INFECTION AND IMMUNITY, Sept. 1982, p. 891-894
0019-9567/82/090891-04$02.00/0

Vol. 37, No. 3

Different Pig Phenotypes Affect Adherence of *Escherichia coli* to Jejunal Brush Borders by K88ab, K88ac, or K88ad Antigen

I. G. W. BIJLSMA,* A. DE NIJS, C. VAN DER MEER, AND J. F. FRIK

Department of Bacteriology, Faculty of Veterinary Medicine, State University, Utrecht, The Netherlands

Received 4 February 1982/Accepted 2 June 1982

At least five different porcine phenotypes were distinguished with the three serological variants of the K88 antigen in the brush border adhesion test. Pigs of one phenotype (A) are susceptible to adherence of all three variants, pigs of three phenotypes are susceptible to only two (B and C) or one (D) of the K88 variants, and pigs of one phenotype (E) are entirely resistant to adhesion of K88 antigen. Preincubation of type A brush borders with an excess of purified K88ad antigen did not interfere with the adhesion of K88ab- or K88ac-positive *Escherichia coli*, whereas in most cases K88ab and K88ac antigen completely blocked the adhesion of K88ad-positive *E. coli*. Likewise, K88ab antigen blocked the adhesion of K88ac-producing *E. coli* to both type A and type B brush borders, and vice versa.

The fimbrial K88 antigen enables pig enteropathogenic *Escherichia coli* strains to adhere to the epithelial cells of the jejunum and thus to colonize the small intestine. The adhesion may be visualized by scanning electron microscopy or in the brush border adhesion test by phase-contrast microscopy. By using the brush border test, Sellwood et al. (8) discovered the existence of at least two porcine phenotypes: "adhesion-positive" and "adhesion-negative" piglets. The adhesion-positive piglets are susceptible to an infection with K88-positive *E. coli* strains, and the adhesion-negative piglets are resistant. A genetic basis appears to exist for both these phenotypes (3, 7, 8).

So far, three serological variants of the K88 antigen have been found: K88ab, K88ac, and K88ad (4, 6). In a preliminary study, a number of pigs were screened for their phenotype with these K88 variants in the brush border adhesion test. In a few cases, some of the K88-positive strains were seen to adhere well, whereas strains possessing other serological K88 variants did not adhere to brush borders of the same preparation. This paper reports the results of further investigations. Attention has also been paid to the nature of the adhesion of K88-positive strains to the receptor sites by inhibiting the adhesion with homologous and heterologous K88 antigen.

MATERIALS AND METHODS

Bacterial strains and media. The *E. coli* strains used in the brush border tests and for the preparation of K88 antigen are listed in Table 1. The standard strains and one field isolate were obtained from P. A. M. Guinée and W. H. Jansen, National Health Institute, Bilthoven, The Netherlands. The other field isolates were collected over a number of years at the Department of Veterinary Bacteriology. Strains H519 and H520 differ from each other in that the K88ac antigen from H519 moves to the anode in immunoelectrophoresis in Noble agar, whereas the K88ac antigen from H520 is cathodic (4). The strains were kept on 5% horse blood agar and inoculated each week onto fresh plates. Brain heart infusion broth (Difco Laboratories) was used for overnight cultures. Diagnostic Sensitivity Test (DST) agar (Oxoid Ltd.) was used as solid medium in 12-cm petri dishes.

Brush border adhesion test. Brush borders were generally prepared from mucosal tissue taken from the middle of the jejunum by the method of Sellwood et al. (8). Segments of the small intestine were taken from 8-month-old pigs immediately after slaughter. A few piglets about 5 weeks old were also available. Three jejunal segments were taken from each of the latter piglets: one from the middle of the jejunum, one from the middle of the anterior third, and one from the middle of the posterior third.

The pigs and piglets were randomly selected at a slaughterhouse from several otherwise unknown herds and litters. In addition, the intestinal segments were gathered over a period of several months.

