Hemorrhagic gastroenteritis caused by *Escherichia coli* in piglets: Clinical, pathological and microbiological findings

Claude Faubert, Richard Drolet

Abstract

A retrospective study (1980–1989) was conducted to describe the clinical, pathological, and bacteriological findings in 55 cases of hemorrhagic gastroenteritis (HGE) caused by *Escherichia coli* in piglets. The condition occurred in weaned and suckling piglets and was associated with several serogroups of *E. coli*. Most of the isolates of *E. coli* possessed the adhesin F4 (K88) and were hemolytic. Only a few of the isolates of *E. coli* tested produced verotoxins. Clinical signs and pathological findings noted in these cases were compatible with shock.

Résumé

Le gastro-entérite à *Escherichia coli* des porcelets : aspects clinique, pathologique et microbiologique

Cette étude rétrospective (1980–1989) fut entreprise dans le but de caractériser les aspects clinique, pathologique et bactériologique de 55 cas de gastro-entérite hémorragique associée à *Escherichia coli* chez les porcelets. Cette maladie affectait les porcelets sevrés ainsi que ceux à la mamelle et était associée à plusieurs sérogroupes d'*E. coli*. La plupart des isolats d'*E. coli* étaient hémolytiques et possédaient l'adhésine F_4 (K88). Seulement quelques isolats d'*E. coli* étaient vérotoxigènes. Les signes cliniques ainsi que les lésions observées étaient compatibles avec un état de choc.

(Traduit par Dr Thérèse Lanthier)

Can Vet J 1992; 33: 251–256

Introduction

Enteric infections caused by *Escherichia coli* in piglets are ubiquitous in the swine industry. Among the clinical manifestations, diarrhea, particularly in suckling piglets, is the most frequent. At the time of weaning, or immediately after, edema disease and postweaning gastroenteritis (PWGE) caused by *E. coli* also occur sporadically (1). The manifestations of PWGE may range from peracute and fatal to chronic with delayed growth (2). In peracute and acute cases of PWGE, there is a shock-like syndrome in which marked gastric and enteric congestion occur, with occasional hemorrhages in the intestinal lumen. Hemorrhagic gastroenteritis (HGE) is a descriptive term referring to this severe form of PWGE (1,3) and is the main subject of the work reported herein.

Hemorrhagic gastroenteritis is generally described as a postweaning condition, and is associated with the serogroups O138, O139, O141, and O149 of *E. coli* (1). Although this condition has been recognized for some time, the pathogenesis of the disease continues to be poorly understood. The purpose of our investigation was to study cases of HGE submitted to our diagnostic laboratory during a period of 10 years and to describe the clinical, pathological, and bacteriological findings that characterize this form of the disease in Québec.

Materials and methods

All cases from pigs diagnosed as enteric *E. coli* infection submitted to the diagnostic laboratory of the Faculté de Médecine vétérinaire de l'Université de Montréal from 1980–1989, inclusive, were considered in the study. Cases selected for further study were those characterized grossly by marked congestion of the gastrointestinal tract, with or without blood-tinged intestinal contents. The pathological diagnoses for these cases varied and included postweaning gastroenteritis, postweaning hemorrhagic gastroenteritis, *E. coli* hemorrhagic gastroenteritis or enteritis, colitoxicosis, *E. coli* enterotoxemia, intestinal colibacillosis, and *E. coli* diarrhea. Clinical information for each case was provided by the attending veterinarian; it included the age and the clinical signs observed for other pigs in the herd as well as those submitted. Tissue sections that were available were reexamined histologically.

For each case, the intestinal population of *E. coli* was estimated based on Gram's-stained direct smears from mucosal scrapings and by the number of lactose-positive colonies cultured on MacConkey agar from the intestinal contents. Methods used to identify and serotype enteric *E. coli* from these cases have been described by Larivière and Lallier (4). Isolates of *E. coli* from cases of HGE were examined for hemolysis after a 24-hour culture on sheep blood agar plates (BAP). Isolates were also examined for production of adhesins K88, K99, and 987P by methods described previously (5).

