

## Gene typing of the colonisation factors F18 of *Escherichia coli* isolated from piglets suffering from post-weaning oedema disease

P. ALEXA, K. ŠTOURAČOVÁ, J. HAMŘÍK, E. SALAJKA

Veterinary Research Institute, Brno, Czech Republic

**ABSTRACT:** Production of verotoxin Stx2e and expression of F18 and K88 colonisation factors were investigated in 222 strains of *Escherichia coli* isolated from weaned piglets. Sixty-two, 30, and 11 of the 129 verotoxigenic strains were classified to the serogroups O139, O141, and O157, respectively. Other serogroups were identified only sporadically and sixteen strains were unclassifiable. No colonisation factors were detectable in 15 (24.2%) of the 62 verotoxigenic strains classified with the serogroup O139. The *fedA* gene shared by the colonisation factors F18 was detected by PCR in 47 of the O139 strains (all but one F18ab). Gene *fedA*, in which the amplification product was digested with endonuclease *Ngo*MI (F18ac), was peculiar to the serogroup O141. No colonisation factors were detected in 19 (14.7%) strains. Genes encoding the colonisation factors F18ac and F18ab were demonstrated in 22 (23.7%) and 11 (11.8%), of the 93 non-verotoxigenic and mostly enterotoxigenic strains, respectively.

**Keywords:** oedema disease; pig; colonisation factors; Stx2e toxin; PCR

Oedema disease of pigs is induced by verotoxigenic strains of *Escherichia coli* (VTEC). The product of these strains is cytotoxic for Vero cells (Konowalchuk *et al.*, 1977) and it was designed as verotoxin. Considering the similarity between verotoxin and the toxin produced by *Shigella dysenteriae*, verotoxins were renamed to Shiga-like toxins (SLT) (O'Brien and LaVeck, 1983). Recently, a new terminology has been proposed designing verotoxins as Shigatoxins (Stx1 and Stx2, including several subtypes) (Calderwood *et al.*, 1996) and the strains producing them as shigatoxigenic *E. coli* (STEC).

As far as causative agents of oedema disease are concerned, the production of verotoxin is most frequently associated with *E. coli* serogroups of O138, O139, and O141. The verotoxin of *E. coli* isolated from piglets affected by oedema disease is the VT2 variant (Marques *et al.*, 1987), or, according to the new designation, Stx2e.

Strains of *E. coli* inducing oedema disease can colonise the piglet's gut (Bertschinger and Gyles, 1994). Wittig *et al.* (1995) supposed that the presence of colonisation factor F18ab and adherence to intestinal

mucosa are essential to induce oedema disease. The susceptibility to infection by strains carrying the colonisation factors F18ab is determined by expression of enterocytic receptors. The sensitivity to adhesion is controlled by the dominant (B) allele ECF18R and resistance to it by the alternative recessive allele (b) on chromosome 6 (Vögeli *et al.*, 1996). The colonisation factor F18ac is found mostly in porcine enterotoxigenic strains of *E. coli*.

Hornich *et al.* (1973) used immunofluorescence staining for the examination of sections of intestinal samples collected from piglets affected by oedema disease and found free bacteria in the intestinal contents, but no bacteria adhered to the intestinal mucosa like in the diarrhoeic form of *E. coli* infections. This finding indicates that colonisation factors can be absent in *E. coli* strains inducing oedema disease.

Experimental induction of oedema disease in pigs is rather difficult. The susceptibility of piglets depends on many factors, particularly genetically controlled resistance, diet, and immunity status (Smith and Linggood, 1971; Bertschinger *et al.*, 1978, 1979; Bertschinger and Gyles, 1994).

The objective of our study was to analyse the prevalence of colonisation factor F18-positive strains of *E. coli* associated with oedema disease in swine herds in the Czech Republic and to classify them in terms of serogroups.

