Managemental influences on the selective proliferation of two strains of haemolytic *Escherichia coli* in weaned pigs

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SUMMARY

In an experimental study on a piggery it was found that haemolytic *Escherichia coli* of O-serotypes 138 or 139 proliferated in the intestinal tracts of pigs following weaning, with *E. coli* of the O-138 type also being occasionally recovered from unweaned pigs, and once from a sow. Organisms of the O-138 type produced heat labile enterotoxin and their presence in weaned pigs was associated with the development of severe post-weaning diarrhoea. *E. coli* of O-139 type produced a vero cell cytotoxin and were associated with a milder diarrhoea in weaned pigs. Under various managemental circumstances the O-138 type *E. coli* almost invariably proliferated after weaning. The O-139 strain of *E. coli* did however proliferate rather than the O-138 strain following the movement of weaned pigs to new accommodation, after weaned pigs were returned to their sow and then weaning again 5 days later, and very occasionally in pigs weaned at 5 weeks of age. In all these cases earlier proliferation of the O-138 *E. coli* had been detected, suggesting that this may be a prerequisite for proliferation of the O-139 strain.

INTRODUCTION

Shortly after pigs are weaned it is common for their coliform flora to alter from one comprising mainly non-haemolytic *Escherichia coli* strains to one dominated by a single haemolytic *E. coli* strain (Kenworthy & Crabb, 1963; Chopra, Blackwood & Dale, 1964; Hinton *et al.* 1985). This alteration is frequently, but not invariably, associated with the subsequent development of diarrhoea in these animals (Miniats & Roe, 1968). Haemolytic isolates may produce heat stable and/or heat labile enterotoxins (Smith, 1976), or vero cell cytotoxins (Smith, Green & Parsell, 1983). The plasmid-mediated haemolysin is itself not essential for entero-pathogenicity (Smith & Linggood, 1971).

It has been noted that two serotypes of potentially pathogenic haemolytic *E. coli* capable of infecting weaned pigs were present in a piggery (Hampson *et al.* 1986). The purpose of the present investigation was to examine more fully the distribution of these two strains among weaned pigs on the piggery, and to

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attempt to influence when they proliferated. It was hoped to gain an insight into the circumstances whereby it is usual for one strain of haemolytic *E. coli* to selectively proliferate after weaning.

MATERIALS AND METHODS

Animals and housing

The piglets used in these experiments were the offspring of Landrace × Large White sows and Landrace boars, from the Pig Research Centre, Massey University. The animal accommodation was routinely pressure-hosed, disinfected with a commercial potassium hydroxide solution, and rested between batches of pigs.

(i) *Experiment 1*

The movement of the pigs studied in expt 1 is summarized in Fig. 1. Fifty piglets from five litters were monitored daily from birth. At 25 days of age, 24 of these piglets were selected at random, weaned and moved into individual wire-mesh cages housed in isolation in a controlled-temperature room maintained at 28 °C. Weaner diet and fresh water were available to them *ad libitum*. Eight of the weaned pigs and four unweaned pigs were slaughtered at 33 days of age. At 47 days of age the remaining 16 caged pigs were transferred to a pen in flat-deck weaner accommodation in a controlled-temperature room. The remaining 22 unweaned pigs were weaned at 35 days of age into similar adjacent pens in another weaner room.

(ii) *Experiment 2*

A summary of pig movements in expt 2 is given in Fig. 2. Sixteen piglets from two litters were cross-suckled from birth, and assigned to one of four groups. Pigs of group 1 (animals 1–4) and group 2 (animals 5–8) were weaned together at 21 days of age and moved to flat-deck weaner accommodation in an otherwise unoccupied weaner room. The unweaned pigs of group 3 (animals 9–12) and group 4 (animals 13–16) remained with one of the two sows. At 26 days of age, the weaned pigs of group 2 were returned to this sow, and the unweaned pigs of group 3 were transferred in their place to the weaner house to join the pigs of group 1. At 31 days of age the unweaned pigs group 4 and the pigs of group 2, which had been weaned and then returned to the sow, were all weaned and moved to a pen adjacent to the other weaned animals.