Escherichia coli isolates available for study were tested for the production of verotoxins using monolayers of Vero cells in 96-well tissue culture plates (Nunclon, Gibco Canada Inc, Burlington, Ontario) as described previously (6,7). Briefly, approximately 10^5 Vero cells suspended in M199 medium (Sigma Chemical

Département de pathologie et de microbiologie, Faculté de Médecine vétérinaire, Université de Montréal, C.P. 5000, Saint-Hyacinthe, Québec J2S 7C6. Present address of Dr. Faubert: Laboratoires Bio-Recherches Ltée., 87, chemin Senneville, Senneville, Québec H9X 3R3.

Reprint requests to Dr. R. Drolet.

Table 1. Age distribution of piglets with HGE and the *E. coli* serogroups of isolates identified in each case

Age (days)	O149: K91:F4	O8: K"4627":F4	O157: K"V17":F4	O138: K81:F4	O45: KE65:F4	O138: K81	O8: KX105	N.A.[a]	Total number of cases (piglets)
1-7	1	2	—	—	—	—	—	—	3 (3)
8-14	6	2	1	—	—	—	—	2	11 (18)
15-21	7	2	2	—	—	—	—	—	11 (20)
22-28	9	1	1[b]	1[b]	—	—	—	2	13 (16)
29-35	4	—	1[c]	—	1	1[c]	1[c]	3	9 (15)
36-42	2	1	—	—	—	—	—	2	5 (7)
43-49	—	—	—	—	—	—	—	1	1 (1)
50-56	—	1	1	—	—	—	—	—	2 (2)
Total	29	9	6	1	1	1	1	10	55 (82)

[a]Not available
[b]Two *E. coli* serogroups were isolated from the same case
[c]Three *E. coli* serogroups were isolated from the same case

Company, St. Louis, Missouri, USA) were placed in each well two days before use. Isolates of *E. coli* grown under agitation in Evan's broth overnight at 37°C were centrifuged and filtered using 0.2 μm pore size filters (Millipore, Mississauga, Ontario) and 0.05 mL of culture filtrate was added to each well. Tissue culture plates were incubated at 37°C in 5% CO_2. Morphological changes of Vero cells were recorded daily for four consecutive days.

In some cases, the fluorescent antibody test (FAT) was used for detection of transmissible gastroenteritis (TGE) virus (TGE virus conjugate supplied by the Institut Armand-Frappier, Laval des Rapides, Québec) and rotavirus (Rotavirus conjugate supplied by Dr. E. H. Bohl, Ohio Agricultural Research and Developmental Center, Wooster, Ohio, USA) using methods described previously (8).

Results

From 1980–1989 inclusive, 55 cases of HGE, involving 82 piglets, were selected from necropsy submissions. Sixty-two of the piglets were submitted dead and 20 were alive. The pigs came from 47 commercial breeding herds. The number of cases of HGE and the age distribution of the piglets affected are shown in Table 1.

Case histories

Clinical information provided by the attending veterinarians often included descriptions of animals submitted as well as of other animals in the herd. Diarrhea associated with *E. coli*, TGE, sudden death, salmonellosis, colitoxicosis, septicemia, and coccidiosis were the diagnoses considered by the practitioners at the times of submission. In 40% of the cases, the major finding was rapid death in apparently healthy pigs with cyanosis of the extremities. In 56% of the cases, a yellow-to-brown diarrhea was observed in some piglets on the farm. Polypnea was evident in five cases, and nervous signs such as trembling, paralysis, and opisthotonos were noticed in five others. In a few cases, vomiting was observed. Slow growth of pigs was preceived as a problem on two farms.

Gross pathological findings

At necropsy, intestinal congestion was noted, especially involving the jejunum or ileum, and sometimes the duodenum or the ascending colon. In 35 cases, blood-tinged intestinal contents, mostly confined to the ileum, was present along with congestion of the intestinal wall.

Intense congestion of the gastric mucosa was noted in 19 cases. In fewer than 20% of the cases, the mesenteric lymph nodes were reported to be congested. Diffuse pulmonary congestion with edema was found in two cases, and necrosis of the ileal mucosa was observed in one case.

