lincosamine streptogramine resistance [13].

The Ørskov's concept, i.e. that the *E. coli* population is composed of widespread clones, was first proven for EPEC strains [88] into which the plasmid responsible for localised adherence (LA) might have been introduced through horizontal spread [122], thereby conferring a selective advantage on these EPEC strains. A similar example is given by EHEC O157:H7 whose clone might have evolved from EPEC O55:H7 by phage transfer of shigella-like toxin genes [122]. A further support for this concept comes from the studies of Contrepois et al. [19] who demonstrated that clonal relationships exist among *E. coli* strains producing CS31A non-fimbrial surface antigens [14] or among ETEC producing F17a fimbriae [19].

In the case of porcine ETEC, however, there is a high level of clonal diversity. For instance, ETEC strains isolated from diarrhoeal suckling pigs (representing the serogroups O9, O20, O101) showed an extensive genetic diversity, without any predominant clone or clonal group [126]. A similar example is given by these Australian authors [44] for ETEC isolates from weaned pigs. In another study we have found that although the serotypes and virulence attributes associated with PWD appear to be limited, they have diverse chromosomal backgrounds [124].

The diversity of porcine ETEC isolates (representing diarrhoeal diseases) may be interpreted as a sign of the causal diversity of diarrhoeal diseases. They may also represent the cases of horizontal spread of virulence factors. This second hypothesis is supported by the fact that most virulence determinants of the ETEC strains are plasmid regulated. We should, however, also remember the complex nature of, for example postweaning diarrhoea of swines (making the divergent causal factors another possibility for divergent clones). This may indicate that the intestinal tracts of farm animals are a powerful 'melting pot' of bacterial genes from which the emergence of new pathotypes, by acquiring new virulence factors, can always be expected [94].

5. CONCLUSION

Enterotoxic enteric colibacillosis of farm animals probably represents the most intensively studied bacterial disease in veterinary medicine. Results of these studies have improved our understanding of pathogenesis of both human and animal diseases due to ETEC and about the biochemical languages that bacteria use in communicating with host cells. They have also improved our knowledge about the molecular evolution of bacterial pathogenesis, in general. Consequently, diagnostic and preventive measures against ETEC have become more efficient.

In spite of these improvements, there is still a series of intriguing questions to be studied in relation to new (so far unknown) virulence factors, to molecular mechanisms of toxigenecity (secretion and export mechanisms of some enterotoxins and mechanisms of stimulation of fluid and electrolite secretion by intestinal absorptive epithelial cells). The further identification of receptors for adhesins and toxins as well as the analysis of genetic determination of resistance to the disease will probably be future explanations to present day's epidemiological experiences.

ACKNOWLEDGEMENTS

Much of our recent experimental work cited here has been supported by the Hungarian basic science grants OTKA T749 and T5478 and A312 as well as by a USDA co-operative agreement 58-5114-9-1005 with the Iowa state University, by IWONL, Brussels, Belgium, and by the Swiss National Science Foundation. The valuable suggestions and comments of Dr Contrepois, Dr Fairbrother, Dr Le Bougouénec and Dr Oswald as well as editorial assistance of Wendy Brand-Williams are gratefully acknowledged.

The authors apologize to those colleagues who feel that some important publications might not have been sufficiently referred to.

REFERENCES

[1] Acres S.D., Isaacson R.E., Babiuk L.A., Kapitany R.A., Immunization of calves against enterotoxigenic colibacillosis by vaccinating dams with purified K99 antigen and whole cell bacterins, Infect. Immun. 25 (1979) 121–126.

[2] Ahren C., Jertborn M., Svennerholm A., Intestinal immune responses to an inactivated oral enterotoxigenic *Escherichia coli* vaccine and associated immunoglobulin A responses in blood, Infect. Immun. 66 (1998) 3311–3316.

[3] Akashi N., Hitotsubashi S., Yamanaka H., Fujii Y., Tsuji T., Miyama A., Joya J.E., Okamoto K., Production of heat-stable enterotoxin II by chicken clinical isolates of *Escherichia coli*, FEMS Microbiol. Lett. 109 (1993) 311–315.

[4] Alexa P., Salajka E., Salajkova Z., Machova A., Combined parenteral and oral immunization against enterotoxigenic *Escherichia coli* diarrhoea in weaned piglets, Vet. Med. (Praha) 12 (1995) 365–370.

[5] Attridge S.R., Hackett J., Morona R., Whyte P., Towards a live oral vaccine against enterotoxigenic *Escherichia coli* of swine, Vaccine 6 (1988) 387–389.

