Phenotypic Characterization of Intestinal *Escherichia coli* of Pigs during Suckling, Postweaning, and Fattening Periods

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A highly discriminatory and standardized biochemical fingerprinting method was used to monitor the persistence and colonization of intestinal *Escherichia coli* isolated from the feces of four sows and their litters (four piglets from each) during the suckling, postweaning, and fattening periods. Altogether, 195 fecal samples were collected and 1,827 *E. coli* strains were tested (mean number of isolates tested per fecal sample per pig, 9.5). Strains were divided into similarity groups on the basis of their biochemical phenotypes (BPTs). The diversity of *E. coli* strains in each sample was measured with Simpson's index of diversity, and similarity between *E. coli* floras of piglets was calculated with a population similarity index. Each fecal sample contained several BPTs of *E. coli*, some of which dominated that population. The intestinal colonization of piglets consisted of successive waves of different *E. coli* BPTs, the tenure of which varied from a few days to 2 weeks. Most of these BPTs disappeared in the succeeding samples and were not recovered again from the same piglets. On the other hand, some *E. coli* strains which colonized piglets early during the suckling period persisted for a long period and were referred to as resident BPTs. Each piglet carried more than one resident BPT (mean of 2.4 BPTs per pig), some of which were also found in other piglets. This finding indicates that both strain and host specificity are important for colonization and persistence of *E. coli* in pigs. During the first week of life, *E. coli* floras of piglets were quite different from those of their dams, suggesting that the dominant *E. coli* strains in piglets might be quite different from those of their sow. Despite the similarity in *E. coli* floras between littermates during suckling, each piglet developed a unique flora during postweaning and fattening periods. Pigs which were kept together in one litter until the end of the study had *E. coli* floras more similar to each other than those mixed with other pigs.

The gastrointestinal tract of the pig is sterile at birth, but within a few hours, it becomes flooded with microorganisms which, despite the relatively low gastric pH, are able to survive. The colonizing bacteria are proposed to be mainly acquired from maternal feces, skin, and teats (1, 2). In fact, it has been reported that suckling piglets eat considerable amounts of their sow's feces (34). *Escherichia coli* cells, together with streptococci of Lancefield groups D and K and *Clostridium perfringens*, are among the earliest bacteria to colonize the gut in piglets (4). The composition of the intestinal *E. coli* floras in pigs is very complex, and it has been reported that different strains may be site specific (9). These bacteria are considered to be normal inhabitants of the intestinal tract, with specific strains being an important cause of diarrhea. The etiology (25–27), epidemiology (21, 30, 32), and pathogenesis (6, 33, 39, 40) of *E. coli* causing pre- and postweaning diarrhea in pigs have been the subject of many studies. However, there is relatively little information about the ecology and behavior of nonenteropathogenic *E. coli* throughout the animal's life. One major problem influencing the study of this complex population is the lack of a suitable method to characterize a large number of strains below the species level with high precision and within a short period. Available studies of the intestinal *E. coli* floras in healthy unweaned and weaned pigs (9, 10), as well as other farm animals (12, 15), rely heavily on O serogrouping (14), biotyping (8), antimicrobial agent resistance patterns (11,

13), or combinations of different methods (12, 15, 37, 38). These methods either are not sufficiently discriminatory to trace the colonization and persistence of a given strain (e.g., O serogrouping) or their typing markers are not genetically stable enough throughout the period of investigation (e.g., antibiotic resistance genes). We have previously reported a computerized biochemical fingerprinting method (the Phene Plate [PhP] system) that uses quantitative measurements of the kinetics of several biochemical reactions of bacteria in liquid media in microtiter plates and that yields, for each strain, a biochemical fingerprint consisting of several quantitative data (28). A personal computer program calculates similarities among the strains tested, and the strains are divided into biochemical phenotypes (BPTs). The system was initially developed for typing *E. coli* strains (19) and has been used to study phenotypic variations among enterotoxigenic *E. coli* strains belonging to certain O serogroups in pigs (21) and in human populations with diarrhea (18, 23). The stability of its biochemical markers upon storage and subculturing has also been evaluated (17). We have recently used the PhP system to study the diversity and stability of the intestinal coliforms in piglets during their first 3 months of life (22). The system was shown to be very discriminatory, and its use enables researchers to monitor the spread of bacterial phenotypes between different samples. Because the PhP system is also very simple to use, it can be applied to studies involving large numbers of isolates and therefore is an excellent tool for studying the persistence of intestinal bacteria over a long period of time.

