Enteritis of Early Weaned Pigs*
I. Enteropathogenic Escherichia coli

by S. L. Chopra[1], A. C. Blackwood and D. G. Dale[2]

Enteritis of pigs due to non-specific causes continues to be a serious economic problem. During the past decade or so the possibility of a pathogenic role of *Escherichia coli* has attracted an increasing amount of attention from many workers (1,2,3,5 — 13). Some quantitative changes in the coliform population associated with enteritis have been reported by Chopra *et al.* (2), Kenworthy and Crabb (7), and Buxton and Thomlinson (1), but these do not present conclusive evidence for incriminating *E. coli* as the primary etiological agent. Reviewing the incidence of enteritis outbreaks in pigs, Stevens (13) indicated three peaks in the life time of the pig, each one of which is associated with numerical increases of *E. coli* population in the intestinal tract. Kenworthy and Crabb (7) also showed that enteritis of early-weaned pigs is associated with large increases of hemolytic *E. coli* populations in the intestinal tract. Saunders *et al.* (9) drew attention to the invasive properties of hemolytic *E. coli* from all viscera of piglets that died with acute symptoms of enteritis. The invasiveness of *E. coli* was particularly noteworthy with Weybridge strain G7 in piglets under one week of age. These findings were confirmed by Sojka *et al.* (12) who compared relative occurrence of different *E. coli* serotypes in edema disease and gastro-enteritis. Strains G7 and E68 were found to be particularly common in piglet enteritis occurring in the first week of life. On the other hand, E68 and E4 were commonly associated with cases of edema disease and G7 was never isolated from the latter. Lecce

and Reep (8) showed that failure to ingest colostrum may result in bacteremia and death due largely to invasion by *E. coli,* strain-G7.

Saunders *et al.* (10, 11) reproduced symptoms of enteritis in "pathogen free" and naturally reared young pigs by feeding strains G7 and E68. Unfortunately, the latter data were confused due to inadvertant inclusion of a pathogenic *E. coli* in control experiments.

In spite of overwhelming evidence which supports direct incrimination of *E. coli* in swine enteritis the association is questioned because the suspected pathogens are often isolated from apparently healthy animals. The present report deals with a study of the occurrence and duration of infectious cycles due to enteropathogenic *E. coli* in natural cases of enteritis and those produced artificially by deliberate infection.

Materials and Methods

Experimental Animals. — Three-week old early weaned pigs were employed for all studies.

Diets. — The animals were weaned on either a commercial early weaning ration (2) or one of its three modifications. These modifications consisted of (i) Basic ration — commercial ration without the incorporation of any chemotherapeutic agent, (ii) Ampicillin ration — the Basic ration supplemented with 100 gm of Ampicillin (6(D(—)-Alpha-Aminophenylacetamide) penicillanic acid) per ton feed, (iii) Hibitane ration — Basic ration supplemented with 100 gm Hibitane (1,6-DI-4'-Chlorophenyl diguanidinohexane) per ton feed. The commercial ration itself contained 15 gm Aureomycin, 10 gm Bacitracin and 5 gm penicillin each per ton ration.