For the adhesion test, equal volumes (0.1 ml each) of brush border suspension in phosphate-buffered saline and phosphate-buffered saline-washed bacteria of an overnight broth culture ($\pm 10^9$ colony-forming units per ml) were mixed in Eppendorff tubes (1.5 ml). The mixtures were incubated for 15 min at room temperature while the tubes rotated in an end-over-end mixer (20 rpm). A drop of each of the mixtures was examined by phase-contrast microscopy for adhesion of the *E. coli* bacteria to the brush borders. At least 20 brush borders were viewed in each preparation.

Preparation of K88 antigen. The technique of Mooi and De Graaf (5) with some modifications was used for

TABLE 1. *E. coli* strains used in the brush border adhesion test

Serotype	Designation of standard strains (reference)	No. of field isolates (reference)
O8:K87:K88ab	G7[a] (7)	1 (4)
O8:K87:K88ac	G205[a] (7)	
O8:K87:K88ad	H56[a] (4)	
O8:K?:K88ad	H70[a] (4)	
O9:K(A)?:K88ad	H110[a] (4)	
O45:K88ac		1
O138:K81:K88ac		1
O141:K85ab:K88ab	E68 (7)	
O149:K91		1
O149:K91:K88ac	H519,[a] H520[a] (4)	1

[a] Also used for preparing the K88 antigens.

isolation of the K88 antigen. The growth of 100 DST agar plates, seeded with an *E. coli* strain (see Table 1), was harvested with 50 mM Tris-hydrochloride buffer (pH 7.4), washed once with the same buffer, and resuspended to a concentration of about 10^{11} cells per ml in the same buffer supplemented with 1.0 M NaCl. Then the bacteria were sheared with an Ystral homogenizer (type X 10/20) at 20,000 rpm for 30 min in ice. Cells were spun down for 60 min at 50,000 × *g*, and the K88 pili were precipitated from the supernatant with 60% ammonium sulfate (16 h at 4°C). The precipitate was spun down at 15,300 × *g*, resuspended in 20 ml of phosphate-buffered saline, and dialyzed against the same buffer. This crude preparation of the K88 antigen was used without further purification.

Blocking of the K88 receptor. Equal volumes (0.1 ml each) of a brush border suspension and of a crude preparation of one of the serological variants of the K88 antigen were incubated for 15 min as described for the brush border adhesion test. Subsequently, 0.1 ml of bacterial suspension was added, and this mixture was treated further as described for the brush border adhesion test.

RESULTS

Brush border adhesion tests. Brush border samples from 65 pigs were tested for adhesion by *E. coli* strains possessing one of the serological variants of the K88 antigen, K88ab, K88ac, or K88ad. All standard strains and the K88-negative control strain (O149:K91) were tested against the brush borders of each pig. The other strains (Table 1) were occasionally used in brush border adhesion tests. The results with the field isolates always corresponded to the results we obtained with the standard strains possessing the same K88 variant. A test was designated as positive if two or more bacteria adhered to each brush border. However, in our experiments, the number of bacteria adhering to a brush border was never less than 10. Absolutely no adhesion was observed in a negative test. The results are presented in Table 2. So far, five phenotypes have been distinguished, provisionally designated A through E. Phenotypes A, B, C, and E were found in the hogs as well as in the 5-week-old piglets, and no significant differences were observed in the brush border adhesion test between the piglets and the older pigs. The less frequently occurring phenotype D was found in the older pigs only. When three different parts of the jejunum of a piglet were examined in the brush border adhesion test, no differences were observed.

Storing the brush borders at −20°C after the addition of an equal volume of glycerol did not affect the adhesion, and the same phenotype was still reproduced, even after storage for 6 months.

Receptor blocking tests. Some of the brush border samples that had been used for the determination of the phenotypes given in Table 2 were also used for receptor blocking tests. To get more information about the specificity of the receptors for the K88 antigen, brush borders were preincubated with an excess of K88 antigen. In separate tests, each of the serological variants of this antigen was used for blocking. Moreover, several preparations of the same K88 variant originating from different strains were used. K88 antigen was prepared from the strains listed in Table 1. With this procedure, each variant of the K88 antigen should block the homologous receptor sites. Adhesion to these blocked brush borders was tested with *E. coli* strains possessing the heterologous variants of the K88 antigen as well as the homologous K88

TABLE 2. Pig phenotypes relating to adhesion of K88ab-, K88ac-, and K88ad-positive *E. coli* strains to brush borders of jejunal epithelium

Phenotype	Adhesion of *E. coli* strains producing[a]:			No. of pigs tested
	K88ab	K88ac	K88ad	
A	+	+	+	18
B	+	+	−	13
C	+	−	+	10
D	−	−	+	5
E	−	−	−	19

[a] All standard strains and the K88-negative control strain (see Table 1) were tested against the brush borders of each pig.

