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K88 VARIANTS K88ab, K88ac AND K88ad IN ORAL VACCINATION OF DIFFERENT PORCINE ADHESIVE PHENOTYPES. IMMUNOLOGICAL ASPECTS.

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ABSTRACT

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Sows of different adhesive phenotypes were vaccinated orally during the last 4 weeks of gestation with K88-positive Escherichia coli. Sows susceptible to adhesion by the K88 variant of the vaccination strain produced a significant IgA-class specific anti-K88 response in colostrum and milk and post-farrowing serum. Indications for an IgM and IgG-class specific anti-K88 response were also found in this group but only in milk. In sows resistant to adhesion by the K88 variant of the vaccination strain only an IgA-class specific anti-K88 antibody response was found in mammary secretions and in post-farrowing sera, but titres did not reach the high values of the former group. The response in the second group was attributed to the frequent administration of large quantities of K88-positive E.coli which to some extent can be compared with a colonization effect. Specificity for the serological components of the K88 variants was detectable in colostral IgA of sows susceptible to the vaccination strain only.

INTRODUCTION

In most cases of neonatal diarrhoea in piglets the disease is caused by enteropathogenic strains of Escherichia coli. Two prerequisites are indispensable for the enteropathogenicity of these strains. Firstly, they must produce enterotoxin, of which two types can be distinguished on the basis of their thermostability: a heat-labile toxin (LT) and a heat-stable toxin (ST). Porcine enterotoxigenic E.coli (ETEC) release one or both of these types of enterotoxin, of which only LT is immunogenic (Wilson et al., 1971). The enterotoxins are the true cause of diarrhoea. Secondly, the presence of an adhesin is essential for virulence of ETEC, by allowing adhesion of the bacteria to the intestinal epithelium and consequent proliferation to high numbers in the jejunum. The fimbrial K88 antigen is the predominant adhesive organelle in porcine ETEC, enabling adhesion to specific receptors on the epithelial brush borders and subse-

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quent colonization of the small intestine.

By using the three serological variants of the K88 antigen in brush border adhesion tests it was possible to distinguish four different kinds of adhesion-positive animals, indicated as phenotypes A to D, and an adhesion-negative phenotype E (Bijlsma et al., 1982). Adhesion-negative animals probably have no receptors for the K88 antigen. This property is inherited as a recessive characteristic, and susceptibility to adhesion is thus dominant over resistance to adhesion (Rutter et al., 1975; Sellwood et al., 1975; Gibbons et al., 1977). Therefore, an adhesion-negative sow may give birth to a partly or completely adhesion-positive litter, dependent on the genotype (heterozygous or homozygous) of the adhesion-positive sire.

Piglets are born without maternal, transplacental immunity, and they have no mature immunoglobulins of their own in their serum (Bourne, 1974). Protection against enteric infections is acquired in colostrum and milk from the nursing dam. To improve the protective capacity of milk and especially of colostrum, sows are vaccinated during pregnancy to raise the content of specific immunoglobulins in mammary secretions. At first the approach to vaccination against neonatal E.coli diarrhoea was rather empirical (Stevens and Blackburn, 1967). Protection against the enterotoxin appeared not to be essential (Rutter et al., 1976), also because ST is not immunogenic (Wilson et al., 1971). A better understanding of the pathogenesis (Jones and Rutter, 1972) prompted investigations on the stimulation of antibodies which prevented the adhesion of ETEC to the intestinal wall. Opsonization might be another activity of antibodies to prevent colonization (Sellwood, 1982). The anti-adhesive protection might be achieved by parenteral (Rutter and Jones, 1973; Kohler, 1974; Rutter et al., 1975; Nagy et al., 1979) or oral vaccination (Kohler et al., 1975; Evans et al., 1980; Moon, 1981). Concerning oral vaccination, Gibbons et al. (1977) postulated that sows lacking the receptor for K88-positive bacteria do not recognize these bacteria as an antigen and do not produce antibodies to K88 to the same extent as adhesion-positive sows. Later studies supported these suggestions (Sellwood, 1979; Kortbeek-Jacobs and van Houten, 1982). Therefore, adhesion-positive offspring of a phenotype E sow will not be protected by colostral and milk antibodies. It would be of interest to determine if, for instance, phenotype A piglets, born to a phenotype D sow, would be protected against infection with K88ac-positive E.coli. The purpose of the present study was to investigate the immune response in relation to the different phenotypes, following oral exposure to a particular K88 variant strain. Combinations of K88 variant strains and sows with a phenotype susceptible to the vaccine strain were chosen. The effect of vaccine strains, differing in K88 variants, was studied in relation to the phenotype of the immunized sow.

