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Development of a FedF Based Enzyme-Linked Immunosorbent Assay for the Detection of Antibodies Against F18$^+$ *Escherichia coli*

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Abstract: Post-weaning diarrhea or edema in piglets caused by F18$^+$ *Escherichia coli* (*E. coli*) is a disease which is spreading worldwide; however, due to the lack of a quick and convenient Assay Method, very little data is available about its epidemiology. In this study, a FedF-based indirect Enzyme-linked Immunosorbent Assay (ELISA) was developed which employed a fragment of the FedF protein of F18$^+$ *E. coli* expressed through genetic engineering. The optimal concentration for the purified protein was found to be 2.25 μg mL^{-1} with an optimal serum dilution of 1:40. The positive cut-off was established to be 0.381. This FedF ELISA showed high specificity toward F18$^+$ *E. coli* positive sera and no cross reactivity with positive sera against a variety of other swine pathogens, especially several other *E. coli* pathogens and other major diarrhea causing pathogens. This FedF-ELISA and an extracted F18 fimbriae antigen based Dot-blot assay were used to assay 146 sera. The two methods showed a total agreement ratio of 93.0%. Seroconversion of experimental infected pigs showed that the sensitivities of the FedF-ELISA and of the Dot-blot assay were approximately equal. The FedF-based ELISA was found to have both specificity and sensitivity for detection of porcine antibodies.

Key words: F18$^+$ *E. coli*, FedF, ELISA, pig, seroconversion

INTRODUCTION

An essential step in the pathogenesis of enteric colibacillosis is the fimbrial receptor interaction facilitating colonization of bacteria in the small intestine (Imberechts *et al*., 1997). F18 fimbrial *E. coli* strains adhere to and colonize the microvilli of small intestinal epithelial cells in piglets and are associated with porcine post-weaning diarrhea and pig edema disease (Imberechts *et al*., 1997; Frydendahl, 2002; Gaastra and Svennerholm, 1996). These are the most widespread causes of death in weaned pigs (Imberechts *et al*., 1997; Bertschinger *et al*., 2000). There are two antigenic variants of F18 fimbriae (Imberechts *et al*., 1992a; Nagy *et al*., 1997), F18ab and F18ac. The F18ab variant is often expressed by Verotoxigenic *E. coli* (VTEC) strains producing Shiga-like Toxin (SLT) and causing edema disease (Imberechts *et al*., 1992b). The F18ac variant often belongs to Enterotoxigenic *E. coli* (ETEC) strains and causes post-weaning diarrhea by expression of enterotoxins (STa or STb) either together with or without Shiga-Like Toxin (SLT) (Nagy *et al*., 1997).

The F18 fimbriae is encoded by the *fed* gene cluster whose genetic organization has been characterized (Imberechts *et al*., 1996; Smeds *et al*., 2001) and is found to be composed of five genes, namely, *FedA FedB*, *FedC*, *FedE* and *FedF* (Smeds *et al*., 2001). The gene *FedA* encodes the major protein FedA which constitutes the backbone of the F18 fimbriae. The gens *FedE* and *FedF* encode the minor proteins FedE and FedF (Imberechts *et al*., 1996, 1992a, b). The *FedB* and *FedC* genes were believed to encode, respectively a putative usher protein (FedB) and a Chaperone (FedC) for the assembly and folding of F18 fimbriae (Smeds *et al*., 2001). The function of the minor protein FedE is still unknown. The FedF protein was recognized as an adhesion molecule responsible for the binding of F18 fimbriae to enterocytes which could be inhibited by antibodies against FedF (Smeds *et al*., 2001). Compared with FedA, FedF protein

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is much more conserved among F18$^+$ *E. coli* strains isolated from different countries and no specific mutations could be found in the *FedF* gene between the antigenic variants F18ab and F18ac (Smeds *et al*., 2001). These facts indicate that FedF would be a good antigen candidate for diagnosis of infections induced by F18$^+$ *E. coli* strains.

