ESCHERICHIA COLI ASSOCIATED WITH COLOSTRUM-FREE NEONATAL PIGS RAISED IN ISOLATION*

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From the early part of the 1900's, workers have speculated concerning the possible causal relationship between *Escherichia coli* and diarrhea in neonates.

Mainly, infants and calves served as focal points for this interest (references 1–3 review the earlier literature). Later, other domestic animals received attention (4–7). Attaching etiological significance to *E. coli* in diarrhea is inherently difficult and complicated since the lesion or affected area (intestines) normally harbors large numbers of *E. coli* as part of the intestinal flora.

Handicapped by this fact, workers sought means for further dividing the species *E. coli* into well characterized subgroups or strains. At first, this was done with differential fermentation reactions followed by the more specific serological typing (4, 8). The rationale of this approach was to demonstrate, in practically pure culture and in large numbers, a strain of *E. coli* in diarrhetic feces that was absent or in low numbers in an asymptomatic individual. More recently, hemagglutination and hemagglutination inhibition titers in patients' sera *versus* suspected pathogenic *E. coli* have been studied (9–11).

From work of this nature, a number of serotypes (usually host-specific) have been classified as enteropathogens (4, 6, 12). However, the precise role of *E. coli* in the etiology of diarrhea remains uncertain since pathogenic serotypes frequently are found in asymptomatic individuals, and equivocal results have been obtained in feeding trials using pathogenic serotypes (13, 14). The need for predisposing factors for the manifestation of disease has been suggested by some authors (12, 15, 16).

Our interest in coliforms and diarrhea grew out of a nutritional problem concerned with the development of serum proteins in the neonatal pig (17). In this work, colostrum-free piglets (obtained by hysterectomy or caught in sterile towels as they were being born, and raised in sanitary, individual, stainless steel cages in an isolation unit) often developed diarrhea when approximately 3 days old. It was not unusual to lose 50 per cent of the pigs by 2 weeks of age. Frequently *E. coli* were isolated from the blood of morbid piglets and from the liver of dead piglets.

In our set-up, sanitation and management were thought to be standardized at a high level, and presumably the piglets were uncontaminated with pathogenic micro-

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organisms from the sow and her environment. Thus, with these variables under control, the role of *E. coli* in diarrhea might be better assessed.

Within this context then, this study was begun with the view of determining:

(*a*) A porcine enteropathogenic serotype(s). By definition, a pathogenic serotype would be one that was isolated frequently from blood and liver of morbid and dead piglets.

(*b*) The distribution of the pathogenic serotype(s) in relation to other serotypes in feces of pigs in the standardized environment.

(*c*) The distribution of antibodies to the pathogenic serotype(s) and other serotypes in various natural fluids (bovine and porcine gamma globulin, cows' and sows' colostrum).

(*d*) The relationship of diet to bacteremia and death.

(*e*) The effect of feeding pathogenic serotype(s) to colostrum-free, gamma globulin–free piglets.

Materials and Methods

Source of Samples.—All of the pigs used in these experiments were born in tiled isolation units. The piglets were taken either by hysterectomy or caught in sterile towels as they were being born and carried into another isolation unit, (with the exception of pigs in the nursing group, these were returned to their dam). Here, they were placed in sterilized, individual, stainless steel cages heated with infrared lamps. Feeding and other details of management were performed by an attendant who donned special clothing in an air lock just prior to entering the isolation unit. All piglets were managed in a like manner with the exception of diet. For the 1st week, piglets were fed 6 times a day and thereafter, 5 times a day. For this series of experiments, piglets in 21 liters were studied.

Bacteriology.—Blood cultures were taken by bleeding piglets from the anterior vena cava at the time of birth and at intervals of the 2nd, 4th, 7th, 10th, 14th, and 21st day thereafter. In the event of death of one of the piglets, a necropsy was performed and liver cultures were taken. A total of 57 coliform isolations were made from blood or liver using these procedures. Piglets with bacteremia usually died within 4 days from the time of the first positive blood culture.

Fecal cultures were taken at random using rectal swabs. In this manner, 82 *E. coli* cultures were isolated from 13 different litters. Usually, this represented a single *E. coli* isolate from 6 pigs in each litter.

All of the specimens (blood, liver, feces) were cultured first on blood agar, using blood agar base medium with 5 per cent bovine blood added. Gram-negative, non–spore-forming rods were picked and identified using standard bacteriological techniques (18, 19). Eosin–methylene blue medium, triple sugar iron agar medium, urease medium, indole, methyl red–Voges Proskauer and citrate, as well as carbohydrate media, were used in the identification scheme.