[6] Bachmann P.A., Baljer G., Gmelch X., Eichhorn W., Plank P., Mayr A., Vaccination of cows with K99 and rotavirus antigen: Potency of K99 antigen combinated with different adjuvants in stimulation milk antibody secretion, Zentralbl. Veterinärm. B. 31 (1984) 660–668.

[7] Bertschinger H.U., Tucker H., Pfirter H.P., Control of *Escherichia coli* in weaned pigs by use of oral immunization combined with a diet low in nutrients, Fortschr. Veterinärmed. 29 (1979) 73–81.

[8] Bertschinger H.U., Bachmann M., Mettler C., Pospischil A., Schraner E., Stamm M., Sadler T., Wild P., Adhesive fimbriae produced in vivo by *Escherichia coli* O139:K12(B):H1 associated with enterotoxaemia in pigs, Vet. Microbiol. 15 (1990) 267–281.

[9] Bertschinger H.U., Stamm M., Vogeli P., Inheritance of resistance to oedema disease in the pig: experiments with an *Escherichia coli* strain expressing fimbriae 107, Vet. Microbiol. 35 (1993) 79–89.

[10] Beutin L., *Escherichia coli* as a pathogen in dogs and cats, Vet. Res. 30 (1999) 285–298.

[11] Bijlsma I.G., de Nijs A., van der Meer C., Frik J.F., Different pig phenotypes affect adherence of *Escherichia coli* to jejunal brush borders by K88ab, K88ac, or K88ad antigen, Infect. Immun. 3 (1982) 891–894.

[12] Billey L.O., Erickson A.K., Francis D.H., Multiple receptors on porcine intestinal epithelial cells for the three variants of *Escherichia coli* K88 fimbrial adhesin, Vet. Microbiol. 59 (1998) 203–212.

[13] Brisson-Noel A., Arthur M., Courvalin P., Evidence for natural gene transfer from gram-positive cocci to *Escherichia coli*, J. Bacteriol. 170 (1988) 1739–1745.

[14] Casey T.A., Herring C.J., Schneider R.A., Bosworth B.T., Whipp S.C., Expression of heat-stable enterotoxin STb by adherent *Escherichia coli* is not sufficient to cause severe diarrhoea in neonatal pigs, Infect. Immun. 66 (1998) 1270–1272.

[15] Contrepois M., Girardeau J.P., Additive protective effects of colostral antipili antibodies in calves experimentally infected with enterotoxigenic *Escherichia coli*, Infect. Immun. 50 (1985) 947–949.

[16] Contrepois M., Guillimin P., Vaccination anti *Escherichia coli* K99 chez la chévre, in: Yvore P., Perrin G. (Eds.), Les maladies de la chévre, Inra, Paris 1984 pp. 473–476.

[17] Contrepois M., Girardeau J.P., Dubourguier H.C., Gouet P., Levieux D., Specific protection

by colostrum from cows vaccinated with the K99 antigen in newborn calves experimentally infected with *E. coli* Ent+ K99+, Ann. Rech. Vet. 9 (1978) 385–388.

[18] Contrepois M., Bertin Y., Girardeau J.P., Picard B., Goullet P., Clonal relationships among bovine pathogenic *Escherichia coli* producing surface antigen CS31A, FEMS Microbiol. Lett. 106 (1993) 217–222.

[19] Contrepois M., Bertin Y., Pohl P., Picard B., Girardeau J.P., A study of relationship among F17-a producing enterotoxigenic and non-enterotoxigenic *Escherichia coli* strains isolated from diarrheic calves, Vet. Microbiol. 64 (1999) 75–81.

[20] Czirok E., Semjen G., Steinruck H., Herpay M., Milch H., Nyomarkay I., Stverteczky Z., Szeness A., Comparison of rapid methods for detection of heat-labile (LT) and heat-stable (ST) enterotoxin in *Escherichia coli*, J. Med. Microbiol. 36 (1992) 398–402.

[21] De Cupere F., Deprez P., Demeulenaere D., Muylle E., Evaluation of the effect of 3 probiotics on experimental *Escherichia coli* enterotoxaemia in weaned piglets, Zentralbl. Veterinarmed. 39 (1992) 277–284.

[22] Dean E.A., Whipp S.C., Moon H.W., Age-specific colonization of porcine intestinal epithelium by 987P-piliated enterotoxigenic *Escherichia coli*, Infect. Immun. 57 (1989) 82–87.

