Folia Microbiol. **49** (1), 59–63 (2004) http://www.biomed.cas.cz/mbu/folia/

Genotyping of Fimbrial Adhesins in *Escherichia coli* Strains Isolated from Slovak Piglets Suffering from Diarrhea

H. VU-KHAC[a,b,c], E. HOLODA[a,b], M. MAJERČIAK[d], G. GAŠPAR[e], E. PILIPČINEC[a,b]

[a]*Institute of Microbiology and Immunology, Department of Food Hygiene and Technology, University of Veterinary Medicine, 041 81 Košice, Slovakia*
[b]*Center for Analysis of DNA, University of Veterinary Medicine, 041 81 Košice, Slovakia*
[c]*Department of Bacteriology, Center of Viet Nam Veterinary Research Institute, Nha Trang, Viet Nam*
[d]*Department of Bacteriology, State Veterinary Institute, 949 01 Nitra, Slovakia*
[e]*Department of Bacteriology, State Veterinary Institute, 842 52 Bratislava, Slovakia*

Received 13 June 2003
Revised version 9 September 2003

ABSTRACT. One-hundred sixty *Escherichia coli* isolates obtained from piglets with diarrhea from different parts of Slovakia were examined for the presence of genes coding for F4, F5, F6 and F41 fimbrial adhesins, and hemolytic activity. According to polymerase chain reaction tests 74 (46 %) *E. coli* isolates were positive for primers that detected genes coding for fimbrial adhesins. Of these 74 isolates, 64 were positive for genes encoding for F4$^+$, four for F5$^+$, five for F6$^+$, and one for both F41$^+$ and F5$^+$ adhesins.

Many strains of *Escherichia coli* are part of the nonpathogenic facultative flora of intestinal tract of humans and other mammals. Some of them are capable of inducing diseases of the gastrointestinal and urinary tracts or may affect the central nervous system (Balows *et al.* 1991). Such effects are ascribed to enterotoxigenic *E. coli* (ETEC) which cause serious economic losses in farm animal herds and are widespread in newborns (Kaper *et al.* 1988) in both developed and developing countries. This is a consequence of a wide range of transmission possibilities of these pathogens including direct contact, food, drinks, environment, atmosphere and others (Ondrašovič *et al.* 1997). Epidemiology and clinical symptoms of the disease are similar in various animal species but the majority of strains are species-specific (Zhu *et al.* 1994). They differ particularly in the type of the expressed surface "adherence" antigen (adhesin or pilus). These microorganisms produce two main types of virulence factors – fimbrial adhesins and enterotoxins (Martins *et al.* 2000).

Adhesin is considered to be a primary component of pathogenicity of enterotoxigenic strains of *E. coli* which allows them to adhere to small-intestine enterocytes (Donnenberg 1995). The first adhesin described, obtained from field isolates of *E. coli* capable of producing diarrheal diseases in piglets, was the K88 antigen (Ørskov *et al.* 1961). Later on serological methods were used to differentiate between three different types of K88 antigens, K88ab, K88ac, and K88ad (Bijlsma *et al.* 1987). In addition, Dykes *et al.* (1985) differing in two subtypes of the K88ab antigen, *viz*. K88ab1 and K88ab2.

In addition to K88 fimbrial antigens, we recognize additional factors of virulence, adhesins of *E. coli* strains, which induce enteric colibacillosis (Hacker 1993). Interaction between bacterial adhesins and tissue receptors, which facilitates colonization of the small intestine, is the primary stage of pathogenesis of enteric colibacillosis. The fimbrial adhesins most frequently diagnosed in *E. coli* strains isolated from piglets with diarrhea were F4 (K88), F5 (K99), F6 (987P), F18 and F41. According to Ojeniyi *et al.* (1994) and Frydendalh (2002), F4 and F18 adhesins are most frequently isolated from weaned piglets while F5, F6 and F41 are commonly isolated from suckling piglets with diarrhea (Wilson and Francis 1986; Wray *et al.* 1993; Kwon *et al.* 1999). The majority of *E. coli* strains expressing F5, F6 and F41 adhesins are nonhemolytic (Nagy and Fekete 1999). The *E. coli* strains expressing the F4 antigen are the most frequent cause of severe neonatal diarrhea (up to day 5 of age) while those expressing other adhesins give rise to milder diarrhea affecting piglets between 4 and 14 d of age (Nagy and Fekete 1999).