MATERIALS AND METHODS

E.coli strains

The strains listed in Table 1 were used for different purposes, namely for

TABLE 1
E.coli strains used for phenotyping, oral immunization and/or preparation of cell-free K88 antigen.

Strain	OK-type	Particulars and (references)
R1	O8:K?:K88ab	(Guinée and Jansen, 1979; Bijlsma et al., 1982)
G7	O8:K87:K88ab	
E68	O141:K85ab:K88ab	
G205	O8:K87:K88ac	
H519	O149:K91:K88ac	
H520	O149:K91:K88ac	
H56	O8:K87:K88ad	
H70	O8:K?:K88ad	
H110	O9:K(A)?:K88ad	
-C	O149:K91	
W11	O149:K91:K88ac	Field isolate
M4	rough	K12, C600, lac$^-$
M4K88ab	rough, K88ab	M4 transconjugants (Guinée et al. 1980)
M4K88ac(l)	rough, K88ac	
M4K88ac(r)	rough, K88ac	
M4K88ad	rough, K88ad	

phenotyping of pigs and piglets in the brush border adhesion assay (Sellwood et al., 1975; Bijlsma et al., 1982) and for oral immunization of sows. The K-12 transconjugants harbouring the K88 variants were used for preparation of cell-free K88 antigen according to the method of Mooi and de Graaf (1979) as described previously (Bijlsma et al., 1982). The cell-free K88 antigen preparations were used in the enzyme-linked immunosorbent assay (ELISA). Strain M4 itself was used for the preparation of a cell-free "M4 antigen". For this purpose M4 growth was treated in the same way as was usual for the preparation of cell-free K88 antigen. The K88ac antigen of G205, H519 and M4K88ac(l) shows anodic mobility in immunoelectrophoresis in Noble agar gels, whereas the K88ac antigen of H520 and M4K88ac(r) moves to the cathode (Guinée and Jansen, 1979).

Animals

The pigs used in this study were crossbred animals of different breeds (Yorkshire, Landrace, Large White, Pietrain). The sows and their adhesive phenotypes were divided into three groups (Table 2). In group I the phenotype of the sows is susceptible to adhesion of the vaccination strain. In group II the phenotype

of the sows is resistant to adhesion of the vaccination strain. In group III vaccination was not employed. Table 2 shows that some of the sows were used two or three times.

TABLE 2
Schedule summarizing the sows, their adhesive phenotype, the individual litter numbers and the E.coli strains in the way they have been used for oral vaccination of the sows during gestation of the different litters.

I Phenotype susceptible to vaccination strain			II phenotype resistant to vaccination strain			III no vaccination	
Sow code		E.coli strain	Sow code		E.coli strain	Sow code	
A10	(1)[a]	M4K88ab	D_C200	(1)	M4K88ab	A613	
A10	(2)	M4K88ab	D_C200	(2)	M4K88ab	A617	
A10	(3)	G7	D_C200	(3)	G7		
A440	(1)	G7	E88	(2)	G7	E88	(3)
A440	(2)	M4K88ad	E123		M4K88ab	E542	
A4234	(1)	H56	E618		W11	E543	
A4234	(2)	M4K88ac(r)				E615	
A4234	(3)	W11					
B439	(1)	G7	B439	(2)	H56		
B437		G7	B439	(3)	H56		
C78	(1)	H110				C78	(2)
D4763	(1)	H56	D4763	(3)	W11		
D4763	(2)	H56					

[a] Figures in parentheses indicate the number of the litter; if no litter number is given, the sow in question produced only one litter.

Blood samples

Blood samples were taken from an ear vein 4 weeks before farrowing, but prior to starting the immunization program, and 2 and 10 days after farrowing. Blood was sampled from the piglets on days 2 and 10 after birth by means of heart puncture. The blood samples were allowed to stand at room temperature for 2 hours, and sera were removed after centrifugation for 15 minutes at 1000 x g and stored at -20°C until use.

Colostrum and milk samples

Colostrum was manually collected from the sows during farrowing. The samples

were pooled from as many teats as possible. Milk samples were collected in the same way on days 2 and 10 after parturition. One hour before milk sample collection the piglets were taken from their dam and the sow was milked after the administration of 10 units of oxytocin into an ear vein after the blood sample had been taken. The mammary secretions were centrifuged at 48,000 x g for 2 hours and the resulting whey was stored at -20°C until use.

Immunization procedure

Starting about 4 weeks before their calculated farrowing dates, the sows were orally immunized by feeding daily approximately 5×10^{11} c.f.u. of one of the E.coli strains in 250 ml tryptone soya broth in their evening food ration.