[23] Dreyfus L.A., Harville B., Howard D.E., Shaban R., Beatty D.M., Moriss S.J., Calcium influx mediated by the *Escherichia coli* heat-stable enterotoxin b (STb), Proc. Nat. Acad. Sci. USA 90 (1993) 3202–3206.

[24] Dubreuil J.D., *Escherichia coli* STb enterotoxin, Microbiology 143 (1997) 1783–1795.

[25] Duchet-Suchaux M., Le Maitre C., Bertin A., Differences in susceptibility of inbred and outbred infant mice to enterotoxigenic *Escherichia coli* of bovine, porcine and human origin, J. Med. Microbiol. 31 (1990) 185–190.

[26] Duguid J.P., Smith I.W., Dempster G., Non-flagellar filamentous appendages (fimbriae) and haemagglutinating activity in Bacterium coli, J. Pathol. Bacteriol. 53 (1955) 335–355.

[27] Duhamel G.E., Moxley R.A., Maddox C.W., Erickson E.D., Enteric infection of a goat with enterohemorrhagic *Escherichia coli* (O103:H2), J. Vet. Diagn. Invest. 4 (1992) 197–200.

[28] Elsinghorst E.A., Weitz J.A., Epithelial cell invasion and adherence directed by the enterotoxigenic *Escherichia coli* tib locus is associated with a 104-kilodalton outer membrane protein, Infect. Immun. 62 (1994) 3463–3471.

[29] Erhard M.H., Kellner J., Eichelberger J., Losch U., New possibilities in oral immunoprophylaxis of newborn diarrhoea in calves – a field study using specific egg antibodies, Berl. Munch. Tierarztl. Wochenschr. 106 (1993) 383–387.

[30] Eslava C., Navarro-Garcia F., Czeczulin J.R., Henderson I. R., Cravioto A., Nataro J.P., Pet, an autotransporter enterotoxin from enteroaggregative *Escherichia coli*, Infect. Immun. 66 (1998) 3155–3163.

[31] Fairbrother J.M., Lariviere S., Lallier R., New fimbrial antigen F165 from *Escherichia coli* serogroup O115 strains isolated from piglets with diarrhoea, Infect. Immun. 51 (1986) 10–15.

[32] Fasano A., Cellular microbiology: how enteric pathogens socialize with their host, ASM News 63 (1997) 259–265.

[33] Felmlee T., Pellett S., Welch R.A., Nucleotide sequence of an *Escherichia coli* chromosomal hemolysin, J. Bacteriol. 163 (1985) 94–105.

[34] Finlay B.B., Falkow S., Common themes in microbial pathogenicity revisited, Microbiol. Mol. Biol. Rev. 61 (1997) 136–169.

[35] Forte L.R., Thorne P.K., Eber S.L., Krause W.J., Freeman R.H., Francis S.H., Corbin J. D., Stimulation of intestinal Cl- transport by heat-stable enterotoxin: activation of cAMP-dependent protein kinase by cGMP, Am. J. Physiol. 263 (1992) 607–615.

[36] Francis D.H., Grange P.A., Zeman D.H., Baker D.R., Sun R., Erickson A.K., Expression of mucin-type glycoprotein K88 receptors strongly correlates with piglet susceptibility to K88(+) enterotoxigenic *Escherichia coli*, but adhesion of this bacterium to brush borders does not, Infect. Immun. 66 (1998) 4050–4055.

[37] Franck S.M., Bosworth B.T., Moon H.W., Multiplex PCR for enterotoxigenic, attaching and effacing, and Shiga toxin-producing *Escherichia coli* strains from calves, J. Clin. Microbiol. 36 (1998) 1795–1797.

[38] Frantz J.C., Jaso-Friedman L., Robertson D.C., Binding of *Escherichia coli* heat-stable enterotoxin to rat intestinal cells and brush border membranes, Infect. Immun. 43 (1984) 622–630.

[39] Gardner J.D., Receptors for gastrointestinal hormones, Gastroenterology 76 (1979) 202–214.

[40] Gibbons R.A., Sellwood R., Burrows M., Hunter P.A., Inheritance of resistance to neonatal *E. coli* diarrhoea in the pig, Theor. Appl. Gen. 51 (1977) 65–70.

[41] Girardeau J.P., Dubourguier H.C., Gouet P., Effect of glucose and amino acids on expression of K99 antigen in *Escherichia coli*, J. Gen. Microbiol. 128 (1982) 2243–2249.

