THE DETECTION OF ENTEROTOXIC *ESCHERICHIA COLI* WITH F41 FIMBRIAL ANTIGEN FROM PIGS IN INDONESIA

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ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) strains are the cause of diarrhoea in newborn piglets. A survey was undertaken in piggeries in the Bogor and Jakarta areas to detect the presence of ETEC infection. Rectal swab samples were collected from both normal piglets and those with diarrhoea. Each sample was cultured on McConkey agar and 5% sheep blood agar. All haemolytic colonies and 4 to 8 representative colonies from each McConkey plate were subcultured onto Minca agar with 1% Iso-Vitalex (VITOX). Specific F41, K99F41, K99, K88 antisera were produced in rabbits and were prepared as coagglutination reagents for fimbrial antigen detection. O-serotypes were identified by coagglutination. *E. coli* F41 serotypes were detected from 16% (138/858) of piglets with diarrhoea, but not from normal animals (0/65). The *E. coli* F41serotypes were associated with O-group 101 (375/378) and O-group 9 (3/378). *E. coli* K99F41 serotype was detected from 4 samples and was associated with O-group 101. *E. coli* K88K99 serotype was isolated from a swab from six-day-old piglet with diarrhoea and was associated with O-group 108. Most of these pathogens were associated with piglets aged between one and ten days which had diarrhoea.

ABSTRAK

Escherichia coli enterotoksigenik merupakan penyebab diare pada anak babi neonatal. Penelitian lapangan dan pengambilan sampel dilakukan di beberapa peternakan babi di daerah Bogor dan Daerah Khusus Ibukota Jakarta. Sampel usap rektal diambil dari anak babi diare dan yang tidak diare atau normal. Tiap sampel dibiakkan ke dalam medium agar McConkey dan agar darah domba 5%. Semua *E. coli* yang bersifat hemolitik dan 4 sampai 8 koloni *E. coli* pada agar McConkey disubkultur pada medium agar miring Minca yang diperkaya dengan 1% Iso-Vitalex (Vitox). Antiserum spesifik F41, K99F41, K88 dan K99 dibuat pada kelinci dan masing-masing antiserum spesifik tersebut dibuat reagen koaglutinasi untuk mendeteksi antigen fimbrial. Beberapa O-antiserum dibuat pada kelinci dan selanjutnya dibuat reagen koaglutinasi untuk O-serotyping. *E. coli* F41 dapat dideteksi sebanyak 16.0% (138/858) dari anak babi diare, tetapi tidak ditemukan pada anak babi normal. *E. coli* F41 termasuk ke dalam O-serogrup 101 (375/378) dan O-serogrup 9 (3/378). *E. coli* K99F41 dapat dideteksi dari 4 sampel anak babi diare dan tergolong ke dalam O-serogrup 101. *E. coli* K88K99 dapat dideteksi dari seekor anak babi diare umur 6 hari dan tergolong O-serogrup 108. Kebanyakan *E. coli* F41 ditemukan pada anak babi diare antara umur 1 sampai dengan 10 hari.

INTRODUCTION

At present, identification of enteropathogenic *Escherichia coli* causing neonatal diarrhoea in piglets and calves relies on the detection of the attachment of fimbrial antigens or pili, namely K88, K99, F41 and 987P (Gaastra and de Graaf, 1982; Morris *et al.*, 1982; Tzipori, 1985). *E. coli* containing K99 fimbrial antigen is the cause of neonatal diarrhoea in calves (Acres *et al.*, 1977), lambs (Sojka, 1971) and piglets (Tzipori *et al.*, 1980; Gaastra & de Graaf, 1982). *E. coli* containing K88 antigen is commonly associated with neonatal and post-weaning diarrhoea of piglets (Tzipori *et al.*, 1980; Harnett and Gyles, 1983; Supar, 1986; Gonzales and Blanco, 1986; Wilson and Francis, 1986; Nakazawa *et al.*, 1987). *E. coli* containing 987P fimbrial antigen has also been associated with neonatal diarrhoea in piglets (Isaacson & Richter, 1981).

