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Porcine proliferative enteritis - characterization of the naturally occurring and experimental disease

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PORCINE PROLIFERATIVE ENTERITIS - CHARACTERIZATION OF THE
NATURALLY OCCURRING AND EXPERIMENTAL DISEASE

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Porcine proliferative enteritis - characterization of

the naturally occurring and experimental disease

by

Larry Gene Lomax

A Dissertation Submitted to the

Graduate Faculty in Partial Fulfillment of the

Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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TABLE OF CONTENTS

GENERAL INTRODUCTION

Preamble

Campylobacter sputorum subspecies mucosalis (CSM)-
associated conditions (intestinal adenomatosis, prolifera-
tive hemorrhagic enteropathy, necrotic enteritis, and regional
ileitis) in swine have been recognized since 1973 in Great
Britain, the Scandinavian countries, Australia, and Canada.
The constant presence of CSM within proliferated mucosal
epithelial cells of the small and large intestine of pigs
with these conditions has been demonstrated. Another CSM-
associated condition (proliferative enteritis) has been
recognized in swine in the United States. The clinical,
epidemiologic, and pathologic findings of pigs with pro-
liferative enteritis have not been described.

The experimental reproduction of all of the CSM-associated
conditions has not been accomplished. Lesions which resemble
mild forms of intestinal adenomatosis have been reported in a
small percentage of pigs orally inoculated with either
homogenized intestinal mucosa from naturally occurring cases
of intestinal adenomatosis or cultures of CSM. However,
the production of CSM-associated conditions that duplicate
those seen in naturally infected swine has not been achieved.
Experimental reproduction must be accomplished before lesion
development can be characterized, and the significance of CSM
as a swine enteric pathogen can be assessed.

OBJECTIVES

The objectives of this investigation were to: 1) characterize the clinical, epidemiological and pathological features of naturally occurring proliferative enteritis in swine, 2) experimentally reproduce proliferative enteritis in Caesarean-derived and colostrum-deprived pigs and characterize the clinical and pathologic features of the disease, and 3) experimentally reproduce proliferative enteritis in specific-pathogen-free pigs and characterize the development of lesions.

DISSERTATION FORMAT

This dissertation is presented in an alternate format which
includes four manuscripts to be submitted to scientific
journals for publication. Three manuscripts will be sub-
mitted to the American Journal of Veterinary Research and
one to the Journal of the American Veterinary Medical
Association. The manuscripts are presented in the format
required by the American Journal of Veterinary Research
except for footnote notation which complies with the Graduate
College Thesis Manual (Iowa State University, 1979).
References are cited at the end of each manuscript and are
in compliance with American Journal of Veterinary Research
format. The manuscripts are preceded by a general intro-
duction which includes a preamble, objectives of the investi-
gations, and a review of the literature. General discussion
and conclusions from the investigations follow the fourth
manuscript. Additional references cited refers to citations
from the general introduction and general discussion, and
they are recorded in American Journal of Veterinary Research
format.

The Ph.D candidate, Larry Gene Lomax, was the principal
investigator for each of the investigations and is the
senior author for each of the manuscripts. Coauthors with

limited involvement and assistance from others are indicated in the by-line and footnotes of each manuscript.

LITERATURE REVIEW

Historical aspects- Descriptive nomenclature pertaining

to lesions in the intestinal tract of swine has appeared in

the veterinary literature for 50 years or more. These terms

have been used to describe lesions for which etiologic agents

could not be found. However, as more of these agents have

been discovered, specific diseases have been established for

them. Unfortunately, older, descriptive nomenclature is

still often used when a specific disease is referred to.

Necrotic enteritis in swine, characterized by mucosal

necrosis and pseudomembrane formation in the ileum,

cecum, and colon has been associated with numerous causes.

Biester (1928)[1] produced these lesions in pigs by orally

inoculating them with Salmonella choleraesuis. Other

authors have demonstrated that intestinal mucosal necrosis

is a characteristic feature of swine infected with

S. choleraesuis.[2,3,4,5,6,7,8] Niacin (nicotinic acid) deficiency

has been described as a cause of necrotic enteritis, but

only the cecum and colon were involved.[9,10,11] Recently,

several authors have considered necrotic enteritis a sequel

to numerous diseases characterized by enteritis in nursing,

growing, and adult swine.[12,13,14] These diseases included

coccidiosis,[14] rotaviral enteritis, transmissible gastro-

enteritis, clostridial enteritis,[14] colibacillosis,[12,15]

salmonellosis,[14,16,17] swine dysentery,[14] and trichuriasis.[14]

Jennings (1959)[18] reviewed the causes of gastroenteritis in the pig and separated fibrinous and ulcerative enteritis from necrotic enteritis. Fibrinous and ulcerative enteritis were characterized by fibrin deposition over the mucosal surface and necrosis of the underlying mucosa, primarily around the ileocecal valve and in the cecum and colon.[18] The causes included hog cholera virus, Erysipelothrix rhusiopathiae and S. choleraesuis.[18] Necrotic enteritis occurred primarily in the cecum and terminal portion of the ileum and was characterized by mucosal thickening with an overlying layer of fibrinous exudation.[18] The affected areas of the intestine were thickened and rigid.

The terms regional or terminal ileitis have been used by Scandinavian authors to describe conditions affecting growing and fattening pigs. Emsbo (1951)[19] studied the gross and microscopic features of the condition which involved only the terminal part of the ileum. There was hypertrophy of the muscular layers of the ileal wall, adenomatous proliferation of the mucosa, and inflammation which included infiltration of epithelioid and multinucleated giant cells of the mucosa and submucosa.[19] Metastases of glandular epithelial elements to the ileocecal lymph node were observed in a few of the cases.[19] Rahko and Saloniemi (1972)[20] described regional ileitis which was similar to Emsbo's description except that

there was transmural granulomatous inflammation of the ileal wall and focal accumulation of epithelioid and multinucleated giant cells in the ileocecal lymph node. Other authors have described the pathologic findings in cases of regional ileitis in weaned pigs.[21,22,23,24,25]

Biester and Schwarte (1931,[2] 1939[26]) described polyploid growths in the intestines of growing pigs and found that these growths consisted of markedly elongated, often branched crypts which contained hyperplastic, immature-appearing epithelial cells but no goblet cells. Dodd (1968)[27] described a nodular proliferation of the ileal mucosa in a 10-week-old pig and used the terms "adenomatous intestinal hyperplasia" and "proliferative ileitis" for his morphologic diagnoses.

The cause of hemorrhage into the intestinal lumen and hemorrhagic inflammation of the small intestine of swine are numerous and include: Bacillus anthracis;[28] Leptospira icterohemorrhagiae;[28] Clostridium perfringens type C[28]; Escherichia coli;[29] Treponema hyodysenteriae;[28] Salmonella spp.; Isospora spp.;[28] Vitamin E deficiency;[30,31] poisoning with warfarin, arsenic, mercury, sodium fluoride, and copper;[28] mycotoxins;[32] torsion of the mesentery;[33] and gastric ulcers.[28,30] In addition, an intestinal hemorrhage syndrome of unknown cause has been associated with the feeding of whey to pigs.[34] This syndrome was also reported

by numerous authors who were unable to associate it with
whey feeding or any other cause.[28,35,36,37,38] However,
these reports were of a superficial nature and their
validity is questionable.

An unfortunate term, "hemorrhagic bowel syndrome",
was introduced into the veterinary literature in 1970 by
O'Neill.[39] He named the condition based on the clinical
observation of discharge of blood from the rectum and the
presence of blood in the lumen of the ileum, cecum, and
colon of 4 to 34 month old pigs.[39] The term was subse-
quently used by other authors throughout the world who
published reports which lacked careful histopathologic
descriptions.[40,41,42,43] The term was also used by authors
who included histopathologic findings in their reports.
O'Hara (1972)[44] described the syndrome which occurred in
growing and adult pigs. The syndrome was characterized
clinically by hemorrhage from the rectum followed by death
in a few hours. Pathologic findings consisted of thickened
ileal walls, clotted blood in ileal lumina, and edema of the
mesentery and ileocecal lymph nodes.[44] Microscopic lesions
were mucosal hyperplasia, superficial mucosal ulceration and
hemorrhage, absence of goblet cells, and infiltration of
inflammatory cells into the lamina propria of the ileum.[44]
Rowland and Rowntree (1972)[45] reported similar pathologic

findings in 54 pigs of all ages in Scotland. However, pigs which survived the hemorrhagic episode were said to develop intestinal adenomatosis[45] (a term apparently synonymous with the term, intestinal adenoma[2]). Other workers described similar conditions, but intestinal adenomatosis did not follow the hemorrhagic syndrome.[46,47,48]

A possible relationship between intestinal adenomatosis, necrotic enteritis, regional ileitis, and the hemorrhagic bowel syndrome (later renamed proliferative hemorrhagic enteropathy[49]) was established when Rowland and Lawson (1973[49,50]) and Rowland et al. (1973,[51] 1976[52]) described the presence of a bacterium within affected epithelial cells of intestinal mucosa from each of these conditions. Lawson and Rowland (1974)[53] and Lawson et al. (1975,[54] 1976,[55] 1979[56]) isolated bacteria with similar biochemical and antigenic characteristics from each of these conditions and named the organisms Campylo-bacter sputorum subspecies mucosalis (CSM). Other workers throughout the world subsequently reported the isolation of CSM from one or more of the above conditions.[57,58,59]

In the United States, a condition was observed in feeder pigs which was similar to the four CSM-associated conditions. Bergeland (1975)[60] called the condition necroproliferative enteritis because it was characterized microscopically by crypt epithelial cell proliferation and mucosal necrosis in the ileum and occasionally also in the cecum and colon.

Epidemiology- Epidemiologic studies pertaining to CSM-
associated conditions in swine are not common in the
veterinary literature. Intestinal adenomatosis has occurred
in pigs of any age past weaning, but it was more common in
growing pigs.[61] Roberts et al. (1979)[61] has observed low average
weight gains in a pig herd where a significant number of
poorly growing pigs had intestinal adenomatosis. Lawson
et al. (1980)[62] measured antibody titers in pigs going to
slaughter from farms where intestinal adenomatosis was known
to occur and found significant antibody titers against various
isolants of CSM in almost all sera examined. Proliferative
hemorrhagic enteropathy has occurred in pigs of any age
after weaning. However, it was reported to be most prevalent
in young replacement gilts and boars which were at least six
months old.[59,63] Regional ileitis and necrotic enteritis have
been observed to occur primarily in young weaned pigs (7-12
weeks old) and in market weight hogs (approximately 6 months
old).[36] Necroproliferative enteritis primarily affected 16
to 45 kg feeder pigs.[60] Morbidity and mortality estimates
in pigs affected with any of the CSM-associated conditions
have varied, but 1-12% morbidities with approximately 50%
mortalities have been reported.[63,45]

Clinical signs and clinical pathology- The clinical signs
of pigs with CSM-associated conditions have been described.
Pigs with intestinal adenomatosis often have wasting with no
other remarkable clinical signs.[61] The clinical signs of
pigs affected with proliferative hemorrhagic enteropathy in-
clude pallor, anorexia, normal to subnormal rectal tempera-
tures, and the passage of blood-tinged or tarry feces 24 to
48 hours prior to death, but some pigs may die without
having been noticed to be ill.[45,59,63] Pigs with necrotic
enteritis or regional ileitis have poor appetites, grow
poorly, have periods of intermittent diarrhea, and may
have mucus, fibrin, or blood in their stools.[23] Pigs
may have fever during the initial phases of the two conditions
but later the rectal temperature is normal or subnormal and
over a course of several weeks affected pigs have atrophy of
skeletal muscles, become emaciated, and die.[23] Fattening
hogs with necrotic enteritis or regional ileitis do not
usually have clinical signs.[23] Feeder pigs with necroprolifera-
tive enteritis have diarrhea and stools which may contain blood
or yellow casts of fibrin and necrotic tissue, and the pigs be-
come emaciated even though they may continue to have a fair
appetite.[60]

Reports describing the clinical pathologic findings in
pigs affected with CSM-associated conditions are uncommon.
Pigs with regional ileitis have anemia and hyperproteinemia,

and kinetic studies using labelled serum albumin have demonstrated that these pigs also have significant protein loss from the intestine.[23,64] Martinsson et al. (1976)[65] reported that wasting pigs with regional ileitis had decreased serum levels of total protein, albumin, transferrin, alkaline phosphatase, and zinc. They also had increased numbers of white blood cells, and increased serum concentration of cortisol and alpha-1-antitrypsin.[65]

Pathology- The characteristic gross pathological finding in pigs affected with any of the CSM-associated conditions is thickening of the wall of the ileum, reticulation of the serosal surface, edema of the mesentery, and enlargement of the ileocecal lymph nodes. Pigs with intestinal adenomatosis may also have involvement of the cecum and proximal colon.[45] The intestinal mucosa of these pigs is thickened and deeply folded, and occasionally focal raised polypoid areas are present, particularly toward the proximal ileum.[45] Luminal exudates are not common in pigs with adenomatosis. Pigs with proliferative hemorrhagic enteropathy have thickened ileal mucosal folds and clotted blood with fibrinous exudate in the lumen of the ileum, cecum, and proximal colon.[47] Necrotic enteritis and regional ileitis in pigs are characterized by thickening, hyperemia, and folding of the ileal mucosa which is also usually covered by pseudomembranes.[19,23,66] These changes are most pronounced at the antimesenteric aspects of the ileum

where large aggregated lymphoid nodules (ALN; Peyer's patches) are present.[23] In addition, pigs with regional ileitis may have marked thickening of the tunica muscularis.[19,20,23] Swine with necroproliferative enteritis have thickening of the ileal mucosa with pseudomembranes and blood in the ileum.[60] Lesions outside the gastrointestinal tract have not been reported in any of the CSM-associated conditions.

The histopathological findings common to all of the CSM-associated conditions in pigs are proliferation of mucosal epithelial cells, absence of goblet cells, flattening of villi, infiltration of inflammatory cells into the lamina propria, and the presence of curved, argyrophilic bacteria within the apical cytoplasm of affected epithelial cells. Pigs with intestinal adenomatosis may have polypoid pro-liferations of epithelial cells in the proximal ileum.[45] Histochemical studies have been conducted on the hyperplastic intestinal epithelium from pigs with intestinal adenomatosis and have shown that the bacteria-infected epithelial cells histochemically resemble immature crypt epithelial cells from the intestinal mucosa of normal swine.[57,67] In addition to mucosal proliferation and intracellular bacteria, there are areas of superficial mucosal necrosis, hemorrhage into the lamina propria, and exudation of fibrin and erythrocytes into the intestinal lumen of pigs with proliferative hemorrhagic enteropathy.[45,47,51,52,59] Pigs with necrotic

enteritis, regional ileitis, and necroproliferative enteritis have coagulative necrosis which often involves the full-thickness of the intestinal mucosa, chronic inflammation of the lamina propria and submucosa, and exudation of large amounts of fibrin into the intestinal lumen.[60,66,68] Hypertrophy of the smooth muscle of the tunica muscularis of the ileum may also be present in swine with regional ileitis.[19,23] The ileocecal lymph nodes from pigs with any of the CSM-associated conditions may have hyperplasia of lymphoid germinal centers.[45] Ileocecal lymph nodes from pigs with regional ileitis may have suppurative to granulomatous lymphadenitis and metastasis of immature intestinal epithelial cells to the lymph node parenchyma.[19,23]

The CSM-associated conditions in pigs have been studied with transmission and scanning electron microscopic techniques. Ultrastructural characteristics common to intestinal tissues from pigs with any of the CSM-associated conditions are the presence of bacteria which are irregularly curved, 2-3 μm in length and 0.3 μm in diameter, and which are not contained within cytoplasmic vacuoles in immature crypt epithelial cells.[50,57,69,70] Bacterial organisms have also been demonstrated free within the subepithelial mucosal region, within phagolysosomes of leukocytes in crypt lumina, and in the lamina propria of pigs affected with proliferative hemorrhagic enteropathy.[71]

Bacteriology- Campylobacter sputorum subspecies mucosalis

has been isolated from the intestinal mucosa of pigs affected

with any of the CSM-associated conditions. Lawson and Rowland

(1974)[53] and Lawson et al. (1975)[54] initially isolated the

organism from cases of intestinal adenomatosis, biochemically

and antigenically characterized it, and named it. Subse-

quently, CSM was isolated from the intestinal mucosa of pigs

with necrotic enteritis, regional ileitis, proliferative

hemorrhagic enteropathy, and necroproliferative enteritis.[55,]

[56,59,72] Gunnarsson (1976)[58] isolated the organism from pigs

in Sweden with regional ileitis. Campylobacter sputorum sub-

species mucosalis has been isolated from the oral cavity of

certain pigs from herds where intestinal adenomatosis was

occurring,[73] but it has not been isolated from the intestines

of normal pigs.[53,54,74]

Lawson et al. (1977)[75], demonstrated that antiserum pre-

pared against one of the initial strains of CSM isolated from

a case of intestinal adenomatosis reacted with heat stable

components of similar organisms from other cases of adeno-

matosis and from cases of regional ileitis and necrotic

enteritis. Antigenic similarity has also been demonstrated

between Lawson's original strain of CSM and isolants of the

organism obtained from cases of proliferative hemorrhagic

enteropathy and regional ileitis occurring in other parts of

the world.[58,75,76] However, Roberts et al. (1977)[77] recovered

a unique isolant of CMS from pigs with intestinal adenomatosis.
This isolant was antigenically different from Lawson's strain.
Indirect fluorescent antibody techniques have been used to
demonstrate the intracellular presence of CSM in intestinal
mucosa from pigs with intestinal adenomatosis, regional
ileitis, necrotic enteritis, and proliferative hemorrhagic
enteropathy[69,53,55,56] when grown in culture, CSM ultra-
structurally has a single polar flagellum, a trilayered cell
wall and a trilaminar cell membrane, and its cytoplasm con-
tains strands of nuclear material and ribosomes.[57]

The genus Campylobacter is composed of Campylobacter
fetus, Campylobacter sputorum, and Campylobacter fecalis.[78]
Campylobacter fetus has the subspecies fetus, intestinalis,
and jejuni.[78] Campylobacter sputorum has the subspecies
sputorum, bubulus, and mucosalis.[78] Members of the genus are
all small gram-negative curved rods (1.5 to 3.5 µm long and
0.2 to 0.4 µm in diameter) which are microaerophilic, and have
a single polar flagellum that is two to three times the length
of the cell and is responsible for its motility.[78,79] The
organisms are motile with a characteristic "corkscrew move-
ment."[78] Campylobacter sputorum and its subspecies are all
catalase negative, and strongly positive for hydrogen sulfide
production in iron-containing media.[78] Campylobacter sputorum
subspecies mucosalis grows on media containing 1.5% sodium
chloride and produces yellow colonies.[54,78]

Treatment and control- The treatment and control of the CSM-associated conditions have largely been conducted on an empirical basis. Growing pigs clinically affected with intestinal adenomatosis and necroproliferative enteritis have been reported to recover without treatment.[60,63] Some authors have reported limited success in treating swine with proliferative hemorrhagic enteropathy, necrotic enteritis, or regional ileitis by the use of antibiotics in the feed or water.[60,80] However, pigs affected by these conditions usually do not respond well to treatment.[63] The antibiotics most frequently used were tylosin,[80] sulphadimidine,[80] furazolidone,[47] penicillin,[80] and tetracycline.[74] In vitro, isolants of CSM have been most sensitive to tetracycline, followed in decreasing order by nitrofurantoin, penicillin, chloramphenicol, erythromycin, neomycin, and Polymyxin B.[57] Love and Love (1977)[80] reported some success in controlling proliferative hemorrhagic enteropathy by exposing susceptible swine to an infected area, supposedly allowing the pigs to become infected with CSM, and then providing them with antibiotics in the feed for a period of time.