[42] Girardeau J.P., Der Vartanian M., Ollier J.L., Contrepois M., CS31A, a new K88-related fimbrial antigen on bovine enterotoxigenic and septicemic *Escherichia coli* strains, Infect. Immun. 56 (1988) 2180–2188.

[43] Guinee P.A.M., Veldkamp M.J., Jansen W.H., Improved Minca medium for the detection of

K99 antigen in calf enterotoxigenic strains of *Escherichia coli*, Infect. Immun. 15 (1977) 676–678.

[44] Hadad J.J., Gyles C.L., Scanning and transmission electron microscopic study of the small intestine of colostrum-fed calves infected with selected strains of *Escherichia coli*, Am. J. Vet. Res. 43 (1982) 41–49.

[45] Hampson D.J., Postweaning *E. coli* in pigs, in: Gyles C.E. (Ed.), *Escherichia coli* in Domestic Animals and Humans, CAB International, Wallingford, 1994, pp. 178–181.

[46] Hampson D.J., Woodward J.M., Connaughton I.D., Genetic analysis of *Escherichia coli* from porcine postweaning diarrhoea, Epidemiol. Infect. 110 (1993) 575–581.

[47] Hirst T.E., Ruddock L.W., Hillary J.B., Lencer W.I., Membrane translocation and targeting of cholera toxin and related enterotoxins in prokaryotic and eukaryotic systems, in: Hacker J., Alouf J.E., Brand B.C., Falmagne P., Fehrenbach F.J., Freer J.H., Goebel W., Gross R., Heesemann J., Locht C., Montecucco C., Olsnes S., Rappuoli R., Reidl J., Wadström T. (Eds.), Bacterial Protein Toxins, Gustav Fischer, Jena, Stuttgart, Lübeck, Ulm, 1998, pp. 113–120.

[48] Hitotsubashi S., Fuji Y., Yamanaka H., Okamoto K., Some properties of purified *E. coli* heat-stable enterotoxin II, Infect. Immun. 60 (1992) 4468–4474.

[49] Hohmann A., Wilson M.R., Adherence of enteropathogenic *Escherichia coli* to intestinal epithelium in vivo, Infect. Immun. (1975) 866–880.

[50] Holland R.E., Sriranganathan N., DuPont L., Isolation of enterotoxigenic *Escherichia coli* from a foal with diarrhoea, J. Am. Vet. Med. Assoc. 194 (1989) 389–391.

[51] Ikemori Y., Peralta R.C., Kuroki M., Yokoyama H., Kodama Y., Research note: avidity of chicken yolk antibodies to enterotoxigenic *Escherichia coli* fimbriae, Poult. Sci. 72 (1993) 2361–2365.

[52] Imberechts H., Van Pelt N., De Greve H., Lintermans P., Sequences related to the major subunit gene fedA of F107 fimbriae in porcine *Escherichia coli* strains that express adhesive fimbriae, FEMS Microbiol. Lett. 3 (1994) 309–314.

[53] Imberechts H.U., Bertschinger B., Nagy B., Deprez P., Pohl P., Fimbrial colonisation factors F18ab and F18ac of *Escherichia coli* isolated from pigs with postweaning diarrhoea and edema disease, in: Paul (Ed.), Mechanisms in the Pathogenesis of Enteric Diseases, Plenum Press, New York, 1997, pp. 175–183.

[54] Isaacson R.E., Nagy B., Moon H.W., Colonization of porcine small intestine by *Escherichia coli*: colonization and adhesion factors of pig enteropathogens that lack K88, J. Infect. Dis. 135 (1977) 531–539.

[55] Isaacson R.E., Moon H.W., Schneider R.A., Distribution and virulence of *Escherichia coli* in the small intestines of calves with and without diarrhoea, Am. J. Vet. Res. 39 (1978) 1750–1755.

[56] Joya J.E., Tsuji T., Jacalne A.V., Arita M., Tsukamoto T., Honda T., Miwatani T., Demonstration of enterotoxigenic *Escherichia coli* in diarrheic broiler chicks, Eur. J. Epidemiol. 6 (1990) 88–90.

[57] Katwa L.C., Parker C.D., Dybing J.K., White A.A., Nucleotide regulation of heat-stable enterotoxin receptor binding and of guanylate cyclase activation, Biochem. J. 283 (1992) 727–735.

[58] Kennan R.M., Monckton R.P., Adhesive fimbriae associated with porcine enterotoxigenic *Escherichia coli* of the O141 serotype, J. Clin. Microbiol. 28 (1990) 2006–2011.