Histopathological findings

Among the 55 cases of HGE selected for study, 42 had tissues available for histological examination. Gastric congestion varied from slight to severe, and was more prominent in the mucosa and submucosa than the muscularis and serosa in all cases examined. A similar pattern of congestion was found in the intestinal wall with occasional hemorrhages in the jejunal or ileal lamina propria mucosae. In most cases, many rod-shaped bacteria were evident on the villus epithelial cells of the small intestine. In many cases there was a moderate infiltrate of neutrophils within the intestinal villus lamina propria mucosae. Necrosis of the villi with marked infiltration of neutrophils occurred in severe cases. In these, microvascular fibrinous thrombi were common and particularly numerous in the mucosa and submucosa of the stomach and intestines. Except for the adhesion of bacteria to epithelial cells, the histological alterations found in the small intestine were also observed in the colon, although less extensively. A summary of the histological changes of the gastrointestinal tract in cases of HGE is shown in Table 2. Different degrees of severity in intestinal lesions are illustrated in Figures 1 and 2.

Diffuse congestion was observed to a variable degree in all other organs examined, particularly in the liver and kidneys. Congestion of the renal medulla was generally marked, with occasional focal hemorrhages. About one-third of the lungs evaluated had slight and

Table 2. Histological lesions of the gastrointestinal tract in 42 cases of HGE

Microscopic findings	Tissue examined				
	Stomach n[a] = 13	Duodenum n = 4	Jejunum n = 47	Ileum n = 45	Colon n = 23
E. coli adhesion to surface epithelial cells	0	4	31	43	0
Infiltration of neutrophils in superficial LPM[b]	0	3	39	45	12
Villus necrosis or superficial LPM necrosis	0	2	39	36	3
Congestion of					
mucosa	13	3	45	45	20
submucosa	13	4	47	45	20
muscularis and serosa	13	4	33	34	12
Thrombosis in					
mucosa	13	4	47	45	23
submucosa	13	2	36	36	20
muscularis and serosa	0	0	0	2	0

[a]Number of tissues examined from different piglets
[b]Lamina propria mucosae

diffuse infiltrates of neutrophils and macrophages within alveolar septa. Slight lymphoid hyperplasia was observed in the white pulp of 23 spleens and, to a more severe degree, in the cortex of a mesenteric lymph node, with focal necrosis of lymphocytes in one case. Foci of small rod-shaped bacteria were observed in the subcapsular sinus of a mesenteric lymph node in one case.

Microbiological findings

In the 55 cases of HGE selected, seven serogroups of *E. coli* were recovered, generally in moderate to large numbers, from cultures of the small intestine. The distribution of each serogroup according to the age of the piglets is shown in Table 1. Ten isolates of *E. coli* were not typable at the time of this study.

In 52 cases, *E. coli* isolated from the small intestine were hemolytic on blood agar plates. In the three other cases, both hemolytic and nonhemolytic colonies of *E. coli* were observed. As shown in Table 1, most of the 48 isolates of *E. coli*, representing 45 cases from which serotyping results were available, produced the adhesin F4 (K88). Of the 48 isolates, 24 (representing 21 cases) were available for further investigation. These consisted of 12 isolates of serogroup O149:K91:F4, four of O157:K"V17":F4, four of O8:K"4627":F4, and one isolate each of serogroups O45:KE65:F4, O8:KX105, O138:K81:F4 and O138:K81. Verotoxic activity was present in culture supernatants of seven of these, from seven different clinical cases. Three isolates were of serogroup O149:K91:F4, two of O8:K"4627":F4, and one each of O157:K"V17":F4 and O138:K81. Four of the VT-producing isolates of *E. coli* came from weaned piglets and three from suckling piglets.

Frozen sections of gut from 21 of the 55 cases were examined for common enteric porcine viruses, such as the rotavirus type A and the TGE virus, using the FAT. Two cases were found positive for the rotavirus and none for TGE. In one case, a few coccidia were present within epithelial cells of the small intestinal mucosa.