## MATERIAL AND METHODS

### Strains of *E. coli*

*E. coli* strain Sw107/86 (O139:F18ab), kindly provided by Prof. H.U. Bertschinger of the Institute of Veterinary Bacteriology, University of Zurich, and our local *E. coli* strain No. 8813 (O147:F18ac) were used as the F18ab-positive and F18ac-positive controls, respectively. The tested set included 222 strains of *E. coli*, either freshly isolated from intestinal content or rectal swabs of weaned pigs in Czech republic, or maintained in our laboratory's collection as suspected causative agent of oedema disease. O-antigens of the strains were identified by agglutination. Genes encoding enterotoxins were detected by PCR (Alexa *et al.*, 1997).

### Detection of Stx2e and colonisation factors

The production of verotoxin was demonstrated in VERO cell cultures as described by Salajka *et al.* (1992) and the Stx2e type was identified by detection of the appropriate gene by PCR (Alexa *et al.*, 2000). Colonisation factors were demonstrated by agglutination in microtitre plates of *intra vitam* stained cultures of the tested strains with specific antisera to K88 and F18. The tested strains were grown in nutrient broth (Imuna, Šarišské Michalany, Slovak Republic) and in the MINCA medium. Adhesion of *E. coli* bacteria to intestinal brush borders was tested by the method described by Sellwood *et al.* (1975) with some modifications (Salajka *et al.*, 1992). The procedure was similar to that of the agglutination test with the difference that serum was replaced with an intestinal brush border suspension in diluted Sørensen's buffer (pH 7.4).

### Detection of the *fedA* gene

The *fedA* gene shared by *E. coli* strains carrying the F18ab and F18ac colonisation factors was detected in pure cultures by PCR using the *fedA1* nucleotide 5'-GTG-AAA-AGA-CTA-GTG-TTT-ATT-TC-3' and the *fedA2* nucleotide 5'-CCT-GTA-AGT-AAC-CGC-

GTA-AGC-3' (Imberechts *et al.*, 1992). The amplification process consisted of the initial 3-min denaturation step at 94°C followed by 30 cycles of 45 s at 92°C, 45 s at 50°C, and 45 min at 72°C and the final 2-min incubation at 72°C. The PCR Master Mix Kit (Qiagen, Hilden, Germany) was used according to the manufacturer's instructions. Each PCR tube contained 10 pmol of each primer and 2 µl of DNA template making together the volume of 20 µl. Positive and negative controls were always included in each PCR run. The amplification products were detected by electrophoresis in 1.5% agarose gel after staining with ethidium bromide and visualisation in a U.V. transilluminator.

The amplified PCR products were digested with the restriction enzyme *Ngo*MI (BioLabs inc. New England) to differentiate between genes encoding F18ab and F18ac. (Imberechts *et al.*, 1994b). For this purpose, 15 µl of the amplification product obtained in the preceding PCR was mixed with 2 µl of the restriction buffer, 1 µl of *Ngo*MI (10 units), and 2 µl of H<sub>2</sub>O. The mixture was incubated at 37°C for 15 min and the digestion products were separated by electrophoresis in 1.5% agarose gel. The *fedA* amplification product specific for F18ab was identified as the 510 bp band whereas the *fedA* type characteristic for the F18ac variant was identified by the presence of two DNA bands of 363 bp and 150 bp in size, respectively (Rippinger *et al.*, 1995).

## RESULTS

The tested set of strains consisted of 129 verotoxin-positive isolates of *E. coli* in which the gene encoding the production of Stx2e was demonstrated and additional 93 strains of *E. coli* belonging to the same serogroups, but negative in tests for Stx2e production. Colonisation factors of the verotoxigenic strains are presented in Table 1. Almost half of the isolates (62 isolates) were classified as the serogroup O139, 30 as the serogroup O141, and 11 as the serogroup O157. O-antigen was not identified in 16 verotoxigenic strains.