The diagnostics of ETEC strains has developed from the tests on intestinal loops of piglets (Evans *et al.* 1973) through immunological tests (latex agglutination, ELISA) (Thorns *et al.* 1989, 1992; Cryan 1990) to DNA tests including the PCR (Stacy-Phipps *et al.* 1995; Holoda *et al.* 1998; Osek 1999; Alexa *et al.* 2001; Bogyiová *et al.* 2002).

The aim of our study was to determine the prevalence of ETEC strains expressing one of the mentioned adherence antigens in three different territories of Slovakia by the polymerase chain reaction (PCR). In parallel, the strains were examined for their ability to express hemolysins.

MATERIALS AND METHODS

Reference strains. PCR diagnosis was done using the following reference strains: *E. coli* 298:K88$^+$ (F4), 329:K99$^+$ (F5), 318:987P$^+$ (F6), and 320:F41$^+$, were kindly supplied by Dr. J. Osek (*National Veterinary Research Institute*, Puławy, Poland); *E. coli* G491:K88ac$^+$, *E. coli* K88ad$^+$ were supplied by Dr. P. Alexa (*Veterinary Research Institute*, Brno, Czechia); *E. coli* M1:K88ab$^+$ (Holoda and Mikula 1994). An apathogenic laboratory strain of *E. coli* HB101 was used as negative control (Ausubel *et al.* 1989).

Field isolates. The screening examination included field isolates of *E. coli* strains obtained from piglets suffering from diarrhea (1–28-d old), originating from nonvaccinated herds from the territory of Bratislava, Nitra and Košice (all Slovakia). The strains were isolated from intestinal contents of dead animals or from rectal swabs of piglets with diarrhea at the *Departments of Bacteriology* (*State Veterinary Institutes* in Bratislava and Nitra, Slovakia) and the *Department of Food Hygiene and Technology* (*Institute of Microbiology and Immunology, University of Veterinary Medicine*, Košice, Slovakia) in the period 2001–2002. The samples were inoculated directly on MacConkey agar (*Oxoid*, England) and the colonies obtained were identified by standard biochemical procedures (Holoda *et al.* 2001). The solitary colonies intended for PCR examination were inoculated on LB agar and cultivated at 37 °C overnight.

Preparation of samples for the PCR determination. One colony grown on the LB agar was resuspended in 100 μL saline. After centrifugation (12 000 ***g***, 30 s), the sediment was resuspended in 100 μL deionized water and incubated at 100 °C for 10 min. Second centrifugation (14 000 ***g***, 5 min) provided a supernatant of which was subjected to PCR examination (2 μL aliquot). Samples with negative PCR results were used to isolate genomic DNA by the Miniprep method (Ausubel *et al.* 1989). The DNA was analyzed again by the PCR.

Reaction conditions for the PCR. The primers used are shown in Table I.

Table I. Primers for diagnosis of *E. coli* bacterial strains expressing adherence antigens

Target gene	Oligonucleotide sequence	Amplified product, bp	Reference
F4	5′-GCT GCA TCT GCT GCA TCT GGT ATG G-3′ 5′-CCA CTG AGT GCT GGT AGT TAC AGC C-3′	–[a]	Holoda *et al.* 2003
F5	5′-TGC GAC TAC CAA TGC TTC TG-3′ 5’-TAT CCA CCA TTA GAC GGA GC-3’	450	Ojeniyi *et al*. 1994
F6	5′-TCT GCT CTT AAA GCT ACT GG-3′ 5′-AAC TCC ACC GTT TGT ATC AG-3′	333	*ditto*
F41	5′-GAG GGA CTT TCA TCT TTT AG-3′ 5′-AGT CCA TTC CAT TTA TAG GC-3′	431	*ditto*

[a]The primers used were specific to all serological variants F4 (K88ab1 792 bp, K88ab2 792 bp, K88ac 786 bp and K88ad 792 bp); amplification products were differentiated by cleavage with restriction endonucleases.