The aim of the present study was to use the PhP system to characterize the intestinal *E. coli* floras of pigs during the suckling, postweaning, and fattening periods.

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MATERIALS AND METHODS

Animals. Altogether, 16 piglets from four litters (4 piglets from each) in the same stable, designated O, P, Q, and R, and their sows were included in this study. All animals were obtained from a herd of Swedish Yorkshire sows crossed with a Swedish Landrace boar at Funbo-Lövsta Research Station, Swedish Agricultural University, Uppsala, Sweden. The piglets were normally not given any antimicrobial drugs, except piglet O1 (from litter O) and piglet P4 (from litter P), who were intramuscularly given a sulfamethoxazole-trimethoprim-containing solution (Borgal, Hoechst, Germany) for 3 days during a diarrhea outbreak which occurred in the stable in the third week of the study. The outbreak resulted in 15 deaths among the piglets in the stable, including 2 piglets in our study from litters P (piglet P3) and R (piglet R4).

Management of the pigs. Piglets of all litters were born on the same day (day 1). They were fed by their dams, and from day 7, a commercially prepared pelleted feed and an electrolyte solution (Ewolyt) were offered ad libitum. All piglets were weaned at 34 days of age. On day 70, pigs of litters O, P, and R were transported for approximately 6 h with other pigs that were to be mixed ($n = 50$). On their return, they were introduced into a fattening stable (stable 2) and mixed according to their weights with pigs of other litters in different boxes. Pigs of litter Q remained together (unmixed group) and were moved along a corridor to stable 2 and put in a box on the other side of a center aisle. The box, however, did not prevent nose-to-nose contact with pigs of the next box. On the same side were four other unmixed groups of pigs. They remained unmixed until the end of the study. (The total number of pigs in the stable was 98.)

Collection of samples. Fecal samples were collected from piglets and their sow by insertion of 3 cm of a charcoal swab into the rectum. Swabs were transported in Cary and Blair transport medium (NBL, Stockholm, Sweden), stored at 4°C, and cultured on MacConkey agar within 24 h. Fecal samples were collected on days 3, 7, 17, 27, and 41; after that, samples were collected weekly for 4 weeks (samples 6 to 9) during the postweaning period and for an additional 2 weeks (samples 10 and 11) after the pigs were transferred to the fattening stable. Finally, two more samples (samples 12 and 13) were collected on days 114 and 145 before the pigs were slaughtered. From the two piglets, P3 and R4, who died during the diarrhea outbreak, only three samples were tested. In all, 179 samples from piglets and 16 samples from sows (collected until the weaning period) were collected.

Isolation and identification of *E. coli*. From each MacConkey agar plate, up to 12 (where possible) lactose-fermenting colonies with the appearance of *E. coli* were randomly selected and identified on the basis of their biochemical profiles by the methods of Edwards and Ewing (5). Altogether, 1,827 isolates were confirmed as *E. coli*. They were stored on nutrient agar slopes at 4°C until tested.