Discussion

In swine, marked congestion of the small intestine which is sometimes associated with blood-tinged intestinal contents has been reported frequently in postweaning colibacillosis (1,2,9,10). Terms such as hemorrhagic gastroenteritis and colibacillary shock have been used to describe this form of the disease (11). Our results suggest that descriptive names for this condition such as "postweaning hemorrhagic gastroenteritis" might be inappropriate, as we found cases of HGE in piglets between one and 56 days of age, and many (69%) were less than 28 days old. Most producers in our area wean their piglets at about 28 days of age.

No pathognomonic clinical sign characterizes HGE (2). Rapid death of apparently healthy piglets with slight cyanosis of the extremities were the most salient clinical signs noted in our study and are considered by some investigators to be indicative of shock (9). Vomiting, polypnea, and terminal nervous signs, observed in some pigs in our study, also occur in animals with shock (12,13). As also observed by others

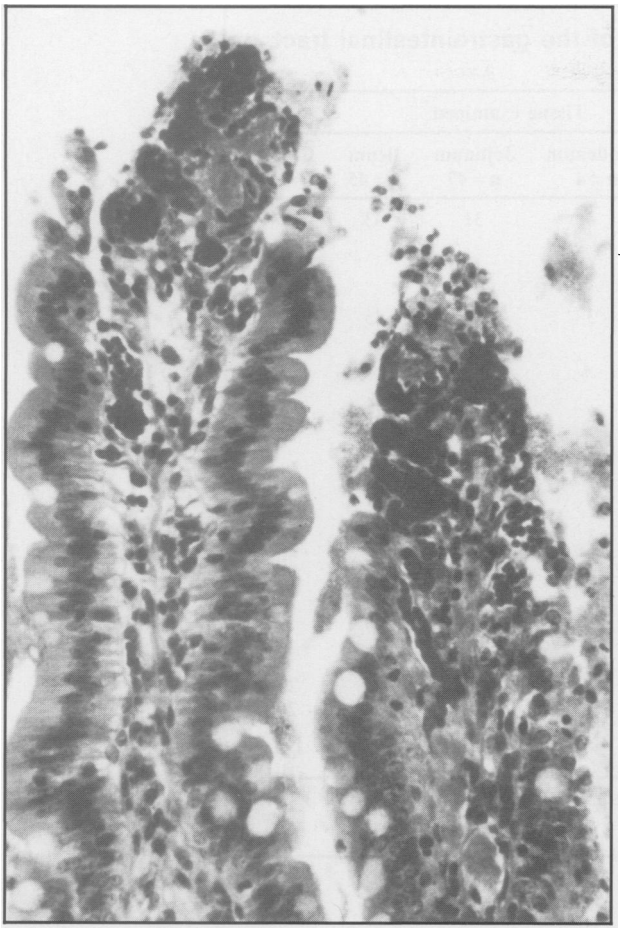

Figure 1. Slight to moderate microscopic changes to the ileal mucosa in a piglet (submitted alive) with *E. coli* HGE. Desquamation of epithelial cells at the tips of the villi with infiltration of neutrophils, congestion, and thrombosis in the lamina propria. Numerous rod-shaped bacteria between the villi.