Sampling

Swabs of rectal faeces were taken daily from all piglets from birth, and from their sows, until the end of the experiments. Occurrence of diarrhoea was recorded. Swabs of mid-jejunal contents and rectal faeces were taken from the 12 slaughtered piglets in expt 1.

Bacteriology

The swabs were used to inoculate split-layer agar plates containing 5% sheep red blood cells in the upper layer and incubated aerobically overnight at 37 °C. The approximate proportion of β-haemolytic coliform colonies amongst the flora
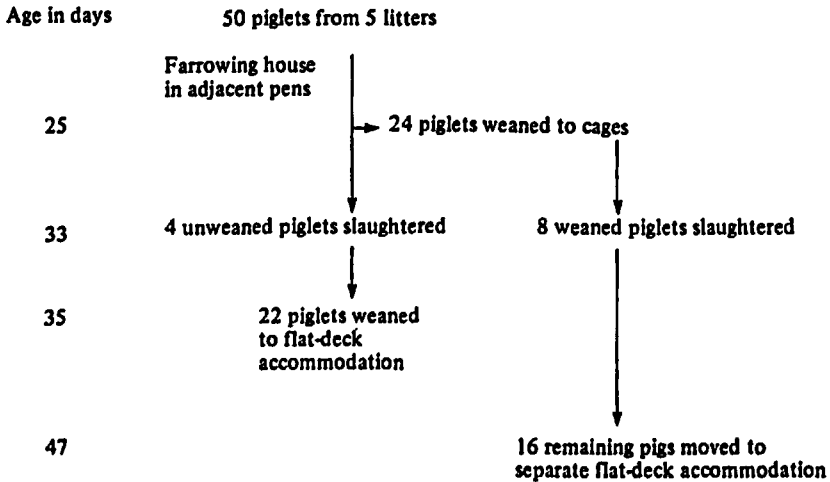
Age in days   50 piglets from 5 litters

                     Farrowing house
                     in adjacent pens

25   ► 24 piglets weaned to cages

33   4 unweaned piglets slaughtered   8 weaned piglets slaughtered

35   22 piglets weaned
            to flat-deck
            accommodation

47   16 remaining pigs moved to
                                                separate flat-deck accommodation

Fig. 1. Movement of piglets in expt 1.

Age in days   16 piglets from 2 litters

            Cross-suckled in
            farrowing house

21   8 piglets weaned to
            8 piglets remain   flat-deck (groups 1 and 2)
            with 1 sow
            (groups 3 and 4)

25   Group 2 returned
                     to sow

                     Group 3 weaned,
                     joins group 1

31   8 piglets weaned to   Groups 1 and 3 in
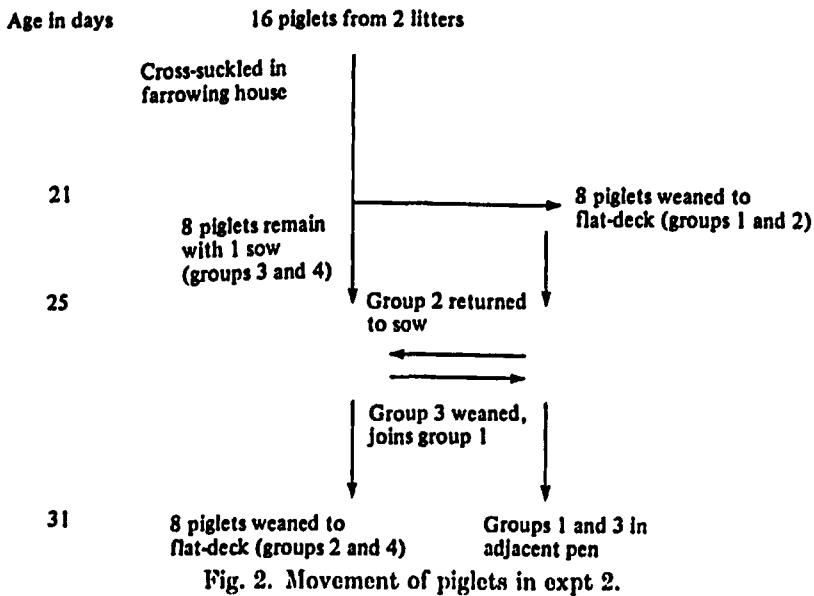   flat-deck (groups 2 and 4)   adjacent pen