Clinical Observations. — General physical fitness of the animals was observed every morning and the consistency of the stool was graded as: formed, loose and diarrhiac. The animals were weighed every week.
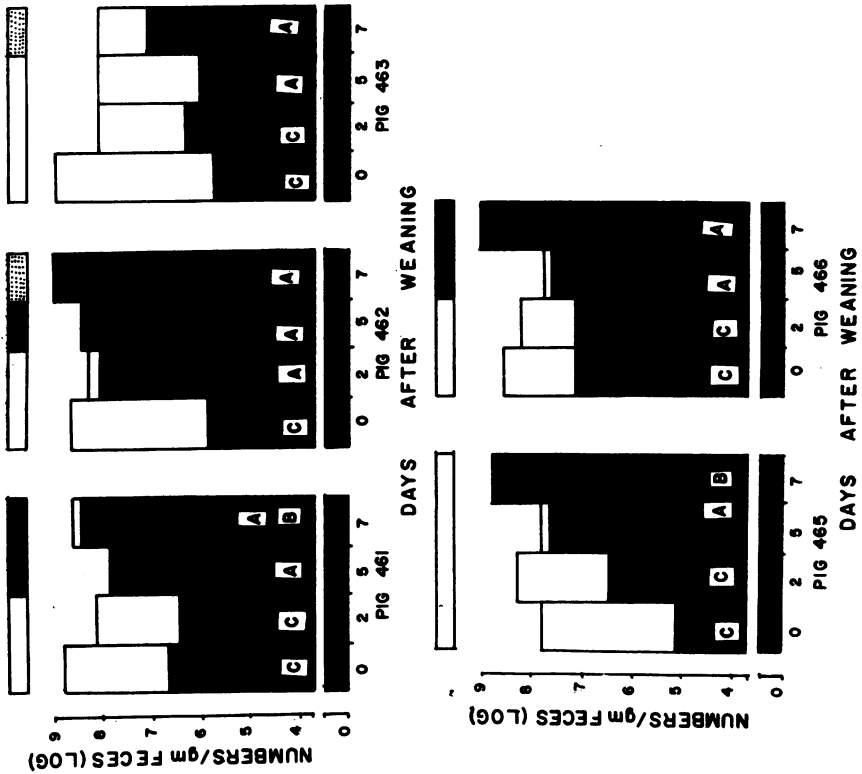
Fig. 1. Changes in the coliform microflora following weaning. Horizontal bar; Blank — no enteritis, solid black — enteritis. Vertical bar: Entire length — total number of colonies growing on penicillin blood agar medium; Black — hemolytic E. coli, C — non-typable, A-G7, B-E145.
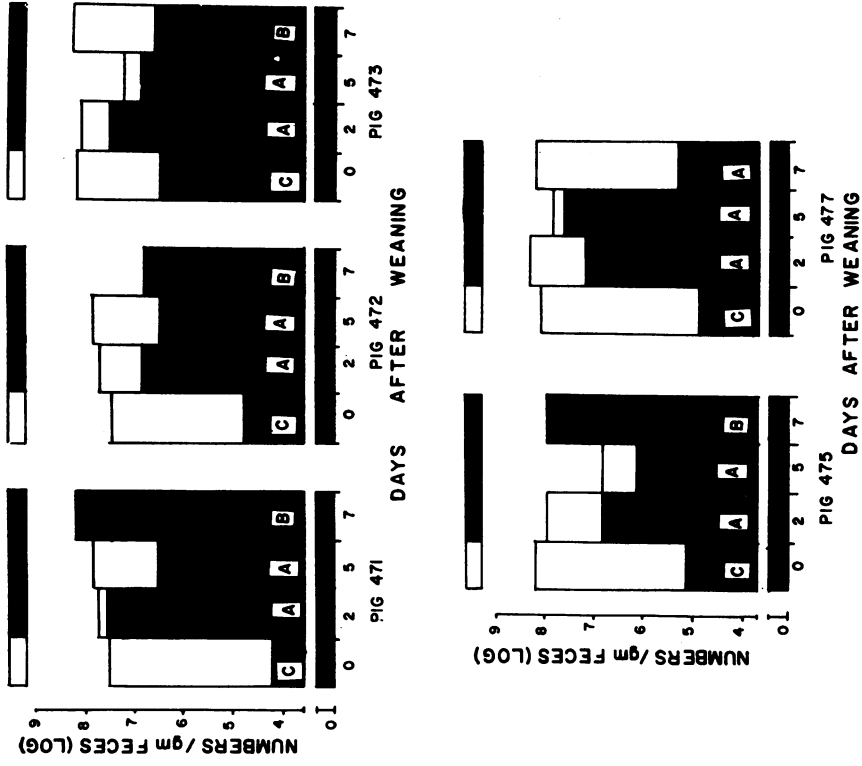

Fig. 2. Changes in the coliform microflora following weaning. Horizontal bar: Blank — no enteritis, Solid black — enteritis, Spotted — mild enteritis. Vertical bar: Entire length — total number of colonies growing on penicillin blood agar medium; Black — hemolytic E. coli, C — non-typable, A-G7, B-E145.

Bacteriological Examination of Fecal Samples. — Fecal samples were obtained from every pig on trial normally at 48 hour intervals. Defecation was induced by introducing a sterile glycerinated swab into the rectum of the animal and the stool was allowed to drop into a sterile glass jar. Tests on the samples were made immediately. A portion of the fecal material was weighed in a micro-blendor and a 1:10 dilution was prepared with distilled water. Further decimal dilutions were prepared in milk dilution bottles. One ml volumes of dilutions 10^{-4} to 10^{-7} were plated in triplicate with 15 to 20 ml of 5% sheep blood agar medium containing 1.0 I.U. of penicillin per ml. Colonies of hemolytic and non-hemolytic bacteria were counted after incubating the plates for 18 - 22 hours at 37°C.