Antigenic Characteristics.—In addition to the identification of these organisms by their tinctorial, colonial, and fermentative reactions, those bacteria that were proved to be *E. coli* were subjected to agglutination tests to divide them further into antigenic strains. Slide and tube agglutination tests using heated and native cell suspension were performed according to Edwards and Ewing (19).

Agglutination in Natural Fluids.—Tube agglutination tests with heated *E. coli* cells were performed to determine whether certain dietary elements used in some of the feeding experiments contained antibodies to *E. coli* strains previously isolated. The fluids tested for ag-

glutinins were fat-free whey from pooled samples of bovine colostrum, porcine colostrum, porcine gamma globulin, and bovine gamma globulin. The whey was prepared according to techniques previously published (20), and the porcine and bovine gamma globulin were from commercial sources.[1] Three different pools of both bovine and porcine colostrum were tested. Each bovine pool was made from the 1st day's post partum secretions of a minimum of 12 cows and the porcine colostral pools from a minimum of 5 sows. Whey was diluted with saline to give a solution containing about 12 mg/ml gamma globulin (approximately the amount in normal serum). The porcine and bovine gamma globulin also were adjusted to this same concentration. Tube agglutinations (19) were carried out using representative cultures of the different *E. coli* strains isolated and each of the 34 *E. coli* 08 isolates. Dilutions of these gamma globulin–rich fluids ranged from 1:40 to 1:1280.

Diets.—Essentially, the variable dietary constituent germain to these experiments was gamma globulin. Thus, the diets used were divided into the following three main groups (based on whether the piglet had access to either bovine or porcine gamma globulin):

(*a*) Sows' colostrum; piglets allowed to nurse in the normal manner (porcine gamma globulin).

(*b*) Cows' colostrum; made from a pool of the 1st day's post partum secretions from a minimum of 12 cows (bovine gamma globulin).

(*c*) Cows' milk or artificial milk made up to simulate cows' milk (no gamma globulin).

In general, the feeding rate per day was 300 ml for the 1st day, increased gradually to 600 ml by the 7th day, with *ad libitum* feeding thereafter. Details of the diets have been published previously (17, 21).

Feeding Experiment.—The disease-provoking capacity of two *E. coli* 08 strains isolated from piglets was tested by feeding these strains to other piglets. One 08 isolate was from liver and the other from feces. 9 colostrum-free pigs, 2 weeks old, that had been raised on cows' milk in an isolation unit served in this experiment. These 9 pigs were obtained from 3 different sows. At the time of feeding the *E. coli* and at 1 and 4 weeks postfeeding, the piglets' sera were examined for gamma globulin by agar immunoelectrophoresis according to techniques reported previously (21), and for agglutinins to the two 08 strains in question.

The inoculum for feeding the pigs was prepared from 24 hour trypticase soy broth cultures of each 08 isolate. 5 ml of each culture (containing approximately 10^8 *E. coli*/ml) was mixed with 10 ml of cows' milk and fed by stomach tube to each pig. Thus, each pig received 5×10^8 *E. coli* 08 of fecal origin and 5×10^8 of liver origin, or a total of 10^9 *E. coli* 08. The pigs were observed carefully for a rise in temperature, diarrhea, and other symptoms of disease.

RESULTS

Distribution of E. coli Serotypes Isolated from Blood or Liver of Piglets.—A total of 57 cultures of Gram-negative bacilli from blood or liver sources were isolated and identified. Of this number, 50 were *E. coli*. The remaining 7 were identified as follows: 4 were *Aerobacter aerogenes*, and 1 each of *Paracolobactrum aerogenoides*, *Pseudomonas* sp. and *Salmonella derby*.

Of the 50 *E. coli* cultures from blood or liver specimens of neonatal pigs, 33 were typable with existing reference antisera. Note in Fig. 1 the distribution of these *E. coli*. Of the 33 typable *E. coli* isolates, 22 (67 per cent) were type 08.

[1] Bovine gamma globulin generously supplied by Dr. E. F. Waller, Sterling-Winthrop Research Institute, Rensselaer, New York, and bovine gamma globulin by Dr. M. E. Davenport. Armour Pharmaceutical Company, Kankakee, Illinois.

The other 11 serotypes were distributed fairly evenly in the remaining 33 per cent (3–6 per cent). In the 15 litters with coliforms in blood or liver, 08 was isolated from 10 of the litters (67 per cent).