Experimental studies- Reports of the experimental reproduction of any of the CSM-associated conditions in swine are uncommon in the veterinary literature. Roberts et al. (1977)[81] apparently produced intestinal adenomatosis and necrotic enteritis in 4 of 9 pigs by intragastric inoculation

with chalk powder, benzetimide, CSM and homogenized mucosa
scraped from the intestine of pigs with naturally occurring
intestinal adenomatosis. Three pigs killed between 53 and
59 days after inoculation had intestinal adenomatosis and
one pig killed 51 days after inoculation had necrotic
enteritis.[81] Focal areas of crypt epithelial cell prolifera-
tion and intracellular CSM were demonstrated in the intesti-
nal mucosa of the mid-jejunum and terminal ileum of pigs
orally inoculated with chalk powder, benzetimidine, and
CSM cultures.[82,83] Experimentally produced intestinal
adenomatosis has been reported in neonatal pigs orally
inoculated with material prepared from the intestinal mucosa
of pigs with naturally occurring intestinal adenomatosis.[68,84]

Comparative enteric diseases- An enteric disease of the
golden hamster (Mesocricetus auratus) is the closest compara-
tive model to the CSM-associated conditions in swine. The
disease in hamsters, like the swine disease, has been called
by various names which include "wet-tail",[85] proliferative
ileitis,[86] regional enteritis,[85] terminal ileitis,[85] enzootic
intestinal adenocarcinoma,[87] transmissible ileal hyperplasia,[88,89] atypical ileal hyperplasia,[87] and hamster enteritis.[85]
The disease is characterized by diarrhea and enteritis, some-
times proliferative enteritis, involving the small intestine of
weanling hamsters.[85] In the acute form, there is often diffuse
hemorrhagic necrosis of the terminal ileal mucosa without

evidence of mucosal hyperplasia.[85,86,87] The proliferative
form is characterized by hyperplastic villi which are wider
and longer than normal.[85,86,90] Hyperplasia is thought to
begin in crypt epithelial cells.[84,88,89] The chronic form
of the disease has muscular hypertrophy of the tunica
muscularis, chronic mucosal inflammation, and partial or
complete occlusion of the ileal lumen.[85]

Causative agents have not been described. Bacteria,
viruses, parasites, and dietary factors all have been impli-
cated but Koch's postulates have never been fulfilled with
any of them.[85,87,89,90,91,92] Strains of Escherichia coli
have received most attention as possible etiologic agents
because when given orally, they will cause acute enteritis
but not proliferative lesions in weanling hamsters.[92] Oral
administration of n-methyl-N-nitrosourea will induce intesti-
nal adenocarcinomas in hamsters; however, these lesions have
no resemblance to the proliferative lesions of hamster
enteritis.[93]

The experimental production of the proliferative
lesions in weanling hamsters have been reported by numerous
authors. This was accomplished by orally inoculating
hamsters with suspensions of ileal mucosa obtained from
hamsters with the naturally occurring disease. Clinical
signs were usually observed approximately three weeks after
oral inoculation.[88,89] In one such experiment, Frisk and

Wagner (1977)[90] observed by electron microscopy two morpho-
logically different bacterial profiles within affected epi-
thelial cells. Intracytoplasmic organisms observed early
in the disease were identified as E. coli and those observed
later in the disease structurally resembled Campylobacter
spp.[90]

Barthold et al. (1978)[94] reported the occurrence of a
natural disease which he called cecal mucosal hyperplasia
in hamsters. It was characterized clinically by diarrhea,
runting, and high mortality in suckling and weanling hamsters
and pathologically by diffuse hyperplasia of cecal crypts
with inflammation of the lamina propria and focal mucosal
necrosis. No relationship between this condition and hamster
enteritis could be found.[94]

Laboratory mice have a naturally occurring infectious
disease called transmissible murine colonic hyperplasia which
is characterized by mucosal hyperplasia, and occasionally ac-
companied by erosion and inflammation of the distal colon.[95]
The condition has been experimentally reproduced with a
variant of Citrobacter freundii (isolant 4280).[95,96] The
authors did not describe the location of this organism
in the colonic mucosa, but presumably it does not invade
and multiply in mucosal epithelial cells. There appear
to be strain differences in mice regarding susceptibility to
and severity of lesions produced with the organism.[97]

Geil and Davis (1968)[98] reported on 64 cases of spontaneous

ileitis in rats. The condition was characterized by seg-
mental dilitation of the ileum, hydropic degeneration and
necrosis of smooth muscle in the wall of the ileum, and
infiltration of mononuclear cells into the ileal wall[98].
There was no mucosal hyperplasia. Limited microbiologic
studies did not reveal an etiologic agent.

A Vibrio sp. (Campylobacter) organism was associated with
diarrheal disease in weanling laboratory rabbits by Moon and
Cutlip (1974).[99] There was degeneration and loss of surface epi-
thelial cells in the cecum accompanied by crypt epithelial
cell hyperplasia and inflammation of the lamina propria and
submucosa.[99] Bacteria resembling Vibrio sp. were observed
by electron microscopy within the cecal lumen and within the
cytoplasm of degenerating epithelial cells.[99]

Vibrio fetus (Campylobacter fetus) has been implicated
as the causative agent in a case of acute necrotic and
hemorrhagic cecocolitis in a baboon (Papio cynocephalus).[100]

Terminal ileitis was diagnosed by Cross and Smith (1973)[101]
in 7 lambs ranging in age from 4 to 6 months. The ileum
of affected lambs was markedly thickened and the mucosa had
accentuated folds.[101] Histopathologic findings were epi-
thelial cell proliferation, which had an adenomatous appear-
ance, and inflammation of the lamina propria.[101]

Isolated reports of conditions affecting the ilea of
other domestic animals have appeared in the literature.

Idiopathic muscular hypertrophy of the ileum has been ob-
served in horses.[102,103,104] The similarity between this
condition and terminal ileitis in swine has been reported;[102]
however, histologic findings in the ilea of these horses were
not described. Regional enterocolitis having some re-
semblance to regional ileitis in swine has been described
in two cocker spaniel dogs.[105]

In man, regional enteritis (Crohn's disease) is a
chronic inflammatory disease of unknown cause affecting pri-
marily the terminal ileum.[106] It occurs in young adults
and frequently produces partial intestinal obstruction.
The inflammatory changes in the ileum are nonspecific and
often granulomatous with ulceration of the mucosa, sub-
mucosa, and subserosa.[106] Ileal mucosal epithelium is not
hyperplastic and bacterial profiles cannot be observed in
the cytoplasm of epithelial cells.

PORCINE PROLIFERATIVE ENTERITIS

(INTESTINAL ADENOMATOSIS): CLINICAL FINDINGS AND

GROSS LESIONS OBSERVED IN 58 PIGS

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This manuscript has been submitted to the

Journal of the American Veterinary Medical Association

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SUMMARY

Epizootics of porcine proliferative enteritis (intestinal adenomatosis) were studied on 44 farms over a two year period (1978-1980). The disease occurred primarily in 18-45 kg (40-100 lb) feeder pigs on farms using conventional housing methods. On farms where confinement housing methods were used, the disease occurred primarily in bred gilts, sows, and boars.

Clinically, feeder pigs usually had chronic diarrhea and wasting. Adult pigs often had hemorrhagic diarrhea of acute onset and died 1 to 3 days later.

Necropsy findings in all age groups were characterized by thickened, firm intestinal walls, primarily in the ileum and distal jejunum. Feeder pigs often had pseudomembranes filling the intestinal lumen, while adult animals often had clotted or unclotted blood filling the intestinal lumen. Histopathologic findings in all age groups were proliferation of crypt epithelium and the presence of curved, Campylobacter sp.-like organisms within the cytoplasm of crypt epithelial cells.

Campylobacter sputorum subspecies mucosalis was isolated from the intestinal mucosa in 20 of 34 affected feeder pigs and from 10 of 24 adult swine.

INTRODUCTION

The Scottish workers, Rowland et al.[1] and Rowland and Lawson[2,3,4] described the intracellular presence of a bacterium in intestinal mucosa epithelial cells from cases of intestinal adenomatosis,[1,2] proliferative hemorrhagic enteropathy,[3,4] necrotic enteritis,[4] and terminal ileitis (regional ileitis).[4] Lawson and Rowland[5] and Lawson et al.[6,7] isolated a species of bacterium from the intestinal mucosa of each of these four conditions and named it Campylobacter sputorum subspecies mucosalis (CSM). Similar organisms were subsequently isolated from like conditions in Australia[8] and the Scandinavian countries.[9]

There are few epidemiologic studies on the four CSM-associated conditions. Intestinal adenomatosis occurs sporadically in post-weaned pigs.[10] Lawson et al.[11] measured antibody titers to CSM in sera from apparently normal pigs going to slaughter from farms where intestinal adenomatosis was known to occur and found significant titers in most sera examined. Roberts et al.[10] observed poor weight gains in a pig herd where intestinal adenomatosis was present. Proliferative hemorrhagic enteropathy appears to be more prevalent in young adult boars and gilts than in swine of other age groups.[12] Up to 50% of the young boars and gilts on a given premise have been reported to be affected and approximately 50% of these pigs died.[8] However, the incidence

of proliferative hemorrhagic enteropathy usually is much lower.[12]

The clinical findings of each of the four CSM-associated conditions have been reported by numerous authors.[3,12,13] Pigs affected with intestinal adenomatosis often have no clinical signs, but emaciation may occur in a few.[3] Pigs with proliferative hemorrhagic enteropathy usually have anemia, hypoproteinemia, normal rectal temperatures, and hemorrhage in the feces 8 to 24 hours prior to death.[12] Young weaned pigs affected with regional ileitis usually have weight loss, anemia, and hypoproteinemia.[13] Market weight hogs with regional ileitis usually have no clinical signs.[13]

Treatment and control of the CSM-associated conditions have been conducted on an empirical basis. A wide variety of antimicrobial drugs have been administered to affected swine in feed, in water, or by parenteral injection and have yielded variable results.[8,12] Pigs clinically affected with proliferative hemorrhagic enteropathy usually do not respond to treatment.[12] Success has been reported in controlling this condition by the exposure of susceptible pigs to infected premises followed by antibiotic administration to these pigs for a period of time.[14] Growing pigs with intestinal adenomatosis have been reported to recover without treatment.[10]

The gross and microscopic lesions of intestinal

adenomatosis, proliferative hemorrhagic enteropathy, necrotic enteritis, and regional ileitis have been described.[2,3,4,15,16]

The objective of this paper is to describe the epidemiologic findings, clinical signs, gross pathologic findings, and treatment of proliferative enteritis (intestinal adenomatosis) as it occurs naturally in midwestern swine.

MATERIALS AND METHODS

Source of animals- Farms in Iowa, Illinois and Missouri
with swine herds experiencing epizootics of proliferative
enteritis were visited during a 2 year period (1978-1980).
The premises were examined and the clinical condition of
affected animals was observed. The term conventional unit
will refer to any swine raising system where pigs are housed
with outside access for any portion of their life. Confine-
ment production unit will refer to swine raised without out-
side access in closed, controlled environment houses.

Pathologic studies- Postmortem examinations of 58 ani-
mals were conducted. Samples taken for histopathologic eval-
uation included duodenum, jejunum (3 levels), ileum (3 levels),
ileocecal valve, cecum, colon, ileocecal lymph nodes, liver,
spleen, kidney, stomach, lung, cardiac and skeletal muscle,
and brain. All tissues were fixed in 10% neutral buffered
formalin. Paraffin-embedded sections were stained with
hematoxylin and eosin. Intestinal sections were also stained
with Warthin-Starry silver stain.[17]

Clinical pathologic studies- Single blood samples were
collected from 20 live clinically affected animals (10 feeder
pigs, 10 adults) by venipuncture of the anterior vena cava
or by cardiac puncture. Hematologic parameters examined
included packed cell volume (PCV), hemoglobin concentration
(Hb), total white blood cell count (WBC), differential

white cell counts, plasma protein concentration, and fibrino-
gen concentration.   Blood urea nitrogen, glutamic-oxalo-
acetic transaminase (SGOT), and serum electrolytes including
inorganic phosphorous, calcium, sodium, potassium, and
chloride were examined from 7 of the 10 adult animals.

Microbiologic examinations- Tissues from the ileum,
cecum, colon, liver and ileocecal lymph node were obtained
for bacteriologic evaluation.   In 15 cases, blood samples and
swabs of gall bladder mucosa were collected for culture.   The
samples were cultured for CSM, Escherichia coli,[18] and
Salmonella spp.   Culturing techniques[19] as described by Songer
et al.[19] were employed for Treponema hyodysenteriae isolation
from swine with cecal and colonic lesions.   Cecal contents
were collected for rotavirus identification by electron
microscopy in cases involving 18-45 kg. feeder pigs.[a]

Selected E. coli isolants were serotyped.[b]

The isolation of CSM was accomplished by scraping the
mucosa from the intestinal wall with a glass slide.   Scrapings
were placed in cold (4 C) brucella broth,[c] glass beads were
added, and the suspension was homogenized using a vortex mixer.
The homogenate was centrifuged for 10 minutes at 60xg to 225xg.
A few drops of the supernatant were inoculated onto brilliant

[a]Conducted by Veterinary Diagnostic Laboratory, Ames, IA.

[b]Conducted by South Dakota State Diagnostic Laboratory,
Brookings, SD.

[c]Difco, Detroit, MI.

green blood agar with novobiocin[a] (tryptose blood agar base
with yeast extract,[b] 5% citrated bovine blood, 5mcg/ml novo-
biocin, and 1:60,000 brilliant green[c]). The plates were
placed in a Gas Pak jar[d] (without catalyst) which was then
evaluated to 650 mm of mercury vacuum and back filled to 120 mm
of mercury with a mixture of equal volumes (50:50 vol/vol) of
hydrogen and carbon dioxide.[20] Incubation was at 37 C for 48
hours. Small grayish-yellow colonies were considered suspects.
Phase contrast microscopy was used to determine motility
and a slide test using 3% hydrogen peroxide was performed to
determine the catalase reaction. Kligler iron agar slants
were inoculated to detect hydrogen sulfide production.
Small, darting or tumbling rods which were catalase negative
and hydrogen sulfide positive were presumed to be CSM. A
slide agglutination test was used as the means for positive
identification.

Antisera to both a Lawson isolant[e] (253/72) and an
Iowa field isolant (53B) of CSM were produced in rabbits by
injections of 1 ml (3x10^{8} colony forming units per ml) of
formalin-inactivated organisms into the lateral ear vein

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[a]Upjohn, Kalamazoo, MI.

[b]Difco, Detroit, MI.

[c]JT Baker Chemical Co., Phillipsburg, NJ.

[d]BBL, Cockeysville, MD.

[e]Isolant obtained from Ms. J. Sue McAllister, University
of Minnesota.

every 4th day for 17 days. Serum was harvested from the
rabbits on day 21.