Most of verotoxigenic *E. coli* strains with F18 fimbriae showed autoagglutination, therefore differentiation between colonisation factor-positive and negative strains by serological methods was not possible. PCR performed with *fedA1* and *fedA2* primers demonstrated that 47 O139 strains (75.8%) possessed the gene encoding the *fedA* subunit and 15 (24.2%) lacked this genetic marker. Forty O139 *E. coli* strains were found to belong to the F18ab type after digestion of

Table 1. Presence of the *fedA* sequences in strains of *E. coli* producing VT2e

Serogroup	Number of strains	<i>fedA</i>	F18ab	F18ac	F18 type not tested	<i>fedA</i> -negative
O108	1	1		1		
O115	1	1		1		
O138	6	6	4	2		
O139	62	47	40		7	15
O141	30	30	1	27	2	
O142	2	2	2			
O157	11	9	1	2	6	2
O?	16	14	2	3	9	2
Total	129	110	50	36	24	19

Table 2. Presence of the *fedA* sequences in VT2e-negative *E. coli* strains of selected serogroups

Serogroup	Number of strains	<i>fedA</i>	F18ab	F18ac	F18 type not tested	<i>fedA</i> -negative
O108	6	6			6	
O138	10	10	4	3	3	
O139	13	6	5	1		7
O141	4	4		3	1	
O157	14	14	1	11	2	
O?	46	16	1	4	11	30
Total	93	56	11	22	23	37

the *fedA* PCR product with *Ngo*MI endonuclease. We were unable to identify the F18 type of the remaining 7 strains because they were no more at disposal. The F18ac and F18ab types were identified in 27 and 1 strain of the 30 verotoxigenic strains of the O141 serogroup, respectively. Both F18ab and F18ac types of this colonisation factor were found among the isolates of the O138 and O157 serotypes and among the strains with unidentified O-antigens. Nineteen verotoxigenic strains (14.7%) lacked the gene encoding the common fragment of *fedA* and 15 of them were classified as O139 serogroup. No verotoxigenic strain showed agglutination with K88 antisera.

The results of the examination of the Stx2e-negative *E. coli* strains belonging to the same serogroups, classified as the verotoxigenic, are shown in Table 2. The colonisation factors F18ab and F18ac were demonstrated in five and one of the 13 O139 strains, respectively. No production of Stx2e or *E. coli* enterotoxins was demonstrated in the strains classified to this serogroup. The strains belonging to the remaining serogroups were enterotoxigenic and produced heat stable or heat labile enterotoxin. F18ac was predominant in strains of all serogroups except for the isolates of

O139 and O138 in which predominance of F18ab was found.

## DISCUSSION

Serological identification of colonisation factors in most of the O139 strains was not possible due to their autoagglutination. Moreover, results of serological typing and *in vitro* testing for adhesion to enterocytes for the demonstration of F18ab colonisation factor are not reliable (Nagy *et al.*, 1997). Our results of the PCR analyses indicate that a relatively large number of strains, particularly those of the serogroup O139 (24.2%) lacked the *fedA* gene which is the major subunit gene of the F18 colonisation factor. Sporadic occurrence of verotoxigenic strains in which F18ab was undetectable by PCR was also reported by Imberechts *et al.* (1994a). Wittig *et al.* (1995) described a number of verotoxigenic strains of the serogroup O139 which lacked any fimbriae. Osek (1999, 2000) reported occurrence of Stx2e positive *E. coli* strains in piglets after weaning, which were fimbria-negative. Isolation of verotoxigenic strains of the serogroup O139 without

the colonisation factor F18 was reported also by Da Silva *et al.* (2001).