Although, postweaning diarrhea or edema in piglets caused by F18$^+$ *E. coli* is experienced worldwide, there is still very little data available about the epidemiology and ecology of F18$^+$ *E. coli*. Several methods have been reported for prevention of these related diseases (Moon and Bunn, 1993; Tsiloyiannis *et al*., 2001) but effective commercial vaccines and drug therapies are still lacking. This makes it difficult to effectively prevent carriers of such diseases from introducing F18$^+$ *E. coli* into a naive herd. Thus, it is very important to be able to effectively monitor the prevalence of infection. Whereas F18$^+$ *E. coli* are presumably present in the gastrointestinal tract of sucking and mature animals, it is uncommon to detect the presence of the bacteria directly however, the antibodies elicited by F18$^+$ *E. coli* infection can be detected in porcine serum and serve as indirect evidence of infection status. Serological testing has a number of advantages including that it is convenient and that it can be handled in a standard and high throughput fashion. It could therefore serve as an effective means to provide herd prevalence data.

In this study, researchers describe the production of a recombinant GST tagged FedF protein in genetically engineered strains of *E. coli* and the establishment of an FedF-based Enzyme-Linked Immunosorbent Assay (ELISA) for detection of FedF specific antibodies in porcine sera.

MATERIALS AND METHODS

Bacterial strains: F18$^+$ *E. coli* Ee strain was isolated by the laboratory (Liu *et al*., 2005). Briefly, it was isolated from pigs in a breeding farm located in Hubei Province which showed the typical clinical signs of edema. The strain was identified by Microstation (Biolog) to belong to O139 serotype and was additionally proved by conventional methods to harbor F18 fimbriae and Shiga-like toxin variant II. F18$^+$ *E coli* Ee strain was cultured on MacConkey agar (Boao Industry, Shanghai, China) for 18 h at 37°C. An individual colony was transferred to tryptone soya broth (Sigma) and incubated at 37°C for an additional 18 h while gently shaking at 100 g.

Preparation of FedF positive sera: Animal experiments were conducted following International Guiding Principles for Biomedical Research Involving Animals. An outbred pig (Landrace±Large White cross) was subcutaneously injected with 80×10^8 cfu of inactivate Ee isolate at intervals of 2 weeks. The first two immunizations were given with Freund's complete adjuvant. The second immunization was given with Freund's incomplete adjuvant. The 3 weeks later, the pig was challenged with a virulent Ee strain at a dose of 100×10^8 cfu in PBS via the ear vein. The challenge was repeated once 3 weeks later. Blood was taken for antiserum preparation on day 21 after the second challenge. Serum prepared in this was used as the positive control serum for developing the FedF-ELISA.

Molecular cloning: Primers for *fedF* gene amplification P1 (5'TTTGAGCTCACTCTACAAGTAGAC-3', SacI site underlined) and P2 (5'-TTAAGCTTTGGTCTACTTAT TACGCGATG-3', HindIII site underlined) were based on the published sequence of *E. coli* 107/86 strain in Genbank (Accession number: Z26520) and had restriction sites added. Oligonucleotides were synthesized by TaKaRa Biotechnology (Dalian). The targeted FedF fragment (897 bp from 64-960 bp, encoding 269 amino acids) was amplified using standard PCR. PCR started at 94°C, 4 min followed by 30 cycles of 94°C, 30 sec; 55°C, 30 sec; 72°C, 1 min. The amplified DNA fragments were purified, digested by SacI and HindIII and then ligated into the SacI and HindIII sites of a prokaryotic expression plasmid pGEX-KG (TaKaRa). The resulting recombinant expression plasmid was named pKF (Fig. 1) and had the FedF fragment (897 bp) inserted downstream of the GST tag protein to thereby express a GST-FedF fusion protein.

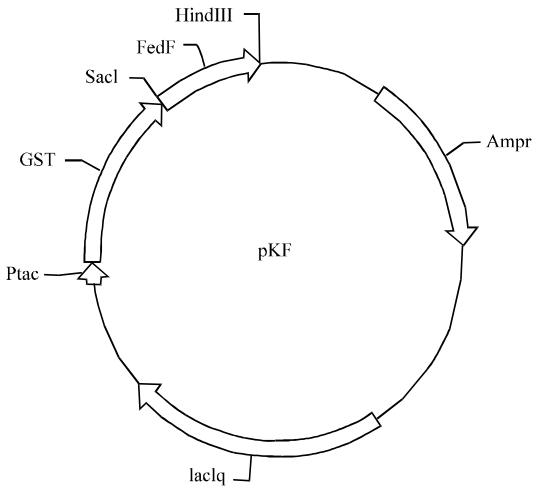

Fig. 1: The expression vector pKF containing the *FedF* gene. The PCR product of *FedF* gene was inserted into the SacI and HindIII sites of plasmid vector pGEX-KG at the C-terminal of the GST tag