Samples of gut homogenate, liver, ileocecal lymph node,
blood and gall bladder were cultured for <u>Salmonella</u> spp. by
inoculation onto brilliant green agar[a] and incubation at 37C
for 48 hours. Suspect colonies were inoculated into Kligler
iron agar and onto urea agar slants. Slide agglutination
tests using commercially prepared group antisera[b] were per-
formed on isolants with appropriate biochemical reactions.[18]

<u>Telephone survey</u>- Ninety-two Iowa veterinarians en-
gaged in limited or extensive swine practice were surveyed.
They were asked what drugs they most often used in treating
laboratory confirmed cases of proliferative enteritis, what
type production unit the disease occurred in, and what age
groups of pigs were primarily involved.

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[a]Grand Island Biological Company, Grand Island, NY.

[b]BBL, Cockeysville, MD.

RESULTS

Epidemiologic findings- Two distinct patterns of
occurrence of porcine proliferative enteritis were observed
(Table 1). The disease primarily occurred in 18-45 kg feeder
pigs and in bred gilts, sows, and young boars. The disease
occurred in these groups of animals regardless of the type
of production operation. The disease occurred more fre-
quently in conventionally housed feeder pigs than in confine-
ment reared feeder pigs. However, confinement housed gilts,
boars, and sows had a higher incidence of disease than did
conventionally housed gilts, boars, and sows. The disease was
uncommon in finishing pigs (50-114 kg) in either housing
situation (Table 1).

The number of swine actually involved in an epi-
zootic on a given farm was difficult to determine but less
than 10% of the animals usually had clinical signs. How-
ever, several epizootics were encountered where up to 50%
of the swine had clinical signs. The disease occurred on
many farms throughout the year, primarily in conventional-
ly housed feeder pigs. Adult breeding swine in confine-
ment often experienced epizootics of short duration.

There was no apparent association between occurrence
of disease and composition of diet or method of feed
delivery to animals. Diets were formulated from a
corn-soybean oil meal base. Disease was observed in

Table 1—Relationship Between Type of Housing and Age of Pig with Porcine

Proliferative Enteritis

	Feeder pig (16-45 kg) (no. of farms)	Finishing pig (50-114 kg) (no. of farms)	Adults (no. of farms) Gilt, Sow	Boar	Total
Conventional	21	0	1	0	22
Confinement	6	1	13	2	22
TOTAL	27	1	14	2	44

some swine fed growth promotant levels of antibiotics in the
feed, in some receiving no antimicrobial drugs in the feed,
in some fed from self-feeders, and in some fed on the pen
floor.

The level of sanitation varied from farm to farm; how-
ever, proliferative enteritis often occurred in convention-
al units with marginal to poor sanitation. Confinement
units generally had a higher level of sanitation, but the
disease was more common in gilts, sows, and boars housed in
this environment.

The movement of animals had a variable but overall im-
portant affect on the occurrence of disease. Feeder pigs pur-
chased at sale barns or moved from building to building on
conventional-type farms frequently developed disease. The
movement of feeder pigs out of nurseries into growing units
in confinement operations was also associated with occurrence
of the disease. The arrival of new boars or gilts onto the
premises of either type operation was associated with the
occurrence of proliferative enteritis in the newly arrived
animals, members of the resident population, or both. The
movement of sows and gilts out of farrowing units into
breeding units or pregnant gilts and sows into farrowing units
was also associated with occurrence of the disease. The
length of time between animal movement and the onset of
clinical signs varied from a few days to four weeks.

Clinical signs- Affected feeder pigs from either conven-
tional or confinement production units usually had diarrhea,
often intermittent for several days to 3 weeks. The feces
were yellow and varied from soft to fluid. The passage of
pseudomembranes was occasionally observed, and there was
infrequent appearance of either bright red or dark tarry
material in the feces. Pigs with protracted diarrhea lost
weight, became dehydrated, and eventually appeared stunted
and emaciated. The rectal temperatures of these animals
varied from normal to elevated (40-41 C). Affected pigs
would usually continue to eat and drink but at decreased
levels. Feeder pigs with acute onset of hemorrhagic diarrhea
were not observed (Table 2).

Gilts, sows, and boars from either type production unit,
usually had anorexia for 1 to 3 days prior to the observation
of soft to watery and bright red, or dark and tarry feces.
There was often blood staining the rump and perineal region.
Within one to three days after the first appearance of blood
in the feces, many animals had marked pallor of the skin (if
light-skinned) and mucous membranes. Affected pigs would
eventually collapse to sternal recumbency, breathe agonally
with mouths open, and die. Loss of condition was not a
prominent feature in affected adult swine. Rectal tempera-
tures taken during episodes of hemorrhagic diarrhea varied
from subnormal to normal. Swine were also occasionally found

dead without observable evidence of hemorrhagic diarrhea.
Four pigs in this group had chronic diarrhea similar to that
observed in feeder pigs (Table 2).

Clinical pathologic data- Average group values for
hemoglobin concentration, packed cell volume, and fibrinogen
concentration were within normal ranges in the 10 feeder
pigs evaluated. Average values for plasma protein concehtra-
tion were decreased and the average plasma protein to fibrino-
gen ratio was 12.0 (Table 3).

Average group values for hemoglobin, packed cell volume,
and plasma protein were decreased in 10 adult swine. Fibrino-
gen levels were within normal range and the average group
plasma protein to fibrinogen ratio was 15.5.

The mean of leukograms from all affected pigs indi-
cated neutrophilia, left shift, and lymphopenia. How-
ever, the left shift was more pronounced in the feeder pig
group (Table 3).

Serum electrolyte and enzyme levels were determined
for 7 of the 10 adults. Group average concentrations of
blood urea nitrogen, inorganic phosphorus, calcium,
glutamic-oxaloacetic transaminase (SGOT), sodium, and
chloride were all within normal ranges. There was a group
average elevation in serum potassium (average value = 7.9
meq/L with a range of 7.2-8.7 meq/L).

Table 2—Clinical Signs of Porcine Proliferative Enteritis

	Chronic or intermittent diarrhea, wasting	Chronic diarrhea with some blood	Hemorrhagic or tarry stools for 1-3 days	Found dead	TOTAL
Conventional					
Feeder pig	20	6	0	1	27
Gilt, sow, boar	0	1	0	0	1
Confinement					
Feeder pig	3	3	0	0	6
Finishing pig	0	1	0	0	1
Gilt, sow, boar	1	3	16	3	23
TOTAL	24	14	16	4	58

Table 3—Average Values for Hematologic Parameters in Two Groups of Pigs with

Proliferative Enteritis (group ranges are in parentheses)

	Feeder pigs (16-45 kg) n=10	(Adults) gilt, sow, boar n=10	Normal[a]
Hemoglobin (g/dl)	12.1(10.9-13.9)	10.0(8.1-11.4)	13.0(10.0-16.0)
Packed cell volume (PCV) (%)	36(32-41)	28(24-35)	42(32-50)
Plasma protein (P.P.) (g/dl)	5.3(4.2-5.6)	5.6(5.0-5.9)	7.0(6.0-8.0)
Fibrinogen (Fib)(g/dl)	440(400-500)	360(200-600)	(100-500)
PP/Fib	12.0(18.5-11.2)	15.5(25.0-9.8)	
White blood cell count[b] (WBC)(n/μl)	24,120(8,400-25,800)	19,580(10,300-32,400)	16,000(11,000-22,000)
Neutrophil(band) (%)	37(24-51)	15(5-25)	1.0(0-4)
Neutrophil(mature) (%)	34(13-52)	36(14-59)	37(28-47)
Lymphocytes (%)	35(16-50)	45(30-76)	53(39-62)
Monocytes (%)	1(0-3)	1(0-3)	5(2-10)
Basophils (%)	0(0-1)	0	.5(0-2)
Eosinophils (%)	0	2.4(0-7)	3.5(.5-11)

[a]Schalm, O. W., 1975.[21]

[b]Corrected for nucleated red blood cells.

Gross lesions- The consistent gross finding in affected
feeder pigs, finishing pigs, gilts, sows, and boars was a
thickening of the ileum and jejunum, and also occasionally
of the cecum and proximal colon. The affected intestine had
a reticulated serosa and felt turgid (Fig 1). There was
also edema and hyperemia of the mesentery. Ileocecal lymph
nodes were moist, pale, and enlarged.

Affected feeder pigs usually had pseudomembranes ad-
herent to the intestinal mucosa (Table 4). Free or clotted
blood was uncommon in the intestinal lumen and if present
was accompanied by fibrinous exudates. The mucosa was
either demonstrably thickened or obliterated by necrosis.
There was also hyperemia of the mucosa, edema of submucosa,
and occasionally thickening of muscular layers.

Affected adult swine (Table 4) had accentuated
mucosal folds, granular appearing mucosal surfaces, and
mucosal hyperemia. These were the only gross intestinal
lesions observed in one adult pig (Fig 2). However, affected
adult swine had large amounts of free or clotted blood alone
or in combination with pseudomembranes in the intestinal
lumen (Fig 3).

Four feeder pigs and one gilt had unilateral or bi-
lateral pulmonary consolidation involving one or multiple
lung lobes in addition to the enteric lesions. One young
boar had chronic ulceration of the esophageal area of
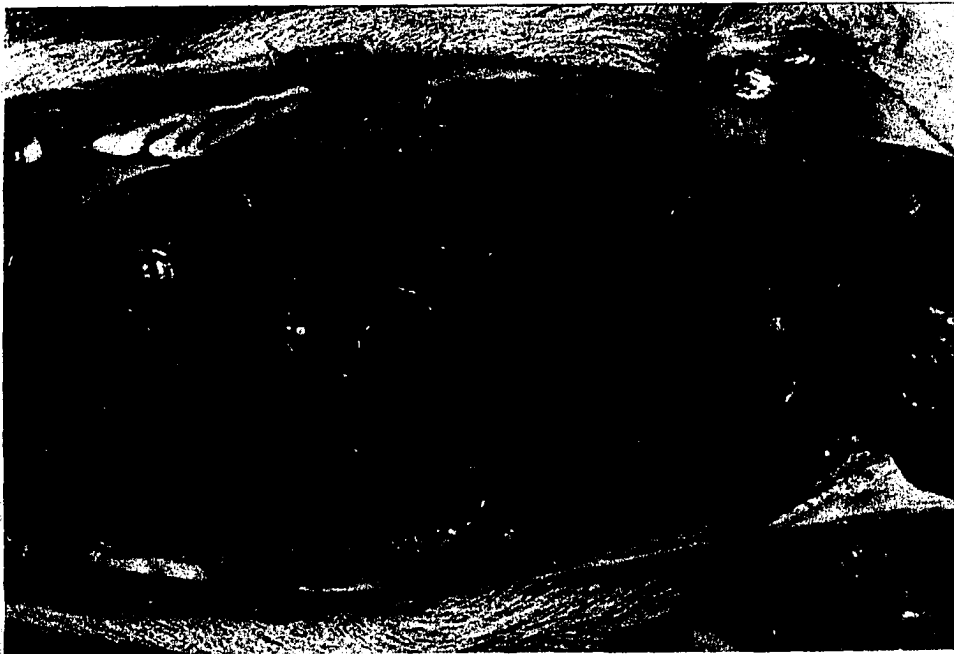
Fig 1—Abdominal viscera of feeder pig with proliferative enteritis. The ileum and jejunum are thick-walled with a reticulated serosal surface.

Table 4—Gross Lesions of Proliferative Enteritis (58 pigs)

	Intestinal wall thickened with pseudomembrane in lumen	Intestinal wall thickened with free or clotted blood in lumen	Intestinal wall thickened with no exudate in lumen	TOTAL
Conventional				
Feeder pig	25	3	0	28
Gilt, sow, boar	1	0	0	1
Confinement				
Feeder pig	4	2	0	6
Finishing pig	0	1	0	1
Gilt, sow, boar	6	15	1	22
TOTAL	36	21	1	58
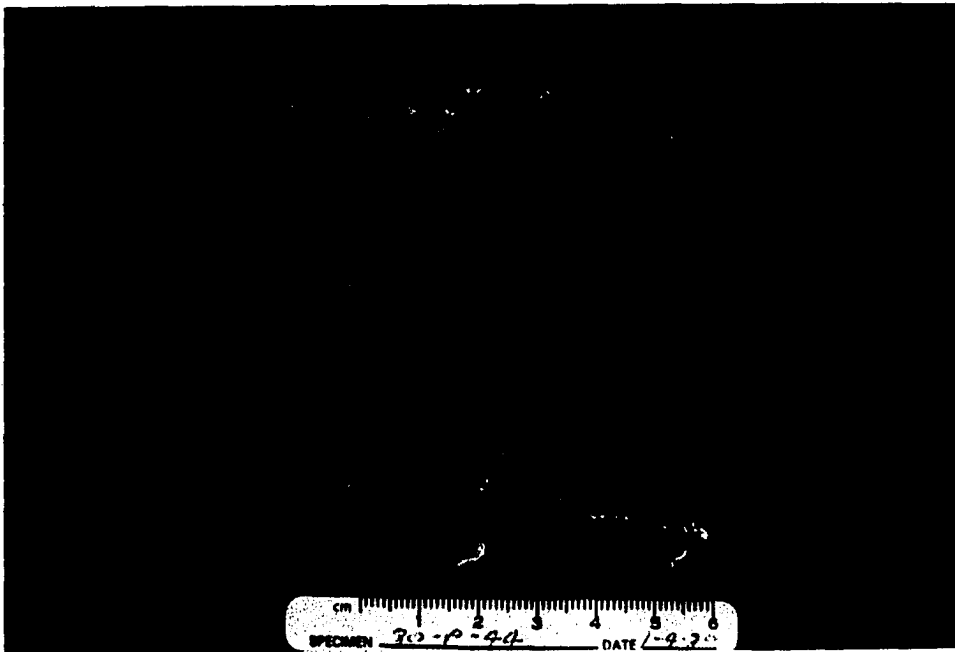
Fig 2—Ileum from young boar with proliferative enteritis.
Mucosal folds are thickened and increased in height. There
is no luminal exudate. The serosa (above) is reticulated,
and the mucosa is hyperemic (arrow).
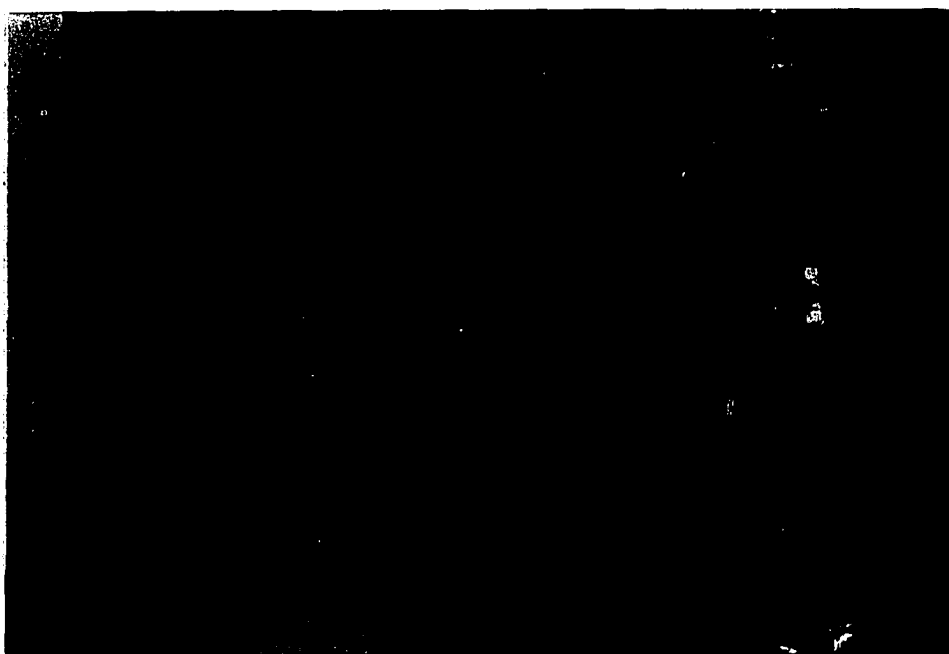
Fig 3—Ileum from gilt with proliferative enteritis. The
ileum contains a cast composed of clotted blood (left),
and a pseudomembrane (center). The serosal surface
(right) is reticulated.

the stomach.

Microscopic lesions- The characteristic microscopic lesion common to all pigs with proliferative enteritis in either management situation was proliferation of crypt epithelial cells and the presence of curved bacteria within the apical cytoplasm of these cells. These bacteria resembled Campylobacter spp. The association of these bacteria with proliferative mucosal lesions is summarized in Table 5.

Microscopic lesions in other organs were rare. The four feeder pigs and one gilt with lung consolidation had focal to diffuse fibrinopurulent bronchopneumonia associated with Pasteurella multocida infection. An additional four feeder pigs had multifocal necrotic hepatitis accompanied by mononuclear cell infiltration.

Bacteriologic findings- Campylobacter sputorum subspecies mucosalis was isolated from affected intestinal mucosa in 20 of 34 feeder pigs from either conventional or confinement type housing. It was also isolated from 10 of 24 affected adults from either type of operation (Table 5). The organism could not be isolated from ileocecal lymph nodes or from blood.