Characterization of E. coli Isolates. — Five representative isolates each of hemolytic and non-hemolytic colonies from fecal cultures of every pig were selected and identified by IMViC reactions. The hemolytic colonies were further identified by plate agglutination reactions with 'OK' and 'O' antisera prepared from the following Weybridge strains obtained by courtesy of Dr. W. J. Sojka:

Weybridge Strain	Antigenic Formula
E68 I	0141: K85 a,b(B)K88(L)
E68 II	0141: K85 a,b—> a,c(B)
E145	0141: K85 a,c(B)
E4	0139: K82(B)
E57	0138: K81(B)
G7	08 : K87(B) K88(L)
M434	0? : K?
G11	0? : K?
G515	054 : K?
E65	045 : K?
E38	0? : K?

Antisera production and agglutination tests were performed by the methods of Edwards and Ewings (4). Representative hemolytic colonies were identified by plate agglutination with 'OK' antisera. Once a few colonies were identified by positive agglutination against any of the above antisera, all hemolytic colonies (30 to 50) were selected from the same plate used for presumptive identification. The final identification of serotypes was confirmed by identifying the 'O' group of 5 to 7 representative colonies.

Artificial Infection. — Pigs were infected by adding an 18 hour broth culture to the drinking water.

Bacteriology of the Dead Animal. — Samples collected from duodenum, ileum and cecum were examined by the same procedure. In addition the inflamed mesenteric lymph nodes were cultured on sheep blood agar medium.

Results

A series of experiments were designed to study the association of *E. coli* with enteritis of early weaned pigs. These studies involved the consideration of the relationship among the weaning process, the weaning rations, and the physiological and serological characteristics of intestinal and fecal *E. coli* to the development of enteritis.

Experiment I. — Two sets of 5 littermate pigs were chosen from two different litters that had been nursed in adjoining pens of the piggery. The litters were allowed to remain in their original pens and were weaned on commercial early weaning ration. Bacteriological examinations were conducted on the fecal samples obtained at intervals of two to three days.

A graphic summary of the results is presented in Figs 1 and 2. The fecal samples obtained on the weaning day from all five members of litter No. 470 contained non-typable hemolytic *E. coli.* The numbers varied from log 4.30 to 6.58 per gram feces (Fig. 1), although none of these pigs had enteritis. Within 48 hours of weaning, all five animals developed diarrhea and the fecal samples obtained on this day contained hemolytic *E. coli* sero-type G7 which comprised 0.06 to 2.3% of the total number of micro-organisms growing on penicillin blood agar medium. None of the pigs recovered from enteritis by the 5th day and *E. coli* — G7 was still present in large numbers. By the 7th day after weaning *E. coli* — G7 was completely replaced by *E. coli* — E145 in four of the five pigs. In pigs No. 471 and No. 475, this strain constituted the entire population growing on the blood agar plates. Pig No. 477 showed signs of recovery on the 7th day and the number of *E. coli* — G7 were log 5.23 per gm feces.