Protein expression and purification: The recombinant plasmid pKF and pGEX-KG was heat transformed into BL21 (DE3) *E. coli* recipient cells. Target protein expression was induced by Isopropyl β-D-1-thiogalactopyranoside (IPTG,1mM) for 3 h at 37°C. The bacterial cells were then collected by centrifugation at 8000 g for 5 min. Cells were lysed with lysozyme (50 μg mL^{-1}) and sonicated three times at 15 kHz for 10 sec in ice with a sonicator (VIRTIS, USA). The pellets were collected after spinning at 12000 g for 10 min. The pellets were treated with polyethylene glycol 4000 (0.2%), GSH (1 mmol L^{-1}) and GSSH (1 mmol L^{-1}). The dissolved supernatant was mixed with saturated $(NH_4)_2SO_4$ placed overnight at 4°C and then spun at 10,000 g for 10 min. The pellet was dissolved with PBS and contained the purified protein. The FedF-GST fusion protein and GST protein concentrations were, respectively, 0.72 and 1.3 mg mL^{-1} as determined by a UV spectrophotometer and calculated according to the equation: C (mg mL^{-1} protein) = $1.45.OD_{280}$-$0.74.OD_{260}$.

The purified protein was examined by SDS-PAGE and Western blot analysis following standard protocols. The SDS-PAGE gel was 12% and visualized with Coomassie brilliant blue R250 staining. For the Western blot analysis, the primary antibody used was the above mentioned positive pig serum diluted at 1:50 with PBS containing 0.1% Tween-20 (PBST). The incubation was 1 h at room temperature which was followed by three washes with PBST for 10 min each time. The membrane was then incubated with goat anti-swine immunoglobulin (Ig) G antibodies conjugated with Horseradish Peroxidase (HRP-IgG) (SouthernBiotech, USA) diluted at 1:2000 with PBST for 1 h at room temperature. After three washes with PBST and twice with PBS, the membrane was developed with the substrate 0.6% 3,3'-diaminobenzidine carbonyl chloride ($CoCl_2$) in Tris.Cl (pH 7.6) containing 0.03% hydrogen peroxide (H_2O_2).

Recombinant FedF protein as antigen in ELISA: FedF protein was adjusted to an optimal concentration as determined by checkerboard titration in carbonate buffer. Briefly, 100 μL of protein solution was added to each well of 96 well immunoassay plates (Jincanhua Industry, Shenzhen, China). The antigen was incubated at 37°C for 1 h and then overnight at 4°C. The fixed plates were washed three times with PBST and subsequently blocked with 150 μL of block buffer for 1 h at 37°C. After one wash with PBST, the plates were incubated with 100 μL serum dilutions (the optimal dilution proportion was determined by checkerboard titration) for 0.5 h at 37°C. The plates were washed four times with PBST and 100 μL of HRP-IgG dilution was added to each well followed by incubation at 37°C for 0.5 h and a subsequent wash with PBST five times. The 100 μL of freshly prepared substrate was added to each well and the plates were allowed to stand for 10 min at 37°C. The reaction was stopped by the addition of 50 μL of 1 N sulfuric acid to each well. The OD630 was measured with an automated plate spectrophotometer. The IgG cut-off value was calculated as the mean OD630-value of the 1/40 dilution of samples from 94 sows plus three standard deviations. The sera of these 94 sows were considered as F18-negative because their OD630 did not decrease upon further dilutions of the serum samples. In addition, all of these sera were negative for $F18^+$ *E. coli* excretion when screened by the Dot-Blot Method described by Verdonck *et al.* (2002).

The ELISA titer for test sera was calculated as the reciprocal of the highest dilution of test sera that gave an OD630 value greater than the cut off value.