Salmonella choleraesuis was isolated from the ileocecal lymph nodes in 4 feeder pigs with necrotic hepatitis (Table 5). Escherichia coli was isolated from the intestine of almost all of the swine studied, but consistent serotypes did

Table 5—Association Between Occurrence of Proliferative Mucosal Lesions, Intra-epithelial Campylobacter sp.-like organisms, and isolation of Campylobacter sputorum subspecies mucosalis (CSM) (58 pigs from 44 farms)

| | Proliferation of immature epithelial cells | Intracytoplasmic Campylobacter sp.-like organisms | Bacterial isolations | |
			CSM	Salmonella choleraesuis
Conventional				
Feeder pig	28	28	16	4
Gilt, sow, boar	1	1	ND[a]	0
Confinement				
Feeder pig	6	6	4	0
Finishing pig	1	1	ND	0
Gilt, sow, boar	22	22	10	0
TOTAL	58	58	30	4

[a]ND = not determined.

not occur (Appendix). Rotavirus was not detected in any feeder pig.

Prevention, control, and treatment- Significant prevention and control measures did not emerge from this study. The association of disease with animal movement or introduction of new stock onto premises prompted the administration of therapeutic levels of antibiotics in feed or water two weeks before animal movement and three to four weeks afterward on farms where the disease had occurred. This procedure appeared to reduce the incidence of new clinical disease. The occurrence of subclinical disease could not be assessed in this study.

After clinical signs were evident, the administration of therapeutic levels of various antimicrobial drugs in feed, in water, or by parenteral injection had variable results. Emaciated feeder pigs with chronic diarrhea were separated from penmates and given parenteral antibiotics for several days. Many of these pigs recovered but many pigs similarly treated did not respond to therapy. The remaining apparently normal penmates of these pigs were placed on therapeutic levels of antibiotics in feed or water for 10 to 25 days.

Adult swine with clinical evidence of hemorrhage in their feces usually responded poorly to treatment which consisted of parenterally administered antibiotics, antihistamines, and corticosteroids. Fluid replacement therapy or blood transfusion was not attempted. When the disease

occurred, all pigs in a pen or enclosure, even those with no clinical signs, were placed on therapeutic antibiotic levels in feed or water.

The ninety-two Iowa veterinarians surveyed by telephone reported that epizootics generally involved 1 to 10% of pigs in a given enclosure on a farm. Seventy-nine percent (412 out of 526) of the epizootics occurred in feeder pigs, housed either conventionally or in confinement. Twenty-one percent (114 out of 526) of the epizootics occurred in bred gilts, sows, boars, or rarely, in finishing pigs, housed either conventionally or in confinement. Each veterinarian treated, on the average, 8 epizootics per year. Regardless of the production method or age groups of pig involved, nitrofurans in feed or water or lincomycin and spectinomycin (L-S 50)[a] in water were the drugs of choice. They also appeared to have the most efficacy. Neomycin in feed or water was also used often with reasonably good results. Less frequently used antimicrobial drugs included sulfas, arsanilic acid, tetracycline, and the nitroimadazoles. Effectiveness of a given drug was often difficult to interpret because if the initial drug chosen did not work a second, third, or even fourth drug was tried.

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[a]Upjohn Company, Kalamazoo, MI.

DISCUSSION

Porcine proliferative enteritis is the proposed name for
Campylobacter sputorum subspecies mucosalis-associated condi-
tions in swine in the midwestern United States. The disease
has somewhat different pathologic and clinical manifesta-
tions in growing and adult swine. The reason for these dif-
ferences is not known.

Feeder pigs in the 18-45 kg category primarily have
chronic diarrhea, loss of weight, and loss of condition.
They are also hypoproteinemic and demonstrate a tissue demand
for neutrophils as evidenced by neutrophilic leukocytosis
with left shift. Lymphopenia may be a manifestation of pro-
longed stress from disease. Workers in Sweden have demon-
strated significant protein loss from the intestine of pigs
suffering from terminal ileitis.[22,23]

Adult swine may have clinical signs and lesions similar
to the feeder pig group. However, more frequently they have
hemorrhaged into the intestinal lumen. Blood loss is demon-
strated by their decreased hemoglobin concentration and
packed cell volume values. These pigs are also hypoprotein-
emic. This is presumably due to the loss of plasma proteins
in addition to erythrocytes into the intestinal lumen. Many
of these pigs also have elevated serum potassium levels.
This may reflect acidosis due to hemorrhagic shock.

This study does not offer effective prevention, control, and treatment procedures. In swine herds where the disease has occurred, the prophylactic use of antibiotics prior to and after moving animals may be of some benefit in preventing or reducing new incidence of disease. There are no effective methods to detect subclinical disease.

There is an urgent need for serologic methods to detect subclinically affected herds. Kurtz et al.[24] have recently reported that a microtiter agglutination test may show promise for detecting humoral antibodies in CSM-exposed swine.

The treatment of clinically affected animals has been on an empirical basis only. Controlled drug studies in field outbreaks are needed. The intracellular nature of CSM may be of significance in the generally poor response to treatment of severely affected pigs. It will probably be difficult to effectively prevent and treat the disease until more is known of the pathogenesis of the disease and the biology of CSM.

An in vitro study to determine the minimum inhibitory concentration of several antimicrobial agents for several field isolants of CSM was conducted by Kurtz et al.[24] They found the organism to be sensitive (in decreasing order) to tetracycline, nitrofurantoin, Penicillin G, chloramphenicol, erythromycin, neomycin, and Polymyxin B.[24]

The diagnosis of porcine proliferative enteritis is made

by gross and microscopic lesion examination. The thickened
ileum is a constant gross finding. Microscopically, the
demonstration of <u>Campylobacter</u> sp.-like organisms within
proliferative mucosal epithelial cells is diagnostic.
Bacteriologic techniques can be used, but they are not
currently refined enough to warrant attempted isolation
on a routine basis. <u>Salmonella</u> <u>choleraesuis</u> was isolated
from 4 feeder pigs in this study. The significance, if any,
of this organism in the disease is not known.

Clinical signs and clinical pathologic findings are not
sufficiently specific to diagnose the disease.

Possible differential diagnoses in feeder pigs,
finishing pigs, and adult animals with clinical signs of
chronic diarrhea, and with passage of pseudomembranes or
blood are numerous and should include swine dysentery,
salmonellosis, trichuriasis, hog cholera, African swine fever,
erysipelas, and proliferative enteritis.

Clinical signs of acute onset of hemorrhage in the feces
of feeder pigs, finishing pigs, or adult animals may also
indicate any number of diseases. Differential diagnoses
should include: salmonellosis; swine dysentery; <u>Clostridium</u>
<u>perfringens</u> enteritis; <u>E</u>. <u>coli</u> enteritis; anthrax; lepto-
spirosis; mycotoxicosis; poisoning with coumarin derivatives,
heavy metals, sodium fluoride, or copper; torsion of the
mesentery; gastric ulcers; and possibly Vitamin E deficiency.

A category of intestinal hemorrhage of unknown etiology should also be considered.

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THE PATHOLOGY OF NATURALLY OCCURRING PORCINE PROLIFERATIVE ENTERITIS

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SUMMARY

The lesions of porcine proliferative enteritis were studied by light and electron microscopy in 18-45 kg (40 to 100 lb) feeder pigs, fattening hogs, bred gilts, sows, and boars.

The characteristic microscopic feature common to all age groups was proliferation of immature crypt epithelial cells, primarily in the ileum and distal jejunum. Similar changes were also observed in the mid-jejunum, cecum, and colon of a few pigs.

The earliest detectable microscopic lesion was focal proliferation of crypt epithelial cells with accompanying inflammation of the lamina propria and leukocytic exudate within affected crypt lumina. Lesions progressed to diffuse crypt cell proliferation, elongation of crypts, and loss of villi.

Immature epithelial cells contained variable numbers of intracytoplasmic, nonmembrane-bound, curved organisms resembling Campylobacter sp. bacteria. Similar organisms were within phagolysosomes of macrophages in the lamina propria, and within the cytoplasm of crypt epithelial cells undergoing mitosis.

Campylobacter sputorum subspecies mucosalis was isolated from the ileal mucosa in 30 of 58 pigs.

INTRODUCTION

Porcine proliferative enteritis is the proposed name for a Campylobacter sputorum subspecies mucosalis (CSM)-associated disease occurring in midwestern swine. The epidemiology, gross lesions, and treatment have been described.[1]

Conditions associated with CSM in swine have been observed throughout the world. Rowland and Lawson[2,3,4] and Rowland et al.[5,6] have associated the intracellular presence of CSM-like organisms with proliferative mucosal lesions in cases of intestinal adenomatosis, proliferative hemorrhagic enteropathy, necrotic enteritis, and regional ileitis (terminal ileitis). Workers in Sweden have observed CSM-like organisms within affected intestinal epithelial cells in cases of regional ileitis.[7] Similar organisms have been observed in cases of proliferative hemorrhagic enteropathy in Australia[8] and Canada.[9]

Lawson and Rowland[10] initially reported the isolation of CSM in 1974 from cases of intestinal adenomatosis. These organisms were antigenically different from other swine enteric Campylobacter spp. and could not be isolated from the intestine of normal swine.[10] Campylobacter sputorum subspecies mucosalis was subsequently isolated from the intestinal mucosa of pigs with necrotic enteritis, regional ileitis, and proliferative hemorrhagic enteropathy.[8,11,12,13]

The gross and microscopic lesions of intestinal adenomatosis, proliferative hemorrhagic enteropathy, necrotic

enteritis, and regional ileitis have been described by Rowland and Lawson[2,3,4], and others.[6-9,14] These four conditions are characterized by proliferation of immature crypt epithelial cells and the presence of CSM-like organisms within these cells. Intestinal adenomatosis has mucosal proliferation without prominent inflammation or necrosis of the small and large intestine.[2,3] Proliferative hemorrhagic enteropathy has mucosal proliferation in the small and large intestine accompanied by superficial mucosal necrosis and hemorrhage into the intestinal lumen.[6] Coagulative necrosis of proliferated mucosa in the ileum and large intestine is characteristic of necrotic enteritis.[6] Regional ileitis has chronic inflammation and ulceration of proliferated mucosa in the terminal ileum.[7] Hypertrophy of smooth muscle of the tunica muscularis has also been described in this condition.[7] Rowland et al.[6] suggested that intestinal adenomatosis is the initial lesion followed by the occurrence of any of the other conditions. Limited experimental evidence tends to support this hypothesis.[15,16]

Campylobacter sputorum subspecies mucosalis-like organisms have been observed by electron microscopy within the apical cytoplasm of immature epithelial cells, and they were not enclosed within cytoplasmic vacuoles.[2,17] The organisms were irregularly shaped, approximately 2-3 μm long, 0.3 μm in diameter, and had an undulating cell wall.[2,17] Love and

Love[14] have demonstrated these organisms within macrophage
phagolysosomes and free within the submucosa in cases of
proliferative hemorrhagic enteropathy.

MATERIALS AND METHODS

Source of animals- Tissues were studied from 58 swine
from 44 farms where porcine proliferative enteritis occurred
The study material consisted of 33 feeder pigs (18-45 kg)
1 finishing pig (85 kg), and 24 bred gilts, sows, and boars.
The pigs were euthanatized by intravenous administration of a
barbiturate or by electrocution. The intestinal tracts
from 400 market weight swine were examined at an Iowa
slaughterhouse.

Pathologic studies- Tissues collected for histopathologic
examination from the 58 animals at necropsy consisted of duo-
denum, jejunum (three levels), ileum (three levels), ileo-
cecal valve, cecum, colon, ileocecal lymph nodes, liver,
spleen, kidney, stomach, lung, cardiac and skeletal muscle,
and brain. Tissues collected from slaughterhouse hogs con-
sisted of ileum (three levels) only. All tissues were fixed
in 10% neutral buffered formalin. Paraffin-embedded sections
were stained with hematoxylin and eosin. Selected sections
of intestine, ileocecal lymph node, and liver were stained
with Warthin-Starry silver stain.[18]

Intestinal tissues for transmission electron micro-
scopy were obtained adjacent to sections selected for
light microscopy. Tissues included jejunum (three
levels), ileum (three levels), cecum, and proximal colon.

All tissues were fixed for a minimum of 48 hours in 3% gluta-raldehyde buffered with 0.1 M Millonig's phosphate buffer (pH 7.2). Tissues were rinsed twice in 0.1 Millonig's buffer and post fixed for one hour in 1% osmium tetroxide buffered with 0.1 M Millonig's phosphate buffer. Fixed tissues were dehydrated through either graded alcohols and propylene oxide or 2,2-dimethoxypropane and embedded in Epon 812.[a] Thick sections (2-3 μm) were stained with alkaline toluidine blue (1% toluidine blue in 1% sodium borate) and examined by light microscopy. Ultra-thin sections were cut with a diamond knife and supported on naked copper grids. Sections were stained with 2% methanolic uranyl acetate and Reynold's lead citrate.[19] They were viewed and photographed with a Hitachi HS-9[b] transmission electron microscope.

Microbiologic studies- Tissues from the ileum, cecum, proximal colon, liver, gallbladder, and ileocecal lymph nodes were collected for bacteriologic evaluation. The mucosa was scraped from each intestinal segment and prepared for CSM isolation.[1] Homogenates of ileocecal lymph node were also cultured for CSM. Antisera to CSM isolants were pro-duced,[1] and used in a slide agglutination test as the means for positive CSM identification.[1] Samples of gut homogenate,

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[a]Ladd Research Industries, Burlington, VT.

[b]Hitachi Scientific Instruments, 328 Eisenhower Lane, Lombard, Ill. 60148.

liver, ileocecal lymph node, and gall bladder were cultured for _Salmonella_ spp.[1]

RESULTS

Histopathologic findings- The microscopic lesions in all
age groups were similar except that in feeder pigs the affected
intestinal mucosa often had severe necrosis and granulomatous
inflammation while adult pigs often had focal areas of
hemorrhage in the lamina propria.

Sequential lesion progression could not be determined
with certainty because tissues were usually taken from pigs
with clinical signs. However, two subclinically affected pigs
(1 bred gilt and 1 feeder pig) had mild multifocal lesions char-
acterized by focal proliferation of immature epithelial cells
within isolated crypts. The affected crypts were often located
over aggregated lymphoid nodules (ALN; Peyer's patches) in the
ileum (Fig 1 and 2). Goblet cells were absent from these crypts
which were surrounded by increased numbers of eosinophils,
neutrophils, and macrophages in the adjacent lamina propria.
Villous epithelium was normal although villi over ALN were
often shortened and irregular (ilea examined from normal
pigs also had similar appearing villi over ALN). There was
also leukocytic debris within the lumina of affected crypts.
Warthin-Starry silver-stained histologic sections from the
same blocks had numerous curved CSM-like organisms within
the cytoplasm of the crypt epithelial cells (Fig 3).

Lesions in all age groups appeared to progress to
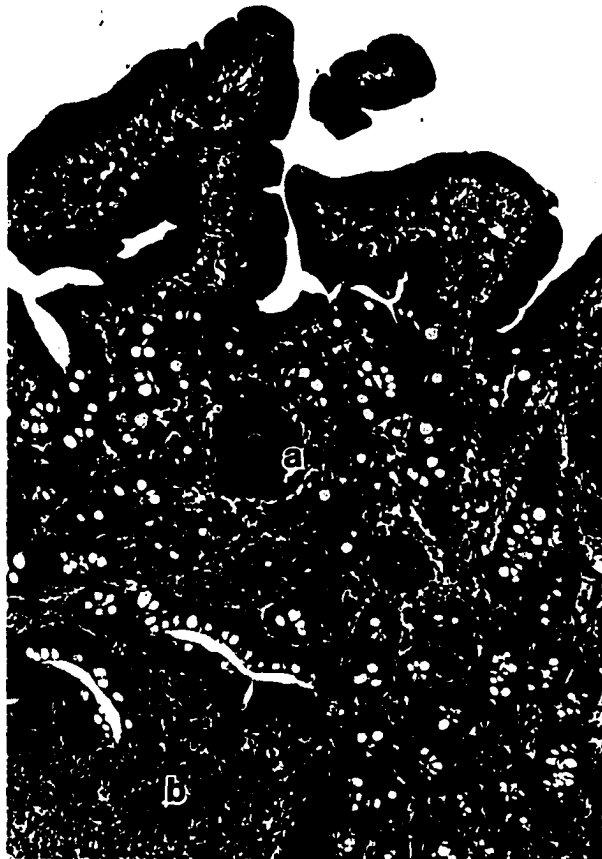
Fig 1—Ileum of gilt with proliferative enteritis. There is
proliferation of crypt epithelial cells (a) in isolated
crypts overlying aggregated lymphoid nodules (b). Leukocytic
exudate is present within crypt lumen (arrow). H&E stain.