The PCR reaction was done by Ampli*Taq* DNA polymerase (*Perkin Elmer*) in an amount of 1.0 U, 2.5 mmol/L dNTPs, PCR buffer solution Perkin Elmer with $MgCl_2$ in a total volume of reaction mixture equal to 50 μL. Additional reaction conditions for PCR corresponded to the references in Table I. With every PCR determination, the DNA isolated from the respective reference strain was used as positive control and DNA from *E. coli* HB101 as negative control.

Agarose gel electrophoresis. The PCR products were identified by agarose gel electrophoresis using 1.5 or 2 % agarose gel in electrophoretic TAE buffer solution (Ausubel *et al.* 1989). The DNA was observed under UV light after staining with ethidium bromide (0.5 mg/L). A 100-bp “ladder” was used as molar-mass standard. The gels were evaluated by the *Kodak Digital Science* system.

Determination of hemolytic activity. The colonies obtained were inoculated on blood agar (Blood agar base; *Oxoid*, England) supplemented with sheep erythrocytes up to a concentration of 5 %, and cultivated at 37 °C for 18 h. The hemolytic activity was evaluated visually.

Genotyping. One-hundred sixty isolates of *E. coli* were collected from samples of intestinal contents from nonvaccinated herds. Genetic variants of fimbrial adhesins F4, F5, F6 and F41 of all isolates were differentiated using PCR. The presence of genes encoding expression of individual fimbrial adhesins was determined.

RESULTS

Seventy-four isolates contained genes coding for some of the investigated adherence adhesins. These 74 positive cases made it possible to obtain 64 PCR-amplified products indicating the presence of $F4^+$ genes, four $F5^+$ genes and five $F6^+$ genes. In one field isolate we found the presence of a gene coding for both F41 and F5 adhesin. None of the isolates contained only the gene encoding F41 adhesin.

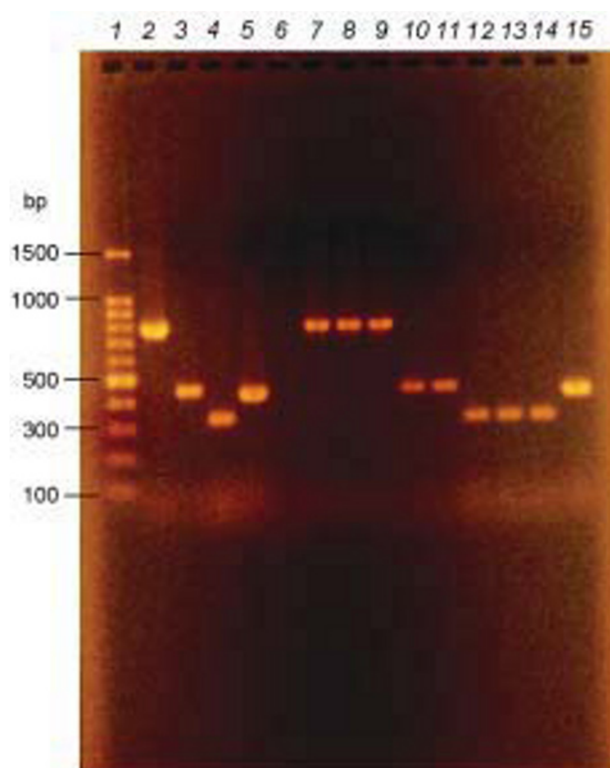

Fig. 1. Agarose gel electrophoresis of PCR amplification products of the *E. coli* isolates; *1* — 100 bp ladder, *2* — F4 positive control, *3* — F5 positive control, *4* — F6 positive control, *5* — F41 positive control, *6* — negative control, *7–9* — F4 positive field isolates, *10*, *11* — F5 positive field isolates, *12–14* — F6 positive field isolates, *15* — F41 positive field isolate.

