THE USE OF ELISA FOR DETECTING ANTI-FIMBRIAL ANTIBODY RESPONSES IN PIGS VACCINATED WITH MULTIVALENT *ESCHERICHIA COLI* CONTAINING K88, K99, F41 AND 987P ANTIGENS

SUPAR, B.E. PATTEN, R.G. HIRST, DJAENURI and NINA KURNIASIH
Research Institute for Veterinary Science, Bogor, West Java, Indonesia

ABSTRACT

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Enteric infections caused by enterotoxigenic *Escherichia coli* (*E. coli*) in piglets are ubiquitous in the pig industry in Indonesia. Mixed infections with different *E. coli* containing either K88, K99, F41 or 987P fimbrial antigen are commonly found both in a group of piglets or from the one piglet. This contributes to the complex nature of enteric colibacillosis in piglets and makes treatment and control difficult. A multivalent whole cell *E. coli* vaccine was developed for the control of colibacillosis. Such a vaccine should contain important fimbrial antigens associated with enteropathogenic *E. coli* as found in the field. Two doses of 2.5 ml vaccine were injected into pregnant sows 6 weeks and again 2 weeks before expected date of farrowing. An enzyme-linked immunosorbent assay (ELISA) was developed for detecting anti-fimbrial antibody responses in serum and colostrum. Small pilot studies of vaccine field trials to control piglet neonatal colibacillosis were undertaken in some piggeries in Jakarta, Bogor, Tangerang and Medan North Sumatera. The relationship between colostral IgA and IgG antibody responses detected by ELISA and piglet diarrhoea and mortality was determined. Under field conditions, the use of two doses of *E. coli* vaccine in pregnant sows dramatically reduced diarrhoea and mortality of their suckling piglets.

Key Words: *E. coli*, vaccine, sows, serum, colostrum, ELISA

ABSTRAK

Supar, B.E. Patten, R.G. Hirst, Djaenuri dan N. Kurniasih. 1993. Penggunaan ELISA untuk deteksi respon antibodi anti fimbrial pada babi yang diimunisasi dengan vaksin *E. coli* multivalen yang mengandung K88, K99, F41 dan 987P antigen. *Penyakit Hewan* 25 (46A): 21-28.

Infeksi enterik pada anak babi yang disebabkan *Escherichia coli* enterotoksigenik yang mempunyai antigen perlekatan atau antigen fimbrial K88, K99, F41 atau 987P banyak terjadi dan tersebar luas pada industri peternakan babi di Indonesia. Infeksi campuran dari beberapa serotipe *E. coli* yang mengandung antigen perlekatan tersebut di atas banyak terjadi pada seekor anak babi atau dalam satu kelompok anak babi dari seekor induk. Keadaan infeksi tersebut menyebabkan enterik-kolibasilosis yang sangat komplek serta mengakibatkan pengendalian dan pengobatan penyakit sangat sulit. Vaksin *E. coli* multivalen dibuat dari isolat lapang dan dikembangkan untuk pencegahan dan pengendalian kolibasillosis. Vaksin tersebut sebaiknya mengandung semua jenis antigen perlekatan yang sesuai dengan serotipe *E. coli* enterotoksigenik yang terdapat di lapangan. Dua dosis vaksin *E. coli* multivalen masing-masing 2,5 ml diinjeksikan pada induk babi bunting 6 minggu dan 2 minggu sebelum partus. Metode enzyme-linked immunosorbent assay (ELISA) dikembangkan untuk deteksi respon antifimbrial antibodi dalam serum dan kolostrum. Studi uji vaksin di lapangan untuk pengendalian neonatal kolibasillosis dilakukan di Jakarta, Bogor, Tangerang dan Medan Sumatera Utara. Dua dosis vaksin *E. coli* multivalen yang diinjeksikan pada induk babi menstimulasi respon antifimbrial IgA dan IgG antibodi. Dalam serum antifimbrial IgG antibodi dan dalam kolostrum atau susu antifimbrial IgA dan IgG antibodi. Induk babi yang divaksin mempunyai anti-K88, K99, F41 dan 987P IgA dan IgG antibodi dalam kolostrum atau susu lebih tinggi dibandingkan dengan kolostrum atau susu dari induk yang tidak divaksin. Dibawah kondisi lapangan, penggunaan 2 dosis vaksin pada induk babi bunting dapat menurunkan secara drastis kasus diare dan kematian anak babi yang menyusu induknya setelah dilahirkan.