Fig. 2. Movement of piglets in expt 2.

was estimated. Three haemolytic coliform-like colonies were selected from these plates from each animal when the following circumstances occurred: (i) when haemolytic coliforms first comprised more than half of the colonies, (ii) again 3 or 4 days after the first sampling if the animals excretion of haemolytic coliforms was continuous, (iii) again when haemolytic coliforms exceeded half of the colonies after there had been a distinct break of several days following a previous period of excretion of haemolytic coliforms, (iv) 3 or 4 days later if the latter period of excretion was continuous and (v) when haemolytic coliforms were present in the jejunal contents of slaughtered piglets. All selected colonies were transferred to bile lactose agar plates to observe their growth on these, and were examined for production of urease on Christensen's urea medium and indole production in peptone water after addition of Kovacs' reagent (Cowan, 1974). Representative

isolates were also examined using API 20E tests to confirm their likelihood of being *Escherichia coli*. Typical β-haemolytic lactose-fermenting colonies were grouped according to their urease and indole reactions.

Serotyping

Most haemolytic *E. coli* colonies could be separated into two distinct groups on the basis of their unusual urease and indole reactions. Representative isolates from these two groups were O-serotyped using antisera against O-types 8, 138, 139, 141 and 149 (Bettleheim & Reeve, 1982), since these are common O-types amongst pathogenic *E. coli* affecting pigs. Thirty-four isolates having O-types 138 and 139 were tested for their ability to produce heat labile toxin using Y1 adrenal cells (Bettleheim *et al.* 1980) and Shiga-like vero cell cytotoxins using vero cells (Knowalchuk, Spiers & Stavric, 1977).

RESULTS

Typing

Urease and indole reactions of the haemolytic *E. coli* isolated gave a useful screening method for identifying isolates as O-types 138 and 139 (Table 1). All but one of the 21 O-138 colonies produced heat labile enterotoxin, whilst all 13 O-139 isolates produced Shiga-like vero cell cytotoxin.

Experiment 1

All 24 pigs weaned into cages at 25 days of age excreted haemolytic *E. coli* in their faeces after weaning, and 16 developed diarrhoea. The haemolytic isolates from these pigs were urease- and indole-positive, and were presumed to be serotype O-138. Colonies of the O-138 type were also recovered from the jejunum of 8 of these pigs when they were slaughtered at 33 days of age. When the surviving 16 piglets from the group were moved to the flat-deck weaner accommodation of 47 days of age, 9 subsequently excreted haemolytic *E. coli* and 2 developed diarrhoea. These isolates were urease negative and either negative or only weakly positive for indole production, with representative isolates proving to be of the O-139 serotype. Colonies of the O-138 type were not found.

Nine (35%) of the 26 unweaned piglets excreted haemolytic *E. coli* of the O-138 type in small numbers (less than 5% of the total coliform organisms) between 3 and $4\frac{1}{2}$ weeks of age. Two of the four unweaned pigs slaughtered at 33 days of age had similar light growths of the O-138 type organism recovered from their jejunal contents. After these pigs were weaned at 35 days of age, 20 excreted haemolytic coliforms of the O-138 type, and two, which were originally from separate litters, excreted the O-139 type *E. coli* as the predominant haemolytic type. The latter two animals had excreted O-138 type *E. coli* before weaning. Seven of the 22 pigs developed diarrhoea, including one of the two animals excreting *E. coli* of the O-139 type.