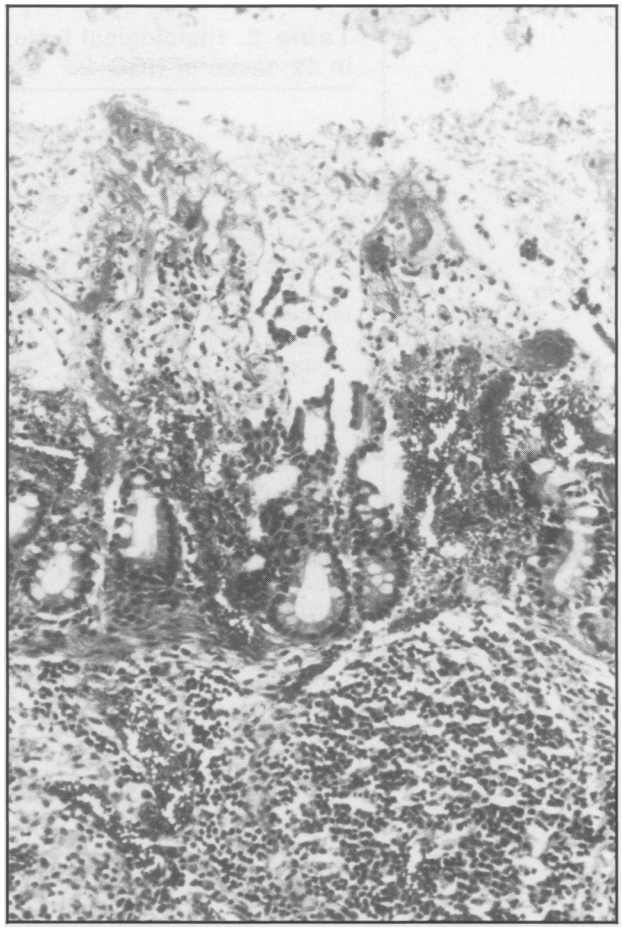

Figure 2. Severe microscopic changes to the ileal mucosa in a piglet (submitted alive) with *E. coli* HGE. Several necrotic villi with infiltration of neutrophils. Diffuse congestion of the mucosa with thrombosis.

(1,2), diarrhea was often observed in the herd, but not necessarily in the piglets submitted for necropsy.

The gross and microscopic lesions observed in this study were similar to those described previously for this condition (1,2,9–11). Splanchnic congestion, often found in organs examined in our study, is also observed in endotoxic shock (12–14) or shock due to other causes (15). Microvascular thrombosis within the mucosa and submucosa of the stomach and intestines was a striking change found in this retrospective study. Severe lesions involved the jejunum and ileum and sometimes the colon. However, in most cases, only slight changes were found in the large intestine. In severe cases, lesions can be confused with those of enteritis caused by *Clostridium perfringens* type C (16). Ischemic necrosis of the villi of the small intestine occurred in many cases, and was associated with the presence of thrombosed vessels and neutrophils in the area. The presence of endotoxemia may explain the observed microscopic lesions, especially fibrinous thrombosis (17).

Although shock appears to be an important component in this disease, its pathogenesis continues to be poorly understood. It has been proposed that pigs are sensitized to lipopolysaccharides of *E. coli* and that rapid absorption of *E. coli* endotoxin from the gastrointestinal tract results in anaphylactic shock (3,18–20). A second theory suggests that HGE is the result of endotoxic shock (1,12,14). After the rapid multiplication of enterotoxic *E. coli* in the gut, endotoxins may be absorbed from the intestinal tract, enter the blood stream, and complicate the enteric disease (21). Several investigators have suggested that the size of the intraintestinal pool of endotoxin, its continuous systemic absorption, and the efficiency of the reticuloendothelial system to detoxify it are important factors influencing the rate of free circulatory endotoxin (22–24). However, this theory remains controversial, because of the difficulty in assessing the intestinal pool of endotoxin and measuring its absorption (15). *In vitro*, the intestinal absorption of endotoxin does not appear to affect significantly the integrity of the intestine (25). However, the pathophysiological circulatory disturbances that occur at the level of the intestine in shock (26,27) may partially explain the lesions observed in piglets affected with HGE.

Various serogroups of *E. coli* were involved in these cases of HGE. Serogroup O149 was the most common isolate, followed by isolates of serogroups O8 and

O157, and a few cases involved O138 and O45. Results of our study suggest that HGE is not commonly associated with the edema disease serogroups (O138, O139 and O141) but rather with the classical enterotoxigenic F4-positive serogroups (O149, O8 and O157). The latter were found in piglets of all ages (Table 1). Serogroups of *E. coli* that have been previously associated with HGE are O138, O139 (1,2,10,11), O141 (1,2,10), O8 (11,14), O157 (11), and O149 (1,11,14).