Distribution of E. coli Serotypes Isolated from Feces of Piglets.—A total of 82 randomly selected *E. coli* cultures were obtained. 50 of these were typable. The distribution of the 50 *E. coli* serotypes is shown in Fig. 2. 18 different serotypes were identified from piglets in 13 litters. There was a wider variety of serotypes present in feces of neonatal pigs than were found in the cultures of blood and livers. Also, these serotypes appeared to be more evenly distributed in the feces of piglets than the serotypes isolated from blood or liver. Strain 08

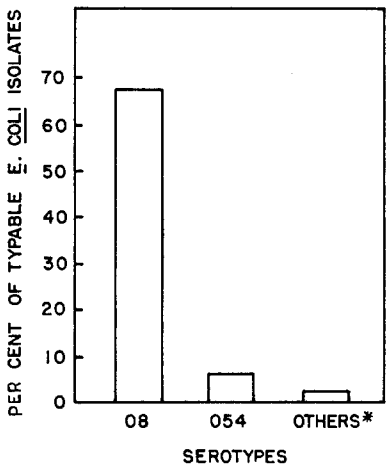

FIG. 1. Distribution of 33 *E. coli* serotypes isolated from blood or liver of piglets from 15 different litters. * = 3 per cent each of OX1, 020ab, 065, 974, 077, 078, 086a, 0119, and 0140.

was isolated 24 per cent of the time, followed by 07 with 14 per cent. The other 16 serotypes were distributed fairly evenly throughout the remaining 62 per cent (2–10 per cent). Strain 08 was found in approximately 62 per cent of the litters sampled.

From the above data, strain 08 was not only the most frequent strain isolated from blood or liver (an indication of pathogenic propensity), but it also was a part of the "normal" fecal flora in our piglet population. The data indicated that approximately one in every four typable *E. coli* organisms in the normal intestinal flora of our piglet population was type 08, since 24 per cent of isolates randomly selected from fecal cultures were of this type. To supply supplementary data regarding the distribution of strain 08 in the normal fecal flora, a healthy, colostrum-free, gamma globulin–free litter was selected for extensive isolation attempts for 08. From this litter of 9 pigs, fecal cultures

were taken at 4 and 8 days of age. From the initial isolation on eosin–methylene blue agar, 10 typical coliform colonies from each piglet were selected and screened for 08 antigens with the slide agglutination test. The identity of all colonies exhibiting presumptive 08 agglutination was confirmed using tube agglutination tests as described above. Out of 135 colonies so examined, 23 (approximately 17 per cent of the total *E. coli* isolated) were serotype 08.

Relationship of Diet to Bacteremia and Death.—It was apparent early that diet was influencing the vigor of piglets, not only in terms of weight gains, but also with respect to the appearance of coliform bacteremia followed by the

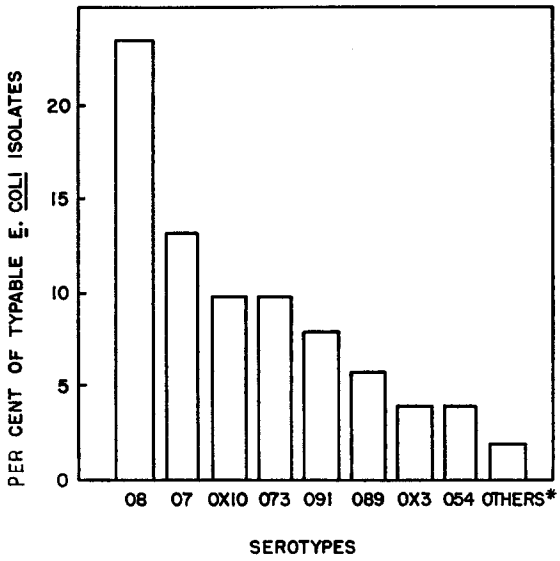

FIG. 2. Distribution of 50 *E. coli* serotypes randomly isolated from feces of piglets from 13 different litters. * = 2 per cent each of 09, 020ab, 055, 058, 018ac, 064, 075, 079, 0100, and 0119.

death of the piglet. Pigs fed diets containing gamma globulin seemed most resistant to bacteremia and death. To lend support to this observation, the findings were analyzed by grouping piglets with coliforms in blood or liver according to whether or not gamma globulin was present in their diet. Fig. 3 substantiates the observation correlating diet and vigor in that 85 per cent of the bacteremia and deaths occurred in those pigs whose diet did not contain gamma globulin. Bovine gamma globulin (cows' colostrum) was just as effective as porcine gamma globulin (sows' colostrum) in conferring resistance on the piglets. In this connection, it was shown that the gut absorption mechanism in the first 36 hours of a piglet's life is non-selective in that piglets can absorb, in addition to homologous porcine proteins, unaltered bovine proteins, avian proteins, and polyvinylpyrrolidone (21).