Biochemical fingerprinting. The principles of the PhP system for biochemical fingerprinting of bacteria and the preparation of the plates have been described before (17–19). The 24 tests used for biochemical fingerprinting of *E. coli* were carefully selected to give the highest degree of discrimination among these bacteria and included tests for D-xylose, maltose, lactose, sorbitol, rhamnose, L-fucose, glycerol, melibiose, deoxyribose, sucrose, raffinose, sorbose, tagatose, D-arabitol, adenitol, dulcitol, ornithine, 5-ketogluconate, melbionate, arbutin, D-arabinose, β -methylglucoside, and lactulose. To check the presence of contaminating species, cellobiose has been included in the panel. A stock culture of each *E. coli* strain was subcultured on a MacConkey agar plate, and a single colony was grown on nutrient agar at 37°C overnight. A loopful of this latter culture was suspended in 8 ml of a sterile 0.1% (wt/vol) Proteose Peptone (Difco) solution containing 0.01% (wt/vol) bromothymol blue. Aliquots (150 μ l) of the suspensions were inoculated into 24 wells in preprepared microtiter plates containing the 24 reagents described above (final reagent concentration of 3.75 g/liter) in each well. For proper rehydration of the reagents, the plates were stored at 4°C overnight and incubated at 37°C the following morning. The A_{620} of each reaction was measured after 4, 7, 24, and 48 h with a microplate reader. The absorbance values were automatically transferred to a personal computer and multiplied by 10, yielding scores ranging from 0 to 30 for each reaction, for which low values mean acidic reactions (yellow) and high values mean alkaline reactions (deep blue). After the final reading, the mean value of four readings was calculated, resulting in 24 different numbers ranging from 0 to 30 for each strain (the biochemical fingerprint). The similarities between the strains were calculated as correlation coefficients as described previously (19) and were clustered according to the unweighted pair group method with arithmetic averages (UPGMA) (36), yielding a dendrogram. From the reproducibility of the method (17, 18), an identity level of 0.975 was chosen. Strains showing similarities to each other at levels higher than this were regarded as identical and were assigned to the same BPT. BPTs with more than one isolate in a fecal sample or with one isolate but in more than one fecal sample from the same piglet were called common BPTs, and those with only one isolate in only one fecal sample were called single BPTs. The phenotypic diversity of the bacterial population was measured with Simpson's index of diversity (Di) (16). Di in the present study depends on the distribution of isolates into different BPTs. It is high (maximum value of 1) for a population consisting of many different BPTs and low (minimum value of 0) if certain BPTs dominate the population.

The phenotypic similarity between the *E. coli* populations in two samples was calculated as the population similarity (Sp) coefficient (20). The Sp coefficient is a measure of the proportion of strains that are identical in two compared

populations. It is high (maximum value of 1) when the populations contain the same dominant strains and is low (minimum value of 0) when the populations compared consist of different strains. Comparisons of *E. coli* populations from different samples yielded a matrix of Sp coefficients that were clustered according to the UPGMA clustering method (36) to yield a dendrogram. In the dendrogram, each sample (consisting of all isolates in that population) is represented on the horizontal axis and the similarities between populations (measured as the Sp coefficient) make up the vertical axis. Different samples are connected with horizontal lines at the level of similarity they show to each other, and thus the higher up this line is the more similar the samples are.

All handling of data, including optical readings, calculations of correlation coefficients, diversity indices, and Sp values, as well as clustering and printing the dendrograms, was performed with the PhP software (BioSys Inova, Stockholm, Sweden).

RESULTS

Variety and diversity of *E. coli* strains in piglets. Altogether, 195 fecal samples were collected and 1,827 *E. coli* strains were examined. The average numbers of isolates tested on each sampling occasion were 9.5 per sow and 9.4 per piglet. Fewer than 10 isolates tested on each occasion were from 5 samples from sows and 62 samples from piglets. In these samples, either the isolates confirmed as *E. coli* failed after initial isolation or some isolates did not survive storage and subcultivation. Nine samples from seven piglets were not tested, and only six samples (three from each) were obtained from piglets P3 and R4, who died because of diarrhea on day 17. Table 1 shows the number of *E. coli* strains tested on each sampling occasion and the number of BPTs found in each piglet. All fecal samples from piglets (except P3 and R4) contained several BPTs of *E. coli* (Table 1). However, the mean variety values (number of BPTs over the number of strains tested) of strains in fecal samples from sows were generally higher than those of their offspring during the suckling period (Table 2).

Altogether, 239 BPTs were found among the isolates ($n = 522$) tested from piglets during the suckling period (mean variety, 0.458). In contrast, 94 BPTs were found among the isolates ($n = 152$) from sows during the same period (mean variety, 0.618; $P = 0.003$). The diversity of the *E. coli* strains, on the other hand, was higher only in the first samples taken from the sows (Table 2).

Two piglets (P3 and R4) died during a diarrhea outbreak which occurred in the stable during the third week of the study. The first samples (on day 3) from these two piglets contained only one BPT (mean variety, 0.095; Di, 0), and although there was an increase in the variety of strains in the next two samples, the majority of the strains were still of one BPT (mean Di, 0.275) during the diarrhea episode (day 17) (Table 1). However, the lowest values for variety and diversity of strains in piglets and sows were found during the diarrhea outbreak (day 17) (Table 2).