All cases of HGE studied were associated with isolates of *E. coli* that were hemolytic on BAP. In a recent study from Nebraska, it was shown that all cases of shock associated with intestinal colibacillosis in swine involved hemolytic strains of *E. coli* (11). Hemolytic *E. coli* can be found in healthy pigs (14,28–30) and the hemolysins of *E. coli* have not been shown to be important in enteric disease (31,32). Both hemolytic and nonhemolytic *E. coli* produce diarrhea and edema disease in pigs and the role of the hemolysin in intestinal infection by this bacterium remains obscure (32,33). It has been suggested that the hemolysin could damage the intestinal mucosa, thereby providing a portal for endotoxins to enter the vascular system (34), but this has not been demonstrated experimentally. The lipopolysaccharides of *E. coli* may be complexed with the alpha-hemolysin of the bacterium and may result in increased toxicity (35). The contribution of the hemolysin in HGE remains speculative but deserves mention in light of our results and those of others (11).

In all 45 cases in which a serotype of *E. coli* was available for this study, the bacteria produced the adhesin F4 (K88). These results agree with those of Moxley *et al* (11) where 94% of the isolates of *E. coli* associated with HGE expressed the F4 pilus antigen. Gram-negative rods were attached to the mucosa of the small intestine in most of the cases in our study. The site of colonization of F4-positive *E. coli* involves almost the whole length of the small intestine, in contrast to F5- or F6-positive *E. coli* which colonize only the distal jejunum and ileum (36). This extensive colonization of the small intestine by F4-positive *E. coli* could hypothetically create a larger pool of endotoxin, hemolysin (or their complex), or another unknown toxin or virulence factor, to be absorbed from the intestinal lumen and result in shock.

Approximately one third of the isolates of *E. coli* available at the time of this study were verotoxin-positive (VT+). Verotoxin-positive *E. coli* isolates have been found in weaned piglets suffering from edema disease (37–40) and diarrhea (6,38,40), in suckling piglets suffering from diarrhea and septicemia (37), as well as in normal pigs (38). In one study, VT+ *E. coli* isolates were cultured from bloody stools of weaned pigs (38). Verotoxins produced by *E. coli* are cytotoxic to Vero cells and may act in a similar way on intestinal epithelial cells *in vivo* by inhibition of protein synthesis (6,41). High concentrations of toxin in the intestine may account for local damage (39). A combination of production of adhesin and verotoxin by the same *E. coli* may contribute to its pathogenicity (42,43). Serogroups producing verotoxin in this study have been shown previously to be occasionally VT+ (38), however O8:K"4627":F4 does not seem to represent a frequent verotoxigenic serogroup. The importance of verotoxins produced by *E. coli* in cases of classical *E. coli* diarrhea of piglets (44) as well as in HGE remains to be demonstrated. Results from our study suggest that verotoxins do not play a significant role in the pathogenesis of HGE because most of the *E. coli* isolates from these cases were nonverotoxigenic.

Our results suggest also that intestinal coccidiosis, porcine coronavirus, and rotavirus are not likely to be predisposing factors for this condition. Septicemia was not a feature noted in the cases of HGE that we examined. Nevertheless, terminal bacteremia has been observed occasionally in this form of enteritis caused by *E. coli* (1,2).

From this study, we conclude that *E. coli* HGE is a condition that is not restricted to the postweaning period, as it affects suckling as well as weaned piglets. Most of the isolates recovered from pigs with HGE were hemolytic and produced the F4 adhesin. The clinical signs and the pathological findings noted in these cases were compatible with shock. Further studies are needed to clarify the pathogenesis of shock that characterizes this often lethal colibacillary enteric infection.

Acknowledgments

We are grateful to Dr. J.M. Fairbrother and Mrs. Clarisse Désautels for their expertise in the verotoxin assay. We also thank the staff of the Département de Pathologie et de Microbiologie de la Faculté de Médecine vétérinaire de Saint-Hyacinthe for their contribution. CVJ