Fig 2—Ileum from gilt with proliferative enteritis. There
is proliferation of crypt epithelial cells with loss of
goblet cells and crypt elongation. Leukocytic exudate is
present in crypt lumina (arrow). Increased numbers of in-
flammatory cells are in the surrounding lamina propria (a).
The villous epithelium is normal. H&E stain.
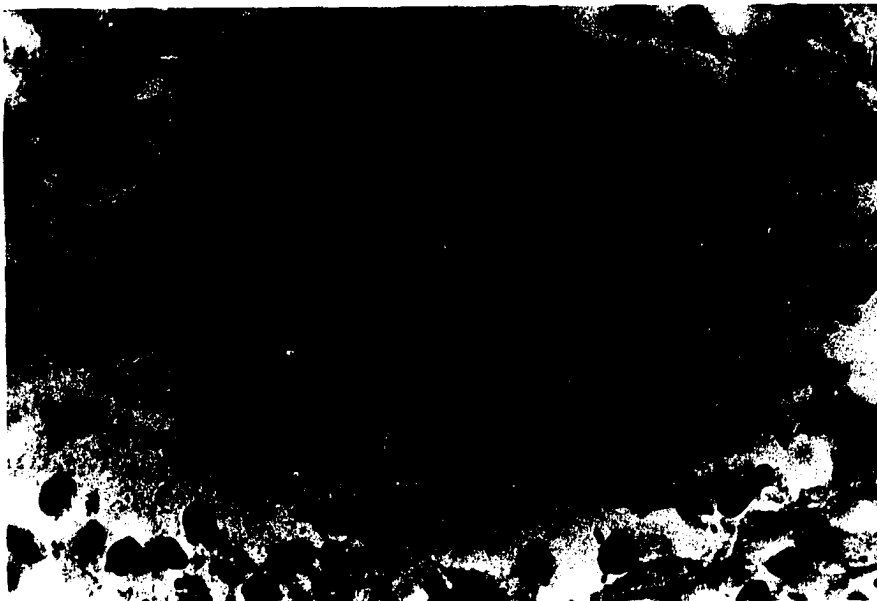
Fig 3—Ileum from feeder pig with proliferative enteritis. Numerous irregularly curved bacteria resembling Campylobacter sp. are in the apical cytoplasm of crypt epithelial cells. Warthin-Starry stain.

diffuse proliferation of crypt epithelial cells, crypt elonga-
tion, dilitation of lacteals, and shortening of villi
(Figs 4a and 4b). The progressive lengthening of crypts
and flattening of the mucosal surface continued until
elongated crypts eventually opened directly onto a flat,
avillous mucosal surface (Fig 5). The epithelial cells
covering the mucosal surface also had an immature appearance
characterized by basophilic cytoplasm and large oval to
oblong basally-oriented, hyperchromatic nuclei. They lacked
a prominent microvillus border. Affected crypts were
surrounded by macrophages, neutrophils, and eosinophils (Fig
6). Leukocytes were also between crypt epithelial cells, and
degenerating leukocytes were in the crypt lumina.

It was difficult to categorize further lesion progres-
sion in any of the affected groups of swine. However, there
was often continued proliferation of immature epithelial
cells which resulted in marked elongation, enlargement, and
branching of crypts (Fig 7). Proliferating crypt epithelial
cells extended into ALN, and there was often necrosis of these
crypt cells. This apparently resulted in microabscess forma-
tion with surrounding infiltration of macrophages (Fig 8).
There was also downgrowth of crypts through the muscularis
mucosa and into the submucosa. An aggregation of immature
epithelial cells forming a gland with a central lumen was with-
in a submucosal lymph channel in the ileum of one pig (Fig 9).

Fig 4a—Ileum from gilt with proliferative enteritis. There
is diffuse proliferation of crypt epithelial cells with
elongated irregular crypts and shortened villi. H&E stain.

Fig 4b—Gilt ileum with proliferative enteritis. There is diffuse proliferation of crypt epithelial cells, elongation of crypts, flattened mucosal surface, and dilitation of lacteals (arrow). H&E stain.

Fig 5—Gilt ileum with proliferative enteritis. Elongated crypts open directly onto a flattened, avillous mucosal surface over aggregated lymphoid nodules (a) in terminal ileum. H&E stain.
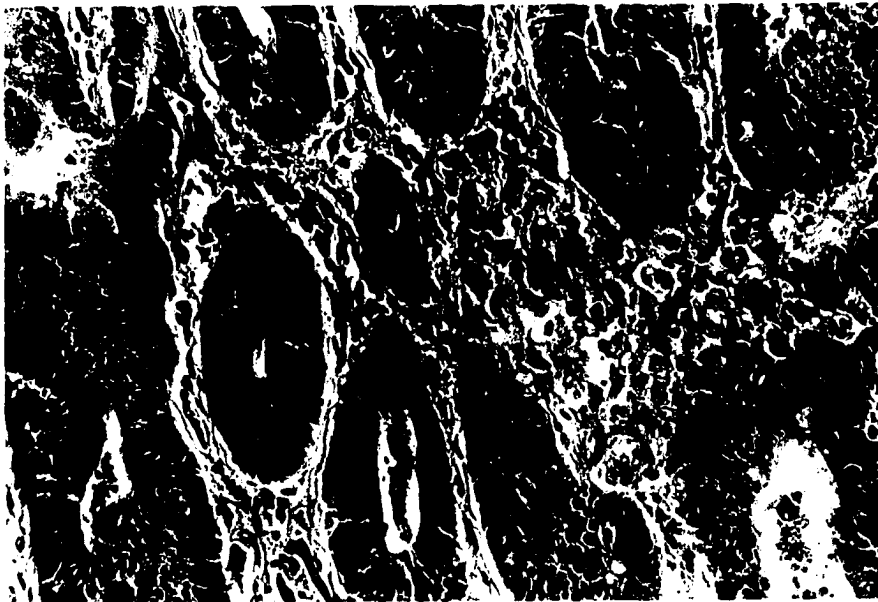
Fig 6—Ileum from young boar with proliferative enteritis. There is diffuse proliferation of crypt epithelium. The lamina propria contains numerous large macrophages (arrow). H&E stain.

Fig 7—Feeder pig ileum with proliferative enteritis.
Large, irregular crypts are lined by immature epithelial
cells. Crypts are often branched (arrow). H&E stain.
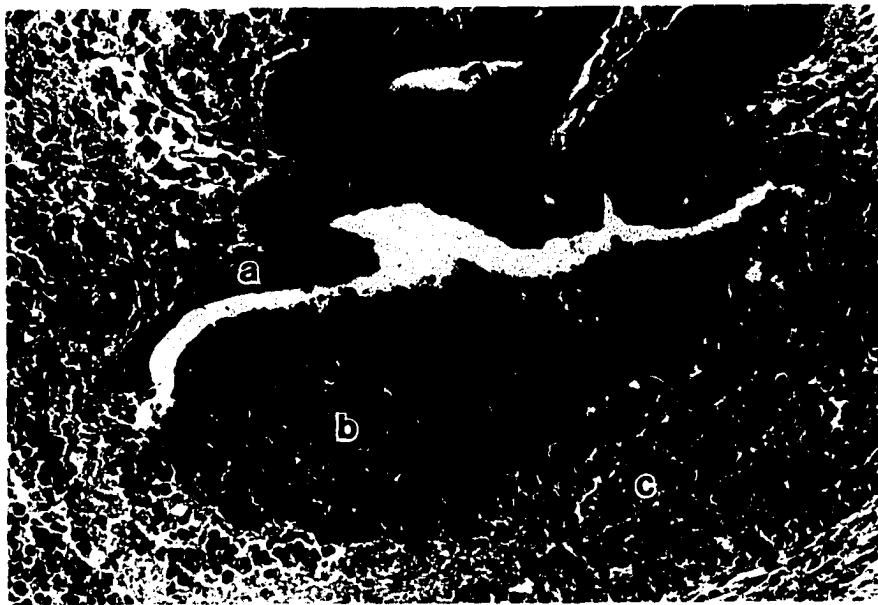
Fig 8—Ileum from feeder pig with proliferative enteritis. There is downgrowth of crypt epithelium (a) into aggregated lymphoid nodules with crypt cell necrosis and microabscess formation (b) surrounded by macrophages (c). H&E stain.

Fig 9—Ileum from feeder pig with proliferative enteritis. Immature crypt epithelial cells forming a gland with a central lumen (small arrow). The cells are within submucosal lymph channel (large arrow). H&E stain.

The intestines of most affected feeder pigs had areas with full-thickness mucosal necrosis which contained scattered islands of enlarged crypts lined with Campylobacter sp.-infected epithelial cells. Variable but usually large numbers of macrophages, lymphocytes, plasma cells, and neutrophils were at the advancing edge of the mucosal necrosis and within the submucosa. Multinucleated giant cells and epithelioid cells were also within the submucosa as was fibrous connective tissue proliferation. Transmucosal necrosis and inflammation were occasionally accompanied by hypertrophy of smooth muscle of the tunica muscularis.

Adult swine had marked congestion of blood vessels in the lamina propria. Affected swine also had superficial mucosal necrosis including necrosis of vascular endothelium and disruption of vascular spaces which resulted in hemorrhage and fibrin exudation into the lamina propria and intestinal lumen. Vascular spaces in deeper regions of the lamina propria were often distended with erythrocytes, and surrounded by macrophages but necrosis of their endothelium could not be detected.

The ileocecal lymph nodes in all affected pigs had inflammatory changes of varying severity characterized by infiltration of neutrophils and eosinophils into the capsular sinus and medullary parenchyma. There was also diffuse hyperplasia of lymphoid germinal centers. Ileocecal lymph nodes

from feeder pigs occasionally had granulomatous foci within
their medullary parenchyma. These foci were infiltrated by
macrophages which often appeared as epithelioid and multi-
nucleated giant cells (Fig 10). The ileocecal node from one
feeder pig had a metastatic focus of immature epithelial
cells which formed irregular crypts containing intraluminal
cellular debris (Fig 11). Campylobacter sp.-like organisms
could not be observed within the cytoplasm of these meta-
static epithelial cells.

The ilea of five out of 400 market weight swine examined
at an Iowa slaughterhouse had gross and microscopic lesions
of proliferative enteritis.

Electron microscopy- Bacterial organisms structurally
similar to CSM were observed within the cytoplasm of most
epithelial cells from proliferative mucosal lesions (Fig
12). They were 1.6 to 2.7 μm long, 0.2 to 0.3 μm in diameter,
and usually S-shaped when longitudinal sections could be
observed. Their cell walls were rugose and trilaminar, and
dense granules were present in their cytoplasm (Fig 12). They
were not surrounded by host cell membranes, and they did not
appear to cause destruction of host cell cytoplasmic
organelles (Fig 12). Bacterial cells undergoing division
were infrequently found within either infected epithelial
cells or macrophages in the lamina propria.

A possible method of entry into crypt epithelial
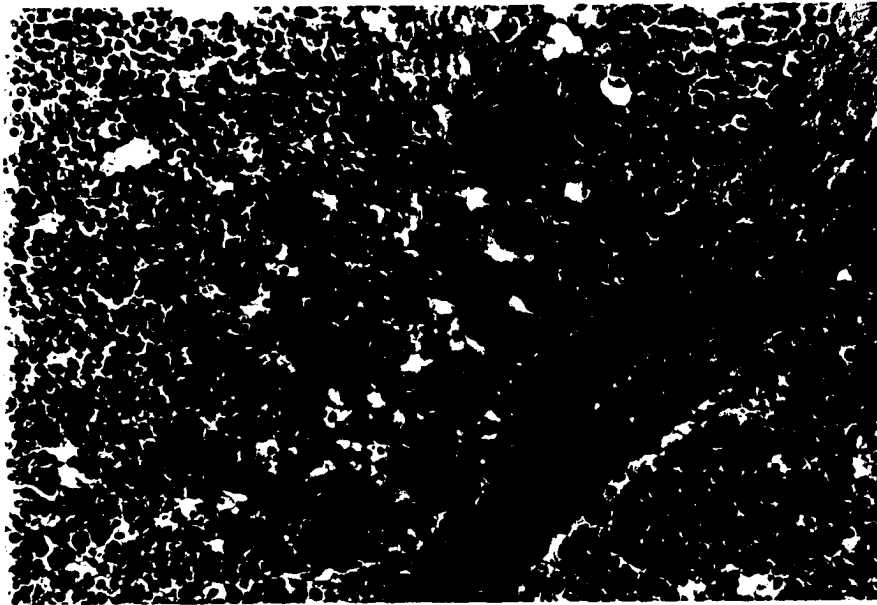
Fig 10—Ileocecal lymph node from feeder pig. There is a focus of multinucleated giant cells (arrow) within the medullary parenchyma. H&E stain.

Fig 11—Ileocecal lymph node from feeder pig. Numerous
irregular spaces containing necrotic debris are lined by
crypt epithelial cells (arrow) and are within the
medullary parenchyma. H&E stain.

Fig 12—Ileum from gilt with proliferative enteritis.
Numerous Campylobacter sp. organisms are free in the
cytoplasm of crypt epithelial cells. There is no apparent
destruction of host cell organelles, and the organisms are
surrounded by a clear space presumed to be fixation arti-
fact. Bar represents .5 µm.

cells was observed in ileal mucosae examined from sub-clinically infected pigs in which the organism appeared to enter through the microvillous border of the cell (Fig 13). There was no apparent distortion of microvilli and invasion was not accompanied by invagination of the host cell plasma membrane. Intercellular junctions appeared intact and the organisms were not observed to enter by this route. Organisms were in the apical and supranuclear regions of host cell cytoplasm and they were usually surrounded by a clear space which was interpreted to be fixation artifact (Fig 12).

Campylobacter sp.-laden epithelial cells were immature crypt cells as judged by their structure (Fig 14). They had short, wide, and irregular microvilli which were less numerous than in mature absorptive epithelial cells. These immature cells also had a poorly developed terminal web and filaments from the cores of microvilli penetrated into the cytoplasm for only a short distance (0.2 to 0.4 μm). Secretory granules and free ribosomes were prominent in the apical cytoplasm while mitochondria were larger than those in mature absorptive cells. Nuclei of immature cells were often indented. Crypt cells undergoing mitosis also had Campylobacter sp.-like organisms within their cytoplasm (Fig 15).

In the lamina propria, Campylobacter sp.-like organisms were often within macrophage phagolysosomes in degenerating

Fig 13—Gilt ileum with proliferative enteritis. Campylo-

bacter sp. bacteria in apical cytoplasm of crypt epithelial

cell. The organism is not surrounded by a host cell membrane

and there is no apparent distortion of microvilli. Bar

represents .5 μm.

Fig 14—Ileum from gilt with proliferative enteritis. Imma-
ture crypt epithelial cells containing numerous Campylobacter
sp. bacteria. The epithelial cells have short irregular
microvilli and numerous secretory granules in the apical
cytoplasm. Bar represents 2.0 μm.
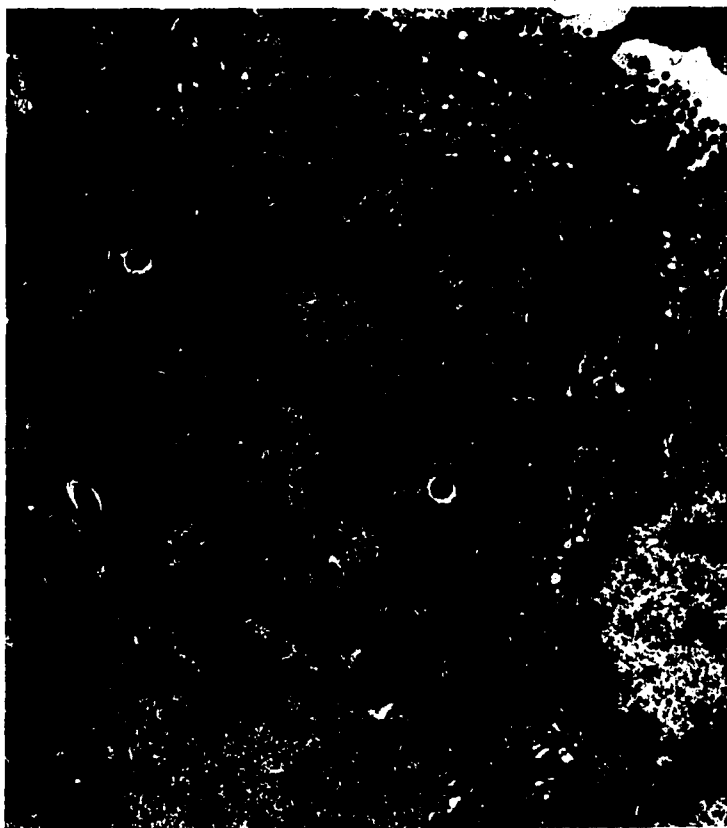
Fig 15—Gilt ileum with proliferative enteritis. A crypt epithelial cell is undergoing mitosis. Numerous bacterial profiles resembling Campylobacter sp. (arrows) are free within the cytoplasm. Bar represents 2.0 µm.

aggregates (Fig 16) surrounded by dense material. Bacterial profiles were not free within the lamina propria, submucosa, or tunica muscularis. Capillaries within the lamina propria had equivocal widening of endothelial cell junctions. Macrophages with numerous bacteria-laden phagolysosomes were occasionally seen lying adjacent to capillary basement membranes.

Microbiologic findings- *Campylobacter* *sputorum* subspecies *mucosalis* was isolated from the affected intestinal mucosa in 30 of 58 pigs. *Salmonella* *choleraesuis* was isolated from the ileocecal lymph nodes in four feeder pigs. *Escherichia* *coli* was isolated from the intestine of almost all pigs studied but consistent serotypes were not identified.

Fig 16—Ileum from feeder pig with proliferative enteritis.
A macrophage in the lamina propria has phagolysosomes which
contain numerous degenerate bacterial profiles resembling
Campylobacter sp. organisms. Bar represents 2.0 μm.