Analysis PCR amplification products of $F4^+$ (Fig. 1) by restriction endonucleases revealed that two out of 64 $F4^+$ ($K88^+$) positive isolates showed the characteristics of genotype K88ab and 62 of genotype K88ac. None of the isolates could be classified as K88ad genotype.

The products of amplification of DNA reference strains were specific in all examinations while amplification of DNA obtained from *E. coli* HB101 provided in all cases negative results.

Hemolytic activity. Cultivation on blood agar of 160 field isolates of *E. coli* showed that 117 of them (73 %) exhibited β-hemolysis; it was detected in all 64 isolates positive for $F4^+$. Isolates that provided positive PCR results for genes coding for other adherence adhesins (F5, F6, F41) were all nonhemolytic. Absence of hemolysis was also observed in a strain which harbored genes coding for both F5 and F41 adhesin (Table II).

DISCUSSION

Enterotoxigenic *E. coli* strains (ETEC) are the frequent cause of acute diarrhea on pig farms. A common feature of these strains is the expression of one or more adherence adhesins (F4, F5, F6, F41, or combination of them) and the subsequent production of enterotoxins. This results in the development of profuse diarrhea which has frequently fatal consequences. The diagnosis of ETEC strains has long relied on the detection of enterotoxins. ELISA tests, radioimmunoassay tests (Cryan 1990) and LT (heat-labile *E. coli* entrotoxin) polynucleotide probe with enzymic, nonisotopic detection systems (Rademaker *et al.* 1993) have been used for this purpose. The effectiveness of serological tests is affected by the level of expression of antigen and the quality of the antiserum used. In the recent period diagnosis of these strains has been carried out increasingly with the help of the PCR method as a quite sensitive and specific method with primers for detection of genes coding for enterotoxin (Stacy-Philips *et al.* 1995) or adherence antigens (Ojeniyi *et al.* 1994; Osek 1999; Alexa *et al.* 2001; Holoda *et al.* 2002; Vu-Khac *et al.* 2003).

We found a prevalence of F4 adherence antigen in ETEC strains isolated from piglets suffering from diarrhea originating from nonvaccinated farms located in three different Slovak territories (east, central, west). In 74 (46 %) of the total number of isolates we detected the presence of genes coding for some adherence antigen. Of these 74 isolates 87 % were F4-positive (40 % of the total number). Also Hampson

(1994) and Nagy and Fekete (1999) detected F4 adherence antigen as the most frequent in ETEC strains isolated from piglets with diarrhea from various countries. Analysis of $F4^+$ amplified products by restriction endonucleases showed unambiguously that F4ac was the predominant variant – as 62 of the 64 F4 positive isolates were F4ac (97 %). A high incidence of F4 adhesin in ETEC strains was also observed by Wilson and Francis (1986) (48 %), Westerman *et al.* (1988) (71 %), Osek and Svennerholm (1991) (56 %) and Ojeniyi *et al.* (1994) (31 %). Also Alexa *et al.* (2001) reported that 233 of 238 F4-positive isolates in Czechia were F4ac (98 %).

Wilson and Francis (1986), Garabal *et al.* (1997) and Kwon *et al.* (1999) emphasized a high prevalence of the F6 adherence antigen in ETEC strains. We found only five to be positive for F6 (3.1 %). A low prevalence of ETEC strains in isolates from piglets with diarrhea which expressed F6 adherence antigen was reported in Sweden (Soderlind *et al.* 1988) and United Kingdom (Wray *et al.* 1993).

High prevalence of ETEC strains expressing F5 and F41 fimbrial adhesions was described in Spain (Garabal *et al.* 1997) and Sweden (Soderlind *et al.* 1988). On the other hand, strains expressing F5 and F41 adherence antigens were isolated rarely in the United Kingdom (Wray *et al.* 1993) and in Poland (Osek and Truszczynsky 1992) which is in agreement with our results (F5-positive were recorded only in four cases, *i.e.* 2.5 %) and none of the isolates coded for F41 alone. One field isolate showed positivity for two adhesins (F5 and F41). These observations support the earlier finding (Morris *et al.* 1980) that adhesins of F41 types were most often found in association with F5.