[59] Koves B., Nagy B., Kulcsar A., Development of a bovine combined Rotavirus-*Escherichia coli* vaccine (in German), Arch. Exp. Veterinarmed. 41 (1987) 904–907.

[60] Kupersztoch Y.M., Tachias K., Moomaw C.R., Dreyfus L.A., Urban R., Slaughter C., Whipp S., Secretion of methanol-insoluble heat-stable enterotoxin (STb): energy- and secA-dependent conversion of pre-STb to an intermediate indistinguishable from the extracellular toxin, J. Bacteriol. 172 (1990) 2427–2432.

[61] Le Bougouénec C., Bertin Y., AFA and F17 adhesins produced by pathogenic *Escherichia coli* strains in domestic animals, Vet. Res. 30 (1999) 317–342.

[62] Lee C.H., Hu S.T., Swiatek P.J., Moseley S.L., Allen S.D., So M., Isolation of a novel transposon which carries the *Escherichia coli* enterotoxin STII gene, J. Bacteriol. 162 (1985) 615–620.

[63] Lintermans P.F., Pohl P., Bertels A., Charlier G., Vandereckerchove J., Van Damme J., Schoup J., Schlickere C., Korhouen T., De Greve H., Van Montagu M., Characterisation and purification of the FY Adhesin present on the surface of bovine enteropathogenic and septicemic *Escherichia coli*, Am. J. Vet. Res. 49 (1988) 1794–1799.

[64] Lortie L.A., Dubreuil J.D., Harel J., Characterization of *Escherichia coli* strains producing heat-stable enterotoxin b (STb) isolated from humans with diarrhoea, J. Clin. Microbiol. 29 (1991) 656–659.

[65] Mainil J.G., Sadowski P.L., Tarsio M., Moon H.W., In vivo emergence of enterotoxigenic *Escherichia coli* variants lacking genes for K99 fimbriae and heat-stable enterotoxin, Infect. Immun. 55 (1987) 3111–3116.

[66] Mainil J.G., Bex F., Jacquemin E., Pohl P., Couturier M., Kaeckenbeeck A., 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 (1990) 187–190.

[67] Mezoff A.G., Jensen N.J., Cohen M.B., Mechanisms of increased susceptibility of immature and weaned pigs to *Escherichia coli* heat-stable enterotoxin, Pediatr. Res. (1991) 424–428.

[68] Moon H.W., Bunn T.O., Vaccines for preventing enterotoxigenic *Escherichia coli* infections in farm animals, Vaccine 11 (1993) 213–219.

[69] Moon H.W., Rogers D.G., Rose R., Effects of an orally administered live *Escherichia coli* pilus vaccine on duration of lacteal immunity to enterotoxigenic *Escherichia coli* in swine, Am. J. Vet. Res. 49 (1988) 2068–2071.

[70] Morein B., Sundquist B., Hoglund S., Dalsgaard K., Osterhaus A., ISCOM, a novel structure for antigenic presentation of membrane proteins from enveloped viruses, Nature 308 (1984) 457–460.

[71] Morona R., Morona J.K., Considine A., Hackett J.A., van den Bosch L., Beyer L., Attridge S.R., Construction of K88- and K99-expressing clones of Salmonella typhimurium G30: immunogenicity following oral administration to pigs, Vaccine 12 (1994) 513–517.

[72] Moseley S.L., Samadpour-Motalebi M., Falkow S., Plasmid association and nucleotide sequence relationships of two genes encoding heat-stable enterotoxin production in *Escherichia coli* H-10407, J. Bacteriol. 156 (1983) 441–443.

[73] Nagy B., Vaccination of cows with a K99 extract to protect newborn calves against experimental enterotoxic colibacillosis, Infect. Immun. 27 (1980) 21–24.

[74] Nagy B., Pathogenesis and prevention of enteric infection of calves and pigs, D.Sc. thesis, Hungarian Academy of Sciences, Budapest, 1993 (in Hungarian).

[75] Nagy B., Nagy Gy., Az enteropathogén *E. coli* törzsek virulenciafaktorainak kimutatása a borjak hasmenésének diagnosztikájában, Magyar Állatorvosok Lapja 37 (1982) 512–517.

[76] Nagy B., Moon H.W., Isaacson R.E., 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 *E. coli*, Infect. Immun. 16 (1977) 344–352.

[77] Nagy B., Bozso M., Palfi V., Nagy Gy., Sahiby M.A., Studies on cryptosporidial infection of goat kids, in: Yvore P., Perrin G. (Eds.), Les maladies de la chèvre, Inra, Paris, 1984, pp. 443–451.