The enzyme-linked immunosorbent assay (ELISA)

A microplate ELISA was used to measure the specific anti-K88 activity of the different immunoglobulin (Ig) classes in serum, colostrum and milk samples. The procedures were followed as described by Hartman et al. (1984a,b).

Coating procedure. The wells of polystyrene microelisa plates (M129A, Cook Dynatech) were coated with one of the variants of the K88 antigen by overnight incubation at room temperature. Each well was filled with 100 µl of K88 antigen. The antigen was diluted (2 µg/ml of protein) in Chang (1947) buffer, pH 7.0. The protein content of the antigen preparation was determined by the method of Lowry et al. (1951). Concurrently, plates were coated with K88ab, K88ac(1), K88ac(r), or K88ad. Control plates were coated with cell-free antigen prepared from strain M4.

Test procedure. Prior to use the antigen-coated plates were rinsed thoroughly three times with tap water containing 0.05% (v/v) of Tween 20. Samples as well as antisera were diluted in phosphate buffered saline (PBS), pH = 7.2, to which 1% egg albumen (Difco 0255-15) and 0.1% Tween 20 had been added. Volumes of 100 µl of each sample were added in serial twofold dilutions, starting with 1 in 20. After an incubation period of 2 hours at 37°C, the plates were washed three times with tap water-Tween 20 as described above. Then, 100 µl of diluted Ig-heavy chain specific goat anti-swine IgA or goat anti-swine IgG, or rabbit anti-swine IgM antiserum (Jacobs et al., 1977; Kortbeek-Jacobs et al., 1984) was added, followed by incubation for 1 hour at 37°C. In parallel tests anti-IgA, anti-IgM and anti-IgG reactivity were assayed.

Incubation with antiserum was followed by three rinses with tapwater-Tween 20 and the addition of 100 µl of diluted conjugate, swine anti-goat IgG (H+L) horse-radish peroxidase conjugate (Tago) or goat anti-rabbit IgG (H+L) horse-radish peroxidase conjugate (Miles Laboratories). The optimum antiserum and conjugate dilution was determined by chequerboard titration with positive and

negative sera. After a further incubation step of 1 hour at 37°C the plates were washed three times as described above.

Enzyme activity bound to the wells was assayed by addition of 100 µl of substrate solution containing 0.01% of ortho-phenylenediamine + 0.003% H_2O_2. After incubation for 1 hour at room temperature in darkness, the reaction was stopped by addition of 25 µl of 8N H_2SO_4. The test was read on a Dynatech Microelisa Minireader MR590 (wavelength 490 nm). Ig class-specific antibody titres were expressed as the $\log_2$ of 1/10 of the reciprocal of the highest sample dilution giving an optical density of at least 0.2 (Hartman et al., 1984b).

Absorption of colostrum

Specific K88-variant antibodies were absorbed from colostra using bacterial cells of M4 transconjugants (Table 1). Growth (24 hrs) on three isosensitest agar (Oxoid) plates was harvested and suspended in 2 ml of saline after two washings. Equal volumes (0.5 ml) of bacterial suspension and colostral whey were mixed and the mixture was incubated for 1 hr at room temperature under continuous rotation. After removal of the cells by centrifugation, the supernatant was placed at 56°C for 30 min to kill possibly remaining bacteria, and subsequently stored in a frozen state (-80°C) until use. The same procedure was followed for absorption of M4-specific antibodies from serum, colostrum and milk using M4 bacterial suspensions. "Unabsorbed" samples were treated in the same way with saline only. IgA-class specific K88-variant antibody titres of unabsorbed and absorbed samples were determined by ELISA.

RESULTS

Table 3 shows the results obtained by ELISA titration of sera, colostrum and milk from sow C78 and of sera from her offspring. Sow C78 was susceptible to adhesion by the K88ad antigen of the vaccination strain H110. So, Table 3 gives an example of the results obtained with a sow from group I in Table 2. The sera of 3 piglets from each litter were assayed. The mean and SD values of piglet serum in Table 3 represent the titres of the whole litter.

In contrast to the low pre-immunization level of IgA anti-K88 antibodies in serum of sow C78, there are rather high pre-existing titres of IgG and IgM anti-K88 antibodies (Table 3). Oral vaccination did not influence IgG and IgM anti-K88 titres. However, there was a marked rise of anti-K88 antibodies of the IgA isotype in serum after oral vaccination. In colostrum high titres of anti-K88 activity were observed in all three of the Ig-classes. Also, rather high anti-M4 antibody titres were found in both the IgG and IgM class in serum and colostrum. The anti-K88 titres in serum of the piglets on the second day post partum are a reflection of the titres found in colostrum. Ten days post partum these titres

in piglets serum had reduced to approximately half of the values on day 2 post partum.