Kata kunci: *E. coli*, vaksin, induk babi, serum, kolostrum, ELISA

INTRODUCTION

Calf and piglet diarrhoea associated with enterotoxigenic *Escherichia coli* (ETEC) is considered important in Indonesia, where dairy cattle and pigs are raised under intensive conditions with improper management (Setiawan *et al.*, 1982; Mudigdo and Peranginangin, 1982; Supar and Hirst, 1985). Intensive studies of neonatal colibacillosis in calves and piglets have been undertaken at the Research Institute for Veterinary Science (BALITVET) Bogor (Supar *et al.*, 1989). Piglet neonatal diarrhoea associated with ETEC was commonly observed in intensive piggeries in the Bogor and Kapuk of Jakarta areas, diarrhoea was found to occur at rates from 13% to 40% within the first two weeks of life with mortality rates from 12% to 30%.

At present antimicrobial drugs given either orally or by intramuscular injection are being used for the treatment of piglets with colibacillosis (Mudigdo and Peranginangin, diarrhoea cases and mortality rates remain high (Unpublished data). Enterotoxigenic *E. coli* possessing either K88, K99, F41 or 987P have now been

shown to be resistant to the commonly used antibiotic in the field. These indicated that the failure of antibiotics to control neonatal diarrhoea and mortality of piglets is readily explained by the high level of multi-resistance of each of the ETEC isolates associated with diarrhoea. These include, ampicillin, streptomycin, oxytetracycline, erythromycin, sulpha drugs, trimethoprim sulphamethoxazole (Supar *et al.*, 1990).

In view of the low efficacy of antibiotics in controlling neonatal colibacillosis, studies on the development of a multivalent whole cell *E. coli* vaccine for swine have been undertaken. This paper reports the use of ELISA for detecting anti-fimbrial antibody responses in sow serum and colostrum and preliminary data on the effect of sow vaccination on reducing piglet diarrhoea and mortality.

MATERIALS AND METHODS

Commercial *E. coli* vaccine

A commercial polyvalent ETEC vaccine containing K88, K99, F41 and 987P fimbrial antigens was supplied by Ausvac Pty Ltd, Bendigo, Victoria, Australia. This vaccine was used for comparative studies and designated as vaccine A.

Local field isolate *E. coli* vaccine

The organisms used for this polyvalent vaccine were: *E. coli* K88 (O groups 108, 138, 149 and 157); *E. coli* K99 (O groups 64 and 101); *E. coli* F41 (O group 101); *E. coli* 987P (O groups 9 and 20); *E. coli* K99F41 (O group 101), and designated as vaccine B.

Preparation of whole cell bacterin of *E. coli* bearing K88 fimbrial antigen

Each *E. coli* K88 isolate was plated onto MacConkey agar and onto 5% sheep blood agar (SBA) and incubated at 37°C overnight. All isolates were haemolytic on 5% SBA. One discrete colony of each isolate verified for the presence of K88 antigen by slide coagglutination was then inoculated onto a 5% SBA plate. After incubation at 37°C for 18-20 hours, the growth was harvested with sterile normal saline (NS). The cell suspension was used to inoculate 12 Roux flasks containing nutrient agar (Difco). Each Roux flask was seeded with 4 mL of the cell suspension prepared for each isolate, then incubated at 37°C overnight. The cell growth on the surface of agar was harvested with NS using sterile glass beads. The cell suspension from each flask of each isolate was pooled and centrifuged at 4000 rpm at 4°C for 20 minutes. The pellet cells were washed three times with sterile NS by centrifugation as described above to remove the medium constituent. The final cell pellet was then resuspended in 20 mL sterile NS. After a purity check, formalin was added to a final concentration of 0.1% . The cell suspension was incubated at 37°C for 3 hours for sterility testing. The cell suspension were stored at 4°C and tested periodically to confirm that all cells were killed. The cell suspension from each *E. coli* K88 isolate at this stage formed the basic stock suspension for the preparation of the vaccine.