At the beginning of the FedF ELISA study, all known positive sera were identified as positive but ~12% of known negative sera also tested positive. This problem was resolved with the addition of an BL21(DE3) lysate to the serum sample dilution buffer to absorb antibodies against *E. coli*. BL21 (DE3) lysate was obtained according to the method of Elgh *et al.* (1998). A concentration of BL21(DE3) lysate of 310 μg mL^{-1} was found to completely eliminate the false-positive reaction without affecting genuine positive sera.

Specificity tests: Positive sera for Pseudo Rabies Virus (PRV), Porcine Parvovirus (PPV), Classical Swine Fever Virus (HCV), Porcine Circovirus (PCV), Porcine Reproductive and Respiratory Syndrome Virus (PPRS), Transmissible Gastroenteritis Coronavirus (TGEV), *Actinobacillus pleuropneumoniae* (APP), *Streptococcus suis* type 2 (SS2), Swine Cholera (SC), $K88^+$ *E. coli*, $K99^+$ *E. coli*, $F41^+$ *E. coli* and $987P^+$ *E. coli* were assayed in the FedF-ELISA. All the above control sera were obtained from Keqian Animal Biotech Product Co., Ltd. Wuhan, China. In addition, the recombinant GST-FedF protein and the GST protein in 0.5% BSA were respectively, mixed with positive and negative sera of $F18^+$*E. coli* and incubated at 37°C for 1 h. The solutions were diluted and assayed in the FedF-ELISA.

Seroconversion of the experimentally infected pigs: Twenty, 5 weeks old piglets were inoculated via the ear vein with 4.0×10^9 cfu $F18^+$ *E. coli* (in 4 mL PBS). Pigs were examined for any typical symptoms in the next week. Sera were collected from 4 days post infection (dpi) to 10 weeks post infection (wpi). The

sera from the 4 dpi to 5 wpi were used to evaluate the sensitivities of two different assays (FedF-ELISA and purified F18 Antigen Dot-blot). Sera samples from 4 dpi to 10 wpi were pooled for each time point and were for the FedF-ELISA. All serum samples were stored at -80°C and tested under code.

RESULTS AND DISCUSSION

GST-FedF fusion protein expression and purification: The GST-FedF protein was expressed in *E. coli* as an expression product of an inclusion body. The expression product was about 56 kDa by SDS-PAGE analysis (Fig. 2) which was confirmed by Western blot analysis with positive pig sera obtained by experimentally immunizing a pig with the F18$^+$ *E. coli* Ee strain.

Development of GST-FedF based ELISA

Condition optimization: Checkerboard titration indicated the optimal concentration for the purified protein was 2.25 μg mL^{-1} (1:320) and the optimal serum dilution was 1:40 (Table 1). Using this as the working concentration, the endpoint cutoff value was determined by measuring 94 F18 dot-blot-negative serum samples and calculated to be their mean OD 630 nm value, plus three times Standard Deviations (mean+3SD) which was 0.195 and 0.062, respectively. Thus, the positive cut-off was established to be 0.381 for a serum sample assaying at a 1:40 or higher dilution.

Comparison of FedF-ELISA and F18 Dot-blot: The FedF-ELISA and extracted F18 fimbriae antigen based Dot-blot were used to assay 146 clinical samples. The two methods shared high agreement ratio which was 91.4% for positive samples and 94.4% for negative samples (Table 2).

Specificity for F18$^+$ *E. coli* FedF protein: The FedF-ELISA was used to detect swine sera positive for various diseases. The FedF-ELISA did not cross react with positive sera for PRV, PPV, HCV, PCV, PRRSV, Hps, SS2, App, TGEV, *Salmonella choleraeuis*, *E. coli* K88, K99, F41 and 987P (all OD<0.340 at 1:40 dilution) however, the F18$^+$SLTEC control positive serum was positive (OD>0.381) at as high as a 1:1280 dilution. Adding GST control antigen to the positive serum did not affect the assay result of the FedF-ELISA while adding GST-FedF to the positive serum caused an antigen dose dependent decrease of the OD reading in the assay indicating that

