

Gene typing of the colonisation factors K88 (F4) in enterotoxigenic *Escherichia coli* strains isolated from diarrhoeic piglets

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ABSTRACT: More than 4 000 *E. coli* strains isolated from diarrhoeic piglets in 111 pig herds in the Czech Republic during the period 1995–2000 were examined for serogroup and virulence factors. Gene typing of the K88 marker by polymerase chain reaction (PCR) was used for the examination of 283 enterotoxigenic strains (ETEC) which agglutinated with antisera against K88 or adhered to intestinal brush borders. The K88 gene was detected in 237 strains; among them 232 strains possessed the K88 variant. Genotype K88ab was found in two strains of the serogroup O8 from one herd and the gene K88ad was detected in three strains of the serogroup O8 originating from another herd. The results show that the type K88ac is predominant in ETEC strains with colonisation factors K88 in pig herds in the Czech Republic.

Keywords: K88; F4; PCR; colonisation factor; adhesins; piglet; diarrhoea

INTRODUCTION

Enterotoxigenic *E. coli* (ETEC) are a frequent cause of diarrhoea of both suckling and weaned piglets. Their pathogenicity is based on two types of virulence factors – colonisation factors and enterotoxins. The colonisation factors (adhesins) are fimbrial antigens which enable adherence of ETEC to intestinal mucosa. K88 was the first adhesin detected in ETEC isolated from piglets (Jones and Rutter, 1972). Later, a series of fimbrial antigens was described in *E. coli*. The following adhesins have most frequently been detected in ETEC isolated from diarrhoeic piglets: K88 (F4), K99 (F5), F41, 987P (F6) and F18 (Ørskov and Ørskov, 1983; Salajka *et al.*, 1992; Ripinger *et al.*, 1995). Wilson and Francis (1986) detected the colonisation factors K88 as the most frequent adhesins in strains inducing diarrhoea especially in newborn piglets. Our previous investigations in the Czech Republic (Alexa *et al.*, 1995) showed that in weaned piglets suffering from colibacillosis infected with ETEC with F18 (8813) colonisation factors prevailed and the second most frequent being the strains harboring K88 fimbriae. Colonisation factors K88 occur in the strains which induce infections in both suckling and weaned piglets, whereas F18 occur almost exclusively in strains isolated from weaned piglets.

Morphologically, the K88 adhesin has a fimbrial structure of 2.1 nm in diameter. Guinée and Jansen (1979) distinguished three antigenic variants which were designated K88ab, K88ac and K88ad with a common epitope “a” and type-specific epitopes “b”, “c” and “d”.

Antibodies directed to fimbrial antigens of ETEC isolated from newborn piglets are considered as protective antibodies. Therefore those antigens are used as a major component of vaccines for immunoprophylaxis of enteric form of colibacillosis in newborn piglets. From the perspective of immunoprophylaxis, vaccines should contain those types of fimbriae which are present in pathogenic strains. The objective of our study was therefore to determine the prevalence of individual genetic variants of K88 fimbriae in enterotoxigenic *E. coli* strains isolated from diarrhoeic piglets in the Czech Republic.

MATERIAL AND METHODS

Escherichia coli strains

During the period of 1995–2000, 4 368 *E. coli* strains isolated from diarrhoeic piglets in 111 pig herds were typed and the presence of colonisation factors was tested. For sub-typing of K88 antigen, 283 enterotoxigenic *E. coli* strains were selected, which were mainly isolated from weaned piglets. The strains were selected based on the basis of positive or dubious results of serological detection of the K88 antigen or negative results of serological examination with positive test of adhesion to intestinal brush borders. The selected strains were mainly of the *E. coli* O8, O108, O138, O141, O147, O149 and O157 serogroups. Some strains were stored in our collection on Dorset egg slants whereas the other were

fresh isolates. The following *E. coli* strains, which were obtained from the International Escherichia and Klebsiella Centre, Statens Serum-institut, Copenhagen, Denmark, were used as controls: G7 (O8 : K87 : K88ab), G205 (O8 : K87:K88ac), G491 (O138 : K81 : K88ac), E68 (O141: K85 : 88ab), G1253 (O147 : K89 : K88ac), Abbotstown (O149 : K88ac), D615 (O149 : K91:K88ac), A2 (O157 : K88ac) and Clo 23/78 (O8 : K87 : K88ad : H19).

Serological typing

Somatic O-antigen was detected by a slow agglutination using specific O-antisera (Salajka *et al.*, 1992). Colonisation factors were detected by a slow agglutination using specific antisera and by a non-specific adhesion to the isolated intestinal brush borders (Salajka *et al.*, 1992). Genes encoding the production of enterotoxins were detected by PCR (Alexa *et al.*, 1997).

K88 differentiation

Differentiation of the K88 genetic variants was performed by PCR. Specific primers for PCR were designed using nucleotide sequences of the K88ab, K88ac and K88ad genes (Dykes *et al.*, 1985) (Gene Bank accession numbers M29374, M29375 and M29376, respectively) (Gaastra *et al.*, 1983) (Table 1).