Preparation of whole cell bacterin of *E. coli* bearing K99 or F41 fimbrial antigen

The *E. coli* bearing either K99, F41 or K99F41 fimbrial antigens isolated from piglets with diarrhoea were all non-haemolytic on 5% SBA. The procedure which was used to prepare each cell suspension from each isolate was as described above. The medium used for the production of K99, F41, or K99F41 was Minca +Iso Vitalex (Vitox, Oxoid) (Guinee *et al.*, 1976).

Preparation of whole cell bacterin of *E. coli* bearing 987P fimbrial antigen

The purity each *E. coli* 987P field isolate was tested on MacConkey agar and 5% SBA and incubated at 37°C overnight. To enhance 987P antigen production, a discrete colony was inoculated into 10 mL tryptic soy broth (TSB) (Difco), 5-10 tubes of each isolate. The inoculated TSB was then incubated statically at 37°C for 7 to 10 days until a heavy pellicle developed, or the pellicle settled at the bottom of the tube. The pellicle or cell sediment was separated from the liquid, then tested for the presence of the 987P antigen by slide coagglutination reaction. If present, the cells were suspended in 20 mL NS. Four mL of this suspension was inoculated onto 5% SBA prepared on 12 Roux flask for the production of large quantities of cells. Each inoculated Roux flask was incubated at 37°C for 18-20 hours. After incubation, the growth was harvested with NS using sterile glass beads. The whole cell bacterin of *E. coli* 987P were obtained as described as for preparation of whole cell bacterin of *E.* coli containing K88 fimbrial antigen.

Preparation of multivalent *E. coli* vaccine

Equal amounts of each stock cell suspension were mixed to give equal proportions of each fimbrial antigen. The final turbidity was adjusted to the No. 10 tube of the MacFarland Standards. The cell suspension was blended with aluminium hydroxide to a final concentration of 25%, then tested for sterility and immunogenicity.

Animal inoculation.

After sterility testing, the immunogenicity of both vaccine A and B was assayed in pregnant sows. Each of eight sows was injected intramuscularly with 2.5 mL of either vaccine in the neck region behind the ear, 7 weeks before the expected date of farrowing. Prior to injection 5 to 7 mL of blood was obtained from each sow. Four weeks later each of the vaccinated sows was bled again then given a booster dose of 2.5 mL of the appropriate vaccine.

Serum collection

Each sow was sampled from the marginal ear vein by use of a sterile disposable syringe, placed into a 5 mL sterile test tube, allowed to clot and kept at 4°C overnight. On the following day the blood samples were centrifuged at 3000 rpm for 20 minutes. The clear supernatant of each sample was put into a sterile Bijou bottle and kept at -20°C until tested by ELISA. A blood sample was drawn from each vaccinated sow once a week up to farrowing.

Colostrum collection

Sow colostrum was collected from farrowing sows during piggery visits. Each of the colostrum samples was drawn from one to five teats from the sow within 1 to 2 days after farrowing and placed in a universal bottle for transport to the laboratory. Fat was removed from the colostrum samples by centrifugation at 9000 rpm for 30 minutes. To each 2 mL of defatted colostrum, 30 µl of 20% (w/v) of rennet (Sigma Chemical Co. R3375, USA) was added, then incubated at 37°C for 2 hours, before centrifuging at 12,000 rpm for 10 minutes. The supernatant, or whey, of each sample was separated and stored at -20°C until tested by ELISA.

Preparation of K88 antigen.