Fecal samples obtained on the weaning day from most of the pigs of litter No. 460 contained relatively large numbers of non-typable hemolytic *E. coli* (Fig. 2). This serotype was found in similar numbers in fecal samples taken after two days
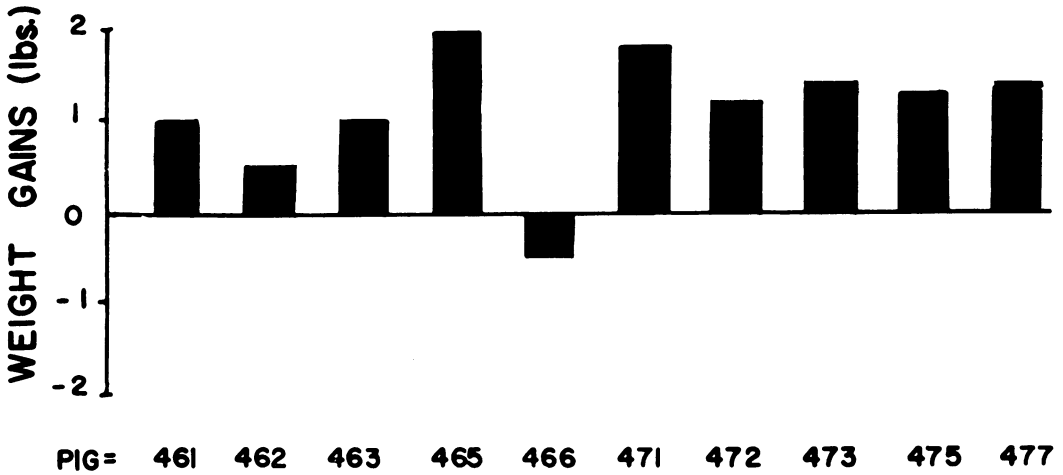
Fig. 3. Weight gains of pigs with and without enteritis by the end of first week after weaning. (See Figs. 1 and 2).
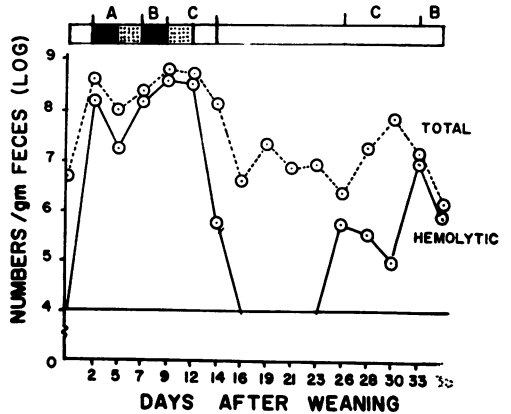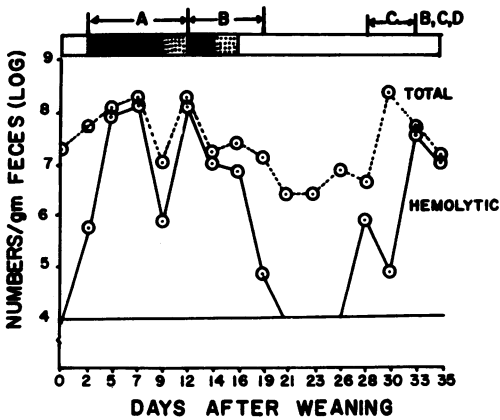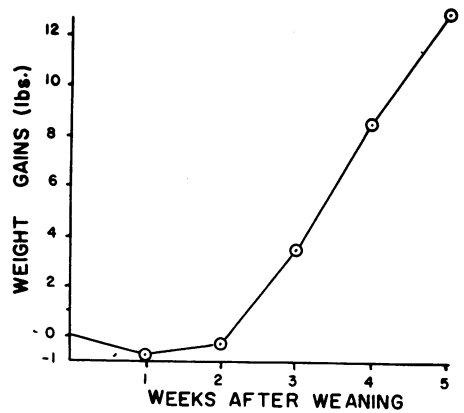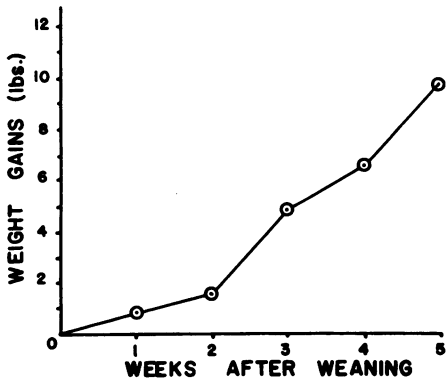

Fig. 4. Effect of enteritis on weekly weight gains (top graph) and fecal E. coli (bottom graph) of a pig fed Basic weaning ration. Horizontal bar: Blank — no enteritis, Solid black — enteritis, Spotted — mild enteritis. A-G7, B-E145, C-non-typable, D-E4.

Fig. 5. Effect of enteritis on weekly weight gains (top graph) and fecal E. coli (bottom graph) of a pig fed Hibitane supplemented early weaning ration. Horizontal bar: Blank — no enteritis, Solid black — enteritis, Spotted — mild enteritis. A-G7, B-E145, C-non-typable.

from all members of this litter except pig No. 462 in which the non-typable hemolytic *E. coli* disappeared and *E. coli* — G7 replaced most of the coliform population. However, none of the five animals showed any signs of enteritis by the 2nd day after weaning. By the 5th day, *E. coli* — G7 was the most numerous organism in four of the five pigs and three of these had enteritis. Strain E145 appeared on the 7th day in the pigs No. 461 and No. 465 and was the only organism isolated (on penicillin blood agar medium) from pig No. 465.

The amounts of weight gained by these animals by the end of the first week after weaning are given in Fig. 3. All animals in this trial except pig No. 465 scoured and this animal gained the maximum amount of weight.

Experiments II and III. — To further substantiate evidence presented in Experiment I, two experiments were conducted in which individually housed three-week-old littermate pigs were weaned on the Basic ration, Ampicillin ration, or Hibitane ration. After four weeks from weaning, the above rations were discontinued, and all the animals were housed together and fed commercial early weaning ration. Bacteriological examinations of the fecal samples obtained at two to three day intervals were carried out throughout the period of observation.