Experiment 2

The excretion patterns of haemolytic *E. coli* presumed to be serotypes O-138 and O-139 are presented in Table 2. Pigs of groups 1 and 2, which were weaned at

Table 1. *Relationship of urease and indole reactions and O-serotype of representative haemolytic* Escherichia coli *isolates*

Urease	Indole	Number of isolates	O-serotype					
			8	138	139	141	149	NT*
+	+	21	–	21	–	–	–	–
–	–or±	13	–	–	13	–	–	–
+	–	1	–	–	–	1	–	–
–	+	14	–	–	–	–	–	14

* Not typable with O-sera 8, 138, 139, 141 and 149.

Table 2. *Excretion of haemolytic* E. coli *of serotypes O-138 and O-139 in piglets from expt 2.*

Age (days)	Pig no.	Group 1				Group 2				Group 3				Group 4				Sow
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
21																		
22																		
23				+														
24		+		+														
25		+		+	+	+	+	+	+									
26		+		+	+	+	+	+	+									
27		+		+	+	+	+	+	+									
28		+	+	+	+	+	+	+	+	+		+				+		*
29		+	+	+	+		+	+	+	+	+	+	+	+	+	+		*
30		+	+	+	+		+			+	+	+	+	+	+	+		*
31		+	+	+	+					+	+	+	+	+	+	+		*
32		+	+	+						+	+	+		+				
33		+	+	+			⊕			+	+	+						
34		+				⊕	⊕			+	+	+						
35		+				⊕	⊕	⊕	⊕	+					⊕			
36		+				⊕	⊕	⊕	⊕	+				+	⊕	⊕	⊕	
37						⊕	⊕	⊕	⊕	+				+	⊕	⊕	⊕	
38				⊕		⊕	⊕		⊕					+	⊕	⊕	⊕	
39				⊕			⊕		⊕		⊕			+	⊕	⊕	⊕	
40				⊕			⊕		⊕		⊕			+	⊕	⊕	⊕	
41				⊕			⊕		⊕		⊕			+	⊕	⊕	⊕	
42				⊕					⊕		⊕			+		⊕		
43											⊕			+				
44											⊕			+				
45											⊕							
46											⊕							

------, day of weaning.
======, day of return to sow.
≡≡≡≡≡, day of re-weaning.

+, excretion of presumptive 0–138 *E. coli* } > 50% aerobic faecal flora.
⊕, excretion of presumptive 0–139 *E. coli* }
*, excretion of presumptive 0–138 by sow (< 50% faecal flora).

21 days of age, all excreted *E. coli* of the O-138 type after weaning, and developed diarrhoea. Pig no. 4 (group 1) also subsequently excreted the O-139 *E. coli* strain, and developed mild diarrhoea. Pigs of group 2, which were returned to the sow, excreted the O-138 *E. coli* for a shorter period than did the animals of group 1, which were not returned. When the four animals of group 2 were weaned for a second time at 32 days of age, all excreted haemolytic *E. coli* of the O-139 type, and all suffered a mild diarrhoea. Pigs of group 3, which were weaned at 26 days of age, all excreted the O-138 type haemolytic *E. coli* after weaning and developed diarrhoea; pig no. 11 from this group also subsequently excreted the O-139 *E. coli* strain, but remained healthy. Following the return of the pigs of group 2 to the sow, both the sow and 3 of the 4 unweaned pigs of group 4 excreted the O-138 type *E. coli*. The three infected piglets developed a mild diarrhoea, but the other piglet and the sow remained unaffected. Following weaning of these pigs the three which had previously been infected with O-138 *E. coli* shed the O-139 type *E coli* and developed a mild diarrhoea. Pig no. 13, which had not excreted haemolytic coliforms before weaning, excreted the O-138 type *E. coli* and suffered from a more severe diarrhoea.