The K88 antigen was prepared from the eference strain *E. coli* G7 (O8:K87,K88ab). The bacterium was grown on nutrient agar and incubated at 37°C overnight. The growth was harvested using PBS. The cell suspension was centrifuged at 3500 rpm for 20 minutes. The cell pellet was washed 3 times with PBS by centrifugation as described previously, the final pellet was resuspended in PBS and the opacity was adjusted equal to the No.10 tube of the MacFarland Standards. It was then heated at 60°C for 20 minutes to detach the fimbrial antigen. After heating it was centrifuged at 3500 rpm for 20 minutes and the supernatant was separated and placed into a sterile bottle after confirming the presence of K88 antigen by slide coagglutination. The supernatant was added with equal volume of saturated ammonium sulphate, then kept at 4°C overnight. The precipitate was pelleted by centrifugation at 3500 rpm for 20 minutes (using preweighed centrifuge tubes). The supernatant was decanted and the pellet was weighed and then resuspended in 10 mL PBS. This process was repeated twice, the final pellet being resuspended in 10 mL PBS and dialyzed against PBS at 4°C overnight, then against normal saline for 3 hours and finally against distilled water for 3 hours. After dialysis, the fimbrial antigen suspension was centrifuged at 3000 rpm for 10 minutes, the supernatant was removed into a sterile bottle and retested for K88 by slide coagglutination. The final antigen suspension was kept at 4°C until used.

Preparation of K99 and F41 antigens

The *E. coli* (IMVS 988, O-:K12,K99) provided by Institute of Medical and Veterinary Science (IMVS) and *E. coli* R55 (O101, K-F41 (local isolate) were used to prepare K99 and F41 antigen respectively. Each serotype was subcultured onto Minca+Is agar slope and incubated at 37°C overnight. This was used to inoculate Minca+Is agar plates. After incubation at 37°C for overnight, the growth was harvested with PBS. The procedure used to prepare the K99 and F41 ELISA antigen was as described above.

Preparation of 987P antigen

The *E. coli* (Moon987P) reference strain was used to prepare 987P ELISA antigen. The organism was subcultured into TSB and incubated statically at 37°C for 7 to 10 days. The pellicle which developed was plated onto 5% SBA and incubated at 37°C overnight and tested for 987P antigen by slide coagglutination. If 987P was present, the pellicle and/or the deposit from the bottom of each tube was suspended into 10 ml NS and used to inoculate 2 Roux flasks containing 5% SBA. After incubation at 37°C overnight, the growth was harvested with PBS using glass beads and then it was centrifuged at

3500 rpm for 30 minutes. The supernatant was decanted and the cell pellet was washed 3 times with PBS by centrifugation. The creamy cell pellet was dissolved in PBS and the turbidity was adjusted to equal to the No. 10 tube of the MacFarland Standards. This suspension was then heated at 60°C for 20 minutes to detach the 987P fimbrial antigen from the cell. The 987P fimbrial antigen fraction was separated by centrifugation and subsequently was precipitated by saturated ammonium sulphate as described previously.

ELISA procedure

The well of round bottom microtitre ELISA plates (Inter Med. Nunc, Denmark) were coated with either K88, K99, F41, or 987P antigen at optimal working dilution at a concentration approximately 2.5 µg/ml, followed by 37°C incubation for 1 hour and 4°C overnight. Each well was coated with 50 µl of antigen of either K88, K99, F41 or 987P diluted in carbonate bicarbonate buffer pH 9.6. The following day, plates were washed four times with phosphate buffered saline pH 7.2 containing 0.05% Tween 20 (PBST). Serum samples were diluted with PBST containing 0.5% ovalbumin (Sigma Chemical Co, USA). Fifty µl volumes of each sample, known positive control serum or colostrum were added in duplicate and serially diluted two-fold *in situ*, ranging from 1:400 to 1:51,200 for sera and 1:8 to 1:1024 for colostrum. After incubating 1 hour at 37°C, the plates were washed with PBST as above. Fifty µl of the appropriate enzyme-conjugate, anti-pig IgG peroxidase (Silenus, Australia) or anti-pig IgA peroxidase (KPL 04-14-01 Maryland USA), diluted in PBST containing 0.5% ovalbumin at optimal working dilution, was added followed by incubation at 37°C for 1 hour. After washing four times with PBST, 100 µl of enzyme substrate containing 1 mM of 2.2-azino-bis-3-ethylbenzthiazoline sulfonic acid (ABTS) plus 2.5 mM H_2O_2 in citric phosphate buffer was added to each well and the plates were incubated at 37°C for 1 hour. Optical densities (OD) were determined on an ELISA plate reader (Titertek Multiskan, MCC, Finland) at a wavelength of 414 nm on each well after the instrument was zeroed with unreacted substrate.