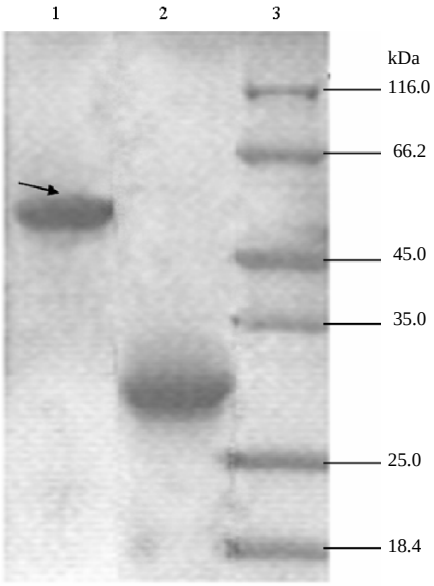

Fig. 2: SDS-PAGE analysis of recombinant GST-FedF protein expressed in *E. coli*, stained with Coomassie brilliant blue R250. The arrows indicated the band of desired GST-FedF (about 56 kD). Lane 1: Inclusion body extracted from *E. coli* cells harboring pKF; Lane 2: Inclusion body extraction from *E. coli* cells harboring pGEX-KG; Lane 3: The molecular weight markers (kDa)

Table 1: OD630 values for the ELISA reactions of serial dilutions of both the coating antigen and the positive or negative sera

	Ag dilution							
Serum dilution	1:20	1:40	1:80	1:160	1:320	1:640	1:1280	1:2560
Positive								
1:20	1.335	1.283	1.254	1.221	1.115	1.115	1.088	0.866
1:40	1.268	1.213	1.201	1.197	1.139	0.838	0.785	0.572
1:80	1.114	0.985	0.955	0.866	0.838	0.766	0.620	0.428
1:160	1.095	0.869	0.869	0.779	0.751	0.601	0.488	0.290
1:320	0.810	0.744	0.700	0.657	0.620	0.413	0.314	0.179
Negative								
1:20	0.310	0.285	0.253	0.224	0.200	0.169	0.139	0.098
1:40	0.267	0.247	0.208	0.198	0.179	0.137	0.115	0.095
1:80	0.236	0.208	0.179	0.155	0.140	0.125	0.093	0.073
1:160	0.204	0.159	0.160	0.154	0.142	0.109	0.092	0.082
1:320	0.186	0.160	0.171	0.140	0.120	0.114	0.102	0.081

Table 2: The comparison between Dot-blot and FedF based ELISA in clinical detection

	FedF-ELISA		
Method	Positive	Negative	Total
Dot-Blot			
Positive	64	4	68
Negative	6	68	74
Total	70	72	142
Coincident ratio	91.40%	94.40%	93.00%

the antibody detected by the FedF-ELISA was specific against FedF but not GST. As expected, the negative serum was not affected by either GST or FedF antigen.

Seroconversion of experimental infected pigs: Four of the 20 pigs died 2 days after the experimental infection their sera were negative in the FedF-ELISA and the F18 Dot-blot assays. Furthermore, the OD values of these sera did not decline when they were been diluted. The sera of the remaining 16 pigs collected at a series of time points after infection (4, 7, 10, 14, 21, 28 and 35 dpi) were tested by the FedF-ELISA and F18 Dot-blot to compare the sensitivity of the two different assays (Fig. 3A). The two methods scored identical positive numbers at all time points except for day 10 dpi where FedF-ELISA detected 13 seroconversions and Dot-blot detected 12 seroconversions. These results indicated that the sensitivity of the FedF-ELISA and Dot-blot assay was approximately equal.

In addition, assaying sera collected from 1-10 weeks post infection using the FedF-ELISA, revealed that the sera FedF specific IgG titers in the infected pigs peaked at 4 weeks post infection and maintained a relatively high level till at least 10 weeks after infection (Fig. 3B).

The FedF (or Adhesin) protein was selected as a diagnostic antigen due to its highly conserved sequence. It has been found to be conserved among strains isolated from different countries (Smeds *et al*., 2001, 2003; Verdonck *et al*., 2004) and between the two antigenic variants F18ab and F18ac (Nagy *et al*., 1997; Rippinger *et al*., 1995). Partial sequencing of the *FedF* gene of Ee strain isolated by the laboratory showed 100% identity with the *FedF* gene of 107/86 strain.