The general pattern of results in both of these experiments was similar in that pigs weaned on Basic and Hibitane rations developed enteritis within the first few days after weaning while those on Ampicillin ration did not. In the pigs with enteritis, *E. coli* — G7 caused the initial infection and was replaced by *E. coli* — E145 except for one pig in which *E. coli* — E145 caused the initial infection.

Figure 4 shows that 48 hours after weaning a pig on Basic ration, the numbers of *E. coli* — G7 which were non-detectable in 10^{-4} fecal dilution at weaning increased to log 5.81 per gram of feces. On the 5th day the animal developed very loose diarrhea and strain G7 increased to log 7.96. This *E. coli* strain remained at the same high level on the 7th day and the animal showed acute symptoms of enteritis. On the 9th day the numbers of *E. coli* — G7 decreased and the clinical symptoms improved. By the 12th day the animal developed a second attack of diarrhea when

almost the entire population of fecal *E. coli* consisted of strain E145. The animal recovered from the clinical symptoms on the 14th day but strain E145 did not disappear until after the 19th day. From the 21st day to the 26th day no hemolytic *E. coli* were isolated. After sampling on the 28th day, this pig was housed with the other two test animals and fed commercial ration. Three different serotype of hemolytic *E. coli* (Fig. 4) were found in the fecal samples between the 28th and 35th day but the animal did not get an attack of enteritis during this period.

The pig fed Hibitane ration developed profuse diarrhea 48 hours after weaning and its fecal sample contained log 8.26 *E. coli* — G7 (Fig. 5). Some signs of clinical improvement were observed on the 5th day and the numbers of *E. coli* — G7 had decreased. On the 7th day this animal developed a second attack of enteritis when strain E145 was found in large numbers. This E145 infection was at a peak on the 9th day (log 8.23) when the animal showed acute symptoms of enteritis. By the 12th day, strain E145 was replaced by a new non-typable hemolytic *E. coli* and symptoms of the disease persisted. After the disappearance of hemolytic *E. coli* on the 16th day, the general condition of the piglet improved. Although a non-typable hemolytic *E. coli* (re)appeared from the 26th day to the 30th day, the animal remained healthy. On the 28th day, this animal was housed with the other test animals and five days later *E. coli* — E145 reappeared in large numbers but the animal did not develop any untoward symptoms. Figure 5 shows that this piglet suffered from acute enteritis during the first two weeks after weaning and at the end of this period weighed less than its original weight on the weaning day but soon after recovery, marked improvement in weight gains was found.

The results from a pig fed Ampicillin ration are presented on Fig. 6. This feed produced a marked influence on the fecal microflora of the animal during the first week after weaning. The total number of bacteria per gm of feces (growing on penicillin blood agar medium) decreased from log 8.73 on the weaning day to log 6.40 on the 5th day after weaning, but increased to the original numbers within the next four days. The 5th day fecal sample contained log 5.41 *E. coli* — G7 but the
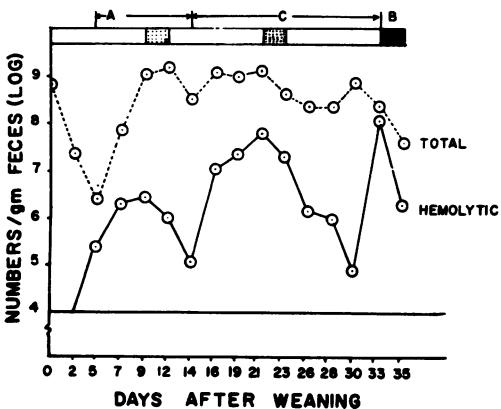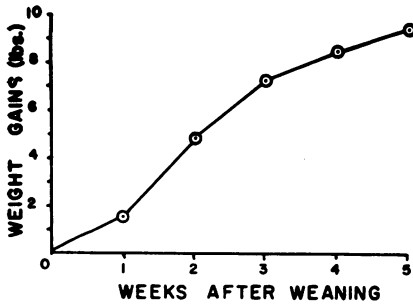
Fig. 6. Effect of enteritis on weekly weight gains (top graph) and fecal E. coli (bottom graph) of a pig fed Ampicillin supplemented ration. Horizontal bar: Blank — no enteritis, Solid black — enteritis, Spotted — mild enteritis. A-G7, B-E145, C-non-typable.