Vaccine field trial

Field testing of the two vaccines was undertaken at a number of locations, initially at a 350 sow piggery in Jakarta. Approximately 30-45 sows and gilts (referred to subsequently as sows) farrowed each month. One group of pregnant sows was injected with vaccine A, one group with vaccine B and a third was left unvaccinated as the control group. Each pregnant sow was injected with 2.5 mL of either vaccine A or vaccine B, 6 weeks and again 2 weeks before the expected date of farrowing. Piglets born from vaccinated and unvaccinated sows were allowed to suckle their own mothers. All other management procedures remained unchanged. Experiments were conducted on the farm over a 9 month period from March to December 1989.

Piggery visits, sample collections and data recording

Farm visits were carried out twice weekly to record the presence of diarrhoea and mortality within the first two weeks post farrowing. During these visits, rectal swabs were taken from all piglets which diarrhoea to confirm the presence of ETEC.

RESULTS

Sow serum antibody responses

The IgG antibody responses in pregnant sows to the fimbrial components of the two vaccines after 2 successive injections of either multivalent vaccine A or vaccine B, measured by ELISA, are shown in Table 1. In general the response to vaccine A was lower than that to vaccine B, particularly that observed for anti-F41. The highest levels were obtained 2 weeks after the second injection. At this point the sows were near to farrowing and the IgG antibody response decreased slightly in most cases. With both vaccines, the greatest response was seen to the K88 component.

Sow colostral whey IgG and IgA antibody responses

A summary of the IgG and IgA responses to fimbrial antigens in colostrum of vaccinated and unvaccinated sows are given in Tables 2 and 3. Vaccination of pregnant sows with both whole cell *E. coli* vaccines, containing K88, K99, F41 and 987P fimbrial antigens, increased the specific IgG levels in the colostrum compared to the unvaccinated controls. Anti-fimbrial IgA was detected at relatively high levels in colostrum and was similar for sows vaccinated with either vaccine A or vaccine B. In both instances the anti-K88 IgA response was the highest. The anti-F41 IgA and anti-987P IgA responses to vaccine A were slightly lower than vaccine B. Anti-fimbrial IgG

and IgA levels in colostrum of unvaccinated sows were [illegible] lower than from vaccinated sows.

Sow serum IgA antibody responses

As shown by data contained in Table 4, anti-fimbrial IgA level to all fimbrial antigens was lower in the serum than in the colostrum (cf Table 3.). Data for unvaccinated controls was not available for comparison.

Vaccine field trials

The results of the trials in the Jakarta piggery G are given in Table 5. There was a substantial reduction in the diarrhoea and piglet mortality rates in both vaccine A and B group litters. Piglet mortality rate in the unvaccinated control group remained high at 26% within the first two weeks post farrowing. The diarrhoea and mortality rates in vaccine B group litters was marginally lower than that in vaccine A group litters.