F41 fimbrial antigen was first reported on the K99 reference strain of *E. coli* F41 by Morris *et al.* (1978) and further characterised by Morris *et al.* (1980). The F41 antigen was also detected on *E. coli* strains from piglets with diarrhoea that lacked K88, K99 and 987P adhesin antigens (Morris *et al.*, 1983, and To, 1984).

The present study is undertaken to survey for the presence of the F41 fimbrial antigen on *E. coli* isolates from piglets with diarrhoea and to determine their relationships with O-serogroups.

MATERIALS AND METHODS

Sample Collection

Rectal swab samples were collected from piglets with diarrhoea and from some normal piglets from 2 piggeries in Bogor and 3 piggeries from Jakarta Capital Territory over a period of 7 weeks (June – July 1988). After collection the swab samples were placed in Amies' transport medium and conveyed to the laboratory.

E. coli isolation was as that described by Supar (1986). Briefly each sample was cultured on McConkey agar and 5% sheep blood agar (SBA) and incubated overnight at 37°C. A selection of 4 to 8 colonies from McConkey agar and all haemolytic colonies on the SBA plates were subcultured on Minca agar enriched with 1% Iso-vitalex (Guinee *et al.*, 1976) and incubated overnight at 37°C.

K88, K99, K99F41 and F41 Antisera Production

K88, K99 antiserums were produced in rabbits according to the method as described by Supar (1986). K99F41 antiserum was prepared from a *E. coli* reference strain B41 (O101, K99F41) and F41 antiserum from a local *E. coli* isolate (R55) which produces only F41 fimbrial antigen. The R55 isolate was characterized using reagents kindly supplied by the Bendigo Veterinary Laboratory, Australia. The method of Murray (1987) was then applied to produce coagglutination reagent from the antiserum.

Detection of F41 Fimbrial Antigen

Each Minca agar culture was first screened by slide coagglutination for K99F41 or K88. Any culture that gave a K99F41 positive coagglutination was then tested with F41 and K99 prepared as coagglutination reagents. If the culture agglutinated with K99F41 and F41 reagents but not with K99 reagent, the culture was recorded as F41 positive. If it did not agglutinate with F41 reagent but agglutinated with K99 reagent, then it was recorded as K99 positive. If the culture agglutinated with all of the three reagents, it was recorded as K99F41 positive. K88 positive samples were not considered further.

Pili suspensions from all isolates which were K99 and F41 positive, were prepared as described by Murray (1987) and were retested as described above.

O-Antisera Preparation

Some O-antisera were prepared in rabbits for O-serotyping using the method of Sojka (1965) with slight modification. O-group antiserum 8, 9, 20, 35, 45, 64, 98, 101, 108, 115, 119, 138, 139, 141, 147, 149, 157 were prepared as coagglutination reagents. A smooth colony of a particular *E. coli* O-serotype was immediately subcultured onto 5% SBA and incubated overnight at 37°C. A cell suspension was then prepared in distilled water. The cell suspension of haemolytic *E. coli* was boiled at 100°C for an hour, whereas suspension of non-haemolytic *E. coli* was autoclaved at 121°C for 2 hours, after which they were centrifuged at 3,000 rpm for 15 minutes and the supernatant removed. The pelleted cells were resuspended in sterile non-pyrogenic saline (P.T. Otsuka Indonesia) for inoculation into New Zealand white rabbits. Each rabbit was inoculated intravenously at 4-day intervals with 0.25 ml, 0.5 ml, 1.0 ml, 1.5 ml and 2 ml of antigens having a turbidity equal to Brown opacity tube no. 1, 2, 3, 5 and 6 respectively. One week after the last inoculation the rabbits were bled and the serums were collected and stored at −20°C. O-coagglutination reagent was prepared by mixing 0.1 ml O-antiserum with 0.9 ml of 10% *Stapylococcus aureus* (Cowan I) cell suspension. The mixture was incubated at room temperature for 3 hours with periodic shaking. Specificity and sensitivity of these reagents were tested using standardised reagents kindly provided by the Department of Agriculture, Regional Veterinary Laboratory Bendigo, Australia.