Table 1. List of PCR primers used in the study

| Primer | Nucleotide sequence | Amplification product |
|---------|----------------------------------|-----------------------|
| K88ab 1 | 5c-TTG CTA CGC CAG TAA GTG GT-3c | |
| K88ab 2 | 5c-CGA AAC AGT CGT CGT CAA A -3c | 296 |
| K88ac 1 | 5c-TTT GCT ACG CCA GTA ACT G -3c | |
| K88ac 2 | 5c-TTT CCC TGT AAG AAC CTG C -3c | 436 |
| K88ad 1 | 5c-GGC ACT AAA GTT GGT TCA -3c | |
| K88ad 2 | 5cCAC CCT TGA GTT CAG AAT T -3c | 169 |

To release DNA from investigated bacteria, one colony of the tested culture was resuspended in 50 µl of deion-

ized water (MiliQ UF, Millipore) and boiled for 10 min. Cell debris was removed by a short centrifugation (10 000 × g for 1 min) and 2 µl of the supernatant was used as the DNA template.

Three different PCR procedures were used individually for each K88 type.

The initial cycle of 3 min at 94°C, common to all K88 types, was followed by:

- 25 cycles of 92°C for 45 s, 65°C for 45 s, and 72°C for 45 s for K88ab;
- 35 cycles of 92°C for 45 s, 54°C for 45 s, and 72°C for 70 s for K88ac;
- 30 cycles of 92°C for 45 s, 50°C for 45 s, and 72°C for 45 s for K88ad.

In the final step of the reaction, the temperature of 72°C was maintained for 3 min and the reaction mixture was cooled to 4°C.

PCR Master Mix Kit (Qiagen, Hilden, Germany) was used throughout the study according to the instructions of the manufacturer. Each PCR tube contained 10 pmol of each primer and 2 µl of template DNA. The total volume of the PCR mixture was 20 µl. The strains *E. coli* G7, G205, G491, E68, G1253, D615, A2 and Clo 23/78 were used as positive controls. Negative control consisted of 2 µl of water instead of DNA. The amplified PCR products were detected by electrophoresis in 2% agarose gel after staining with ethidium bromide and visualisation in a UV transilluminator.

The results obtained by this protocol were subsequently compared with those obtained by PCR, in a modified procedure according to Franklin *et al.* (1996). However, to receive the reliable results, cycling conditions of the amplifications had to be modified in the following way.

The initial cycle of 3 min at 94°C was followed by:

- 30 cycles of 92°C for 45 s, 44°C for 45 s, and 72°C for 45 s for K88ab and K88ad;
- 30 cycles of 92°C for 45 s, 63°C for 45 s, and 72°C for 45 s for K88ac.

In the final step of the reaction, the temperature of 72°C was maintained for 2 min and the PCR mixture was cooled to 4°C.

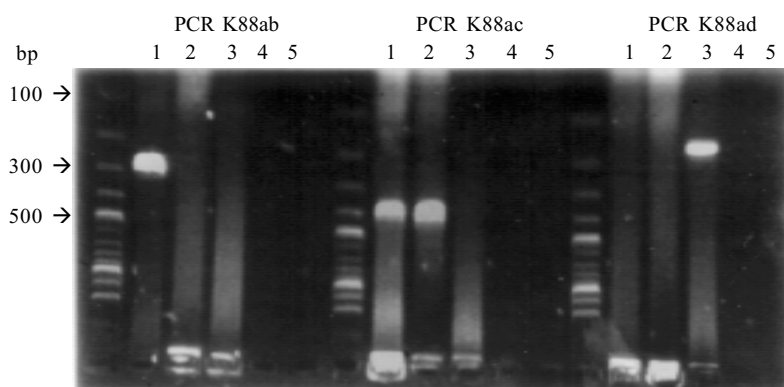


Figure 1. Gel electrophoresis indicating K88 gene after PCR amplification

1 – *E. coli* strain with K88ab variant, 2 – strain with K88ac, 3 – strain with K88ad, 4 – PCR negative control (water), 5 – K88 negative strain. K88ab positive strain – amplified products with both K88ab and K88ac primers (lanes 1)

RESULTS

When primers shown in Table 1 were used, the strains possessing the gene for K88ab antigen amplified the product with both primers K88ab and K88ac (Figure 1). Both reactions were performed for differentiation of these genetic variants (Table 2). Products of amplification of DNA K88ac and K88ad were specific in all reference *E. coli* strains.

The results of the genetic typing were identical by both PCR methods. Of 283 ETEC strains suspected to possess the adhesins, 238 were K88 positive. The remaining 45 strains had another adhesin type (predominantly F18 as determined by the slide agglutination test) or were adhesin free. No K88 genes were found in ETEC of the serogroups O108, O138 and O141. The results of subtyping are shown in Table 3. Most of the ETEC strains possessing the K88 gene belonged to the serogroup O149 ($n = 167$) and these strains had genetic markers for K88ac fimbriae. Two of the examined ETEC strains of the serogroup O149 were without adhesins and 13 strains harbored F18ac fimbriae. The K88 adhesin of the ETEC strains of the O147, O149 and O157 serogroups belonged to the K88ac genotype. ETEC strains of the serogroup O8 were exceptional. K88 genes were detected in 34 out of 46 strains examined, and of them 29 belonged to the type K88ac, 2 strains to the type K88ab and 3 to the type K88ad (Table 3). Both strains with the K88ab subtype produced heat labile enterotoxin (LT), and they were isolated from piglets of the same herd. Three strains with

K88ad genetic variant produced heat stable enterotoxin (STa) and they were originated from another single herd.