Tabel 1. Sow fimbrial serum antibody responses to vaccination with multivalent vaccine

Time[1] (weeks)	K88 response[2]		K99 response		F41 response		987P response	
	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B
0	0.76±0.11	0.64±0.20	0.67±0.07	0.63±0.07	0.51±0.13	0.44±0.08	0.65±0.09	0.65±0.18
1	1.00±0.11	0.84±0.19	0.85±0.15	0.74±0.18	0.65±0.19	0.65±0.18	0.85±0.11	0.84±0.12
2	1.22±0.11	0.98±0.21	0.90±0.18	0.80±0.15	0.78±0.17	0.65±0.12	1.00±0.08	0.98±0.14
3	1.38±0.16	1.09±0.19	0.95±0.12	0.90±0.22	0.85±0.21	0.66±0.17	1.10±0.17	1.02±0.10
4	1.55±0.17	1.16±0.22	1.08±0.16	0.90±0.25	1.00±0.25	0.75±0.21	1.29±0.14	1.07±0.11
5	1.60±0.14	1.20±0.18	1.19±0.22	0.90±0.26	1.18±0.26	0.82±0.22	1.30±0.16	1.17±0.07
6	1.71±0.20	1.20±0.14	1.33±0.23	1.01±0.23	1.27±0.26	0.83±0.23	1.49±0.16	1.17±0.10
7	1.70±0.14	1.25±0.24	1.31±0.21	1.01±0.23	1.41±0.34	0.80±0.27	1.49±0.15	1.17±0.19

[1] First dose of vaccine at 0 weeks, second dose at 4 weeks
[2] OD±SD (N=8) measured at serum dilution of 1:400

Tabel 2. Sow fimbrial colostral IgG antibody levels

Colostral whey dilution	K88 response[1]		K99 response		F41 response		987P response	
	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B
8	2.54±0.08	2.50±0.12	1.76±0.14	1.93±0.14	1.31±0.06	1.73±0.21	1.25±0.18	1.40±0.24
16	2.45±0.08	2.32±0.12	1.54±0.24	1.55±0.22	1.10±0.07	1.26±0.26	1.02±0.15	1.13±0.29
32	2.20±0.17	2.03±0.19	1.35±0.28	1.36±0.25	1.02±0.08	1.06±0.13	0.98±0.12	0.90±0.19
64	1.86±0.12	1.86±0.01	1.17±0.28	1.04±0.27	0.93±0.08	0.80±0.08	0.90±0.12	0.70±0.16
128	1.40±0.16	1.56±0.15	1.00±0.26	0.79±0.23	0.80±0.04	0.63±0.08	0.79±0.10	0.65±0.08
256	1.24±0.19	1.29±0.15	0.71±0.20	0.62±0.18	0.55±0.03	0.46±0.04	0.68±0.09	0.52±0.07
512	0.78±0.10	0.7±0.15	0.53±0.11	0.46±0.11	0.43±0.08	0.31±0.02	0.40±0.14	0.38±0.10
1024	0.38±0.09	0.35±0.06	0.37±0.09	0.26±0.06	0.25±0.06	0.16±0.04	0.20±0.07	0.18±0.05
8[2]	1.16±0.14		0.89±0.28		0.92±0.26		0.90±0.22	
16	0.78±0.19		0.60±0.23		0.64±0.22		0.60±0.21	
32	0.65±0.17		0.43±0.20		0.49±0.19		0.48±0.15	
64	0.44±0.16		0.29±0.18		0.34±0.17		0.35±0.11	
128	0.31±0.13		0.20±0.09		0.19±0.10		0.21±0.08	
256	0.17±0.08		0.12±0.08		0.10±0.03		0.13±0.08	
512	0.10±0.05		0.07±0.05		0.06±0.03		0.06±0.03	
1024	0.06±0.03		0.04±0.03		0.04±0.02		0.04±0.02	