[78] Nagy B., Casey T.A., Moon H.W., Phenotype and genotype of *Escherichia coli* isolated from pigs with postweaning diarrhoea in Hungary, J. Clin. Microbiol. 28 (1990) 651–653.

[79] Nagy B., Hoglund S., Morein B., Iscom (immunostimulating complex) vaccines containing mono- or polyvalent pili of enterotoxigenic *E. coli*; immune response of rabbit and swine, Zentralbl. Veterinarmed. 37 (1990) 728–738.

[80] Nagy B., Casey T.A., Whipp S.C., Moon H.W., Dean E.A., Pili and adhesiveness of porcine postweaning enterotoxigenic and verotoxigenic *Escherichia coli*, in: Proc. 12th Cong. Int. Pig Vet. Soc., The Hague, The Netherlands, 1992 p. 240.

[81] Nagy B., Casey T.A., Whipp S.C., Moon H.W., Susceptibility of porcine intestine to pilus-mediated adhesion by some isolates of piliated enterotoxigenic *Escherichia coli* increases with age, Infect. Immun. 60 (1992) 1285–1294.

[82] Nagy B., Awad-Masalmeh M., Bodoky T., Munch P., Szekrenyi M.T., Association of shiga-like toxin type II (SLTII) and heat stable enterotoxins with F18ab, F18ac, K88 and F41 fimbriae of *Escherichia coli* from weaned pigs, in: Proc. 14th Cong. Int. Pig Vet. Soc., Bologna, Italy, 1996, p. 264.

[83] Nagy B., Nagy G., Meder M., Mocsari E., Enterotoxigenic *Escherichia coli*, rotavirus, porcine epidemic diarrhoea virus, adenovirus and calici-like virus in porcine postweaning diarrhoea in Hungary, Acta Vet. Hung. 44 (1996) 9–19.

[84] Nagy B., Whipp S.C., Imberechts H., Bertschinger H.U., Dean-Nystrom E.A., Casey T.A., Salajka E., Biological relationship between F18ab and F18ac fimbriae of enterotoxigenic and verotoxigenic *Escherichia coli* from weaned pigs with oedema disease or diarrhoea, Microb. Pathog. 22 (1997) 1–11.

[85] Nakazava M., Sugimeto C., Isayama Y., Kashiwazaki M., Virulence factors *Escherichia coli* isolated from piglets with neonatal and postweaning diarrhoea in Japan, Vet. Microbiol. 13 (1987) 291–300.

[86] Nataro J.P., Kaper J.B., Diarrheagenic *Escherichia coli*, Clin. Microbiol. Rev. 11 (1998) 142–201.

[87] O'Brien A.D., Holmes R.K., Protein toxins of *Escherichia coli* and Salmonella, in: Neidhart F.C., Curtiss III R., Ingraham J.L., Lin E.C.C., Low K.B., Magasanik B., Reznikoff W.S., Riley M., Schaechter M., Umbarger H.E. (Eds.), *Escherichia coli* and Salmonella: Cellular and Molecular Biology, ASM Press, Washington DC, 1996, pp. 2788–2802.

[88] Ochman H., Selander R.K., Evidence for clonal population structure in *Escherichia coli*, Proc. Natl. Acad. Sci. USA 81 (1984) 198–201

[89] Olasz F., Boldogkoi Zs., Nagy B., Genetic modification of STa and STb production of porcine enterotoxic *Escherichia coli*, in: Hacker J., Alouf J.E., Brand B.C., Falmagne P., Fehrenbach F.J., Freer J.H., Goebel W., Gross R., Heesemann J., Locht C., Montecucco C., Olsnes S., Rappuoli R., Reidl J., Wadström T. (Eds.),

Bacterial Protein Toxins, Gustav Fischer, Jena, Stuttgart, Lübeck, Ulm, 1998, pp. 405–406.

[90] Ørskov I., Ørskov F., Serology of *Escherichia coli* fimbriae, Prog. Allergy 33 (1983) 80–105.

[91] 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.

[92] Pohl P., Lintermans P., Moury J., Facteurs de virulence chez les *E. coli* septicemies et saprophytes du veau, Ann. Méd. Vét. 130 (1986) 515–520.

[93] Pohl P., Lintermans P., Mainil J., Daube G., Kaeckenbeeck A., ETEC-like strains from cattle, Vet. Rec. 125 (1989) 382.

[94] Pupo G.M., Karaolis D.K., Lan R., Reeves P.R., Evolutionary relationships among pathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and mdh sequence studies, Infect. Immun. 65 (1997) 2685–2692.