References

1. Barker IK, Van Dreumel AA. The alimentary system. In: Jubb KVF, Kennedy PC, Palmer N, eds. Pathology of Domestic Animals. Orlando: Academic Press, 1985: 128–135.
2. Richards WPC, Fraser CM. Coliform enteritis of weaned pigs. A description of the disease and its association with hemolytic *Escherichia coli*. Cornell Vet 1961; 51: 245–257.
3. Thomlinson JR, Buxton A. Anaphylaxis in pigs and its relationship to the pathogenesis of oedema disease and gastroenteritis associated with *Escherichia coli*. Immunology 1963; 6: 126–139.
4. Larivière S, Lallier R. *Escherichia coli* strains isolated from diarrheic piglets in the province of Quebec. Can J Comp Med 1976; 40: 190–197.
5. Fairbrother JM, Larivière S, Johnson WM. Prevalence of fimbrial antigens and enterotoxins in nonclassical serogroups of *Escherichia coli* isolated from newborn pigs with diarrhea. Am J Vet Res 1988; 49: 1325–1328.
6. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. Infect Immun 1977; 18: 775–779.
7. Scotland SM, Gross RJ, Rowe B. Laboratory tests for enterotoxin production, enteroinvasion and adhesion in diarrhoeagenic *Escherichia coli*. In: Sussman M, ed. The Virulence of *Escherichia coli*: Reviews and Methods. Montréal: Academic Press, 1985: 395–405.
8. Morin M, Morehouse LG, Solorzano RF, Olson LD. Transmissible gastroenteritis in feeder swine: Clinical, immunofluorescence and histopathological observations. Can J Comp Med 1973; 37: 239–248.
9. Stevens AJ. Coliform infections in the young pig and a practical approach to the control of enteritis. Vet Rec 1963; 75: 1241–1246.
10. Svendsen J. Enteric *Escherichia coli* diseases in weaned pigs. Nord Vet Med 1974; 26: 226–238.