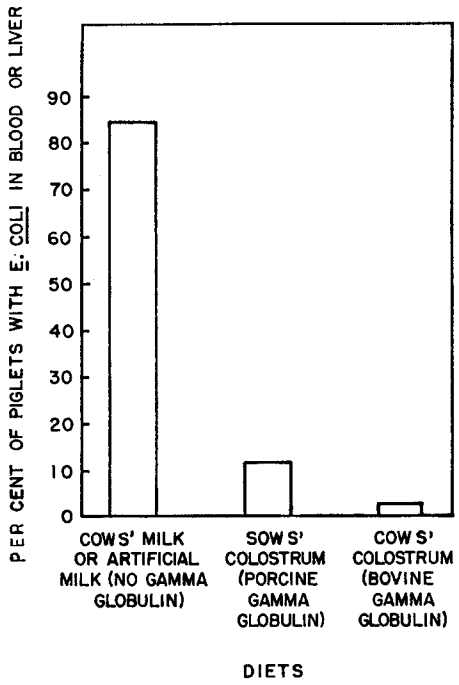

FIG. 3. Relationship of piglets with *E. coli* in blood or liver to presence of gamma globulin in diet.

TABLE I

*Agglutinins in Colostrum and Gamma Globulin to Representative E. coli Serotypes**

Serotypes	Bovine colostrum	Bovine gamma globulin	Porcine colostrum	Porcine gamma globulin
058‡	160	—	—	—
0100	640	—	160	80
086a	160	—	160	—
064	640	—	80	—
08§	160	—	80	—
08	1280	80	320	80
08	40	—	—	—
08	320	—	—	—
08	80	—	40	—
08	1280	—	80	—

055, 077, 073, 0140, 0X3 were negative.

* Results expressed as reciprocal of highest dilution showing agglutination.

‡ The results of 0X1, 0X10, 054, 018ac, 07, 079, 091, 089, 074, 020ab, 078, 065, and 09 were similar to 058.

§ Results of the six 08 isolates selected were representative of the 32 08 isolates tested.

Agglutinins in Natural Fluids.—Our results indicated that resistance to bacteremia and death were closely paralleled by the presence of gamma globulin in the diet and the absorption of unaltered gamma globulin by the piglet. It was of interest to determine whether protection might be attributed, in part at least, to agglutinins to *E. coli* in the gamma globulin found in natural fluids. Accordingly, 87 different *E. coli* isolates, representing 19 different O serotypes, were tested against cows' and sows' colostrum, as well as purified gamma globulin obtained from porcine and bovine serum. The results (Table I) show that bovine colostrum contained a wealth of agglutinins to *E. coli*. Porcine colostrum, in comparison, was not so well endowed. The reason for the poor results obtained with the purified fractions of gamma globulin is not apparent to us at this time.

Feeding Experiment.—The 9 colostrum-free pigs which received the inoculum of 10^9 *E. coli* 08 did not develop signs of disease attributable to the fed *E. coli*. In Fig. 4, agar immunoelectrophoresis of the two piglets' sera indicated that gamma globulin still was absent at 2 weeks (the time of feeding the *E. coli*) but present by 3 weeks. Blood serum samples taken 3 times (2, 3, and 6 weeks of age) were negative at serum dilutions of 1:10 and 1:40 for agglutinins to the 08 isolates fed. These results suggested a resistance to infection that was not dependent on antibody–gamma globulin.

DISCUSSION

One of the purposes of this investigation was to define a pathogenic type(s) of *E. coli* in colostrum-free piglets raised under extremely sanitary conditions. Since our results showed that 67 per cent of the typable coliform isolates from the blood or liver of morbid and dead pigs were *E. coli* 08, this strain was considered the main porcine coli enteropathogen in our isolated environment. Interestingly, in England piglets raised under normal farm conditions in the 1st week of their lives were especially susceptible to *E. coli* 08. Later, other strains seemed more significant with respect to an association with disease (5, 12). Because of the techniques used in obtaining and raising our piglets, it appeared that the source of strain 08 was indigenous and was not derived from contamination from the sow or her environment.

Random sampling of feces of piglets in 13 litters as well as a directed effort to isolate *E. coli* 08 from the feces of piglets in a healthy, colostrum-free litter showed that this enteropathogen makes up approximately 20 per cent of the *E. coli* in the gut of the piglets. It seems, then, that *E. coli* 08, as well as many other *E. coli* types, are a part of the normal microbial ecology of the gut of the piglet raised under our conditions. However, *E. coli* 08, although accounting for only 20 per cent of the fecal coliforms, have the propensity or selective advantages to invade from the gut.