[95] Rapacz J., Hasler-Rapacz J., Polymorphism and inheritance of swine small intestinal receptors mediating adhesion of three serological variants of *Escherichia coli*-producing K88 pilus antigen, Anim. Genet. 17 (1986) 305–321.

[96] Rippinger P., Bertschinger H.U., Imberechts H., Nagy B., Sorg I., Stamm M., Wild P., Wittig W., Designations F18ab and F18ac for the related fimbrial types F107, 2134P and 8813 of *Escherichia coli* isolated from porcine postweaning diarrhoea and from oedema disease, Vet. Microbiol. 45 (1995) 281–295.

[97] Robertson, D.C., Pathogenesis and enterotoxins of diarrheagenic *Escherichia coli*, in: Roth J.A. (Ed.), Virulence Mechanisms of Bacterial Pathogens, American Society for Microbiology, Washington DC, 1988, pp. 241–263.

[98] Rose R., Whipp S.C., Moon H.W., Effects of *Escherichia coli* heat-stable enterotoxin b on small intestinal villi in pigs, rabbits, and lambs, Vet. Pathol. 24 (1987) 71–79.

[99] Runnels P.L., Moon H.W., Schneider R.A., Development of resistance with host age to adhesion of K99+ *Escherichia coli* to isolated intestinal epithelial cells, Infect. Immun. 28 (1980) 298–300.

[100] Salajka E., Salajkova Z., Alexa P., Hornich M., Colonization factor different from K88, K99, F41 and 987P in enterotoxigenic *Escherichia coli* strains isolated from postweaning diarrhoea in pigs, Vet. Microbiol. 32 (1992) 163–175.

[101] Savarino S.J., Fasano A., Watson J., Martin B.M., Levine M.M., Guandalini S., Guerry P., Enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 represents another subfamily of *E. coli* heat-stable toxin, Proc. Nat. Acad. Sci. USA 90 (1993) 3093–3097.

[102] Sellwood R., Gibbons R.A., Jones G.W., Rutter J.M., Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes, J. Med. Microbiol. 8 (1975) 405–411.

[103] Seriwatana J., Echeverria P., Taylor D.N., Rasrinaul L., Brown J.E., Peiris J.S.M., Clayton C.L., Type II heat-labile enterotoxin-producing *Escherichia coli* isolated from animals and humans, Infect. Immun. 56 (1988) 1158–1161.

[104] Sherman D.M., Acres S.D., Sadowski P.L., Springer J.A., Bray B., Raybould T.J., Muscoplat C.C., Protection of calves against fatal enteric colibacillosis by orally administered *Escherichia coli* K99-specific monoclonal antibody, Infect. Immun. (1983) 653–658.

[105] Smith H.W., Halls S., Studies on *Escherichia coli* enterotoxin, J. Pathol. Bacteriol. 93 (1967) 531–543.

[106] Smith H.W., Gyles C.L., The relationship between two apparently different enterotoxins produced by enteropathogenic strains of *Escherichia coli* of porcine origin, J. Med. Microbiol. 3 (1970) 387–401.

[107] Smith H.W., Linggood M.A., Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *Escherichia coli* with particular reference to porcine diarrhoea, J. Med. Microbiol. 4 (1971) 467–485.

[108] Smith H.W., Green P., Parsell Z., Vero cell toxins in *Escherichia coli* and related bacteria: transfer by phage and conjugation and toxic action in laboratory animals, chickens and pigs, J. Gen. Microbiol. 129 (1983) 3121–3137.

[109] So M., McCarthy B.J., Nucleotide sequence of the bacterial transposon Tn1681 encoding a heat-stable (ST) toxin and its identification in enterotoxigenic *Escherichia coli* strains, Proc. Nat. Acad. Sci. USA 77 (1980) 4011–4015.

[110] Sperandio V., da Silveira W.D., Comparison between enterotoxigenic *Escherichia coli* strains expressing F42, F41 and K99 colonization factors, Microbiol. Immunol. 37 (1993) 869–875.

[111] Stevens J.B., Gyles C.L., Barnum D.A., Production of diarrhoea in pigs in response to *Escherichia coli* enterotoxin, Am. J. Vet. Res. 33 (1972) 2511–2526.

[112] Thomlinson J.R., Lawrence T.L., Dietary manipulation of gastric pH in the prophylaxis of enteric disease in weaned pigs: some field observations, Vet. Rec. 109 (1981) 120–122.