the animals fed Hibitane and Ampicillin rations (Figs. 5 and 6). However, the pig fed Basic early weaning ration died of peracute enteritis on the 7th day after weaning. Figure 7 shows that this pig developed profuse diarrhea accompanied by a very heavy infection of E. coli — G7 (log 9.90) on the second day following weaning. The symptoms of enteritis continued on the 4th day. By the 6th day, the G7 strain was superimposed by strain E145 and the animal became extremely ill and died of peracute symptoms on the 7th day. The numbers of E. coli strains G7 and E145 obtained from samples of the intestinal contents of duodenum, ileum, and cecum are presented in Fig. 7. Hemolytic E. coli constituted most of the bacterial population (growing on penicillin blood agar medium) in the duodenal and cecal samples, but these were the only organisms isolated from the contents of the ileum. The serological identification of the hemolytic E. coli showed that strain E145 which appeared on the 6th day (10% of the total hemolytic population) was the predominant strain found on the 7th day (70 to 100%). The mesenteric lymph glands of this ani-

animal remained clinically healthy. The numbers of E. coli — G7 reached a maximum (log 6.50) on the 9th day and the animal had a very mild diarrhea. The enteritis symptoms disappeared on the next day and the numbers of E. coli — G7 in later fecal samples showed a gradual decline. Between the 16th and 30th days after weaning, the fecal samples of this pig contained an Ampicillin resistant, nontypable hemolytic E. coli. On the 21st day when the numbers of this strain were at a peak (log 7.90) the piglet developed mild diarrhea which disappeared a day later. Five days after this animal was housed with the other two test animals and fed commercial ration, E. coli — E145 appeared in large numbers (log 8.16) and this microbial change was followed by an attack of diarrhea on the next day. This pig gained the most weight during the period of the trial (Fig. 6).

The results obtained from Experiment III were similar to those given above for
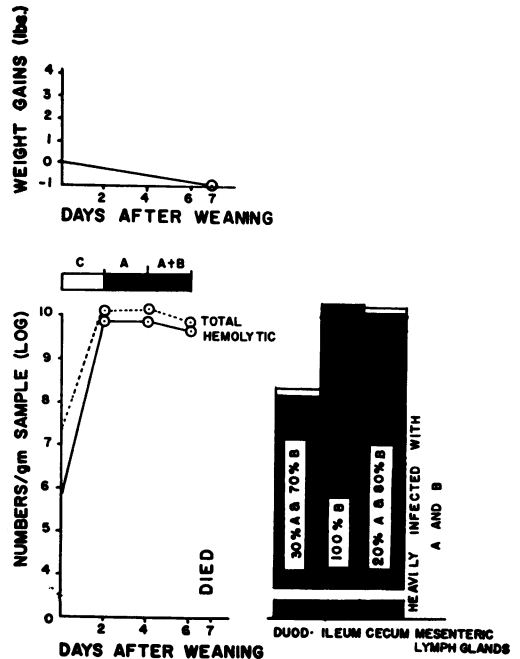

Fig. 7. Effect of enteritis on weekly weight gains (top graph) and fecal and intestinal E. coli (bottom graphs) of a pig fed Basic early weaning ration. Horizontal bar: Blank — no enteritis, Solid black — enteritis; Vertical bars: entire length — total number of colonies growing on penicillin blood agar medium, Solid black — hemolytic E. coli; A-G7, B-E145, C-non-typable.

mal were acutely inflamed and appeared hemorrhagic. Heavy growth of strains G7 and E145 was isolated from several of these inflamed nodes. Figure 7 shows that the carcass weight of this pig was 1 lb less than its original weight at weaning.

These three experiments provided very strong evidence of the infectious nature of two hemolytic strains of *E. coli*. A fourth experiment was then planned to assess the validity of these results on a larger number of pigs. A total of 10 three-week-old early weaned pigs were obtained from three different litters which had been reared in adjacent pens of the piggery. Three of these pigs (one from each litter), were fed commercial early weaning ration and the other seven were fed Ampicillin ration. Each group of animals was housed in separate box stall. Neither the pigs fed commercial ration nor those on Ampicillin ration contracted infections of pathogenic serotypes of *E. coli* and none of the ten animals developed clinical signs of enteritis during the first week after weaning. On the 6th day after weaning, the animals on commercial early weaning ration were infected by adding 10 ml of the 18-hour broth culture of *E. coli* — E145 to their drinking water. The pigs fed Ampicillin ration were left uninfected. By the 9th day, the fecal samples obtained from all three infected animals contained *E. coli* — E145 in large numbers (Fig. 8) but none of the animals had developed enteritis. All three animals had diarrhea between the 9th and 13th days and strain E145 was found in large numbers. The animals recovered slowly from the infection and from the clinical symptoms during the next week.