The prevalence of fimbrial types found in our experiments is similar to results published by Wittig *et al.* (1995). Our verotoxigenic strains of *E. coli* belonging to the serogroup O139 carried only the F18ab type, while the F18ac type was predominant in the isolates of other *E. coli* serogroups. Only one of the F18ab-positive O139 strains failed to produce verotoxin. Colonisation of the gut by pathogenic *E. coli* strains is generally regarded as one of the conditions for the development of the diarrhoeic form of the infection or oedema disease. Explanation is needed for the relatively high percentage of verotoxigenic O139 strains isolated from typical cases of oedema disease in which F18 colonisation factor was not demonstrated. This finding is in agreement with the results of Hornich *et al.* (1973) who did not observe fluorescein-labelled O139-positive *E. coli* cells adhering to the intestinal mucosa of piglets affected by oedema disease. Although histological lesions were typical for oedema disease, labelled *E. coli* O139 were seen only in the intestinal contents without forming mucosal coatings. Our results are also consistent with the earlier findings of Sojka *et al.* (1957), Gilka *et al.* (1959), and Kretzschmar (1961) who observed massive occurrence of haemolytic *E. coli* in the intestinal contents and isolated them in pure culture from swollen and oedematous lymph nodes, but not from other tissues or the blood, of piglets suffering from oedema disease. Mechanisms and structures allowing verotoxigenic strains to colonise lymph nodes are unknown. Interestingly, most of the papers dealing with the pathogenesis of oedema disease disregard possible colonisation of lymph nodes. If verotoxigenic *E. coli* strains colonise lymph nodes, they probably propagate there and produce verotoxin which can, via lymph, reach the blood circulation. This mechanism could explain cases of oedema disease in which no colonisation of the intestinal mucosa was observed. Although this hypothesis has not been supported by exact data, we find it appropriate to draw attention to this possible way of penetration of verotoxin into the blood stream.

## REFERENCES

- Alexa P., Hamřík J., Salajka E., Štouračová K., Rychlík I. (2000): Differentiation of verotoxigenic strains of *Escherichia coli* isolated from piglets and calves in the Czech Republic. *Vet. Med. – Czech*, **45**, 39–43.
- Alexa P., Rychlík I., Nejezchleb A., Hamřík J. (1997): Identification of enterotoxin-producing strain of *Escherichia coli* by PCR and biological methods. *Vet. Med. – Czech*, **42**, 97–100.
- Bertschinger H.U., Gyles C.L. (1994): Oedema disease of pigs. In: Gyles C.L.: *Escherichia coli* in Domestic Animals and Humans. CAB International, Wallingford, Oxon OX10 8DE, UK. 193–219.
- Bertschinger H.U., Eggenberger E., Jucker H., Pfirter H.P. (1978/1979): Evaluation of low nutrient, high fibre diets for the prevention of porcine *Escherichia coli* enterotoxaemia. *Vet. Microbiol.*, **3**, 281–290.
- Calderwood S.B., Acheson D.W.K., Keusch G.T., Berret T.J., Griffin P.M., Strockbine N.A., Swaminathan B., Kaper J.B., Levine M.M., Kaplan B.S., Karch H., O'Brien A.D., Obrig G.T., Takeda Y., Tarr P.I., Wachsmuth I.K. (1996): Proposed new nomenclature for SLT (VT) family. *ASM News*, **62**, 118–119.
- Da Silva A.S., Valadares G.F., Penatti M.P.A., Brito B.G., da Silva Leite D. (2001): *Escherichia coli* strains from edema disease: O serogroups, and genes for shiga toxin, enterotoxins, and F18 fimbriae. *Vet. Microbiol.*, **80**, 227–233.
- Gilka F., Nesvadba J., Salajka E. (1959): Enterotoxemie prasat vyvolaná hemolytickou variantou *Escherichia coli*. *Veterinářství*, **9**, 41–51.
- Hornich M., Salajka E., Šarmanová Z., Ulmann L. (1973): Enteric *Escherichia coli* infections in piglets. Morphological findings and localization of the causative agent by immunofluorescence. *Acta Vet., Brno*, **42**, 413–422.
- Imberechts H., De Greve H., Schlicker Ch., Bouchet H., Pohl P., Charlier G., Bertschinger H., Wild P., Vanderkerkhove J., Van Damme J., Van Montegu M., Lintermans P. (1992): Characterization of F107 fimbriae of *Escherichia coli* 107/86, which causes edema disease in pigs, and nucleotide sequence of the F107 major fimbrial subunit gene, *fedA*. *Infect. Immun.*, **60**, 1963–1971.
- Imberechts H., Bertschinger H.U., Stamm M., Sydlér P., Pohl P., De Greve H., Hernalsteens J.-P., Van Montagu M., Lintermans P. (1994a): Prevalence of F107 fimbriae on *Escherichia coli* isolated from pigs with oedema disease or postweaning diarrhoea. *Vet. Microbiol.*, **40**, 219–230.
- Imberechts H., Van Pelt N., De Greve H., Lintermans P. (1994b): Sequences related to the major subunit gene *fedA* of F107 fimbriae in porcine *Escherichia coli* strains that express adhesive fimbriae. *FEMS Microbiol. Lett.*, **119**, 309–314.
- Konowalchuk J., Speirs J.I., Stavric S. (1977): Vero response to a cytotoxin *Escherichia coli*. *Infect. Immun.*, **18**, 775–779.
- Kretzschmar Ch. (1961): Untersuchungen zur Coli-Enterotoxämie des Schweines. *Monatsh. Veterinärmed.*, **16**, 891–901.