O-Serotyping

The O-serotype of *E. coli* F41 or K99F41 isolates were determined by slide agglutination with monospecific antisera prepared as coagglutination reagents (Connaughton, 1987, personal communication). Each *E. coli* isolate was grown on SBA overnight at 37°C. O-somatic antigens were released by suspending a loopful of cells in 2 ml sterile 0.9% NaCl and then autoclave the suspension at 121°C for 2 hours. Haemolytic *E. coli* isolates were prepared in the similar manner, but were boiled at 100°C for 1 hour.

A loopful of each heat treated cell suspension was put on a glass slide and mixed with a loop of O-coagglutination reagent, the mixture was gently shaken and then observed for the presence of an agglutination reaction within 15 to 30 seconds.

Heat-Stable Toxin (ST) Detection of *E. coli* F41

The presence of heat-stable toxin in *E. coli* F41 organisms was assayed in 1 to 3-day old mice according to the method of Giannella (1976) which was modified by Supar (1987).

RESULTS AND DISCUSSION

One hundred and thirty eight of 858 (16%) rectal swabs from piglets with diarrhoea were positive for

E. coli F41 fimbrial antigen, whereas F41 antigen was not detected in *E. coli* from any of the 65 swabs from piglets without diarrhoea (Table 1). K99F41 fimbrial antigen was also detected in the *E. coli* isolates from 4 swab samples taken from one piggery (Table 1).

Table 1. Detection of *E. coli* fimbrial antigen from piglets with and without diarrhoea

Piggery	Piglets sampled	*E. coli* fimbrial antigen		
		F41	K88K99	K99F41
		No	No	No
R	145 diarrh.	19	–	–
	22 normal	–	–	–
IB	98 diarrh.	9	–	–
	8 normal	–	–	–
BT	179 diarrh.	3	–	–
	7 normal	–	–	–
L	64 diarrh.	1	–	–
	5 normal	–	–	–
G	344 diarrh.	106	1	4
	23 normal	–	–	–
5 small holdings	28 diarrh.	–	–	–
Total	858 diarrh.	138	1	4
	65 normal	–	–	–

Notes: No = number of piglets from which organism type isolated
5 small holdings: AT, SH, AS, BS and TM

F41, K99 and K99F41 pili suspension when tested by coagglutination gave the same results as the cell suspension. In the case of a culture that auto-agglutinated, the preparation of a pili suspension permitted the culture to be serotyped using the coagglutination method.

The prevalence of *E. coli* containing F41 antigen in 16% of the pigglets sampled was much higher than in diarrhoea due to other *E. coli* serotypes as reported previously by Supar *et al.* (1988). They found a prevalence of *E. coli* containing fimbrial antigen K88 in 2.2% (19/863) and for *E. coli* K99 antigen in 10.8% (93/863) from the samples collected in the similar manner. The higher prevalence of diarrhoea caused by *E. coli* containing F41 antigen is in agreement with that found in other countries (Morris *et al.*, 1983; To, 1984; Nakazawa *et al.*, 1987).

E. coli F41 were mostly isolated from piglets up to 2 weeks of age which had diarrhoea while after this age only 6 out of 138 piglets were positive (Table 2). Detection of this strain in post-weaning diarrhoeal cases was uncommon and *E. coli* F41 strain was not

Table 2. Non-haemolytic *E. coli* F41 strains isolated from piglets with diarrhoea and their relationship with O-serogroups

Piggeries	Age (days)	Piglets sampled	Samples positive F41 antigen	O-serogroup
			No	
G	1 – 3	167	63	O101
	4 – 6	71	23	O101
	7 – 9	42	9	O101
	10 – 12	37	7	O101
	13 – 15	19	4	O101
	16 – p.w.	8	–	–
Sub-total		344	106	
B	1 – 3	62	1	O101
	4 – 6	24	1	O9
	7 – 9	43	1	O101
	10 – p.w.	70	–	–
Sub-total		179	3	
L	1 – 3	39	1	O101
	4 – 33	25	–	–
Sub-total		64	1	
IB	1 – 3	11	1	O9
			4	O101
	4 – 6	14	2	O101
	7 – 49	73	–	–
	p.w.	14	2	O101
Sub-total		112	9	
R	1 – 3	17	7	O101
	4 – 6	20	5	O101
	7 – 10	34	2	O101
	11 – 20	51	3	O101
	21 – 30	23	2	O101
Sub-total		145	19	