The results of our study and published data indicate that the frequency of occurrence of individual types of adherence antigens is related to geographical location.

Table II. The occurrence of fimbrial adhesins and hemolytic activity in *E. coli* strains isolated from suckling piglets with diarrhea

Fimbrial adhesin	Number of isolates	Hemolytic activity	
		+	–
F4ab	2	2	0
F4ac	62	62	0
F4ad	0	0	0
F5[a]	4	0	4
F6	5	0	5
F41[a]	0	0	0
F41 + F5	1	0	1
F[b]	86	53	33
Total number	160	117	43
percentage	100	73.1	26.9

[a]Individually.
[b]F4, F5, F6, and F41 negative.

All F4-positive isolates formed β-hemolysis on blood agar (*see* Table II). This corresponds to the findings of Wittig *et al.* (1994) who reported that all ETEC strains $F4^+$ or $F18^+$ adhesins were capable of producing hemolysin. They assumed the presence of pathogenicity DNA islands on plasmids or on chromosomes which can explain the correlation of the linkage gene clusters coding for hemolysin and fimbriae. Frydendahl (2002) suggested the hemolytic activity as a possible marker for pathogenic potential as defined by the presence or absence of virulence factor genes. Fifty-three of the remaining field isolates exhibited hemolytic activity but none of them was positive for the presence of any of the adherence antigens investigated in this study.

This work was supported by VEGA grant 1/8025/01 from the *Slovak Grant Agency* and an internal grant of the *University of Veterinary Medicine* in Košice (Slovakia). The authors thank Dr. J. Osek (*National Veterinary Institute*, Puławy, Poland) and Dr. P. Alexa (*Veterinary Research Institute*, Brno, Czechia) for providing reference strains.

REFERENCES

ALEXA P., ŠTOURAČOVÁ K., HAMŘÍK J., RYCHLÍK I.: Gene typing of the colonization factors K88 (F4) in enterotoxigenic *Escherichia coli* strains isolated from diarrheic piglets. *Vet.Med.Czech.* **46**, 46–49 (2001).

AUSUBEL F.M., BRENT R., KINGSTON R.E., MOORE D.D., SEIDMAN J.G., SMITH J.A., STRUHL K.: *Current Protocols in Molecular Biology*. Greene Publishing Association–Wiley-Interscience, New York 1989.

BALOWS A., HAUSLER W.J., HERRMANN K.L., ISENBERG H.D., SHADOMY H.J.: *Manual of Clinical Microbiology*, 5th ed. American Society for Microbiology, Washington (DC) 1991.

BIJLSMA I.G.W., VAN HOUTEN M., FRIK J.F., RUITENBERG E.J.: K88 variants K88ab, K88ac and K88id in oral vaccination of different porcine adhesive phenotypes. Immunological aspects. *Vet.Immunol.Immunopathol.* **16**, 235–250 (1987).

BOGYIOVÁ E., KMEŤOVÁ M., BIROŠ E., SIEGFRIED L.: Detection of *pap*-, *sfa*- and *afa*-specific DNA sequences in *Escherichia coli* strains isolated from extraintestinal material. *Folia Microbiol.* **47**, 723–726 (2002).

CRYAN B: Comparison of the assay systems for detection of enterotoxigenic *Escherichia coli* heat-stable enterotoxin. *J.Clin.Microbiol.* **28**, 792–794 (1990).

DONNENBERG M.S.: Enteropathogenic *Escherichia coli*, pp. 709–726 in M.J. Blaser, P.D. Smith, J.I. Ravdin, H.B. Greenberg, R.L. Guerrant (Eds): *Infection of the Gastrointestinal Tract*. Raven Press, New York 1995.

DRAHOVSKÁ H., KOCÍNCOVÁ D., SEMAN M., TURŇA J.: PCR-based methods for identification of *Enterococcus* species. *Folia Microbiol.* **47**, 649–653 (2002).

DYKES C.W., HALLIDAY I.J., READ M.J., HOBDEN A.N., HARFORD S.: Nucleotide sequences of four variants of the K88 gene of porcine enterotoxigenic *Escherichia coli*. *Infect.Immun*. **50**, 279–283 (1985).