The other sows of group I gave comparable results both in serum and mammary secretions, irrespective of the K88 variant used for vaccination. Therefore, we combined the titration results obtained with sows from group I in Figures 1, 2 and 3. The same has been done with the results obtained with the sows from groups II and III (see Table 2). In the diagrams the mean and standard error of the mean (SEM) values of specific anti-K88 Ig-levels are given as determined in the samples of all sows from the respective groups. The figures allow comparison of the effect of oral immunization in groups I and II and no immunization in group III.

TABLE 3
Titres of Ig-class specific anti-K88 antibodies in sera and mammary secretions of sow C78 and in sera of three piglets per litter in response to oral dosing of K88ad antigen (strain H110) during the last four weeks of gestation.

Samples		Sow C78			Piglets				
		serum			colostrum/milk serum				
Days before/after parturition		-28	+2	-10	0	+2	+10	+2	+10
IgA titres	anti-K88ab	2	5	4	9	8	8	7 ± 1	3.7 ± 0.6
	anti-K88ac(1)	2	5	4	9	8	8	7 ± 1	3.3 ± 1.2
	anti-K88ac(r)	2	4	3	9	8	8	6.7 ± 0.6	2.7 ± 0.6
	anti-K88ad	1	3	3	7	7	7	5.7 ± 0.6	2 ± 1
	anti-M4	0	0	0	2	0	0	n.d.	n.d.
IgM titres	anti-K88ab	7	6	6	9	8	8	6.3 ± 0.6	3.7 ± 0.6
	anti-K88ac(1)	6	6	6	9	9	8	6.7 ± 0.6	3.7 ± 0.6
	anti-K88ac(r)	6	6	6	9	8	8	6 ± 1	3.7 ± 0.6
	anti-K88ad	6	6	6	7	6	7	5 ± 1	2 ± 1
	anti-M4	6	6	5	6	3	3	n.d.	n.d.
IgG titres	anti-K88ab	5	4	5	7	5	5	5.7 ± 0.6	4
	anti-K88ac(1)	5	5	5	7	6	5	5.7 ± 0.6	3.7 ± 0.6
	anti-K88ac(r)	3	4	3	6	5	5	5 ± 1	2.7 ± 0.6
	anti-K88ad	3	3	4	5	4	3	4 ± 1	1.3 ± 0.6
	anti-M4	5	4	5	6	1	0	n.d.	n.d.

Anti-K88 antibody in serum

High titres of IgG and IgM anti-K88 reactivity were already present in pre-immunization serum of sows in groups I and II (Fig. 1 and 2). These titres