TABLE 3. Serological variants of K88 antigen blocking receptor sites of brush borders of different phenotypes and preventing adhesion of *E. coli* strains that possess the homologous or a heterologous K88 variant

Brush border phenotype	Variants of K88 antigen interfering with adhesion of *E. coli* strains producing:			No. of pigs tested
	K88ab	K88ac	K88ad	
A	ab, ac	ab, ac	ab,[a] ac,[a] ad	6
B	ab, ac	ab, ac	—[b]	4
C	ab, ad	—	ad	5
D	—	—	ad	3

[a] The K88ad-positive strains adhered well to the phenotype A brush borders from one pig, in contrast to the other five pigs, despite blocking with both K88ab antigen and anodic K88ac antigen from strains G205 and H519, respectively. Cathodic K88ac antigen from strain H520 blocked adhesion of K88ad-positive strains, as expected.

[b] —, The effect of blocking cannot be observed here, because the brush borders of these phenotypes are resistant to adhesion of the *E. coli* strains possessing the relevant K88 antigen (see also Table 2).

variant (Table 3). Blocking the brush border receptor sites with cell-free K88 antigen always inhibited the adhesion of *E. coli* strains producing the homologous variant of the K88 antigen, as might be expected. Blocking with K88ab antigen also inhibited the adhesion of K88ac-positive *E. coli* strains to phenotype A and B brush borders, and vice versa. Antigens K88ab and K88ac both prevented the adhesion of K88ad-positive strains to phenotype A brush borders, with one exception (see Table 3). In this exceptional case, the adhesion of K88ad-positive *E. coli* to the K88ad receptor sites of phenotype A brush borders was not prevented by cell-free K88ab antigen or anodic K88ac antigen, whereas cathodic K88ac antigen still inhibited adhesion of K88ad-positive strains. On the other hand, K88ad antigen inhibited the adhesion of *E. coli* strains possessing a heterologous K88 antigen (K88ab) only in phenotype C brush borders.

DISCUSSION

Until now, two porcine phenotypes have been recognized with regard to the susceptibility of brush borders to K88 adhesion (8). Our results show that by using the three serological variants of the K88 antigen, a further subdivision can be made within the group of "adhesive" animals, and five pig phenotypes were discerned provisionally. Some of the adhesion-positive pigs are susceptible to all three K88 variants (phenotype A), whereas others show a positive brush border adhesion test with two (phenotypes B and C) or only one (phenotype D) of the serological variants of the K88 antigen. The adhesion-negative phenotype of Sellwood et al. (8) includes our phenotypes E and D, for the K88ad variant was not yet known at that time.

Although the K88 variants share the K88a component, this does not influence their adhesive properties. For instance, K88ab and K88ac adhere to phenotype B brush borders; however, K88ad shows no adhesion to phenotype B brush borders, even though it is the only K88 variant that adheres to phenotype D brush borders. Therefore, the existence of at least four different variants of the "adhesive" pig phenotype is a further confirmation of the results of Wilson and Hohmann (9), who demonstrated that the K88b and K88c components were associated with adhesion, whereas this association did not apply to the K88a component. (The K88d component was unknown at that time.) Despite this association, it is uncertain and even unlikely that the immunogenic components K88b, K88c, and K88d are identical to the adhesion component of the K88 antigen. This may be deduced from the fact that, e.g., the K88ac antigen was able to block the K88ab and K88ad receptors of phenotype A brush borders (Table 3), yet K88c-induced antibodies did not react with K88b and K88d.