EVANS D.G., EVANS D.J., PIERCE E.F.: Differences in the response of rabbit small intestine to heat-labile and heat-stable enterotoxins of *Escherichia coli*. *Infect.Immun*. **7**, 873–880 (1973).

FRYDENDAHL K.: Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhea and edema disease in pigs and a comparison of diagnosis approaches. *Vet.Microbiol*. **85**, 169–182 (2002).

GARABAL J.L., VÁZQUEZ F., BLANCO J., BLANCO M., GONZÁLEZ E.A.: Colonization antigens of enterotoxigenic *Escherichia coli* strains isolated from piglets in Spain. *Vet.Microbiol*. **54**, 321–328 (1997).

HACKER J.: Role of fimbrial adhesins in the pathogenesis of *Escherichia coli* infections. *Can.J.Microbiol*. **38**, 720–727 (1993).

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

HOLODA E., MIKULA I.: Construction of recombinant K88 DNA with P_{tac} promoter. *Folia Microbiol.* **39**, 171–175 (1994).

HOLODA E., BIANCHI A.T.J., SMITS M., DEGEUS B., BERGEN H., BRIARE J., MIKULA I.: Utilization of recombinant DNA technologies in immunoprophylaxis, p. 20 in *Proc. 21st Congr. Czechoslovak Society for Microbiology*, Hradec Králové (Czechia) 1998.

HOLODA E., PISTL J., PILPČINEC E.: *General Microbiology and Genetic of Microorganism.* (In Slovak) Vienala, Košice (Slovakia) 2001.

HOLODA E., HUNG VU KHAC, BERGEN H., HOLEČKOVÁ B., WANTRUBOVÁ A., ANDRAŠKOVÁ S., PILIPČINEC E.: Primers for the detection of *Escherichia coli* K88ab^{+}. *Folia Vet.* **46**, 119–122 (2002).

HOLODA E., VU-KHAC H., ANDRAŠKOVÁ S., CHOMOVÁ Z., PILIPČINEC E.: PCR assay for detection of F4ab1, F4ab2, F4ac and F4ad fimbrial adhesins in *E. coli* strains. *Folia Microbiol.* **49**, in press (2004).

ISAACSON R.E., MOON H.W., SCHNEIDER R.A.: Distribution and virulence of *Escherichia coli* in the small intestines of calves with and without diarrhea. *Am.J.Vet.Res.* **39**, 1750–1755 (1978).

KAPER J.B., LEVINE M.M.: Progress towards a vaccine against enterotoxigenic *E. coli*. *Vaccine* **6**, 197–199 (1988).

KWON D., KIM O., CHAE C.: Prevalence of genotypes for fimbriae and enterotoxins and of O serogroups in *Escherichia coli* isolated from diarrheic piglets in Korea. *J.Vet.Diagn.Invest*. **11**, 146–151 (1999).

MARTINS M.F., MARTINEZ-ROSSI N.M., FERREIRA A., BROCCHI M., YANO T., CASTRO A.F.P., SILVEIRA W.D.: Pathogenic characteristics of *Escherichia coli* strains isolated from newborn piglets with diarrhea in Brazil. *Vet.Microbiol*. **76**, 51–59 (2000).

MORRIS J.A., THORN C.J., SOJKA W.J.: Evidence for two adhesive antigens on the K99 reference strains *Escherichia coli* B41. *J.Gen. Microbiol*. **118**, 107–113 (1980).

MULLANEY C.D., FRANCIS D.H., WILLGOHS J.A.: Comparison of seroagglutination, ELISA, and indirect fluorescent antibody staining for the detection of K99, K88, and 987P pilus antigens of *Escherichia coli*. *J.Vet.Diagn.Invest*. **3**, 115–118 (1991).

NAGY B., FEKETE P.Z.: Enterotoxigenic *Escherichia coli* (ETEC) in farms animals. *Vet.Res*. **30**, 259–284 (1999).