In this research, an ELISA was developed based on a recombinant GST-FedF fusion protein containing the full FedF protein sequence except for the signal sequence and transmembrane region. This recombinant GST-FedF antigen showed high immunoreactive specificity toward F18$^+$ *E. coli* positive sera and did not react with positive sera against a variety of other swine pathogens,

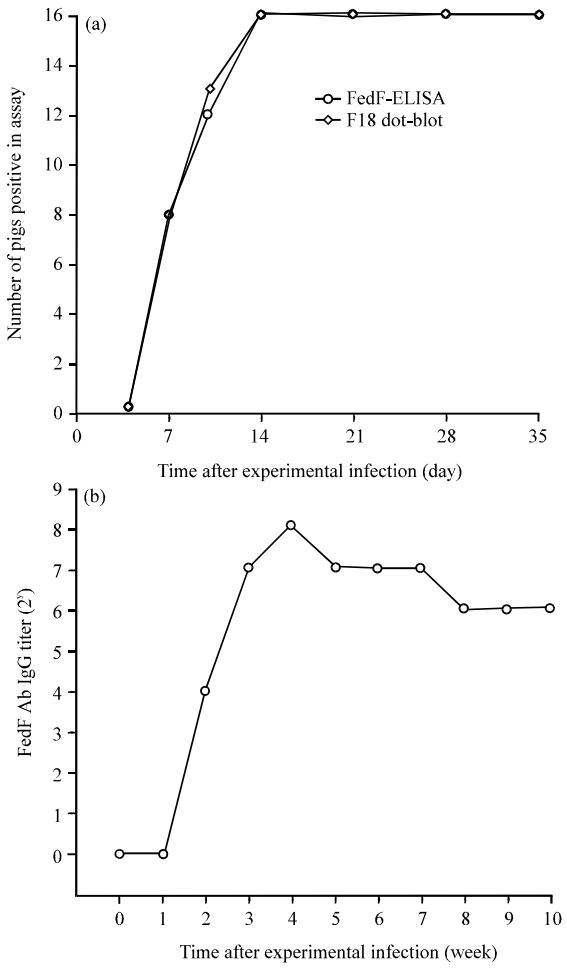

Fig. 3: a) Number of FedF antibody positive pigs and b) FedF titers in pigs at various time points after experimental infection with F18$^+$ *E. coli*

especially several other *E. coli* pathogens (such as K88, K99, F41, 987P) and other major diarrhea causing pathogens (SC, TGEV).

An ELISA based on natural F18 fimbriae was reportedly used to assess the association between the open status of swine farms and the seroprevalence of F18$^+$ *E. coli* in Northern Belgium (Verdonck *et al*., 2003). A difference has to be pointed out between this study and Verdonck's. In this study for experimental infection, the pigs were infected via the ear vein while the study by Verdonck used intragastric administration which is the natural route of infection. Ear vein infection was chosen for the reason that F18 specific serum antibodies would not be elicited in F18R-pigs by intragastric administration (Bertschinger *et al*., 1993). Thus, the seroconversion data in the study needs to be viewed in this light with this fact in mind.

CONCLUSION

All the 16 F18$^+$ *E. coli* experimental infected pigs developed FedF specific antibody as detected by FedF-ELISA. The two methods scored identical positive numbers at all but day 10 dpi where FedF-ELISA detected 13 seroconversions and dot-blot detected 12 seroconversions. This result indicated that the sensitivity of FedF-ELISA displayed a slightly higher sensitivity. The antibody level peaked around 4 weeks post-infection and remained at a relatively high level for the next 10 weeks. This result indicates that FedF-specific IgG antibodies can serve as an infection marker for F18$^+$ *E. coli* in pigs (for differential diagnosis) as no commercial vaccines contain F18 fimbriae. Thus, methods and results reported here may find application in F18$^+$ *E. coli* infection epidemiology and ecology surveys.

Collectively, FedF-specific IgG antibody could serve as a marker for F18$^+$ *E. coli*. Infection of pigs (except piglets which have passive immunity). An ELISA based recombinant FedF antigen showed both high sensitivity and specificity.

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REFERENCES

Bertschinger, H.U., M. Stamm and P. Vogeli, 1993. Inheritance of resistance to oedema disease in the pig: Experiments with an *Escherichia coli* strain expressing fimbriae 107. Vet. Microbiol., 35: 79-89.