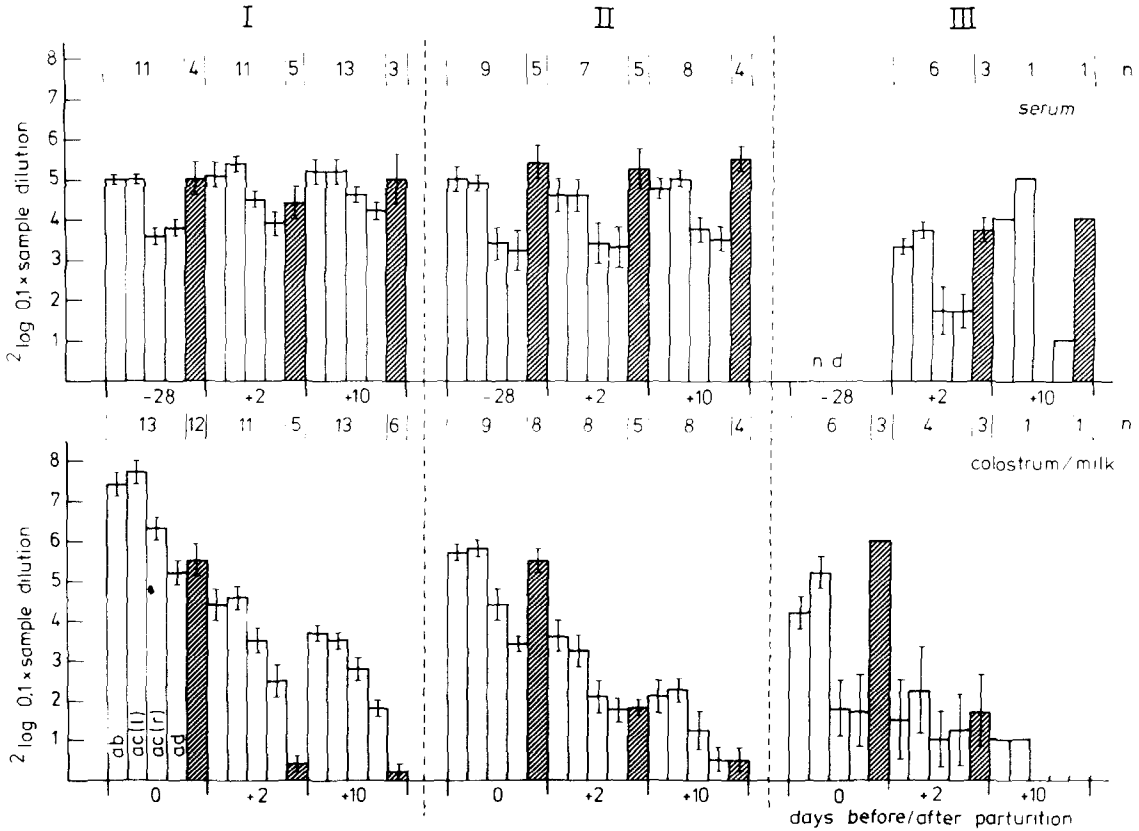

Fig. 1. IgG-class specific titres for the K88 variants K88ab, K88ac(l), K88ac(r) and K88ad (open bars) and for the K88-negative control strain M4 (hatched bars) in serum (above) and in mammary secretions (below) from sows susceptible to the K88 variant of the vaccination strain (group I), from sows resistant to the K88 variant of the vaccination strain (group II) and from unvaccinated sows (group III). The same order is maintained for the anti-K88 variant titres on the consecutive days as indicated in the lower left corner. The number of samples titrated for anti-K88 activity or anti-M4 activity is indicated by n.