Colonization and persistence of *E. coli* strains. A pattern of successive waves of *E. coli* BPTs was observed in all piglets. Phenotypes that were found in one sample were almost completely replaced by another on the next sampling occasion, which in turn were also replaced by a new type(s), and so forth (Fig. 1a). Normally, fecal samples were dominated by a few *E. coli* BPTs. Most of these strains were maintained for a period of a few days to 2 weeks and were not recovered again from the same piglet. These strains were regarded as transient, and their proportion in each sample varied greatly. However, each piglet also carried BPTs which were found on several occasions, and some persisted until the animals reached their market weight and were slaughtered (day 145) (Fig. 1b). In the present study, BPTs which were found over a 1-month period and which were found in at least three fecal samples from the same piglet were considered resident BPTs. Most piglets carried more than one resident BPT. Altogether, 34 resident BPTs were found in all

TABLE 1. Number of *E. coli* strains tested and number of BPTs found in each fecal swab culture of sows and their litters

Pig	No. of strains tested (no. of BPTs found) ^a on sampling day:													Total no. tested ^b
	3	7	17	27	41	48	56	63	70	77	84	114	145	
Litter O														
Sow	12 (6)	14 (5)	11 (7)	10 (3)										47
Piglet 1	11 (4)	11 (2)	10 (5)	NT ^c	10 (5)	4 (2)	9 (5)	4 (4)	11 (10)	5 (5)	4 (4)	NT	7 (7)	86
Piglet 2	11 (7)	10 (7)	9 (6)	10 (1)	9 (7)	8 (5)	11 (6)	10 (7)	11 (9)	10 (2)	6 (4)	NT	11 (4)	116
Piglet 3	8 (3)	8 (6)	11 (3)	6 (4)	10 (3)	11 (7)	11 (2)	12 (4)	5 (5)	10 (6)	10 (9)	6 (6)	6 (6)	114
Piglet 4	10 (4)	10 (4)	5 (1)	4 (3)	11 (3)	11 (7)	11 (2)	11 (4)	5 (5)	10 (6)	10 (9)	5 (5)	4 (4)	107
Litter P														
Sow	8 (7)	10 (8)	11 (7)	9 (7)										38
Piglet 1	11 (5)	8 (8)	7 (7)	11 (4)	9 (3)	11 (7)	12 (9)	12 (8)	11 (3)	11 (5)	14 (5)	11 (2)	11 (2)	139
Piglet 2	8 (4)	8 (5)	8 (2)	10 (3)	11 (5)	10 (5)	11 (8)	10 (4)	9 (3)	9 (4)	10 (10)	7 (2)	10 (3)	121
Piglet 3	10 (1)	10 (3)	10 (3)	† ^d										30
Piglet 4	11 (7)	11 (3)	4 (4)	11 (2)	NT	10 (6)	6 (5)	6 (5)	12 (6)	12 (7)	10 (8)	10 (8)	4 (3)	107
Litter Q														
Sow	10 (7)	6 (3)	4 (4)	5 (3)										25
Piglet 1	8 (3)	NT	10 (1)	9 (7)	8 (4)	8 (8)	10 (5)	10 (8)	10 (2)	11 (7)	4 (1)	8 (4)	11 (7)	107
Piglet 2	7 (7)	4 (4)	11 (2)	9 (3)	4 (2)	11 (7)	5 (3)	7 (2)	13 (7)	4 (3)	9 (5)	11 (5)	11 (3)	106
Piglet 3	6 (5)	8 (6)	9 (1)	10 (5)	11 (8)	11 (4)	5 (3)	12 (4)	10 (4)	12 (9)	12 (9)	10 (7)	11 (6)	127
Piglet 4	6 (4)	4 (4)	11 (2)	7 (7)	11 (4)	11 (2)	11 (4)	10 (9)	8 (7)	11 (7)	16 (11)	11 (7)	10 (4)	127
Litter R														
Sow	10 (9)	10 (9)	12 (2)	10 (7)										42
Piglet 1	7 (7)	10 (4)	11 (5)	11 (8)	8 (4)	11 (5)	10 (7)	11 (2)	11 (5)	10 (4)	10 (6)	NT	12 (7)	122
Piglet 2	9 (4)	NT	10 (7)	10 (5)	10 (9)	11 (9)	12 (9)	8 (6)	NT	11 (6)	12 (6)	10 (2)	10 (4)	113
Piglet 3	NT	10 (2)	10 (4)	11 (4)	12 (3)	9 (5)	11 (8)	11 (3)	11 (6)	11 (3)	4 (3)	11 (5)	10 (5)	121
Piglet 4	11 (1)	10 (2)	11 (4)	†										32