DISCUSSION

There was constant association between proliferation of immature crypt epithelial cells and the presence of non-membrane bound intracytoplasmic Campylobacter sp.-like organisms in the intestinal mucosa of pigs with proliferative enteritis. This association has also been demonstrated in cases of intestinal adenomatosis, necrotic enteritis, proliferative hemorrhagic enteropathy, and regional ileitis.[4,5,6]

Porcine proliferative enteritis as it occurs in midwestern swine has features similar to all of the above conditions. The apparent initial lesion of proliferation of immature crypt epithelial cells resembles intestinal adenomatosis. However, inflammation does appear to be a component of the early proliferative enteritis lesion. Extensive hemorrhage into the intestinal lumen often accompanied by superficial mucosal necrosis is a feature of proliferative hemorrhagic enteropathy. Similar lesions also occur in proliferative enteritis, especially in bred gilts, sows, and boars. The reason for this culminating manifestation of the disease in this age group is not known. Porcine proliferative enteritis also has pathologic features common to necrotic enteritis and regional ileitis especially in 16-45 kg feeder pigs. Necrotic enteritis is characterized by diffuse necrosis of proliferated intestinal mucosa and

granulomatous mucosal inflammation.[20,21] Hypertrophy of smooth

muscle of the tunica muscularis, and (rarely) metastasis of

glandular mucosal elements to the ileocecal lymph node have

been described for regional ileitis.[6,7,20,21] All of these

lesions were observed in proliferative enteritis.

The effect of CSM in the intestinal lumen on crypt

epithelial cells is not known. In experimental studies with

rabbits orally inoculated with Salmonella typhimurium, the

organism had to be within a "critical proximity" of intesti-

nal microvilli in order for fibrillar cores of microvilli and

tight junctions to undergo degeneration.[22] Destruction of

microvilli or intercellular junctions could not be observed

in the intestines of pigs with proliferative enteritis.

Campylobacter sp. organisms appeared to penetrate through

crypt epithelial cells at the microvillous border and they

did not cause invagination of host cell membranes. Once in-

side the cell, they were not membrane bound. In contrast,

Salmonella typhimurium has been shown to penetrate intestinal

epithelium, and a cavity forms in the apical cytoplasm in

front of the advancing bacteria.[23] The cavity eventually

pinches off from the apical cytoplasm and forms a vacuole

around the organism.[23] Salmonella typhimurium may also

penetrate through the intercellular junctional complex and

become enclosed by the lateral cell membranes of two adjacent

epithelial cells.[23] Salmonella enteritidis has been shown to

penetrate similarly in experimentally infected chickens.[24]

Takeuchi[25] has stated that enteric microorganisms may be classified into four groups based on their invasive potential. The first group is characterized by the ability to readily penetrate epithelial cells, multiply in the lamina propria, and eventually produce systemic infection.[25] Salmonella spp. organisms are in this group. The second group has the ability to reside and multiply in epithelial cells and produce severe epithelial damage, but they are infrequent in the lamina propria and rarely produce systemic disease.[25] This group is represented by Shigella spp. organisms. The third group is primarily found in the micro-villous border and does not invade, or at least not deeply, into the epithelial cell cytoplasm.[25] Members of this group include Cryptosporidia spp., Giardia spp., certain spirochetes, and others. The last group of organisms does not penetrate epithelium but adheres to the epithelial surface, and produces disease by elaboration of an enterotoxin.[25] This group is represented by Vibrio cholerae and certain strains of pathogenic Escherichia coli. Campylobacter sputorum subspecies mucosalis does not appear to belong to any of these classifications.

Many other enteric microorganisms, both pathogenic and nonpathogenic, have been identified. Most of these have been identified within or associated with mature villous

absorptive epithelial cells or goblet cells.[25] Pathogens displaying a preference for immature crypt epithelial cells are not common, but Parvoviruses of the cat and dog are examples of such pathogens.

Campylobacter sputorum subspecies mucosalis appears to have a predilection for crypt epithelium. Limited experimental studies in neonatal piglets orally inoculated with cultures of CSM tends to substantiate this observation.[26] However, instead of causing crypt epithelial cell destruction as does the parvovirus of feline panleukopenia, CSM appears to directly or indirectly stimulate production of crypt cells.

The ileocecal lymph node reacts rapidly to inflammation in its drainage area.[27] In a study in germ free mice orally inoculated with Salmonella panama, inflammation and formation of germinal centers could be observed in early stages of infection.[27] Inflammation and lymphoid hyperplasia of the ileocecal lymph nodes were constant findings in all pigs studied with porcine proliferative enteritis; however, CSM was not isolated from these lymph nodes.

Under normal conditions, epithelial cells produced in crypts migrate up the villi, mature into absorptive epithelial cells and are extruded at the villus tip. Radioactive thymidine studies of Salmonella typhimurium infected rabbits have shown that the generation time of epithelial cell renewal is

significantly increased.[28] It is believed that crypt cells
undergo accelerated mitosis to replace increased cell loss
along the villous surface. Excessive loss of mature in-
testinal epithelium has been documented in several enteric
diseases and appears to be a general reparative response of
the mucosa to injury.[25] Kinetic studies have not been reported
on naturally occurring CSM-associated conditions in swine.
Observations indicate that crypt cell proliferation precedes
absorptive epithelial cell loss leading eventually to a
flattened mucosal surface covered with epithelium which has
morphologic characteristics of immature epithelial cells.

The possibility that CSM-associated conditions are a
manifestation of immediate or delayed hypersensitivity has not
been studied, but Love and Love[14] have suggested that prolifera-
tive hemorrhagic enteropathy resembles an immediate hypersensi-
tivity reaction. Ferguson and co-workers[29-31] and MacDonald
and Ferguson[32] have studied the effects of local delayed hyper-
sensitivity on the small intestine in mice with heterotopically
transplanted grafts of fetal small intestine (allografts and
isografts) by a variety of methods. They were able to
demonstrate a cell-mediated (thymus dependent) reaction
characterized by proliferation of immature crypt epithelial
cells, mononuclear cell infiltration into the lamina propria
and increased cell loss from villi resulting in a flattened
mucosal surface.[33] Interestingly, crypt cell proliferation

preceded villus atrophy by several days.[33] The authors postulated that the effects may be produced by lymphokines which are mitogenic for immature crypt epithelial cells.[33]

Normal rats infected with the intestinal nematode Nippostrongylus brasilensis developed crypt hyperplasia and villus atrophy in those areas of the small intestine where the parasites were present.[29,33] Thymectomized rats given similar infective doses of the parasite did not develop these lesions.[25] Therefore, the epithelial damage was thought to be due to a thymus dependent immune response and not directly to the parasite itself.[33]

Crypt epithelial cells within lymphatics in the submucosa and within the ileocecal lymph node were observed in one pig in this study. Emsbo[20] encountered similar metastatic foci of epithelial cells within ileocecal lymph nodes in a few cases of regional ileitis in Denmark. It was his opinion that they represented nonneoplastic metastasis of rapidly-dividing immature epithelial cells.[20] It is not uncommon to observe downgrowth of proliferating crypt epithelial cells into ALN and into the muscularis mucosa. These epithelial cells could enter an afferent lymphatic channel, be carried to the regional lymph node, and retain their proliferative capacity in that location. The presence of anaplastic-appearing epithelial cells has not been documented in CSM-associated conditions in the pig.

The pathogenesis of CSM-associated conditions in swine awaits characterization and clarification. The role of CSM either as a primary or secondary pathogen in porcine intestinal adenomatosis, porcine proliferative enteritis, proliferative hemorrhagic enteropathy, necrotic enteritis, and regional ileitis has not yet been convincingly demonstrated. However, the intracellular presence of the organism in proliferated crypt epithelial cells has been repeatedly demonstrated and documented.

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PORCINE PROLIFERATIVE ENTERITIS:

THE EXPERIMENTAL DISEASE IN CAESAREAN-DERIVED

AND COLOSTRUM-DEPRIVED PIGS

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SUMMARY

The hypotheses that porcine proliferative enteritis is an infectious disease and that Campylobacter sputorum subspecies mucosalis (CSM) is involved in the development of this disease were experimentally tested.

Three experiments were conducted with 10-week-old Caesarean-derived and colostrum-deprived pigs. Fifteen of twenty-two pigs which were given homogenized mucosal scrapings (crude inoculum) intragastrically had gross and/or microscopic lesions of proliferative enteritis. Ten pigs were inoculated with cultures of both CSM and Salmonella choleraesuis, and two of these pigs had evidence of proliferative enteritis. Pigs (4) treated with S. choleraesuis only had a diffuse fibrinous gastroenteritis without evidence of mucosal proliferation. Proliferative enteritis was produced in one of five pigs inoculated with pure cultures of CSM.

Proliferative lesions in the intestine were characterized by the proliferation of immature crypt epithelial cells. Affected cells contained variable numbers of curved, intracytoplasmic Campylobacter sp. organisms. Campylobacter sputorum subspecies mucosalis was isolated from the intestinal mucosa of 8 pigs treated with either crude inocula or cultures of the organism.

INTRODUCTION

Porcine proliferative enteritis is the proposed name for a disease which occurs naturally in midwestern swine and is associated with the intracellular presence of <u>Campylo-bacter</u> sp.-like organisms. The clinical, epidemiologic, and pathologic findings of the naturally occurring disease in midwestern swine have been described.[1,2] It occurs in pigs of weaning age or older, but it is primarily observed in 14-45 kg feeder pigs and in bred gilts, sows, and boars. Gross lesions are characterized by thickening of the wall of the ileum primarily, but also of the jejunum, cecum and colon. There is reticulation of the serosa, hyperemia and edema of the mesentery, and enlargement of the ileocecal lymph nodes. The affected intestinal segment may contain variable amounts of fibrin, necrotic material, and blood within its lumen. The underlying mucosa has prominent mucosal folds, a granular appearance, and is hyperemic. Occasionally, there are diffuse areas of mucosal necrosis and prominent thickening of the tunica muscularis.

Microscopically, there is diffuse proliferation of crypt epithelium, elongation of crypts, and flattening of the villous surface. Variable numbers of macrophages, eosino-phils, and neutrophils are present in the lamina propria. There is superficial to full thickness mucosal necrosis

accompanied by exudation of fibrin, leukocytes, and erythrocytes into the intestinal lumen. Silver-stained histologic sections of affected mucosa have curved organisms within the cytoplasm of affected crypt epithelial cells.

The electron microscopic features of naturally occurring proliferative enteritis have been described.[2,3] Campylobacter sp.-like organisms are free within immature epithelial cell cytoplasm, are 2 to 3 μm long and 0.2 to 0.3 μm in diameter, and have a trilaminar, undulating cell wall.[3] The organism apparently preferentially penetrates immature crypt epithelial cells by passing through the microvillous border of these cells.[2]

The disease has clinical and pathologic resemblance to other CSM-associated diseases which have been reported throughout the world.[4] Rowland and Lawson[5] and others[6,7] have observed the intracellular presence of the organism in intestinal mucosa of pigs with intestinal adenomatosis, necrotic enteritis, proliferative hemorrhagic enteropathy, and regional ileitis. Lawson and Rowland[8] biochemically and antigenically characterized, and named the organism. Campylobacter sputorum subspecies mucosalis has been isolated from the intestinal mucosa in pigs with each of these conditions.

The experimental reproduction of any of the CSM-associated conditions in swine given oral inoculations of

either CSM or homogenized intestinal mucosa from naturally occurring cases has not been convincingly demonstrated.[9-12] Focal microscopic proliferative lesions in the mucosa of the small and large intestine have been produced, and intracellular _Campylobacter_ sp.-like organisms have been demonstrated in a small percentage of neonatal pigs orally inoculated with either type inoculum.[10,13]

The objective of the following experiments was to experimentally produce and characterize proliferative enteritis in 10-week-old Caesarean-derived and colostrum-deprived pigs.

MATERIALS AND METHODS

Experimental animals- Four crossbred sows were obtained
from an Iowa swine herd not known to have experienced an
epizootic of proliferative enteritis. The sows were trans-
ported on the 112th day of pregnancy to Iowa State University
where they were individually housed. Caesarean sections were
performed at 114 days of gestation in a formaldehyde-fumigated
surgery suite equipped with a receiving room for newborn
piglets. The piglets were transported as a litter in a
sterile chamber to a twice-fumigated isolation building where
they remained throughout the course of the experiments. The
pigs received only SPF-lac[a] milk replacer until 5 weeks of
age when an 18% protein unmedicated starter ration was
gradually introduced to them. They were vaccinated with
Salmonella choleraesuis, Erysipelas rhusiopathiae, Pasteurella
multocida, and Escherichia coli bacterins at 8 weeks of age.
At nine weeks of age they were gradually placed on a 16%
unmedicated grower ration. The pigs were intragastrically
inoculated at 10 weeks of age and weekly thereafter for three
weeks. Thirty pigs were obtained in this manner and were
used in experiments one and two.

Twenty-four 8-week-old Caesarean-derived and colostrum-
deprived pigs were purchased for use in experiment 3. They

[a]Borden Chemical, Borden Inc., Box 419, Norfolk,
Virginia.

were housed, fed, vaccinated, and orally challenged similarly to pigs in experiments one and two. Food was removed from all pigs in all experiments twenty-four hours prior to intragastric inoculation.

Source of inoculum

Crude inoculum- The mucosa from pigs with naturally occurring proliferative enteritis was scraped from the intestinal wall using a glass slide, mixed with an equal volume of sterile phosphate buffered saline (pH 7.1),[16] and homogenized for three minutes in a blender. This homogenate was either used fresh or stored at -62C until needed. Pre-inoculation culture of crude inoculum was negative for Salmonella spp.

Campylobacter sputorum subspecies mucosalis inoculum-
An isolant was obtained from the intestinal mucosa of a pig given crude inoculum in experiment one (isolant no. 14-4p). This isolant was stored in 10 ml aliquots at -56C and used as the source of inoculum for experiments two and three. The inoculum was grown in flasks of brucella broth[a] supplemented with 10% fetal calf serum[b] under deoxygenated hydrogen and carbon dioxide (50:50 vol/vol). After flushing with gas, flasks were stoppered and incubated at 37C for 24 or 48 hours. In order to increase growth, the flasks were gently agitated (50 rpm)[c].

[a]Difco, Detroit, MI.

[b]Sterile Systems Inc., Logan, Utah.

[c]Junior Orbit Shaker, Lab-Line Instruments Inc., Melrose Park, Ill.

Colony counts of approximately $1x10^8$ colony forming units (CFU) per ml were obtained.

Salmonella choleraesuis inoculum- Salmonella choleraesuis used as inoculum in experiment 3 was isolated from the ileocecal lymph node of a pig in experiment 1. Flasks of trypticase soy broth[a] were seeded with the growth from one agar slant and incubated aerobically at 37C overnight with gentle agitation. Colony counts were approximately $1x10^8$ to $1x10^9$ CFU per ml.

Clinical examinations- The color and consistency of feces of all pigs were noted. Rectal temperatures of all pigs in experiments two and three were determined daily.

Clinical pathologic examinations- A single blood sample was obtained from all pigs in experiment one by intracardiac puncture immediately prior to euthanasia. Serial blood samples were collected from all pigs in experiments two and three. Blood was obtained from the anterior vena cava on days 0,3,5,8,12,15,20,24,28, and 32 after initial inoculation. Hemoglobin concentration, packed cell volume, total and differential leukocyte counts, plasma protein concentration, and plasma fibrinogen concentration were determined.

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[a]BBL, Cockeysville, MD.

Pathologic examinations- Postmortem examinations were
conducted on all pigs. Duodenum, jejunum (3 levels), ileum
(3 levels), ileocecal valve, cecum, colon (3 levels), ileo-
cecal lymph nodes, liver, spleen, kidney, stomach, lung,
cardiac and skeletal muscle, and brain were taken for
histopatholic examination. All tissues were fixed in 10%
neutral buffered formalin. All paraffin-embedded sections
were stained with hematoxylin and eosin, and all intestine
sections were also stained with Warthin-Starry silver
stain.[17]

Intestinal tissues selected for electron microscopy were
obtained adjacent to sections selected for light microscopy.
The procedures for tissue preparation for transmission
electron microscopy have been described.[2]

Microbiologic examinations- Samples of intestinal
mucosa from the ileum, cecum, and colon, and ileocecal lymph
nodes were obtained from all pigs and were cultured for CSM
and S. choleraesuis. Swabs of gallbladder mucosa, blood
samples, and samples from the liver were collected from all
pigs for S. choleraesuis isolation by methods previously
described.[1] Samples of jejunal mucosa were collected for
indirect fluorescent antibody examination for transmissible
gastroenteritis virus.[a] Cecal contents were collected for

[a]Procedures performed by the Iowa Veterinary
Diagnostic Laboratory, Ames, IA.

rotavirus identification by direct electron microscopy.[a]

Statistical procedures- An analysis of variance procedure was performed on clinical pathologic data obtained in experiments two and three. Significant treatment group differences were examined for each blood collection date and an F-value and probability statement were obtained.

Experimental design-

Experiment one- Fifteen ten-week-old pigs were randomly allotted to three groups of five pigs each. Each group was housed in a separate controlled environment room. Each pig in groups one and two was given 100 ml of crude inoculum (CI) and 50 ml of 10% sodium bicarbonate[b] intragastrically by a stomach tube. Group three pigs (control) were given 100 ml of phosphate buffered saline and 50 ml of 10% sodium bicarbonate intragastrically (Table 1). Necropsies were conducted one to four days following onset of clinical diarrhea or at termination of the experiment (5 weeks).