[1] OD+SD (N=8)
[2] Unvaccinated controls

Tabel 3. Sow fimbrial colostral IgA antibody levels

Colostral Whey Dilution	*K88 response*[1]		*K99 response*		F41 response		987P response	
	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B
8	2.00±0.24	2.00±0.27	1.73±0.39	1.80±0.28	1.22±0.34	1.68±0.15	1.44±0.24	1.48±0.15
16	1.76±0.16	1.75±0.41	1.48±0.39	1.44±0.32	0.86±0.32	1.23±0.19	1.05±0.18	1.00±0.18
32	1.51±0.13	1.51±0.41	1.26±0.43	1.19±0.17	0.70±0.31	1.02±0.21	0.86±0.12	0.82±0.22
64	1.21±0.14	1.33±0.31	1.04±0.26	0.98±0.17	0.55±0.30	0.76±0.18	0.69±0.09	0.63±0.20
28	0.99±0.22	0.99±0.30	0.80±0.21	0.74±0.14	0.38±0.21	0.49±0.16	0.53±0.13	0.44±0.19
256	0.73±0.23	0.69±0.23	0.63±0.18	0.53±0.10	0.24±0.14	0.32±0.05	0.41±0.09	0.32±0.18
512	0.42±0.21	0.40±0.22	0.35±0.17	0.34±0.06	0.15±0.07	0.20±0.03	0.23±0.09	0.21±0.09
1024	0.20±0.11	0.22±0.11	0.27±0.08	0.22±0.02	0.10±0.03	0.12±0.02	0.12±0.05	0.12±0.04
8[2]	0.75±0.18		0.65±0.21		0.60±0.28		0.60±0.22	
16	0.58±0.18		0.50±0.21		0.42±0.31		0.46±0.20	
32	0.42±0.13		0.45±0.16		0.31±0.11		0.32±0.09	
64	0.29±0.11		0.35±0.16		0.22±0.08		0.25±0.07	
128	0.19±0.07		0.28±0.07		0.14±0.06		0.19±0.04	
256	0.14±0.04		0.19±0.03		0.10±0.04		0.15±0.04	
512	0.09±0.02		0.14±0.07		0.08±0.03		0.06±0.03	
1024	0.08±		0.09±0.05		0.07±0.02		0.04±0.02	

[1] OD+SD (n=8)
[2] Univaccinated controls

Tabel 4. Sow fimbrial serum IgA antibody responses to vaccination

Serum dilution	K88 response[1]		K99 response		F41 response		987P response	
	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B	Vaccine A	Vaccine B
8	0.68±0.16	0.61±0.18	0.38±0.07	0.30±0.08	0.64±0.16	0.57±0.06	0.55±0.12	0.59±0.22
16	0.49±0.18	0.43±0.11	0.31±0.07	0.22±0.06	0.48±0.09	0.52±0.07	0.39±0.07	0.39±0.09
32	0.35±0.13	0.33±0.09	0.24±0.04	0.16±0.04	0.38±0.07	0.36±0.07	0.31±0.07	0.27±0.06
64	0.26±0.09	0.26±0.07	0.18±0.04	0.10±0.03	0.18±0.07	0.26±0.06	0.24±0.07	0.21±0.04
128	0.20±0.03	0.19±0.06	0.14±0.05	0.09±0.03	0.18±0.05	0.17±0.04	0.16±0.03	0.16±0.03
256	0.12±0.02	0.12±0.04	0.07±0.03	0.05±0.02	0.12±0.02	0.11±0.04	0.11±0.03	0.09±0.02
512	0.09±0.02	0.07±0.02	0.05±0.02	0.03±0.01	0.06±0.01	0.06±0.03	0.05±0.03	0.05±0.01
1024	0.05±0.02	0.04±0.01	0.03±0.02	0.03±0.00	0.04±0.01	0.03±0.02	0.05±0.01	0.03±0.00

[1] OD +SD (n=8)

Tabel 5. Effect of vaccination on morbidity and mortality

Treatment	No. sows	No. piglets	Week 1		Week 2		Total deaths week 1 + 2 (%)
			Scours (%)	Deaths (%)	Scours (%)	Deaths (%)	
Vaccine A	19	159	14	6	5	5	11
Vaccine B	23	202	6	5	5	4	9
Unvaccinated	29	264	27	16	27	12	26

DISCUSSION

In this study the immune response to a multivalent killed whole cell *E. coli* vaccine, containing four important fimbrial antigens, was evaluated in pregnant. The choice of a killed vaccine was made as it was easy to developed and would avoid the spread of organisms, or the potential for vaccine failure should a live vaccine be used improperly in the field.