None of the seven pigs on Ampicillin supplemented ration was cross infected during the week following artificial infection of the pigs fed commercial ration although both groups were housed in the same experimental barn and were looked after by the same attendant. On the 13th day, two of the seven pigs on Ampicillin ration were removed and housed with the three pigs which were recovering from *E. coli* — E145 infection. On the same day, the drinking water of the remaining five pigs on Ampicillin ration was contaminated by adding 10 ml of an 18-hour old broth culture of *E. coli* — E145. Both pigs which were housed with the recovering animals contracted *E. coli* — E145
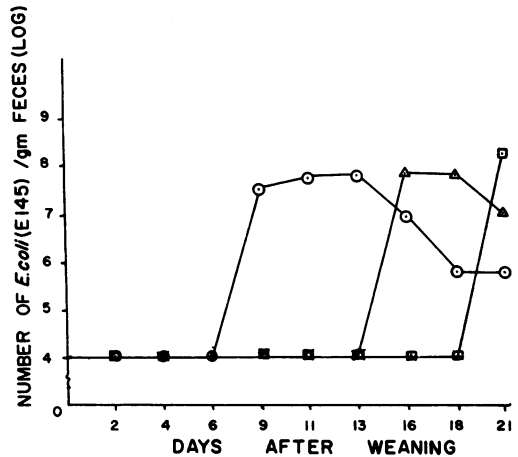

Fig. 8. Effect of artificial infection with E. coli — E145. Circles . . . Three animals fed commercial early weaning ration throughout the experiment. These animals were artificially infected with E. coli — E145 by contaminating their drinking water on the 6th day after weaning; Triangles . . . Two animals fed Ampicillin ration until the 13th day after weaning when they were housed with the group on commercial ration; Squares . . . Five animals fed Ampicillin ration until the 18th day after weaning, drinking water of these animals was contaminated with E. coli — E145 on the 13th day after weaning and on the 18th day they were housed with the other two groups and fed commercial ration. The data presented on this graph pertains to only one representative animal of the group on each treatment.

infection within three days and developed enteritis which continued during the following week (Fig. 8). Artificial infection of the pigs fed Ampicillin ration was unsuccessful as strain E145 was not detected in the feces of these animals and nor did these animals show any visible symptoms of enteritis. On the 18th day, all ten animals were housed together and fed commercial ration. Within the next three days, all five of the newly introduced animals contracted *E. coli* — E145 and developed enteritis (Fig. 8).

Discussion

Certain strains of *E. coli* are associated with enteritis of early weaned pigs. These strains are so virulent that they replace the previously existing coliform microflora within a matter of a few hours. The virulence and pathogenicity of *E. coli* strains G7 and E145 were demonstrated and proven conclusively in a series of experiments involving 26 early weaned pigs.

The pigs weaned on Basic early weaning ration and the one supplemented with Hibitane were infected with these strains shortly after weaning. When the infection due to either of the two strains was at a peak, the animals developed diarrhea.

The clinical symptoms of enteritis were alleviated gradually with the decline of each infection, which indicated that both strains were actively involved in the etiology of baby pig enteritis. The fact that an abrupt multiplication of pathogenic strains was not the effect of clinical enteritis itself is apparent from the results presented in Fig. 7 which shows that a pig on Basic ration died while harbouring 10^{10} viable cells of pathogenic E. coli. Both pathogenic strains were isolated from the inflamed mesenteric lymph glands of the dead animal which showed that these E. coli possess an invasive capacity. Furthermore, in the last experiment where these strains did not appear in the fecal samples of pigs, none of the animals developed enteritis during the entire period of observation. In most of the animals, the symptoms of enteritis appeared only when the total counts of the pathogenic E. coli were more than 10^7 per gm of feces.

The symptoms of enteritis were successfully reproduced in the pigs by contaminating their drinking water with E. coli — E145. Within three days after the artificial infection, the organism was able to replace almost all other coliforms in the alimentary tract of the infected pigs. However, the animals showed symptoms of enteritis four days after drinking the contaminated water, which indicates that the organism has to establish itself and multiply in the gastro-intestinal tract before it can cause any visible symptoms of the disease.