The structure of the receptor sites remains to be elucidated with regard to the three known serological variants of the K88 antigen. Possibly, there are separate receptors specific for each of the three K88 variants. This, however, does not explain the fact that blocking with a particular K88 antigen also inhibits the adhesion of other K88 variants; e.g., blocking of phenotype A brush borders with K88ab antigen inhibits the adhesion of K88ac- and K88ad-positive strains as well. A more likely possibility is the existence of only one receptor which, depending on its modification, represents phenotype A, B, C, or D. The latter hypothesis also offers a better explanation for the results obtained with the brush border adhesion tests after the receptor sites were blocked with one of the serological variants of the K88 antigen.

In this context, it is worth mentioning the phenotype A brush border variant that showed different behavior in the receptor blocking test

(Table 3). Adhesion of K88ad-positive *E. coli* to these phenotype A brush borders was not inhibited by K88ab or anodic K88ac antigen. The results suggest that the K88ad receptor site of phenotype A brush borders might have two configurations. This differentiation within phenotype A can only be detected by the receptor blocking test. In addition, the results from the latter technique clearly demonstrated a functional difference between the serologically identical anodic and cathodic forms of the K88ac antigen.

Recent studies (1, 2; J. M. C. Kortbeck-Jacobs, PH.D. thesis, State University, Utrecht, The Netherlands, 1981) have described the use of oral vaccination of the dam with K88 antigen or K88-positive bacteria. The existence of the immunological gut-mammary link supplies the piglets with passive local protection against K88-positive *E. coli* infections via colostrum and milk from the immunized sow. Genetic studies (3) suggest that homozygous recessive dams (our phenotype E) do not recognize K88-positive *E. coli* bacteria as antigens and do not produce antibodies against them. Therefore, the immunological consequences of the existence of four different "adhesive" phenotypes (A through D) in connection with the three serological variants of the K88 antigen must be studied further to obtain an effective oral vaccination of the sow. By refinement and extension of the genetic model of Gibbons et al. (3, 7, 8), the genetics of the different porcine phenotypes must be clarified.

LITERATURE CITED

1. **Chidlow, J. W., and P. Porter.** 1979. Intestinal defence of the neonatal pig: interrelationship of gut and mammary function providing surface immunity against colibacillosis. Vet. Rec. **104**:496–500.
2. **Evans, P. A., T. J. Newby, C. R. Stokes, D. Patel, and F. J. Bourne.** 1980. Antibody response of the lactating sow to oral immunization with *Escherichia coli*. Scand. J. Immunol. **11**:419–429.
3. **Gibbons, R. A., R. Sellwood, M. Burrows, and P. A. Hunter.** 1977. Inheritance of resistance to neonatal *E. coli* diarrhoea in the pig: examination of the genetic system. Theor. Appl. Genet. **51**:65–71.
4. **Guinée, P. A. M., and W. H. Jansen.** 1979. Behavior of *Escherichia coli* K antigens K88ab, K88ac, and K88ad in immunoelectrophoresis, double diffusion, and hemagglutination. Infect. Immun. **23**:700–705.
5. **Mooi, F. R., and F. K. de Graaf.** 1979. Isolation and characterization of K88 antigens. FEMS Microbiol. Lett. **5**:17–20.
6. **Ørskov, I., F. Ørskov, W. J. Sojka, and W. Wittig.** 1964. K antigens K88ab(L) and K88ac(L) in *E. coli*. Acta Pathol. Microbiol. Scand. **62**:439–447.
7. **Rutter, J. M., M. R. Burrows, R. Sellwood, and R. A. Gibbons.** 1975. A genetic basis for resistance to enteric disease caused by *E. coli*. Nature (London) **257**:135–136.
8. **Sellwood, R., R. A. Gibbons, G. W. Jones, and J. M. Rutter.** 1975. Adhesion of enteropathogenic *Escherichia coli* to pig intestinal brush borders: the existence of two pig phenotypes. J. Med. Microbiol. **8**:405–411.
9. **Wilson, M. R., and A. W. Hohmann.** 1974. Immunity to *Escherichia coli* in pigs: adhesion of enteropathogenic *Escherichia coli* to isolated intestinal epithelial cells. Infect. Immun. **10**:776–782.