OJENIYI B., ARHENS P., MEYLING A.: Detection of fimbrial and toxin genes in *Escherichia coli* and their prevalence in piglets with diarrhea. The application of colony hybridization assay, polymerase chain reaction and phenotypic assays. *J.Vet.Med.B* **41**, 49–59 (1994).

ONDRAŠOVIČ M., ONDRAŠOVIČOVÁ O., VARGOVÁ M., KOČIŠOVÁ A.: *Environmental Problems in Veterinary Practice.* University of Veterinary Medicine, Košice (Slovakia) 1997.

ØRSKOV I., ØRSKOV F., SOJKA W.J., LEACH J.M.: Simultaneous occurence of *E. coli* B and L antigens in strains from diseased swine. *Acta Pathol.Microbiol.Scand.Sect.B* **53**, 404–422 (1961).

OSEK J.: Prevalence of virulence factors of *Escherichia coli* strains isolated from diarrheic and healthy piglets after weaning. *Vet. Microbiol.* **68**, 209–217 (1999).

OSEK J., SVENNERHOM A.M.: Determination of K88 antigens and enterotoxins of *Escherichia coli* strains isolated from Polish piglets with diarrhea by the use of enzyme-linked immunosorbent assays. *Vet.Microbiol*. **29**, 299–307 (1992).

OSEK J., TRUSZCZYNSKY M.: Occurrence of fimbriae and enterotoxins in *Escherichia coli* strains isolated from piglets in Poland. *Comp. Immunol.Microbiol.Infect.Dis*. **15**, 285–292 (1992).

RADEMAKER C.M.A., MARTINEZ-MARTINEZ L., PEREA E.J., JANSZE M., FLUIT A.C., GLERUM J.H., VERHOEF J.: Detection of enterovirulent *Escherichia coli* with diarrhea in Sevilla, southern Spain, with nonradioactive DNA probes. *J.Med.Microbiol.* **38**, 87–89 (1993).

SODERLIND O., THAFVELIN B., MOLLBY R.: Virulence factors in *Escherichia coli* strains isolated from Swedish piglets with diarrhea. *J.Clin.Microbiol*. **26**, 879–884 (1988).

STACY-PHILIPS S., MECCA J.J., WEISS J.B.: Multiplex PCR assay and simple preparation methods for stool specimens detect enterotoxigenic *Escherichia coli* DNA during the course of infection. *J.Clin.Microbiol.* **33**, 1054–1059 (1995).

THORN 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**, 36–43 (1992).

THORNS C.J., SOJKA M.G., ROEDER P.L.: Detection of fimbrial adhesins of ETEC using monoclonal antibody-based latex reagents. *Vet. Res*. **125**, 91–92 (1989).

VU-KHAC H., HOLODA E., WANTRUBOVÁ A., ANDRAŠKOVÁ S., CHOMOVÁ Z., ČURLÍK J., MAJERČIAK M., PILIPČINEC E.: Using for PCR detection of F18 fimbrial antigen in *E. coli* strains isolated from piglets with diarrhea in Slovakia. *Folia Vet.* **47**, 102–106 (2003).

WESTERMEN R.B., MILLS K.M., PILLIPS R.M., FORTNER G.W., GREENWOOD J.M.: Predominance of the ac variant in K88-positive *Escherichia coli* isolated from swine. *J.Clin.Microbiol.* **26**, 149–150 (1988).

WILSON R.D., FRANCIS D.H.: Fimbriae and enterotoxin associated with *Escherichia coli* serogroups isolated from pigs with colibacillosis. *Am.J.Vet.Res.* **47**, 213–217 (1986).

WRAY C., MCLAREN I.M., CAROLL P.J.: *Escherichia coli* isolated from farm animals in England and Wales between 1986 and 1991. *Vet.Rec*. **133**, 439–442 (1993).

ZHU C., HAREL J., JAQUES M., DESAUTELS C., DONNENBERG M.S., BEADRY M., FAIRBROTHER J.M.: Virulence properties and attaching-effacing activity *Escherichia coli* O45 from swine postweaning diarrhea. *Infect.Immun*. **62**, 4153–4159 (1994).