DISCUSSION

Escherichia coli are generally considered to be negative for urease activity and positive for indole production, although serotypes associated with porcine post-weaning diarrhoea may have urease activity (Larsen, 1976). Investigation of the distribution of *E. coli* of O-types 138 and 139 amongst weaned pigs in this study was facilitated because these two haemolytic strains had distinct and atypical urease activity and indole production, which could be used to distinguish isolates, and which correlated with O-type.

Haemolytic *E. coli* of the O-138 serotype were widely distributed amongst the animals sampled. Approximately one third of pigs which were weaned at 5 weeks of age excreted low numbers of these bacteria in their faeces before weaning. A sow which was exposed to weaned piglets which were shedding the organism also became infected, but, like most of the infected unweaned piglets, did not develop diarrhoea. The majority of the pigs which had been recently weaned at various ages and under different circumstances excreted large numbers of the O-138 strain of *E. coli*, and many developed diarrhoea. These observations confirm the role of the O-138 *E. coli* strain as an enteric pathogen of weaned pigs on this piggery.

Certain weaned animals suffered two distinct continuous periods of excretion of haemolytic *E. coli*, but in all these cases the O-138 strain was shed in the first period, and the O-139 strain in the second. In the first experiment, 2 of 22 pigs which were weaned at 5 weeks of age excreted *E. coli* of the O-139 strain immediately after weaning, and 9 of 16 did so after they were moved to new accommodation: in the second experiment 9 of 16 pigs excreted this strain after being weaned or reweaned. Again all these animals had excreted *E. coli* the O-138 serotype at some time before they excreted the O-139 type. No clear explanation for this selective proliferation of the two strains was obtained. The two strains might have required different trigger mechanisms allowing them to proliferate, but the only consistent circumstance preceding proliferation of the O-139 strain was prior proliferation of the O-138 strain. The latter strain, which readily

proliferated after weaning, may in some unexplained way have altered the environment of the gastrointestinal tract so as to facilitate multiplication of the O-139 strain. Some insight into this could have been gained by examining the sites and extent of proliferation of the O-139 strain within the gastrointestinal tract, but unfortunately none of the pigs which were slaughtered were infected with this strain. Another possible explanation for the appearance of the two strains at different times in the same individual involves the strains actively competing with each other for establishment within the gastrointestinal tract of susceptible, recently weaned pigs, with the O-138 strain predominating. Development of active immunity against the O-138 strain would then favour proliferation of the O-139 strain when suitable conditions for rapid growth occurred for a second time (following reweaning or movement to new accommodation). A possible mechanisms for competition between the two strains would involve their production of colicines (Craven & Barnum, 1971) since most *E. coli* strains associated with post-weaning diarrhoea are colicinogenic (Vasenius 1967; Larsen, 1976). The two *E. coli* types in this study were incompatible when grown *in vitro* (Hampson *et al.* 1986), but further work is required to examine the basis of this incompatibility, and to investigate the relative growth rates of the two strains when grown both alone and together.

The O-139 strain of *E. coli* produced a vero cell cytotoxin, but such toxins by themselves have not been shown to cause diarrhoea in weaned pigs. No attempt was made to assay for production of heat stable toxin 'b', which causes fluid accumulation in ligated intestinal loops of weaned pigs (Burgess *et al.* 1978). Production of this or some uncharacterised toxin by the O-139 strain could explain the diarrhoea occasionally seen in pigs infected with this strain. Also since heat stable toxins tend to produce a less severe diarrhoea than does heat labile toxin (Morris & Sojka, 1985), this could explain why infection with the O-138 strain produced a more severe clinical disease than did infection with the O-139 strain. Other differences between the two strains in extent and site of proliferation in the gastrointestinal tract may also have contributed to apparent differences in their virulence, but again this matter was not investigated in the present study.

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