11. Moxley RA, Erickson ED, Breisch S. Shock associated with enteric colibacillosis in suckling and weaned swine. Proc George A. Young Swine Conference and Annual Nebraska SPF Swine Conference, 1988: 33-38.
12. Nielsen NO, Clugston RE. Comparison of *E. coli* endotoxin shock and acute experimental edema disease in young pigs. Ann N Y Acad Sci 1971; 176: 176-189.
13. Clugston RE, Nielsen NO. Experimental edema disease of swine (*E. coli* enterotoxemia). 1. Detection and preparation of an active principle. Can J Comp Med 1974; 38: 22-28.
14. Nielsen NO. Edema disease. In: Leman AD, Straw B, Glock RD, Mengeling WL, Penny RHC, Scholl E, eds. Diseases of Swine. Ames: Iowa State University Press, 1986: 528-541.
15. Cheville NF. Blood and vascular system. In: Cheville NF, ed. Cell Pathology. Ames: Iowa State University Press, 1983: 191-197.
16. Dam A, Knox B. Haemolytic *Escherichia coli* associated with enteritis and enterotoxaemia in pigs in Denmark. Nord Vet Med 1974; 26: 219-225.
17. Morrison DC, Ulevitch RJ. The effects of bacterial endotoxins on host mediation systems. Am J Pathol 1978; 93: 527-605.
18. Buxton A, Thomlinson JR. The detection of tissue-sensitizing antibodies to *Escherichia coli* in oedema disease, haemorrhagic gastro-enteritis and in normal pigs. Res Vet Sci 1961; 2: 73-88.
19. Thomlinson JR, Buxton A. A comparison of experimental anaphylactic shock in guinea pigs with naturally-occurring oedema disease and haemorrhagic gastro-enteritis in pigs. Res Vet Sci 1962; 3: 186-202.
20. Shreeve BJ, Thomlinson JR. Absorption of *Escherichia coli* endotoxin by the neonatal pig. J Med Microbiol 1972; 5: 55-59.
21. Moon HW. Pathogenesis of enteric diseases caused by *Escherichia coli*. Adv Vet Sci Comp Med 1974; 18: 179-211.
22. Ravin HA, Rowley D, Jenkins C, Fine J. On the absorption of bacterial endotoxin from the gastro-intestinal tract of the normal and shocked animal. J Exp Med 1960; 112: 783-792.
23. Wiznitzer T, Schweinburg FB, Atkins N, Fine J. On the relation of the size of the intraintestinal pool of endotoxin to the development of irreversibility in hemorrhagic shock. J Exp Med 1960; 112: 1167-1171.
24. Greene R, Wiznitzer T, Rutenburg S, Frank E, Fine J. Hepatic clearance of endotoxin absorbed from the intestine. Proc Soc Exp Biol Med 1961; 108: 261-263.
25. Nolan JP, Hare DK, McDevitt JJ, Vilayat Ali M. In vitro studies of intestinal endotoxin absorption. 1. Kinetics of absorption in the isolated everted gut sac. Gastroenterology 1977; 72: 434-439.
26. Lillehei RC, Dietzman RH, Movsas S. The visceral circulation in shock. Gastroenterology 1967; 52: 468-471.
27. Lundgren O. Studies on blood flow distribution and countercurrent exchange in the small intestine. Acta Physiol Scand Suppl 1967; 303: 1-42.
28. Campbell SG. Studies on strains of haemolytic *Escherichia coli* isolated from normal swine after weaning. Vet Rec 1959; 71: 909-911.
29. Smith HW. The haemolysins of *Escherichia coli*. J Pathol Bacteriol 1963; 85: 197-211.
30. Wilson MR. Enteric colibacillosis. In: Leman AD, Straw B, Glock RD, Mengeling WL, Penny RHC, Scholl E, eds. Diseases of Swine. Ames: Iowa State University Press, 1986: 520-528.
31. Smith HW, Linggood MD. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *Escherichia coli* with particular reference to porcine diarrhea. J Med Microbiol 1971; 4: 467-485.
32. Cavalieri SJ, Bohach GA, Snyder IS. *Escherichia coli* alpha-haemolysin: characteristics and probable role in pathogenicity. Microbiol Rev 1984; 48: 326-343.
33. Welch RA, Dellinger EB, Minshew B, Falkow S. Haemolysin contributes to virulence of extra-intestinal *E. coli* infections. Nature 1981; 294: 665-667.
34. Kurtz HJ, Short EC Jr. Pathogenesis of edema disease in swine: pathologic effects of hemolysin, autolysate, and endotoxin of *Escherichia coli* (O141). Am J Vet Res 1976; 37: 15-24.
35. Bohach GA, Snyder IS. Chemical and immunological analysis of the complex structures of *Escherichia coli* alpha-hemolysin. J Bacteriol 1985; 164: 1071-1080.
36. Gaastra W, De Graaf FK. Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol Rev 1982; 462: 129-161.
37. Kashiwazaki M, Ogawa T, Isayama Y, Akaika Y, Tamura K, Sakazaki R. Detection of vero cytotoxic strains of *Escherichia coli* isolated from diseased animals. Natl Inst Anim Health Q (Tokyo) 1980; 20: 116-117.
38. Gannon VPJ, Gyles CL, Friendship RW. Characteristics of verotoxigenic *Escherichia coli* from pigs. Can J Vet Res 1988; 52: 331-337.
39. Gannon VPJ, Gyles CL, Wilcock BP. Effects of *Escherichia coli* shiga-like toxins (verotoxins) in pigs. Can J Vet Res 1989; 53: 306-312.
40. Smith HW, Green P, Parsell Z. Vero cell toxins in *Escherichia coli* and related bacteria: transfer by phage and conjugation and toxic action in laboratory animals, chickens and pigs. J Gen Microbiol 1983; 129: 3121-3137.
41. Brown JE, Rothman SW, Doctor BP. Inhibition of protein synthesis in intact HeLa cells by *Shigella dysenteriae 1* toxin. Infect Immun 1980; 29: 98-107.
42. O'Brien AD, Laveck GD, Thompson MR, Formal SB. Production of *Shigella dysenteriae* type 1-like cytotoxin by *Escherichia coli*. J Infect Dis 1982; 146: 763-769.
43. Pai CH, Kelly JK, Meyers GL. Experimental infection of infant rabbits with verotoxin-producing *Escherichia coli*. Infect Immun 1986; 51: 16-23.
44. O'Brien AD, Laveck G. Purification and characterization of a *Shigella dysenteriae* 1-like toxin produced by *Escherichia coli*. Infect Immun 1983; 40: 675-683.