^a The total number of BPTs found in pigs was 967.^b The total number of isolates tested was 1,827.^c NT, not tested.^d †, piglet died.

piglets (mean, 2.4 BPTs per piglet); all (except one) appeared in fecal samples for the first time during the suckling period (Fig. 2). These strains, however, were not recovered from samples collected during the diarrhea outbreak or a week after weaning (Fig. 1). To investigate whether the resident strains in each piglet were also common in others, we compared these strains. Figure 2 shows a dendrogram derived from a UPGMA clustering of similarity coefficients obtained after pairwise comparisons of the 34 resident BPTs. Several common groups

were found among these strains, with some piglets sharing the same resident phenotypes (Fig. 2).

***E. coli* flora in piglets.** The fecal *E. coli* floras of piglets and their dams were compared with each other. Normally, each piglet carried a unique population of *E. coli* strains during the first week of life (samples on days 3 and 7) (Fig. 3), except two piglets from litter R (i.e., R2 and R4 on day 3) and two from litter O (i.e., O1 and O4 on day 7) who shared similar floras (Fig. 3). Similarity between the *E. coli* floras of piglets and

TABLE 2. Variety^a and Di of *E. coli* strains found on different sampling days in piglets of four litters and their dams

Sampling day	Litter O			Litter P			Litter Q			Litter R			Mean variety		Mean Di	
	Variety		Di (piglets)	Variety		Di (piglets)	Variety		Di (piglets)	Variety		Di (piglets)	Sows	Piglets	Sows	Piglets
	Sow	Piglets		Sow	Piglets		Sow	Piglets		Sow	Piglets					
3	0.50	0.45	0.855	0.87	0.43	0.917	0.70	0.70	0.993	0.90	0.44	0.628	0.72	0.49	0.925	0.848
7	0.36	0.49	0.831	0.80	0.51	0.928	0.50	0.87	1.00	0.90	0.27	0.802	0.62	0.54	0.851	0.890
17	0.64	0.43	0.750	0.64	0.55	0.889	1.0	0.15	0.537	0.17	0.48	0.835	0.53	0.39	0.737	0.752
27	0.30	0.40	0.647	0.78	0.28	0.876	0.60	0.63	0.939	0.70	0.53	0.854	0.59	0.47	0.816	0.829
41		0.45	0.794		0.40	0.811		0.53	0.871		0.53	0.910		0.48		0.846
48		0.62	0.849		0.58	0.976		0.51	0.877		0.61	0.927		0.58		0.907
56		0.36	0.923		0.76	0.983		0.71	0.946		0.73	0.727		0.61		0.894
63		0.51	0.946		0.61	0.830		0.59	0.794		0.37	0.889		0.52		0.864
70		0.91	0.952		0.38	0.914		0.49	0.960		0.50	0.831		0.57		0.914
77		0.54	0.797		0.50	0.922		0.68	0.976		0.41	0.815		0.54		0.877
84		0.87	0.879		0.68	0.941		0.63	0.963		0.58	0.950		0.69		0.933
114		1.0	0.953		0.43	0.696		0.58	0.860		0.33	0.862		0.58		0.837
145		0.75	0.911		0.32	0.778		0.47	0.643		0.50	0.895		0.51		0.806

^a Variety represents the number of BPTs over the number of strains tested.