Experiment two- Fifteen 10-week-old pigs were randomly allotted to three groups. Seven pigs (group 1) were given 100 ml of crude inoculum and 50 ml of 10% sodium

[a]Procedures performed by the Iowa Veterinary Diagnostic Laboratory. Ames, IA.

[b]Fischer Scientific Scientific Company. Fair Lawn, Ner Jersey 97410.

bicarbonate. Three pigs (group 2) were given 100 ml $(1 \times 10^8$ CFU per ml) of CSM in brucella broth plus 50 ml of 10% sodium bicarbonate. Five control pigs (group 3) were given 50 ml phosphate buffered saline and 50 ml brucella broth. The route of inoculation and dosage schedule were the same as in experiment one (Table 1).

Experiment three- Twenty-four 10-week-old pigs were purchased and randomly allotted to five treatment groups. Group one (6 pigs) was given 100 ml of crude inoculum plus 50 ml of 10% sodium bicarbonate. Group two (10 pigs) was given a combined initial inoculation composed of 100 ml $(1 \times 10^8$ CFU per ml) of CSM, 25 ml $(1 \times 10^8$ CFU per ml) of S. choleraesuis (SC), and 50 ml of 10% sodium bicarbonate. They then were given the same dosage of CSM once a week for three additional weeks. Group three (4 pigs) was given 25 ml $(1 \times 10^8$ CFU per ml) of S. choleraesuis plus 50 ml of sodium bicarbonate as a single initial inoculation. The two pigs in group four were given 100 ml $(1 \times 10^8$ CFU per ml) of CSM and 50 ml of 10% sodium bicarbonate. Group five (2 pigs) served as uninfected controls and was given 25 ml of brucella broth, 25 ml of trypticase soy broth, and 50 ml of 10% sodium bicarbonate once weekly for three weeks (Table 1).

Pigs in experiments two and three were killed when they became moribund or at the termination of the experiment (5 weeks).

Table 1—Design of Experiments 1, 2 and 3

Experiment	Group	No. of pigs	Week 1	Week 2	Week 3	Week 4
I	1	5	CI[a]	CI	CI	CI
	2	5	CI	CI	CI	CI
	3	5	C[b]	C	C	C
II	1	7	CI	CI	CI	CI
	2	3	CSM[c]	CSM	CSM	CSM
	3	5	C	C	C	C
III	1	6	CI	CI	CI	CI
	2	10	SC[d]&CSM	CSM	CSM	CSM
	3	4	SC	-	-	-
	4	2	CSM	CSM	CSM	CSM
	5	2	C	C	C	C

[a]CI = crude inoculum.

[b]C = control group.

[c]CSM = Campylobacter sputorum subspecies mucosalis.

[d]SC = Salmonella choleraesuis.

RESULTS

Experiment one- Five of ten pigs given crude inocula
developed diarrhea 23 to 28 days post initial inoculation
(PI). The feces were watery, yellow, and occasionally mixed
with blood. At the time of necropsy, the pigs had neutro-
philic leukocytosis with a left shift (average white
blood cell count = 26,3000 with 23% band neutrophils and
28% segmented neutrophils). They were also hypoproteinemic
(average plasma protein concentration = 3.8 g/dl). They had
thickening of the ileum and distal jejunum with reticula-
tion and hyperemia of the serosa, edema of the mesentery
(Fig 1), and enlargement of ileocecal lymph nodes. The
ileum and distal jejunum had abundant brown-yellow fibrinous
exudates which formed intraluminal casts (Fig 2a). Removal
of the cast material revealed diffuse hyperemia of the mucosa
and thickened mucosal folds (Fig 2b).

Microscopically, there was diffuse proliferation of im-
mature epithelial cells, absence of goblet cells, elongation
of crypts, and loss of villi in the ileum, jejunum, cecum,
and proximal colon (Fig 3). Large numbers of macrophages,
eosinophils, and lymphocytes were present in the lamina
propria, and crypt lumina contained leukocytic debris.
Elongated crypts often opened directly onto a flattened,
avillous, mucosal surface. There was also downgrowth of
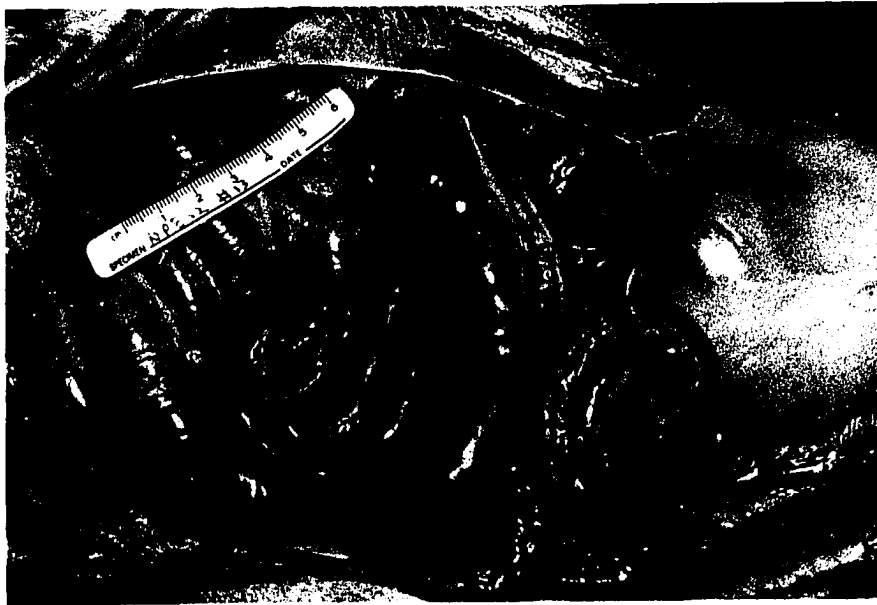
Fig 1—Thickened small intestine (arrow) from pig given crude
inocula in experiment one. There is also hyperemia and
reticulation of the serosal surface.

Fig 2a—Ileum from pig given crude inocula in experiment one.
Casts composed of fibrinous exudates are in the lumen.
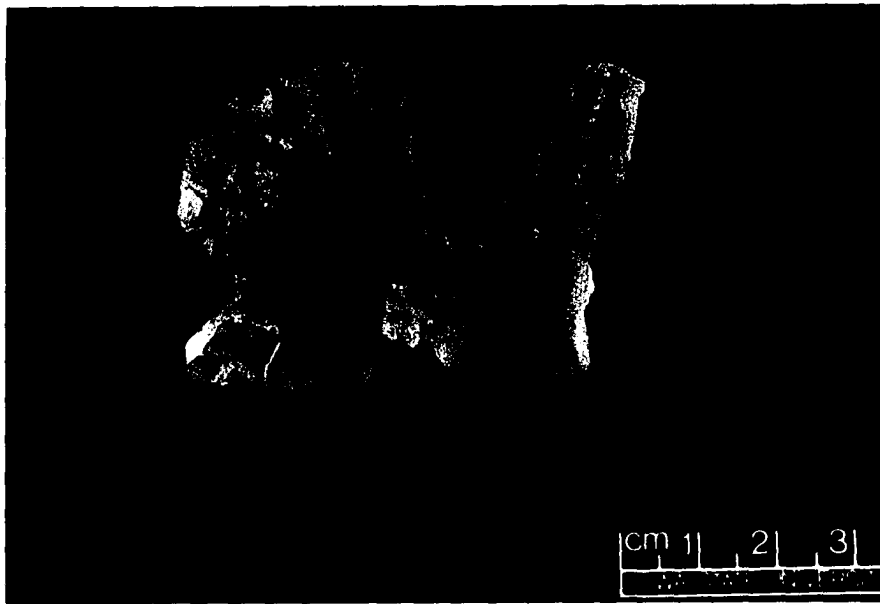
Fig 2b—Ileum from pig given crude inocula in experiment one. Upon removal of the exudate, prominent thickened mucosal folds are apparent.

Fig 3—Ileum from pig given crude inocula in experiment one. There is diffuse proliferation of immature crypt epithelial cells, absence of goblet cells, elongation of crypts, and loss of villi. Inflammatory cells, primarily mononuclear, are in the lamina propria. H&E stain.

crypt epithelium into aggregated lymphoid nodules (ALN: Peyer's patches) and through the muscularis mucosa into the submucosa. There was focal to diffuse mucosal necrosis varying from superficial to full thickness accompanied by diffuse infiltration of neutrophils and macrophages into the lamina propria and submucosa. The necrosis often left only scattered islands of proliferated crypts within the mucosa (Fig 4). Organized fibrin thrombi were occasionally observed in submucosal blood and lymph vessels. One pig had marked hypertrophy of smooth muscle of the tunica muscularis. Silver-stained sections of affected mucosa from all pigs had large numbers of curved <u>Campylobacter</u> sp.-like organisms within the apical cytoplasm of immature crypt epithelial cells (Fig 5).

Other microscopic lesions in the 5 pigs consisted of focal necrotic hepatitis with accompanying infiltration of mononuclear cells. There was also diffuse infiltration of neutrophils into medullary portions of ileocecal lymph nodes, diffuse hyperplasia of lymphoid germinal centers, and multiple foci of coagulative necrosis in cortical and medullary parenchyma. The pigs also had focal nonsupportive interstitial pneumonia.

Transmission electron microscopy confirmed the presence of <u>Campylobacter</u> sp.-like bacteria within the apical cytoplasm of crypt epithelial cells. The organisms were 2 to 3 μm long,
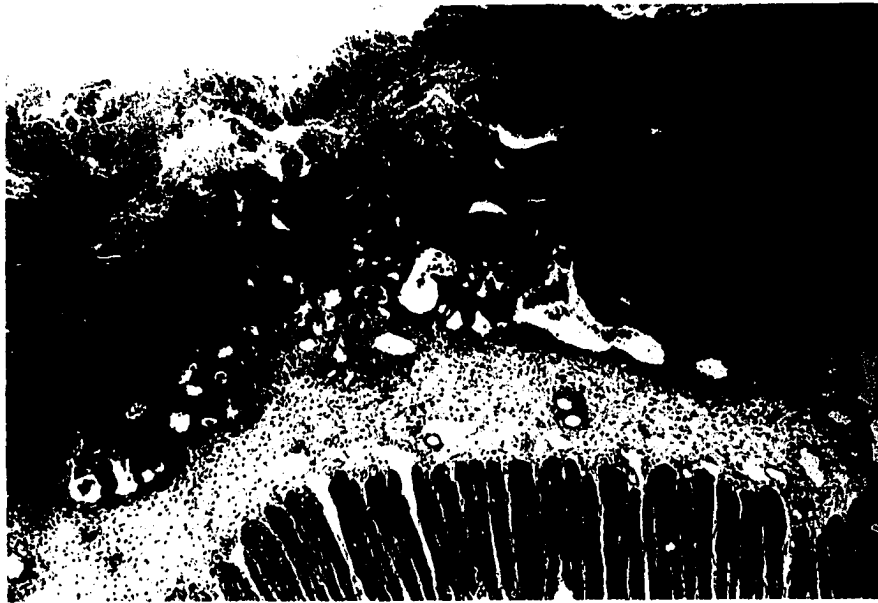
Fig 4—Ileum from pig given crude inocula in experiment one. There is diffuse, severe, mucosal necrosis and inflammation of the mucosa and submucosa. Islands of proliferated crypts remain in the mucosa (arrow). H&E stain.
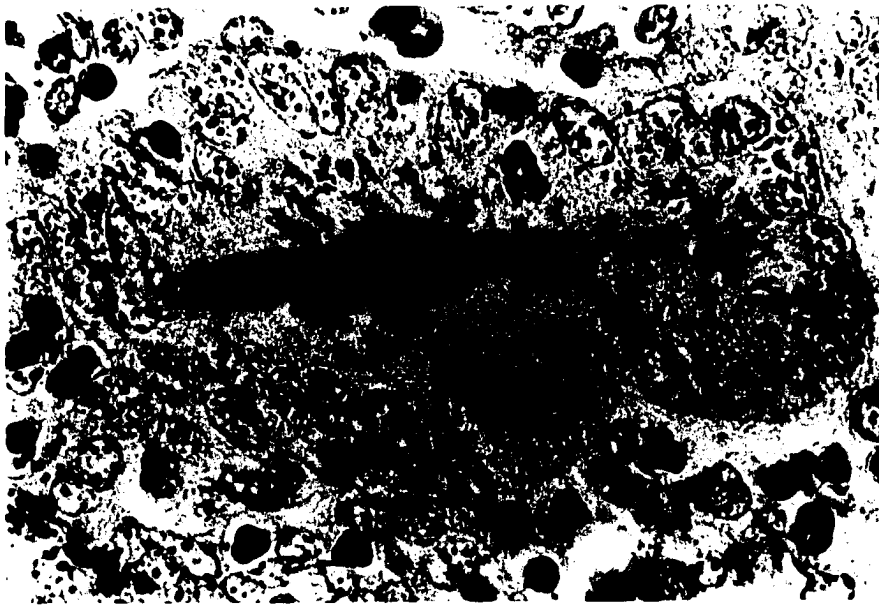
Fig 5—Ileum from pig given crude inocula in experiment one.
Crypt epithelial cells contain numerous curved Campylobacter
sp.-like organisms within their apical cytoplasm. Warthin-
Starry stain.

0.2 to 0.3 µm in diameter, and S-shaped when observed in longitudinal plane of section. They also had an undulating trilaminar cell wall and dense granules in their cytoplasm. They appeared to enter through the microvillous border, caused no apparent microvillous destruction, and were not surrounded by invaginated host cell plasma membrane (Fig 6). Inside the crypt epithelial cell, the organisms were not enclosed in cytoplasmic vacuoles but were free in the cytoplasm (Fig 7), however, they did not cause apparent destruction of cytoplasmic organelles. Similar bacterial profiles were observed within macrophage phagolysosomes in the lamina propria.

Campylobacter sputorum subspecies mucosalis was cultured from the ileal mucosa, and S. choleraesuis was isolated from the ileocecal lymph nodes and liver of the 5 affected pigs. All pigs were negative for transmissible gastroenteritis virus and rotavirus.

An additional two pigs in experiment one had only microscopic lesions in the ileum and cecum characterized by focal to diffuse areas of crypt cell proliferation, crypt elongation, loss of villi, and inflammation of the lamina propria. Campylobacter sp.-like organisms were observed within the cytoplasm of affected crypt epithelial cells. There were no microscopic lesions in other organs examined except for focal suppurative lymphadenitis in the ileocecal lymph nodes.

Fig 6—Ileum from pig given crude inocula in experiment one.
There is a Campylobacter sp.-like organism (arrow) partially
in the apical cytoplasm of a crypt epithelial cell. Micro-
villi around the organism appear intact. There is no apparent
invagination of host cell plasma membrane. Bar represents
.5 µm.
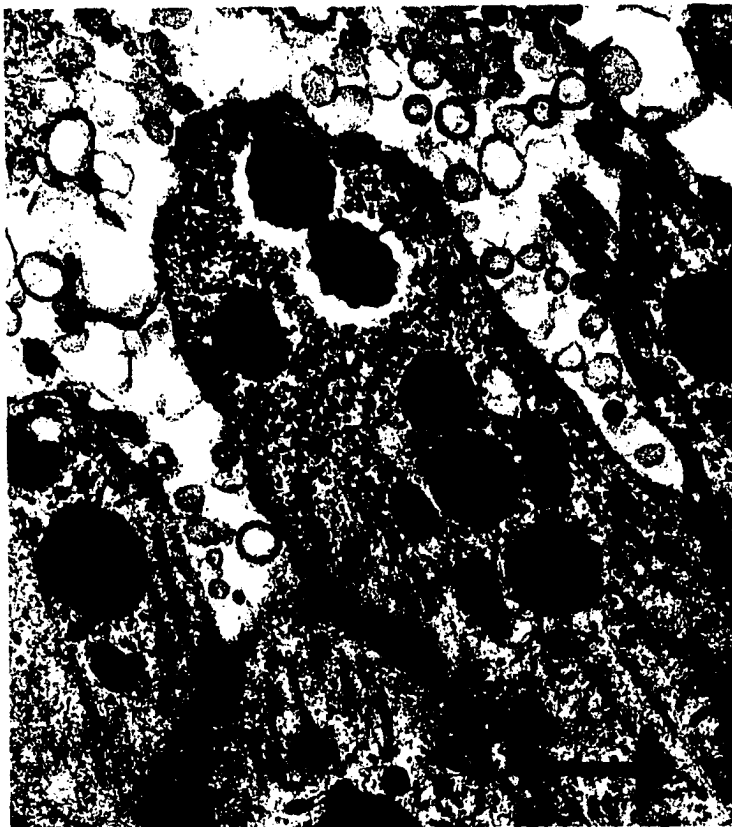
Fig 7—Ileum from pig given crude inocula in experiment one. _Campylobacter_ sp.-like organisms are present in the apical cytoplasm of crypt epithelial cells (arrows). Bar represents 0.5 μm.

Diarrhea was not observed, and CSM and S. choleraesuis were not isolated from these pigs. They had hematologic parameters similar to the control pigs.

Control pigs did not develop clinical signs, had no lesions, and neither CSM nor S. choleraesuis could be isolated from them (Table 1).

