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Research Article

Adhesive Patterns of *Escherichia coli* F4 in Piglets of Three Breeds

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Abstract: *Escherichia coli* expressing F4 fimbriae is the major pathogenic bacteria that causes diarrhea in piglets before weaning. The adhesion of *E. coli* to the brush borders of the epithelial cells of piglets is the precondition leading to diarrhea, which in turn is due to the presence of the F4 receptors determined by an autosomal recessive gene on the brush borders of the epithelial cells. In order to clarify the genetic mechanism of the adhesion, an *in vitro* adhesion experiment was carried out for three variants of *E. coli* F4 (ab, ac, and ad) in 366 piglets of three pig breeds [Landrace (LR), Large White (LW), and Songliao Black (SB)]. The results showed that there existed significant differences ($P<0.001$) in the adhesion percentage among the three breeds. Most SB piglets were nonadhesive for all the three variants, whereas most LR piglets were adhesive. Within each breed except for LR, the proportions of the three F4 variants adhering to the brush borders differed significantly. According to the patterns of the adhesion of the three F4 variants in the three breeds, it is very likely that the three F4 variants F4ab, F4ac, and F4ad have different receptors that are controlled by three different loci.

Keywords: *E. coli*; F4; piglets; adhesion patterns; receptors

Diarrhea has consistently been identified as a primary infectious disease resulting in poor health and death losses among pigs. This disease accounts for 11.5%–29.5% of death in piglets. *Escherichia coli* is the major pathogenic bacteria causing diarrhea in swine, which is responsible for 56.2% of the incidence of piglet diarrhea and 24.7% of the mortality from diarrhea [1]. Based on the type of its fimbriae, *E. coli* strains are classified as F4 (K88), F5 (K99), F6 (987P), F18, F17, F41, and F42, among which F4 is the most prevalent one that causes piglet diarrhea during the pre-weaning period. Furthermore, the three variants of the F4 strain, F4ab, F4ac, and F4ad, can be distinguished serologically according to its antigen type.

Through its fimbriae, *E. coli* can adhere and colonize to the brush border membranes of the epithelial cells of a piglet's small intestine [2]. The organism secretes one or two enterotoxins. An enterotoxin is a heat-stable toxin (ST) and/or a heat-labile toxin (LT), which stimulates the small intestine secreting massive fluid and electrolyte into the gut lumen and results in diarrhea. Therefore, adhesion to the epithelial cells of the small intestine is the essential precondition for the bacteria to lead to diarrhea among piglets. However, not all piglets are susceptible to *E. coli* F4. Certain piglets are innately resistant to *E. coli* F4, which can prevent the adhesion of *E. coli* F4 to their small intestine's epithelial cells. The adhesion could be due to

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the presence or absence of specific bacterial adhesin receptors in the small intestine's epithelial cells. Genetically, the adhesive phenotype behaves in a simple dominance Mendelian fashion [3]. When viewing the adhesive phenotypes of the three *E. coli* F4 variants jointly, Bijlsma *et al*.[4] identified five adhesive patterns, designated A through E, that is, A: F4ab+/F4ac+/F4ad+, B: F4ab+/F4ac+/F4ad–, C: F4ab+/F4ac–/F4ad+, D: F4ab–/F4ac–/F4ad+, and E: F4ab–/F4ac–/F4ad–, where + means adhesive and – means nonadhesive. Later, Bonneau *et al*. [5] and Baker *et al*. [6] found two additional patterns, that is, G: F4ab–/F4ac+/F4ad– and F: F4ab+/F4ac–/F4ad–, respectively.

In this study, the adhesive patterns of the three *E. coli* F4 variants in pre-weaning piglets of three breeds, Large White (LW), Landrace (LR), and Songliao Black (SB) are investigated. The results help in understanding the molecular mechanisms of the presence of the *E. coli* F4 receptors, which is fundamental for developing a strategy of breeding for resistance to piglet diarrhea.

1 Materials and Methods

1.1 Variants of *E. coli* F4

The three variants of the *E. coli* F4 strain, 195 (F4ab, C83901, O8:K87), 200 (F4ac, C83907, O149:K91), and 216 (F4ad, C83923, O8:K87) were provided by the China Institute of Veterinary Drug Control, Beijing, China. A bovine-origin *E. coli* strain 238 (C83286, O38:K99) was used as the negative control.

1.2 Animals

A total of 366 pure bred piglets of different breeds were used in the study, among which 189 were LW, which were offspring of 7 boars and 31 sows, 87 were LR and offspring of 5 boars and 15 sows, and 90 were SB and offspring of 4 boars and 15 sows. All piglets were raised at the Pig Breeding Farm of the Institute of Animal, Chinese Academy of Agricultural Sciences, Beijing, China. They were all slaughtered at 35-day-old and their jejunum samples were collected.

1.3 Preparation of the epithelial cells

A 10 cm segment was taken from the jejunum sample that was collected within two hours after slaughter, cut open along its longitudinal axis, and cleaned with cold hypotonic EDTA solution (5 mmol/L EDTA, pH 7.4). The epithelial cells were obtained by scraping the mucosal surface of the tissue with a glass microscope slide, and then placed in 50 mL cold hypotonic EDTA solution. After incubation for 30 min at 4℃, the solution was centrifuged at 1200 g for 10 min to pellet the epithelial cells. The pelleted epithelial cells were dissolved in 50 mL cold phosphate-buffered saline (PBS, pH 7.4) and centrifuged again under the same conditions. The settled epithelial cells were dissolved in 5 mL of cold PBS with addition of 100 μL gentamicin sulfate (1 mg/mL) and 100 μL sodium azide (3 mmol/L) and stored at 4℃.

1.4 Preparation of bacterial suspension

The *E. coli* strains were removed from the cryostorage and cultured in Ordinary Broth Agar for 24 h at 37℃ for three generations, and then cultured in LB medium (Trypton, Yeast extration, NaCl, pH 7.0–7.2) at 37℃ for 10–16 h. The solution was centrifuged at 4,000 r/min for 15 min to pellet the bacterial cells. The pelleted cells were dissolved in PBS (pH 7.4) with an optical density of approximately 1.0 at 520 nm.

1.5 Verification of the F4 variants

The three variants of the F4 strain were verified by PCR using variant-specific primers [7] (Table 1). These primers were applied together to each of the F4 variants. For every correct and unpolluted variant, the PCR product corresponding to it is obtained.

The bacterial DNA amplification was performed