FIG. 1. Examples of variations in BPTs of *E. coli* showing resident and transient strains in fecal samples from two piglets, O3 (a) and P4 (b). Common BPTs are BPTs with more than one isolate in a fecal sample or with one isolate that is found in more than one fecal sample. Numbers in boxes show the number of isolates of each BPT. BPTs found on more than one occasion are connected with dotted lines. Single BPTs are BPTs with only one isolate found in only one fecal sample. N (boxed), not tested.

those of their littermates increased in samples taken during the diarrhea outbreak on day 17 (e.g., piglets R3 and R4, Q1 and Q2, and Q3 and Q4) (Fig. 3). In samples taken during the first week, there were no similarities between the *E. coli* floras of piglets and those of their dams (Fig. 3), but similar floras were found in some piglets and their dams on days 17 (i.e., piglets R3 and R4) and 27 (i.e., piglets P2 and R3).

The *E. coli* floras of littermates were also compared on days 41, (i.e., a week after weaning), 77 (a week after pigs were transferred to the fattening stable), and 145 (before animals

FIG. 2. Dendrogram showing similarity among 34 resident BPTs from all piglets and their first day of appearance in the fecal sample. The dotted line indicates the identity (ID) level above which BPTs are considered identical.

FIG. 3. Dendrograms derived from the UPGMA clustering of Sp coefficients obtained from comparisons of *E. coli* populations of sows and their litters during the suckling period. The vertical axis represents the similarities between different samples. Pigs from litters O, P, and R were mixed with others during the fattening period, whereas pigs in litter Q were kept together (unmixed group) until the end of the study.

were slaughtered) (Fig. 4). Four pigs, O4, Q2, R1, and R3, developed mild diarrhea after weaning. Generally, there was little similarity between the *E. coli* floras of these pigs and those of their littermates (Fig. 4). On the other hand, the *E. coli* floras of pigs in almost all litters were very similar 1 week after the pigs were mixed with others in the fattening stable (Fig. 4). This high level of similarity disappeared in samples taken on day 145, except for pigs in litter Q, who were kept together during this period (Fig. 4).

Comparisons of *E. coli* populations between litters. *E. coli* populations of all litters were compared with each other on different sampling occasions (strains from sows were not included). Each litter had its own populations on day 3 and continued to do so, except during the diarrhea episode (day 17), for which a high Sp value between litters O and Q was

FIG. 4. Dendrograms derived from the UPGMA clustering of Sp coefficients obtained from comparisons of *E. coli* floras of piglets in each litter 1 week after weaning (day 41), 1 week after pigs were transferred to a fattening stable (day 71), and before pigs were slaughtered (day 145). The vertical axis represents the similarities between different samples.

FIG. 5. Dendrograms derived from the UPGMA clustering of Sp coefficients obtained from comparisons of *E. coli* populations found in different litters throughout the study. *E. coli* strains from sows were not included. The vertical axis represents the similarities between different samples.

observed (Fig. 5). During the rest of the study, the levels of similarity among the *E. coli* populations of litters were generally low and varied regularly until 1 week after pigs were transferred to the fattening stable (day 71). Samples taken after this period constantly showed a high level of similarity among the *E. coli* floras of litters which had been mixed with others (i.e., O, P, and R) (Fig. 5).

DISCUSSION

In the present study, we used a highly discriminatory and standardized biochemical fingerprinting method to characterize *E. coli* strains isolated from fecal samples of piglets and their dams. The variety values of *E. coli* BPTs differed among samples, but in general, the mean number of *E. coli* BPTs in piglets increased as animals aged. Sows normally had a higher number of BPTs than their offspring, which indicates that, during the suckling period, only a few phenotypes of *E. coli* strains colonize the gut from the beginning but they increase in number as the piglets grow older. This could be due to redifferentiation of the mucosal epithelium in piglets during the suckling period as postulated by others (29, 31). There is also a dramatic change in the intestinal environment of piglets after weaning because of dietary changes, which in turn results in an alteration of the composition of the indigenous flora.