With no shortage of type 08 in the environment, it follows that the conditions poising the susceptibility of the piglet to invasion seem more pertinent

to the health of the litter than does the ubiquitous enteropathogen, *E. coli* 08. Our data revealed that when gamma globulin was in the diet of piglets (either in the form of sows' or cows' colostrum) the early invasion by *E. coli* was markedly reduced. Since we have shown that the piglet easily absorbs gamma globulin from his gut into his blood stream (21) and that the colostrum fed the piglets was rich in antibodies to coliforms (08 included), it was tempting to assume that resistance or protection arises from circulating antibody antagonizing invading *E. coli*. Such an explanation seems consonant with the results obtained from calves with diarrhea (23–25).

Assuming resistance does result from antigen-antibody interaction, it seems improbable, however, that antibody inhibition of bacteria in the blood circulation is the main and only means whereby gamma globulin functions in the resistance of piglets to diarrhea and bacteremia. A more complete and meaning-

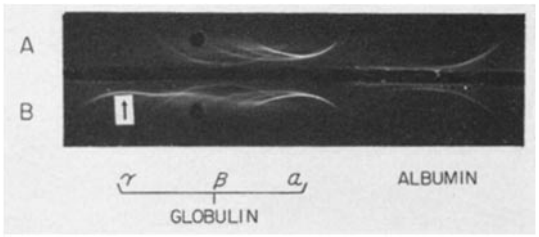

FIG. 4. Immunoelectrophoresis of serum from a piglet infected *via* stomach with 10^9 *E. coli* 08. *A.*, Serum obtained at 2 weeks (time of infection); *B.*, Serum obtained at 3 weeks (1 week postinfection); Center slit, rabbit antiporcine serum; Arrow points out gamma globulin arc.

ful explanation should take into account the observation that little trouble is encountered in raising colostrum-free, gamma globulin–free piglets after they are 2 weeks old (22). Furthermore, it must be remembered that feeding overwhelming numbers of pathogenic *E. coli* 08 to 2-week-old, colostrum-free, gamma globulin–free, 08 agglutinin–free piglets resulted in no signs of disease. Thus, if antibodies function in the piglets' resistance to diarrhea, bacteremia, and death as caused by *E. coli*, then such antibodies are necessary only very early in the life of the piglet.

This early dependency on gamma globulin suggests that the piglet is born susceptible to invasion by bacteria normally found in the gut and a period of time must pass before the piglet becomes resistant to invasion. It is during this interval (coincident with the time period in which piglets absorb large molecules) that the presence of gamma globulin in the diet is critical. Within this critical period, then, gamma globulin might function in resistance in three ways: firstly, by promoting the localized inhibition of the bacteria in the gut. Secondly, since gamma globulin is absorbed into the blood stream, it might

benefit the piglet by inhibiting the stray bacteria that passed the first line of defense. And, thirdly, in a less defined way, gamma globulin might act in resistance by influencing the rapid maturation of the piglet serum protein profile (22).

SUMMARY

Escherichia coli 08 was the most frequent coliform isolated from the blood and liver of morbid and dead neonatal, colostrum-free piglets raised under extremely sanitary conditions. This strain accounted for 67 per cent of the typable *E. coli*. The next most numerous strain occurred at a frequency of 6 per cent. Hence, *E. coli* 08 was considered the main coli enteropathogen in our experimental, isolated environment.

In random samples of the feces of healthy and diarrhetic neonatal piglets, 24 per cent of the typable *E. coli* was type 08. When a directed effort was made to isolate *E. coli* 08 from the feces of neonatal piglets in a healthy, colostrum-free litter, this strain was isolated from 17 per cent of the total *E. coli* colonies examined. Thus, the enteropathogen *E. coli* 08 was ubiquitous in the feces of piglets in our environment, making up approximately 20 per cent of the fecal *E. coli*.

85 per cent of the bacteremia and death in which *E. coli* was isolated from blood or liver occurred in piglets fed diets void in bovine and porcine gamma globulin.

Tube agglutination tests demonstrated that agglutinins to *E. coli* 08, and other serotypes as well, were present in bovine colostrum and to a lesser extent in porcine colostrum. These agglutinins were practically lacking in solutions of porcine and bovine gamma globulin.

Feeding 10^9 *E. coli* 08 bacteria to 2-week-old, colostrum-free, gamma globulin–free, 08 agglutinin–free piglets did not produce visible disease.

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