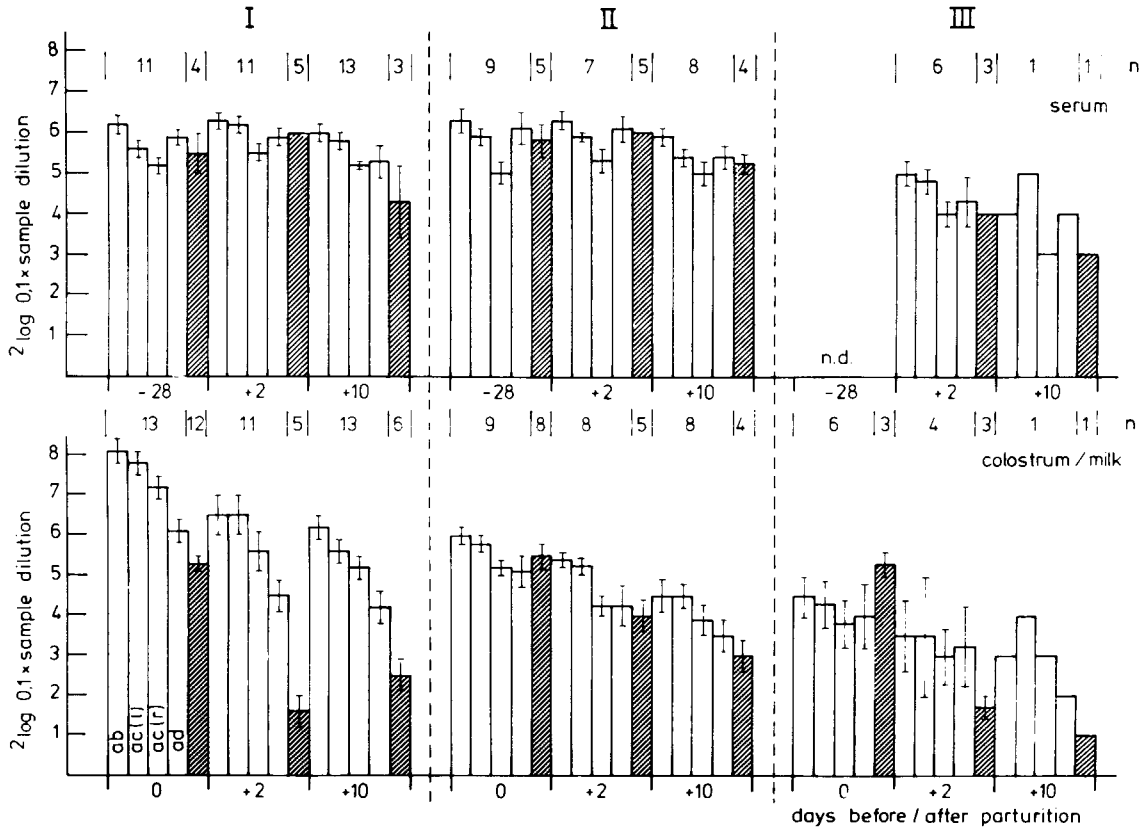

Fig. 2. IgM-class specific titres for the K88 variants K88ab, K88ac(l), K88ac(r) and K88ad (open bars) and for the K88-negative control strain M4 (hatched bars) in serum (above) and in mammary secretions (below) from sows susceptible to the K88 variant of the vaccination strain (group I), from sows resistant to the K88 variant of the vaccination strain (group II) and from unvaccinated sows (group III). See Fig. 1 for further explanation.

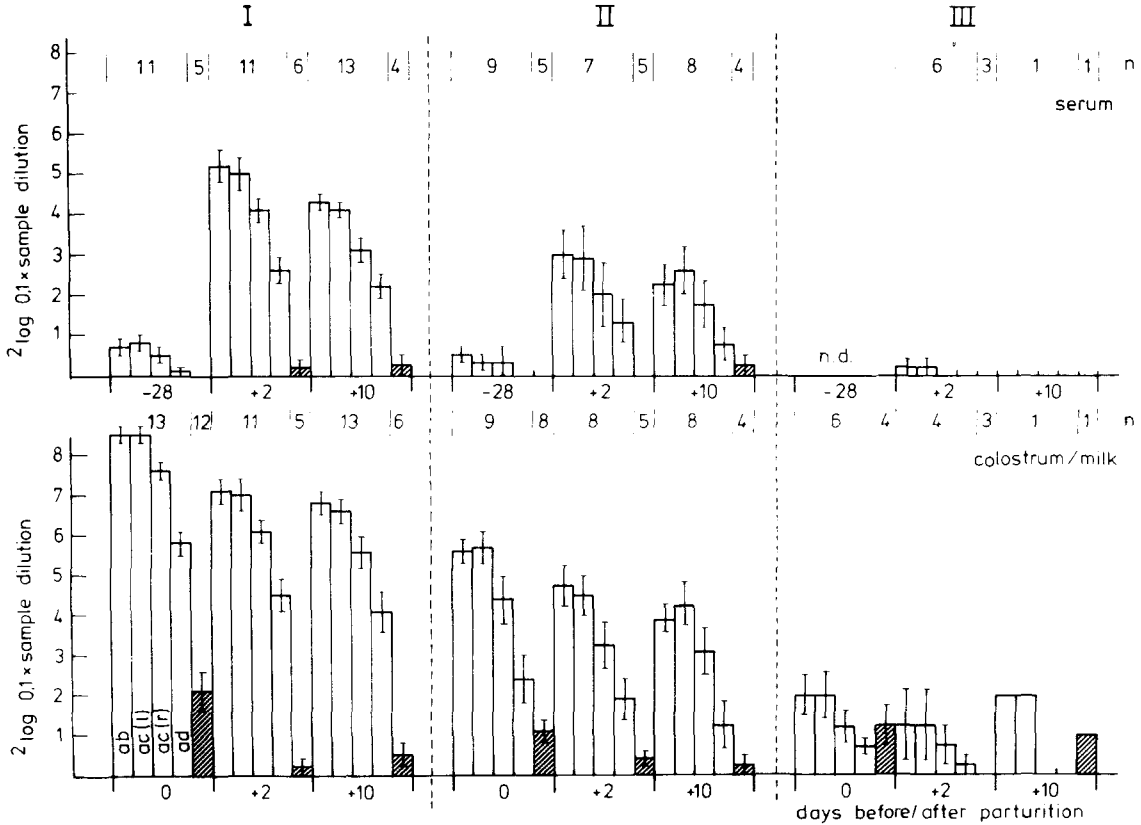

Fig. 3. IgA-class specific titres for the K88 variants K88ab, K88ac(l), K88ac(r) and K88ad (open bars) and for the K88-negative control strain M4 (hatched bars) in serum (above) and in mammary secretions (below) from sows susceptible to the K88 variant of the vaccination strain (group I), from sows resistant to the K88 variant of the vaccination strain (group II) and from unvaccinated sows (group III). See Fig. 1 for further explanation.

remained high after parturition. The anti-M4 titres in these Ig-classes were almost as high as the anti-K88 titres. The 2 and 10 day serum IgG and IgM anti-K88 titres in group III sows were slightly lower than in group I and II sows.