The diversity of *E. coli* floras was high in most samples, except for those taken during the diarrhea episode. If the outbreak was due to a diarrheogenic strain, we should have found identical strains in all infected piglets and therefore a reasonably low level of diversity in affected litters, a situation which is often seen in outbreaks of bacterial infections. However, this was not the case, because both litters P and R, in which two piglets died, showed a higher level of diversity than litters Q and O. To rule out the possibility of sampling error, we measured similarities among BPTs found in duplicate fecal samples from piglets with diarrhea (P3 and R4) and found similar BPTs (data not shown). Another interesting finding was that the two piglets who died because of diarrhea had a very low level of diversity of *E. coli* floras from the beginning, and this remained so until the last sample was taken on day 17 (Table 1). In another study, we analyzed the diversity and

stability of the intestinal coliform floras among six of our piglets and also found that piglets who died had a lower level of diversity of coliforms than those who survived (22). In all samples, *E. coli* floras of piglets consisted of more than one BPT. Most of these BPTs were transient and replaced by new types in the succeeding samples. By using an O antigen typing method, Craven and Barnum (3) also found a similar pattern of successive colonization of *E. coli* serogroups in healthy piglets during the suckling and early postweaning periods. However, in our study, we also found resident BPTs which persisted for longer periods. All of these strains (except one) colonized the piglets during the suckling period (Fig. 2). Bacterial colonization of the intestinal tract occurs almost immediately after birth, with *E. coli* being among the earliest bacteria. However, in order to grow and maintain the population, the colonizing *E. coli* strains must be able to compete for the limiting nutrients that can be utilized under the physiological conditions prevailing in the gut (7). The fact that each piglet carried certain BPTs of *E. coli* for a long period indicates that these *E. coli* strains have a certain ability which enables them to colonize the gut and maintain their population more easily than others. By using the same biochemical fingerprinting technique, Kühn et al. (24) monitored the colonization and persistence of *E. coli* phenotypes in the intestines of infants until 18 months of age. They also found that the strains which colonized infants soon after birth persisted longer than those which colonized them later. Biochemical fingerprinting of the resident strains in our study showed that, although they belonged to different BPTs, some piglets (even those from different litters) carried the same phenotypes, indicating that both host specificity and strain specificity are important for colonization of piglets by *E. coli*.

Most resident strains were not found in the samples taken during the diarrhea outbreak and 1 week after weaning. Both diarrhea and weaning are critical phases in terms of bacterial colonization because high-level peristaltic movements due to diarrhea and changes in nutritional status of the animal after weaning may greatly affect the physiological status of the gut and hence colonization of bacteria. Therefore, it is possible that, during these periods, the resident phenotypes were present in small numbers in fecal samples and escaped detection.

The *E. coli* floras in fecal samples collected during the fattening period were different in all piglets except samples from litter Q on day 145 (Fig. 4). Piglets in this litter were kept as a litter in a separate box between other intact litters of unmixed pigs until the end of the study. Under these conditions, in which a few animals are in close and constant contact with each other, the chance of acquiring bacteria from each other is high. It was therefore expected to find identical BPTs in large numbers and thus high Sp coefficients between *E. coli* populations of pigs in this litter.

The fecal flora contents are assumed to reflect those of the large intestine, although the intestinal contents are more dilute (35). Suckling piglets are postulated to acquire their first bacterial flora directly from the teats, skin, and feces of their dams (1, 2, 34). Therefore, one would expect to find similar bacterial floras in piglets and sows during the early stage of the animals' life. However, we did not find much similarity between the *E. coli* floras of piglets and those of their dams in samples collected during the first week of the study. This finding shows that the dominant *E. coli* strains in piglets during the first week of life are quite different from those of their sows. *E. coli* populations in different litters were also compared with each other. Two litters, P and R, which were infected with the diarrheogenic agent had *E. coli* floras completely different

from each other and from those of other litters on day 17. In contrast, litters O and Q showed *E. coli* floras similar to each other (Fig. 5). These results once again indicate that the cause of diarrhea during the outbreak was not a pathogenic *E. coli* strain.

In conclusion, we found that the intestinal colonization of piglets by *E. coli* was represented by successive waves of different phenotypes with persistence times that varied from a few days to 2 weeks. However, certain resident phenotypes which colonized piglets during the suckling period persisted longer. Many of the resident BPTs were found in several piglets, indicating that both host specificity and strain specificity are important for the colonization of piglets with *E. coli*. The diversity of *E. coli* phenotypes increased as the piglets aged, probably because of changes in the physicochemical characteristics of the intestinal tract. Piglets who died because of diarrhea had a lower level of diversity of *E. coli* floras than those who survived. Furthermore, different populations of *E. coli* were observed among piglets and their dams during the first week of the piglets' life, indicating that the dominant *E. coli* floras of piglets were quite different from those of their sows.

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