- Marques L.R.M., Peiris J.S.M., Cryz S.J., O'Brien A.D. (1987): *Escherichia coli* strains isolated from pigs with edema disease produce a variant of Shiga-like toxin II. FEMS Microbiol. Lett., 44, 33–38.
- Nagy B., Whipp S.C., Imberechts H., Bertschinger H.U., Dean-Nystrom E.A., Casey T.A., Salajka E. (1997): Biological relationship between F18ab and F18ac fimbriae of enterotoxigenic and verotoxigenic *Escherichia coli* from weaned pigs with oedema disease or diarrhoea. Microb. Pathogenesis, 22, 1–11.
- O'Brien A.D., LaVeck G.D. (1983): Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect. Immun., 40, 675–683.
- Osek J. (1999): Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. Vet. Microbiol., 68, 209–217.
- Osek J. (2000): Clonal analysis of *Escherichia coli* strains isolated from pigs with post-weaning diarrhea by pulsed-field gel electrophoresis. FEMS Microbiol. Lett., 186, 327–331.
- Rippinger P., Bertschinger H.U., Imberechts H., Nagy B., Sorg I., Stamm M., Wild P., Wittig W. (1995): 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, 281–295.
- Salajka E., Salajková Z., Alexa P., Hornich M. (1992): Colonization factor different from K88, K99, F41 and 987P in enterotoxigenic *Escherichia coli* strains isolated from postweaning diarrhoea in pigs. Vet. Microbiol., 32, 163–175.
- Sellwood R., Gibbons R.A., Jones G.W., Rutter J.M. (1975): Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: The existence of two pig phenotypes. J. Med. Microbiol., 8, 405–411.
- Smith H.W., Linggood M.A. (1971): The effect of antisera in protecting pigs against experimental *Escherichia coli* diarrhoea and oedema disease. J. Med. Microbiol., 4, 487–493.
- Sojka W.J., Erskine R.G., Lloyd M.K. (1957): Haemolytic *Escherichia coli* and "Oedema Disease" of Pigs. Vet. Rec., 69, 293–301.
- Vögeli P., Bertschinger H.U., Stamm M., Stricker C., Hagger C., Fries R., Rapacz J., Stranzinger G. (1996): Genes specifying receptors for F18 fimbriated *Escherichia coli*, causing oedema disease and postweaning diarrhoea in pigs, map to chromosome 6. Anim. Genet., 27, 321–328.
- Wittig W., Klie H., Gallien P., Lehmann S., Timm M., Tschäpe H. (1995): Prevalence of the fimbrial antigens F18 and K88 and of enterotoxins and verotoxins among *Escherichia coli* isolated from weaned pigs. Zentbl. Bakt., 283, 95–104.

Received: 02–02–14

Accepted after corrections: 02–04–11

---

*Corresponding Author*

MVDr. Pavel Alexa, CSc., Veterinary Research Institute, Hudcova 70, 621 32 Brno, Czech Republic  
Tel. +420 5 41 32 12 41, fax +420 5 41 21 12 29, e-mail: alexa@vri.cz

---