When the pigs fed Ampicillin ration were offered drinking water infected with E. coli — E145, neither the organism appeared in the fecal samples nor did any of the seven animals develop enteritis, confirming the previous report from this laboratory (3) on the in vivo inhibitory action of Ampicillin on the growth of pathogenic E. coli. On the other hand when these animals were housed with other animals showing clinical symptoms of enteritis, each one of the seven pigs contracted E. coli — E145 infection and developed enteritis. Presumably, none of the ten animals in this experiment had harboured E. coli — E145 prior to weaning, and therefore unlike the first three experiments where the stress of weaning induced a quick multiplication of the pathogenic E. coli and caused enteritis, such stress alone could not precipitate an attack of enteritis in the absence of the pathogenic organisms in the intestinal tract of these pigs.

The origin of pathogenic E. coli in naturally occurring cases of enteritis is difficult to ascertain. Conceivably, acquired from the mothers, these strains exist in non-detectable numbers in the alimentary tracts of the piglets prior to weaning, and flare up suddenly due to the stress of weaning. All pigs in the first three experiments were initially infected with E. coli — G7 which was soon replaced by E. coli — E145. Whether this succession of infections is of any real significance is difficult to determine from these experiments. Assuming that both strains are present in the animal prior to weaning in subinfective numbers, the strain possessing a greater virulence is able to multiply first and after the animal acquires some degree of immunity to this organism, another pathogenic strain, probably of the next level of virulence, multiplies and replaces the former strain.

During a five week observation period, none of the pigs suffered from a second attack of diarrhea cuased by the same strain of E. coli. The results indicate that after an animal recovers from an attack of enteritis caused by a particular strain of E. coli, it remains immune to further attacks of the same organisms. When the pigs on Ampicillin ration were housed with those on commercial ration, the latter were already recovering from artificially produced E. coli — E145 infection. Only the newly introduced animals which were still susceptible to E. coli — E145 contracted this infection and developed enteritis but the recovering pigs neither showed any increases in number of E. coli — E145 in their feces nor did they develop a second attack of enteritis. These observations indicate the development of active immunity to E. coli infections and support the findings of Gordon and Luke (5) who successfully immunized piglets by vaccinating pregnant sows. Jones et al. (6) were unsuccessful in reducing the incidence of clinical enteritis by employing live or dead E. coli monobacterins. The data found in the present studies show that an infection with one strain of E. coli does not immunize the animal to further infections with different serotypes and could explain the lack of success experienced by Jones et al. (6).

Conclusions

By employing Koch's postulates, certain strains of *Escherichia coli* have been proven to cause enteritis of early weaned pigs.

1. Two strains of hemolytic *E. coli* (G7 and E145 Weybridge) were isolated repeatedly in large numbers from the fecal samples of early weaned pigs that developed enteritis shortly after weaning.

2. Symptoms of enteritis were reproduced artificially by contaminating the drinking water of the pigs with a live culture of *E. coli* strain E145.

3. The same strain of *E. coli* was isolated in very large numbers from the fecal samples of artificially infected animals.

4. The infection of this strain of *E. coli* was transmitted naturally by housing susceptible animals with the diseased animals.

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The Influence of Frequency of Semen Collection, Fractionation of the Ejaculate, and Dilution Rate on the Survival of Stored Dog Sperm.

Semen from nine beagle dog trained for routine semen collection was used to compare survival of extended sperm during storage from 8 to 16 days at 5°C. The following were compared: (1) the first two fractions of semen versus three fractions, (2) semen obtained after dogs were sexually rested for 1 versus 5 days, (3) first versus third ejaculates collected within 24 hours, and (4) an extension of one part of semen to three parts of extender versus 1:30. The third fraction did not affect the survival of dog sperm in most extenders but was of possible benefit when the p.H. of the extender was below 6.0. Sperm in semen samples obtained after 5 days of sexual rest appeared to maintain a higher percentage of motility during storage than did those obtained after a 1-day rest period. However, the sperm concentration was higher in samples collected infrequently, and when this was taken into consideration in extending the semen to standard numbers of sperm for storage little difference was observed. The first ejaculate in a series of three collected in a 24 hour period averaged 66, 61, 57, and 29 percent motile sperm when examined after 1, 4, 8, and 12 days of storage at 5°C. Corresponding values for the third ejaculate were 74, 67, 60, and 23 percent. Sperm motility was higher during storage when semen was extended 1:30 to give 20 million sperm per ml. than when extended 1:3 to give 200 million sperm per ml. (P .01). The average percentage of motile sperm when nine ejaculates of semen were extended 1:30 with a yolk-citrate-glycine-glucose extender was 71, 70, 69, 62, and 43 after 1, 2, 4, 8, and 16 days of storage, respectively.

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