[113] Thorns C.J., Sojka M.G., Roeder P.L., Detection of fimbrial adhesins of ETEC using monoclonal antibody-based latex reagents, Vet. Rec. 125 (1989) 91–92.

[114] Thorns C.J., Bell M.M., Chasey D., Chesham J., Roeder P.L., Development of monoclonal antibody ELISA for simultaneous detection of bovine coronavirus, rotavirus serogroup A, and *Escherichia coli* K99 antigen in feces of calves, Am. J. Vet. Res. 53 (1992) 36–43.

[115] Tsen H.Y., Jian L.Z., Development and use of a multiplex PCR system for the rapid screen-

ing of heat labile toxin I, heat stable toxin II and shiga-like toxin I and II genes of *Escherichia coli* in water, J. Appl. Microbiol. 84 (1998) 585–592.

[116] Tsuji T., Joya J.E., Honda T., Miwatani T., A heat-labile enterotoxin (LT) purified from chicken enterotoxigenic *Escherichia coli* is identical to porcine LT, FEMS Microbiol. Lett. 55 (1990) 329–332.

[117] Tzipori S., Withers M., Hayes J., Robins-Browne R., Ward K.L., Attachment of *E. coli*-bearing K88 antigen to equine brush-border membranes, Vet. Microbiol. 9 (1984) 561–570.

[118] Vazquez F., Gonzalez E.A., Garabal J.I., Blanco J., Fimbriae extracts from enterotoxigenic *Escherichia coli* strains of bovine and porcine origin with K99 and/or F41 antigens, Vet. Microbiol. 48 (1996) 231–241.

[119] Vogeli P., Bolt R., Fries R., Stranzinger G., Co-segregation of the malignant hyperthermia and the Arg615-Cys615 mutation in the skeletal muscle calcium release channel protein in five European Landrace and Pietrain pig breeds, Anim. Genet. 1 (1994) 59–66.

[120] Whipp S.C., Intestinal responses to enterotoxigenic *Escherichia coli* heat-stable toxin b in non-porcin species, Am. J. Vet. Res. 47 (1991) 615–617.

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

[122] Whittam T.S., Genetic variation and evolutionary processes in natural population of *Escherichia coli*, in: Neidhart F.C., Curtiss III R., Ingraham J.L., Lin E.C.C., Low K.B., Magasanik B., Reznikoff W.S., Riley M., Schaechter M., Umbarger H.E. (Eds.), *Escherichia coli* and Salmonella: Cellular and Molecular Biology, ASM Press, Washington DC, 1996, pp. 2708–2720.

[123] Wiedemann V., Linckh E., Kuhlmann R., Schmidt P., Losch U., Chicken egg antibodies for prophylaxis and therapy of infectious intestinal diseases. V. In vivo studies on protective effects against *Escherichia coli* diarrhoea in pigs, Zentralbl. Veterinarmed. 38 (1991) 283–291.

[124] Wilson R.A., Nagy B., Whittam T.S., Clonal analysis of weaned pig isolates of enterotoxigenic and verocytotoxigenic *E. coli*, in: Proc. 13th IPVS Congr. Bangkok, Thailand, 1994, p. 199.

[125] Wittig W., Prager R., Stamm M., Streckel W., Tschape H., Expression and plasmid transfer of genes coding for the fimbrial antigen F107 in porcine *Escherichia coli* strains, Int. J. Med. Microbiol. Virol. Parasitol. Infect. Dis. 281 (1994) 130–139.

[126] Woodward J.M., Connaughton I.D., Fahy V.A., Lymbery A.J., Hampson D.J., Clonal analysis of *Escherichia coli* of serogroups O9, O20, and O101 isolated from Australian pigs with neonatal diarrhoea, J. Clin. Microbiol. 31 (1993) 1185–1188.

[127] Woodward M.J., Wray C., Nine DNA probes for detection of toxin and adhesin genes in *Escherichia coli* isolated from diarrhoeal disease in animals, Vet. Microbiol. 25 (1990) 55–65.

[128] Yamanaka H., Nomura T., Fujii Y., Okamoto K., Extracellular secretion of *Escherichia coli* heat-stable enterotoxin I across the outer membrane, J. Bacteriol. 179 (1997) 3383–3390.

[129] Yokoyama H., Peralta R.C., Diaz R., Sendo S., Ikemori Y., Kodama Y., Passive protective effect of chicken egg yolk immunoglobulins against experimental enterotoxigenic *Escherichia coli* infection in neonatal piglets, Infect. Immun. 60 (1992) 998–1007.