Bertschinger, H.U., V. Nief and H. Tschape, 2000. Active oral immunization of suckling piglets to prevent colonization after weaning by enterotoxigenic *Escherichia coli* with fimbriae F18. Vet. Microbiol., 71: 255-267.

Elgh, F., M. Linderholm, G. Wadell, A. Tarnvik and P. Juto, 1998. Development of humoral cross-reactivity to the nucleocapsid protein of heterologous hantaviruses in nephropathia epidemica. FEMS Immunol. Med. Microbiol., 22: 309-315.

Frydendahl, K., 2002. Prevalence of serogroups and virulence genes in *Escherichia coli* associated with postweaning diarrhoea and edema disease in pigs and a comparison of diagnostic approaches. Vet. Microbiol., 85: 169-182.

Gaastra, W. and A.M. Svennerholm, 1996. Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). Trends Microbiol., 4: 444-452.

Imberechts, H., H. De Greve and P. Lintermans, 1992a. The pathogenesis of edema disease in pigs: A review. Vet. Microbiol., 31: 221-233.

Imberechts, H., H. De Greve, C. Schlicker, H. Bouchet and P. Pohl *et al*., 1992b. 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. Immunity, 60: 1963-1971.

Imberechts, H., H.U. Bertschinger, B. Nagy, P. Deprez and P. Pohl, 1997. Fimbrial colonisation factors F18ab and F18ac of *Escherichia coli* isolated from pigs with postweaning diarrhea and edema disease. Adv. Exp. Med. Biol., 412: 175-183.

Imberechts, H., P. Wild, G. Charlier, H. De Greve, P. Lintermans and P. Pohl, 1996. Characterization of F18 fimbrial genes *FedE* and fedF involved in adhesion and length of enterotoxemic *Escherichia coli* strain 107/86. Microb. Pathog., 21: 183-192.

Liu, G., B. Wang, M. Liu, Q. He, X. Tang and H. Chen, 2005. Detection, identification and biological characterization of *Escherichia coli* eiciting edema of piglet. Chin. J. Vet. Sci., 25: 31-33.

Moon, H.W. and T.O. Bunn, 1993. Vaccines for preventing enterotoxigenic *Escherichia coli* infections in farm animals. Vaccine, 11: 213-220.

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

Rippinger, P., H.U. Bertschinger, H. Imberechts, B. Nagy and I. Sorg *et al*., 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.

Smeds, A., K. Hemmann, M. Jakava-Viljanen, S. Pelkonen, H. Imberechts and A. Palva, 2001. Characterization of the adhesin of *Escherichia coli* F18 fimbriae. Infect. Immunity, 69: 7941-7945.

Smeds, A., M. Pertovaara, T. Timonen, T. Pohjanvirta, S. Pelkonen and A. Palva, 2003. Mapping the binding domain of the F18 fimbrial adhesin. Infect. Immunity, 71: 2163-2172.

Tsiloyiannis, V.K., S.C. Kyriakis, J. Vlemmas and K. Sarris, 2001. The effect of organic acids on the control of post-weaning oedema disease of piglets. Res. Vet. Sci., 70: 281-285.

Verdonck, F., E. Cox, B. Ampe and B.M. Goddeeris, 2003. Open status of pig-breeding farms is associated with slightly higher seroprevalence of F18$^+$ *Escherichia coli* in Northern Belgium. Prev. Vet. Med., 60: 133-141.

Verdonck, F., E. Cox, E. Schepers, H. Imberechts, J. Joensuu and B.M. Goddeeris, 2004. Conserved regions in the sequence of the F4 (K88) fimbrial adhesin FaeG suggest a donor strand mechanism in F4 assembly. Vet. Microbiol., 102: 215-225.

Verdonck, F., E. Cox, K. van Gog, Y. van der Stede, L. Duchateau, P. Deprez and B.M. Goddeeris, 2002. Different kinetic of antibody responses following infection of newly weaned pigs with an F4 enterotoxigenic *Escherichia coli* strain or an F18 verotoxigenic *Escherichia coli* strain. Vaccine, 20: 2995-3004.