Intramuscular administration of both vaccine A and vaccine B to sows stimulated the production of anti-fimbrial IgG in serum and colostrum as well as anti-fimbrial IgA in the colostrum and, to a lesser extent serum. This indicates that secretory IgA antibodies to K88, K99, F41 and 987P were produced preferentially in the mammary gland rather in the blood. Following oral vaccination with live ETEC vaccine, it was reported that there was anti-K88 antibody of the IgA isotype in the serum and milk of sows (Kortbeek-Jacobs and van Houten, 1982; Bijlsma *et al.*, 1987). The increase in anti-fimbrial IgA in the colostrum or milk, is from the local production of the IgA isotype in the mammary gland (Bourne, 1977).

Both vaccine A and B produced good antibody responses and, therefore, the potential to control collibacillosis caused by ETEC K88, K99, F41 and 987P carrying *E. coli*. These results demonstrated that a such vaccine should provide passive protection to piglets able to suckle colostrum at birth. In addition, the initial study of the ETEC vaccine in pregnant sows demonstrated that neither vaccine A nor vaccine B had undesirable side effects, including site reactions and abortion. This finding supports a previous report from the USA where pregnant sows which received fimbriated *E. coli* vaccine of either 2 mL, 4 mL or 10 mL doses did not show unacceptable reaction at any of the dose levels (Schnackle, 1983).

Vaccination of pregnant sows prior to farrowing with whole bacterial cell of *E. coli* containing K88, K99, F41, and 987P fimbrial antigens under field conditions markedly reduced the neonatal diarrhoea and mortality rate in the suckling piglets in the first two weeks of life up to weaning (Table 5.). These findings are greater than those previously reported following the use of a combined oral and intramuscularly administered *E. coli* vaccine (Intagen, BOCH Silcock, UK) where the mean of mortality rate decreased from 13.8% to 7% (Chidlow *et al.*, 1979). However, they support those reported by Schnackel (1983). In addition, the neonatal diarrhoea and mortality rates in piglets born from unvaccinated groups decreased after the first trial (results not shown). This was probably due to the effect of vaccination which reduced dramatically the number of diarrhoea cases and thus the level of ETEC contamination in the environment.

Other studies have shown that the predominant challenge organism isolated from piglets of vaccinated sows was *E. coli* 987P (Supar *et al.*, 1991). This suggests that the 987P component of the vaccines is less antigenic than the other components and that acquired immunity is unable to control the heavy challenge the *E. coli* 987P. Serological assays have confirmed that both serum IgG and colostral IgG and IgA levels to 987P were slightly lower than for the other antigens (Tables 2,3,4).

Piglets are born without maternal transplacental immunity (Bourne, 1974). For protection against neonatal diarrhoea, newborn piglets rely initially on maternal antibodies obtained from the colostrum or milk. It was demonstrated by Evans *et al.* (1980) that anti-K88 IgG in the milk is indicative of anti-adhesive activity. However, Sellwood *et al.* (1975) found that IgM was associated with opsonic activity in the colostrum of adhesin-susceptible sows and IgG appeared to have a similar function, but with lower efficiency. More recently, it was reported that oral vaccination with live *E. coli* K88 significantly increased secretory IgA in the colostrum but not in the serum of farrowing sows (Kortbeek-Jacobs and van Houten, 1982). Bijlsma *et al.* (1987) also reported that oral immunisation of pregnant sows with live *E. coli* K88 induced anti-K88 specific IgA, IgM and IgG responses in colostrum but not in serum.

Further studies have been undertaken with the multivalent *E. coli* vaccine in piggeries in other locations in Indonesia. In all cases either vaccine A or vaccine B were able to significantly reduce the incidence of neonatal diarrhoea and death. Small differences which were observed in the efficacy of the two vaccines could be readily explained in terms of the levels of fimbrial-specific antibodies which were produced. ELISA was found to be a simple and convenient method of carrying out these determinations in both serum and colostral whey samples.

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