Pre-immunization titres of IgA anti-K88 antibodies were practically absent in group I and II sow's sera (Fig. 3). A significant rise was observed in the serum on day 2 post partum, particularly in group I, whereas the anti-M4 titre remained almost undetectable. Even the sows in group II, which were resistant to adhesion by the K88 variant of the vaccination strain, showed enhancement of the IgA anti-K88 antibodies, albeit to a lesser degree. IgA anti-K88 antibodies could not be detected in sera of the unvaccinated group III sows.

Anti-K88 antibody in mammary secretions

In mammary secretions of all three groups of sows, the titres of anti-K88 antibodies in all three Ig-classes were highest in colostrum. A gradual decrease of these titres was observed in milk samples of day 2 and day 10 in all three groups. The decrease was most obvious in the anti-K88 antibody titres of the IgG-class. These titres in the day 10 milk samples had been reduced to 50% or less of the colostral titres. Another decreasing tendency can be observed between the groups I, II and III. The respective Ig anti-K88 titres were highest in group I, lowest in group III, and intermediate in group II for all three Ig-classes.

Comparing anti-M4 titres to anti-K88 titres in colostral and milk samples of group I sows, the anti-M4 antibody titres of the IgG isotype (Fig. 1) as well as of the IgM isotype (Fig. 2) remained markedly lower (especially in the milk samples) than the anti-K88 antibody titres. This was not observed in groups II and III. The IgA anti-K88 antibodies (Fig. 3) reached very high titres in mammary secretions of group I, while the anti-M4 titres remained very low. The same is valid for IgA anti-K88 titres in colostrum and milk of group II sows. IgA antibodies against the K88 variants were hardly present in colostral and milk samples of group III sows.

Anti-K88 variant specificity

We could not find differences in Ig titres for the different K88 variants that might be attributed to the vaccination strains (Figs. 1, 2 and 3), in particular to the serological components of the K88 variants in the vaccines. Neither could we find these differences in the results of the individual sows, as shown in Table 3. However, anti-K88ad and anti-K88ac(r) titres were always lower than anti-K88ab and anti-K88ac(1) titres. Only serum IgM titres did not show this effect obviously (Fig.2). Some colostral IgA-class specificity for the serological components of the K88 variants used for oral immunization of sows

can be deduced from Table 4. After absorption of colostral whey with M4K88ac(1) there was a residual titre which was present only with regard to the K88 variant used for immunization. However, M4K88ac(1) absorbed all anti-K88 reactivity from the colostral whey when the sow had been immunized with a k88ac-positive strain.

TABLE 4
IgA-class specific titres of antibodies to the different K88 variants as determined in unabsorbed (-) and absorbed (+) colostrum of a number of orally immunized sows.

Sow code (litter no.)	vaccination strain	absorption M4K88ac(1)	IgA titres for K88 variants ab	ac(1)	ac(r)	ad
A10 (2)	M4K88ab	-	8	8	6	6
		+	3	0	0	0
A10 (3)	G7(K88ab)	-	8	8	8	6
		+	4	0	0	0
A4234	W11(K88ac)	-	7	8	7	6
		+	0	0	0	0
B437	G7(K88ab)	-	8	8	5	3
		+	7	0	0	0
C78	H110(K88ad)	-	7	8	6	5
		+	2	0	0	4
D4763 (1)	H56(K88ad)	-	6	6	5	4
		+	1	0	0	4
D_C200 (2)	M4K88ab	-	4	4	3	1
		+	1	0	0	0

0 = titre was too low to be perceptible

Also, practically no residual titre was observed in colostrum from sow D_C200, which was not susceptible to adhesion by the vaccination strain. Strain M4K88ac(1) gave satisfactory results in absorption, whereas the other transconjugants appeared to be less suitable in absorption experiments.

Removal of the anti-M4 activity from serum, colostrum and milk samples by means of absorption with the E.coli K-12 strain M4 did not change the IgG and IgM titres of anti-K88 activity in the absorbed samples, when compared with anti-K88 titres in "unabsorbed" samples. This indicates that the IgG and IgM anti-K88 antibody activity in serum and mammary secretions is also rather specific.

DISCUSSION

For protection against neonatal diarrhoea the new-born piglet relies initially on maternal antibodies obtained in colostrum and milk. The present study concerned the protective capacity of the mammary secretions resulting from oral immunization with a particular K88 variant and the adhesive phenotype of the dam. Ig-class specific anti-K88 titres were determined by the ELISA technique.