Notes: No = number of swabs from which organism type isolated
p.w. = post-weaning diarrhoea

detected in any piglets sampled which did not have diarrhoea. *E. coli* possessing both K88 and K99 fimbrial antigens was isolated from a piglet in a piggery (Table 3). This finding is interesting as there are no

Table 3. *E. coli* K99 F41 and K88 K99 strains and their relationships with O-serogroups

Farm	Age of piglets	*E. coli* serotypes	No. isolates	O-group
G	1 day	K99F41 (NH)	1	O101
	2 days	K99F41 (NH)	1	O101
	3 days (2 piglets)	K99F41 (NH)	5	O101
	4 days	K99F41 (NH)	1	O101
	6 days	K88K99 (H)	8	O108

Notes: H – haemolytic on 5% sheep blood agar
NH – non-haemolytic on 5% sheep blood agar

other reports on these fimbrial antigens being found in a single organism.

E. coli containing F41 or K99F41 fimbrial antigen were all non-haemolytic on 5% SBA. *E. coli* possessing K88 and K88K99 antigens were haemolytic on 5% sheep blood agar whereas an isolate containing only K99 antigen was non-haemolytic. Haemolytic *E. coli* strains lacking of K88, K99, F41 and 987-P fimbrial antigens were detected in a limited number of swab samples from piglets 1-day old up to 10 days post-weaning which had diarrhoea. Hoblet *et al.* (1986) detected haemolytic *E. coli* lacking K88, K99, 947P and F41 fimbrial antigens associated with post-weaning diarrhoea in pigs. These *E. coli* strains contained K85ab and K85ac capsular antigens. Although similar serotypes were found in the piggeries studied, post-weaning diarrhoea did not appear to be a serious problem towards piggeries in the Bogor or Jakarta areas (unpublished data).

The majority of *E. coli* F41 strains were associated with O-group 101 and a small number only were associated with O-group 9 (Table 2). *E. coli* K99F41 isolates were also associated with O-group 101 (Table 3), whereas *E. coli* possessing K88K99 antigen serotype were associated with O-group 108 (Table 3). *E. coli* possessing only K88-adhesin which were detected from the same sample also associated with O-group 108. An *E. coli* possessing K99 antigen associated with O-group 101 was also detected.

Three isolates of *E. coli* with F41 fimbrial antigen obtained from 3 different piggeries were tested for their production of enterotoxin by means of a suckling-mouse bioassay. The 3 isolates tested were positive for heat-stable toxin (STa) (Table 4) which agrees with that reported in the United States of America by Wilson and Francis (1986), and in Japan by Nakazawa *et al.* (1987) for *E. coli* containing F41 antigen.

The findings indicate that *E. coli* containing F41 fimbrial antigen is an important enteric pathogen of newborn piglets up to 2 weeks of age in piggeries in Indonesia. Most F41 strains were detected on *E. coli* lacking K88, K99 and 987P but some were associated with K99 antigen. The *E. coli* F41 strains were only associated with O-serogroups O9 and O101.

In conclusion, enteropathogenic *E. coli* containing adhesive antigens F41 may be a major problem in neonatal diarrhoea which leads to death of infected animals and so can cause major economic losses in the pig industry. Further work needs to be carried out to determine the ways of minimising the effects of enteropathogenic *E. coli*.

Table 4. Detection of heat stable enterotoxin (STa) by means of suckling mouse bioassay of *E. coli* F41 strains isolated from piglets

	E. coli strain	Ratio G:C	STa evaluation
1.	Non inoculated baby mice	0.0502	–
2.	Non inoculated medium	0.0515	–
3.	*E. coli* negative for all pili types from normal piglet	0.0508	–
4.	Reference strains *E. coli*		
	Compt K12K99	0.0927	+
	B41 (O101 K99 F41)	0.0887	+
5.	*E. Coli* from piglets with diarrhoea		
	Isolate G1164 (O101 K– F41)	0.1078	+
	Isolate IB53 (O101 K– F41)	0.0826	+
	Isolate R99 (O101 K– F41)	0.0832	+

Notes: G : gut weight
C : carcass weight
G, IB, R : piggery codes
K– : capsular antigen not identified

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