DISCUSSION

Serological typing of K88 antigens is dependent on the expression of antigen *in vitro* and on the antisera specificity. The PCR-based technique is not dependent on the level of expression of the antigen and simultaneously eliminates the inaccuracy of serological methods. Therefore we have tested suspected ETEC isolates for the K88 genes by PCR. This enabled subtyping of K88ab, K88ac and K88ad genetic variants.

The results obtained showed the antigenic structure of ETEC strains in herds of the Czech Republic. The proportion of individual serogroups in the strain collection studied was influenced by the fact that in most herds the sows were vaccinated against *E. coli* infections of newborn piglets. Therefore strains isolated from weaned piglets were predominant in the set. This corresponds with the highest findings of the strains of the O149 serogroup in piglets of this age (Alexa *et al.*, 1995; Hampson, 1994). Surprisingly enough, the K88ab-positive strains were found in a single herd and K88ad gene was found in another strains collected from another herd of the 111 herds tested. Similar results were published by Choi and Chae (1999) who analysed 44 strains positive for K88 isolated in Korea. Forty-two strains were K88ac and 2 strains were K88ab positive whereas the K88ad genotype was not detected. Our results show that K88ac is the major K88 adhesin type in pig herds in the Czech Republic, which corresponds to previously published results indicating that the incidence of the K88ac type would be similar around the world.

Table 2. PCR results for determination of the K88 genotype

| Strain with antigen | Amplification with primers | | |
|---------------------|----------------------------|-------|-------|
| | K88ab | K88ac | K88ad |
| K88ab | + | + | – |
| K88ac | – | + | – |
| K88ad | – | – | + |

Table 3. Relationship between the K88 genotype and *E. coli* serogroup

| O-serogroup | K88 variant | | | K88 negative | Number of strains tested |
|-------------|-------------|-------|-------|--------------|--------------------------|
| | K88ab | K88ac | K88ad | | |
| O8 | 2 | 29 | 3 | 12 | 46 |
| O108 | | | | 5 | 5 |
| O138 | | | | 5 | 5 |
| O141 | | | | 3 | 3 |
| O147 | | 18 | | 1 | 19 |
| O149 | | 167 | | 15 | 182 |
| O157 | | 4 | | | 4 |
| Other | | 15 | | 4 | 19 |
| Total | 2 | 233 | 3 | 45 | 283 |

REFERENCES

- Alexa P., Salajka E., Hamřík J., Nejezchleb A. (1995): Pathogenic strains of *Escherichia coli* in weaned piglets in the Czech Republic. *Pig News Info.*, 16, 81N-84N.
- 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.
- Choi CH., Chae CH. (1999): Genotypic prevalence of F4 variants (ab, ac and ad) in *Escherichia coli* isolated from diarrheic piglets in Korea. *Vet. Microbiol.*, 67, 307–310.
- Dykes C.W., Halliday I.J., Read M.J., Hobden A.N., Harford S. (1985): Nucleotide sequences of four variants of the K88 gene of porcine enterotoxigenic *Escherichia coli*. *Infect. Immun.*, 50, 279–283.
- Franklin M.A., Francis D.H., Baker D., Mathew A. (1996): A PCR-based method of detection and differentiation of K88⁺ adhesive *Escherichia coli*. *J. Vet. Diagn. Invest.*, 8, 460–463.

- Gaastra W., Klemm P., De Graaf F.K. (1983): The nucleotide sequence of the K88ad protein subunit of porcine enterotoxigenic *Escherichia coli*. FEMS Microbiol. Lett., 18, 177–183.
- Guinee P.A., Jansen W.H. (1979): Behavior of *Escherichia coli* K antigens K88ab, K88ac, and K88ad in immunoelectrophoresis, double diffusion, and hemagglutination. Infect. Immun., 700–705.
- Hampson D.J. (1994): Postweaning *Escherichia coli* diarrhoea in pigs. In: Gyles C.L.: *Escherichia coli* in domestic animals and humans. CAB International, Wallingford, Oxon OX10 8DE, 171–191.
- Jones G.W., Rutter J.M. (1972): Role of the K88 antigen in the pathogenesis of neonatal diarrhea caused by *Escherichia coli* in piglets. Infect. Immun., 6, 918–927.
- Ørskov I., Ørskov F. (1983): Serology of *Escherichia coli* fimbriae. Prog. Allergy, 33, 80–105.
- Rippinger P., Bertschinger H.U., Imberechts H., Nagy B., Sorg. I., Stamm M., Wild P., Wittig W. (1995): Designation 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., Salajkova 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.
- Wilson R.A., Francis D.H. (1986): Fimbriae and enterotoxins associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. Am. J. Vet. Res., 47, 213–217.

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