During the incubation of the sample with solid phase K88 antigen in the ELISA there is competition between antibodies of the different Ig-classes. Antibodies of a particular Ig-class having a high affinity might to some extent prevent binding of antibodies with low affinity. Therefore, Ig-class specific titres might be over- or underestimated. The values obtained are not absolute, but the method does indicate increase or decrease of anti-K88 antibodies in each Ig-class. Thus, conclusions may be drawn from changes in antibody titres, and from differences in antibody titres between the three groups of sows. The changes and differences reflect on the one hand the effect of oral immunization, and on the other the influence of the phenotype.

In the present study the Ig-class specific anti-K88 antibody titres, as determined in serum of two day old piglets, appeared to be a reflection of the antibody profile in colostrum. This is in accordance with the observations of Yabiki et al. (1974) and Kortbeek-Jacobs and van Houten (1982). An overall reduction of the serum immunoglobulins to about 50% was observed in ten day old piglets. There is no demonstrable production of immunoglobulins by the piglet itself up to that time (Yabiki et al., 1974; Jacobs et al., 1977). So during the first two weeks of life the piglet is completely dependent on colostral antibodies for its humoral immunity.

Experimental studies have demonstrated that intestinal colonization of the sow prepartum (Bohl et al., 1972; Kortbeek-Jacobs and van Houten, 1982) or during lactation (Evans et al., 1980) will selectively stimulate a specific IgA response in mammary secretions. In the present experimental design colonization was imitated by the administration of large quantities of live K88-positive E.coli culture for a prolonged period. This design approximated the most effective immunization schedule as given by Kortbeek-Jacobs et al. (1984). Also Kohler et al. (1975) and Moon (1981) administered large quantities of K99-positive and P987-positive E.coli prepartum and observed a protective effect, but they did not study the Ig-class(es) of the protective antibodies.

The results of the present study confirm the link between the immune systems of the gut and mammary gland. Oral administration of K88-positive E.coli to the sow induced in the mammary secretions a specific K88 antibody response, which was primarily of the IgA-class. This response was observed in sows of both group I and group II. The susceptible pigs of group I possessed receptors for the K88

variants of the vaccination strains, facilitating colonization of the intestinal surface. The frequent administration of large doses of K88-positive bacteria to the sows of group II, which were resistant to adhesion by the K88 variants of the vaccination strains, caused a colonization effect apparently sufficient to produce an immune reaction. Yet, the IgA anti-K88 titres were obviously higher in group I compared to group II. Also, significant IgA anti-K88 titres were found in post-farrowing sera of the sows of groups I and II.

In the present study no IgM-class and IgG-class specific anti-K88 response to oral vaccination was observed in serum, since prevaccination serum already contained practically the same anti-K88 antibody titres as post partum sera of day 2 and day 10. The IgG and IgM-class specific anti-K88 titres in colostrum were accompanied by almost equally high anti-M4 titres. The nature of the M4-antigen has not been investigated, but "M4" probably represents impurities such as type 1 fimbriae or cell wall fragments remaining in the rather crude K88 antigen preparations. The titres of anti-M4 and anti-K88 antibodies of the IgG and IgM isotype in colostrum might be derived from serum by transudation. However, the IgG and IgM-class specific anti-K88 titres in milk of group I sows remained comparatively high, whereas the anti-M4 titres fell markedly. These indications for an IgM anti-K88 and an IgG anti-K88 response in milk might suggest that the selectivity of the response to oral immunization in the gut-mammary link is not confined to IgA. There is evidence for an IgM response to oral immunization (Evans et al., 1980) and that IgM also plays a role in surface immunity (Porter et al., 1974), but an IgG response has never been observed. The IgG anti-K88 titres in milk of group I sows are probably due to the prolonged continuous supply of large quantities of K88 antigen, and might not be observed if administered less frequently.

Oral immunization with a particular K88 variant yielded high specific anti-K88 titres for the homologous K88 variant in the relevant cases, e.g. in the IgA class in mammary secretions of group I sows. However, approximately the same high titres were found for the heterologous K88 variants. This might be attributed to the common a-component of the K88 variants. A weak specificity for the specific component of the K88 variants (b, c or d) was only detectable in colostral IgA of group I sows after absorption with a heterologous variant.

Evans et al. (1980) demonstrated a good correlation between the IgA anti-K88 titre in milk and an IgA mediated anti-adhesive activity. Sellwood (1982) found an IgM associated opsonic activity in colostrum of adhesion susceptible sows, whereas even IgG appears to function similarly but with lower efficiency. It is not known whether IgM K88-antibodies and IgG K88-antibodies in milk are still active after passage through the stomach. In any case, specific anti-K88 antibodies in mammary secretions, obtained by oral immunization, will offer the

piglets a good passive protection against E.coli diarrhoea, although they will probably not reach the titres found under the experimental conditions of this study.

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