Experiment two- Three of seven pigs given crude inocula developed a mild watery, yellow diarrhea 6 to 10 days PI. One animal died on day 2 PI with gangrenous pneumonia due to placement of the stomach tube in the trachea. Campylobacter sputorum subspecies mucosalis was isolated from the affected lungs. Three of the six remaining pigs developed a chronic intermittent diarrhea which persisted throughout the course of the experiment (5 weeks). These pigs ate sparingly and grew poorly, but daily rectal temperatures were normal throughout the experiment. There were no significant differences in hematologic parameters between this group and control pigs (Appendix).

At necropsy, one pig had mild thickening and serosal hyperemia of the terminal ileum. There was also mucosal hyperemia and thickened, elongated mucosal folds, which were most noticeable over ALN. However, there was no intraluminal exudate. Ileocelcal lymph nodes were slightly enlarged, moist, and pale. There were no gross lesions in other

animals examined.

Microscopically, all of the pigs (3) with chronic diarrhea had focal to diffuse areas of proliferation of immature crypt epithelium, crypt elongation, shortening of villi, and macrophage infiltration into the lamina propria of the ileum. Goblet cells were absent in elongated crypts. Flattened mucosal surfaces were covered by immature crypt epithelium and elongated crypts often opened directly onto this surface (Fig 8). Mucosal necrosis was rarely observed and if present, it was only superficial. There was mild focal suppurative lymphadenitis in the ileocecal lymph nodes. Silver-stained ileal sections had numerous Campylobacter sp.-like organisms with similar structure to that described in experiment one. Organisms were observed free within immature crypt epithelial cells by electron microscopy. They were also observed within macrophage phagolysosomes in the lamina propria.

Group three animals given cultures of CSM had no clinical signs, gross or microscopic lesions, and hematologic parameters did not differ significantly from control animals.

Campylobacter sputorum subspecies mucosalis isolation was not attempted, and S. choleraesuis, rotavirus, and transmissible gastroenteritis virus were not detected in any animal (Table 1).

Fig 8—Ileum from pig given crude inocula in experiment two.
Elongated crypts, lined with proliferated immature epithelium,
open directly onto a flattened avillous mucosal surface.
Large numbers of macrophages are present in the lamina propria.
The mucosal surface is covered by immature epithelial cells.
H&E stain.

Experiment three- A third experiment was designed in order to test the importance of S. choleraesuis (isolated from pigs in experiment one) in the development of proliferative enteritis.

Five of six animals in group one developed a watery, yellow-green diarrhea 3 to 6 days after initial oral inoculation with crude inoculum. They continued to have intermittent diarrhea throughout the course of the experiment (5 weeks) but rectal temperatures remained normal. Hematologic parameters measured on days 0,3,5,8,12,15,20,24,28, and 32 PI did not differ significantly from control values.

At necropsy (35 days PI) three pigs had mild thickening, serosal reticulation, and hyperemia of the terminal ileum. Ileocecal lymph nodes were slightly enlarged, pale, and moist. The ilea had no luminal exudates, but, there was mucosal hyperemia and prominent thickened mucosal folds, especially over ALN.

Focal to diffuse proliferative mucosal lesions similar to those described in pigs given crude inoculum in experiment 2 were present in the ilea of these pigs. The presence of Campylobacter sp.-like organisms in proliferated crypt epithelial cells was confirmed by silver-stained histosections and by electron microscopy. Campylobacter sputorum subspecies mucosalis was isolated from the ileal mucosa of two pigs, but S. choleraesuis was not isolated from any of the pigs.

Nine of ten pigs in experimental group two which were given CSM and S. choleraesuis cultures developed yellow, watery diarrhea three to five days PI. These pigs had anorexia and elevated rectal temperatures (40-42C) beginning on day 3. The temperature elevation remained fairly constant for 7 days, after which time the pigs either became moribund and were euthanatized or their rectal temperatures returned to normal and they began to eat sparingly. Seven of nine pigs were killed when they became moribund during the first 12 days of the experiment. Two of the nine pigs survived and had intermittent diarrhea throughout the experiment (5 weeks).

All nine pigs had neutrophilic leukocytosis with left shift on days 3,5,8 and/or 12 PI. These values were significantly (P<.05) increased above control pig values (Appendix). The total white blood cell counts and differential counts taken on days 15,20,24,28, and 32 PI in the two surviving pigs did not differ significantly from control pigs. However, the surviving pigs were hypoproteinemic (average plasma protein concentration = 4.8 g/dl on day 32 PI).

The clinically ill pigs had enteric lesions, characterized by serosal hyperemia and diffuse gastroenteritis with fibrinous casts in the glandular stomach, distal jejunum, cecum, and colon. The two pigs which survived until termination of the experiment also had thickened ilea and irregular,

enlarged, mucosal folds over ileal ALN. All pigs had slight enlargement of the mesenteric lymph nodes.

Microscopic lesions in those pigs which were killed within three to four days PI were diffuse blunting of villi in the duodenum, jejunum, and ileum accompanied by diffuse infiltration of neutrophils and macrophages into the lamina propria and submucosa (Fig 9). There was also focal necrosis of lymphocytes in ALN (Fig 9), and focal areas of superficial mucosal necrosis with exudation of fibrin and leukocytes into the glandular stomach, jejunum, ileum, cecum, and colon. These pigs did not have proliferation of crypt epithelium, and Campylobacter sp.-like organisms could not be identified in either crypt lumina or in crypt epithelial cells. Those pigs killed between days 5 and 12 PI had lesions similar to those of pigs killed on days 3 to 4, except that mucosal necrosis was more diffuse and severe. The two pigs killed at the termination of the experiment had focal to diffuse areas of proliferation of crypt epithelium in the distal jejunum, ileum, ileocecal valve, and cecum. There was also crypt elongation, villous blunting, and inflammation of the lamina propria. Campylobacter sp.-like organisms were observed in the cytoplasm of affected immature epithelial cells by both silver-stained histosections and electron microscopy.

Other microscopic lesions in this group included

Fig 9—Ileum of pig given cultures of CSM and S. choleraesuis
(4 days PI). There is loss of villi, and diffuse infiltra-
tion of neutrophils and macrophages into the lamina propria
and submucosa. Note focal necrosis of lymphocytes in
aggregated lymphoid nodules (arrow), and lack of prolifera-
tion of crypts. H&E stain.

moderate to severe suppurative lymphadenitis (mesenteric lymph nodes), multifocal necrotic hepatitis, and focal to diffuse interstitial pneumonia. There was also fibrinopurulent pericarditis, epicarditis, and pleuritis in two pigs.

Salmonella choleraesuis was isolated from the intestine, liver, and ileocecal lymph node from five of the ten pigs. Campylobacter sputorum subspecies mucosalis was isolated from the ileal mucosa of one of the two pigs with proliferative lesions.

The four pigs (group 3) given cultures of S. choleraesuis developed watery, yellow-green diarrhea within three to five days PI. They also had elevated rectal temperatures (41-42,5C) which began on the second day PI and persisted until day 7 PI. Two of the four pigs were killed when they became moribund between days 3 and 7. The remaining two pigs were killed at the termination of the experiment (5 weeks).

All of the pigs had significant (P<.05) neutrophilic leukocytosis with left shift on days 3,5, and 8 PI (Appendix). There were no significant differences in hematologic param- eters monitored on days 15,20,24,28 and 32 between the two surviving pigs and control pigs.

The two pigs killed between days 3 and 7 PI had fibrinous gastroenteritis. Microscopic lesions were loss of villi, inflammation of the lamina propria, mucosal necrosis, and exudation of fibrin and leukocytes into the

intestinal lumen. Gastric lesions were superficial mucosal
necrosis and fibrin exudation into the gastric lumen. There
was also focal to diffuse suppurative lymphadenitis
(mesenteric lymph nodes), focal necrotic hepatitis, and focal
to diffuse interstitial pneumonia. One pig had diffuse
suppurative meningitis. Salmonella choleraesuis was iso-
lated form the intestine, liver, and ileocecal lymph nodes
of both pigs.

The two pigs which survived to termination of the experi-
ment had no significant gross or microscopic lesions and
S. choleraesuis was not isolated from their tissues.

One of the two pigs given cultures of CSM (group 4)
developed a watery green-yellow diarrhea three days PI which
persisted intermittently throughout the experiment (5 weeks),
but the pig continued to eat. Rectal temperatures of both
pigs remained normal throughout the experiment. There were
no significant differences in their hematologic data when
compared with control pigs. At necropsy the pig with
diarrhea had mild thickening and serosal hyperemia of the
terminal 8 cm of the ileum which had no mucosal exudate.
However, there was mucosal hyperemia, and thickened mucosal
folds, especially over ALN.

Microscopically, there was diffuse proliferation of
immature crypt epithelial cells, crypt elongation, loss of
villi, and diffuse infiltration of macrophages into the lamina

propria (Fig 10). Leukocytic exudate was also present within crypt lumina. Silver-stained histologic sections had Campylobacter sp.-like organisms within the cytoplasm of crypt epithelial cells. They were also demonstrated free within the cytoplasm of these cells and within the phagolysosomes of phagocytic cells in the crypt lumen (Fig 11). The organisms were occasionally observed undergoing division within infected crypt epithelial cells (Fig 12).

Neither CSM nor S. choleraesuis were isolated from either pig in group 4 (Table 2). The control pigs (group 5) did not develop diarrhea, had no lesions, and CSM and S. choleraesuis could not be isolated from them.

Fig 10—Ileum of pig given cultures of CSM. There is diffuse proliferation of immature crypt epithelial cells, absence of goblet cells, crypt elongation, loss of villi, and inflammation of the lamina propria. H&E stain.

Fig 11—Ileum from pig given CSM cultures. Campylobacter
sp.-like organisms are free within the cytoplasm of crypt
epithelial cells (arrow). Bar represents 4 μm.

Fig 12—Ileum from pig given CSM culture. <u>Campylobacter</u> sp.-like organism is free within crypt epithelial cell and is undergoing division. Bar represents .5 µm.

Table 2—Summary of Three Experiments Using Either Crude Inoculum (CI), C. sputorum subspecies mucosalis (CSM) and/or S. choleraesuis (SC)

Experiment	Treatment[a] (no. of pigs)	Proliferative mucosal lesions		Intracellular Campylobacter sp.-like organisms (no. of pigs)	CSM cultured from (no. of pigs)	SC cultured from (no. of pigs)
		Gross (no. of pigs)	Microscopic (no. of pigs)			
I	CI (10)	5	7	7	5	5
II	CI (6)	1	3	3	ND[b]	0
	CSM (3)	0	0	0	ND	0
III	CI (6)	4	5	5	2	0
	SC+CSM (10)	1	2	2	1	5
	SC (4)	0	0	0	0	2
	CSM (2)	1	1	1	0	0
TOTAL	41	12	18	18	8	12

[a]Control groups not included.

[b]ND = not determined.

135

DISCUSSION

This series of experiments using 10-week-old Caesarean-derived and colostrum-deprived pigs indicates that prolifertive enteritis is an infectious disease of swine and that CSM is associated with proliferative intestinal mucosal lesions.

Salmonella choleraesuis was isolated from animals in experiment one with the most diffuse proliferative intestinal mucosal lesions, but mucosal necrosis, often severe, was also present. The severe inflammation and necrosis may have been associated with concurrent S. choleraesuis infections. The gross and microscopic appearance of intestinal lesions in these pigs very closely resembled those seen in naturally occurring cases of proliferative enteritis especially in 16-45 kg feeder pigs.[1,2] Salmonella choleraesuis along with CSM has been isolated from some of these naturally infected animals,[1,2] Hematologic data from the experimental pigs also resembled that from naturally infected pigs.[1] There was neutrophilia with left shift and hypoproteinemia at the time of necropsy in five pigs in experiment one.

The origin of the S. choleraesuis cultured from pigs in experiment one was not determined. Since strict isolation and sanitation procedures were adhered to throughout the experiment, it was thought that S. choleraesuis was present

in the crude inoculum. However, preinoculation culture of
this material did not detect S. choleraesuis. When this
isolant was used in experiment three, it proved highly viru-
lent, but the two pigs which were also given cultures of CSM
and survived to termination of the experiment had prolifera-
tive lesions and mucosal necrosis. Pigs given cultures of
S. choleraesuis alone did not develop proliferative intesti-
nal lesions.

The administration of crude inoculum which was apparently
uncontaminated with Salmonella sp. produced focal to diffuse
proliferative lesions and inflammation of the lamina propria
without prominent mucosal necrosis. Campylobacter sp.-like
organisms were consistently observed within proliferated im-
mature crypt epithelial cells. Lesions in the single pig
that developed proliferative mucosal lesions after being
given culture of CSM resembled those lesions in pigs given
crude inoculum (apparently without S. choleraesuis). These
results suggest that CSM is able to infect crypt epithelial
cells without the presence of another known enteric pathogen.

The explanation for the apparent variability in
virulence of CSM used in experiments two and three is un-
known. It was noticed by phase contrast microscopy that
cultured organisms were not as motile as organisms taken
directly from naturally infected intestinal mucosa.

It is possible that motility of the organism is an important virulence factor.

The morphologic features of <u>Campylobacter</u> sp.-like organisms in infected immature epithelial cells resembled those previously described for naturally-occurring cases of porcine proliferative enteritis,[1,2,3] intestinal adenomatosis,[4,14] proliferative hemorrhagic enteropathy,[15] regional ileitis,[5,6] and necrotic enteritis.[5] It was usually S-shaped, 2 to 3 μm long, 0.2 to 0.3 μm in diameter, and had a rugose trilaminar cell wall. The organism was infrequently observed partially embedded in crypt epithelial cells at the microvillus border, and it did not cause apparent destruction of host cell membranes or cytoplasmic organelles. There was no apparent invagination of host cell plasma membranes and no formation of a phagocytic vacuole around invading organisms.

The sequential progression of lesion development could not be determined in these experiments. In areas where mild lesions existed, immature crypt epithelial cells were involved and mature villous absorptive epithelial cells were not. However, villi eventually became flattened and covered with immature epithelial cells. The reason for the apparent lack of mature epithelial cells is unknown. It may be that

there is arrested crypt cell maturation or that immature cells are shed from the mucosal surface before they mature.

The typical disease of intestinal mucosal proliferation with massive intraluminal hemorrhage seen in bred gilts, sows, and boars[1,2] was not reproduced in the pigs used in these experiments. It is possible that these conditions could be produced in fully susceptible adult swine given cultures of CSM. The pathogenesis of the CSM-associated conditions in pigs and the virulence factors of CSM are poorly understood. Future research efforts should be directed toward these problems.

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PORCINE PROLIFERATIVE ENTERITIS:

THE EXPERIMENTAL DISEASE

IN SPECIFIC-PATHOGEN-FREE PIGS

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SUMMARY

Thirty-three 10-week-old specific-pathogen-free pigs were randomly placed into three treatment groups. Group one was given intragastrically administered homogenized intestinal mucosa (crude inoculum) from naturally occurring cases of proliferative enteritis. Group two was given cultures of Campylobacter sputorum subspecies mucosalis. Group three served as controls. One pig from each group was killed 4,7,10,14,18,21,24,28,31,36, and 38 days after inoculation. The earliest intestinal lesion observed in groups one and two was leukocytic exudate within crypt lumina and focal inflammation of the surrounding lamina propria. The lesions occurred primarily over ileal aggregated lymphoid nodules (ALN: Peyer's patches). These changes were followed by focal proliferation of immature crypt epithelial cells and infiltration of increasing numbers of macrophages into the lamina propria. Campylobacter sp.-like organisms were observed within the cytoplasm of affected epithelial cells by light and electron microscopy. Lesions progressed to diffuse crypt cell proliferation, elongation of crypts, and loss of villi. Mucosal necrosis was not a prominent feature.

INTRODUCTION

Porcine proliferative enteritis is a naturally occurring enteric disease of swine characterized by proliferation of immature crypt epithelial cells, crypt elongation, loss of villi, and inflammation of the lamina propria.[1,2] Lesions occur primarily in the ileum but the jejunum, cecum, and colon may also be involved. Campylobacter sp.-like organisms have been consistently present within the cytoplasm of proliferating crypt epithelial cells.[1,2]

The experimental disease has been described in Caesarean-derived and colostrum deprived pigs.[3]

Campylobacter sputorum subspecies mucosalis (CSM) is a member of the genus Campylobacter which includes curved, gram-negative, monotrichous rods that have microaerophilic growth requirements.[4,5,6] Campylobacter sputorum has the subspecies sputorum, mucosalis, and bubulus, all of which are catalase negative.

The objective of this paper is to characterize the sequential development of lesions of porcine proliferative enteritis in specific-pathogen-free pigs intragastrically inoculated with material prepared from the intestinal mucosa of pigs with the naturally occurring disease (crude inoculum) and with cultures of CSM.

MATERIALS AND METHODS

Source of animals- Thirty-three 10-week-old pigs were
obtained from specific-pathogen-free sows[a] by natural
farrowing. All experimental pigs were housed in isolation
rooms. Feed consisted of a 16% protein ration to which no
antibiotics or other drugs had been added.

Source of inoculum- Crude inoculum was obtained from pigs
with naturally occurring proliferative enteritis by scraping
the intestinal mucosa from the intestinal wall using a glass
slide. This material was mixed with an equal volume of
sterile phosphate buffered saline (pH 7.1),[7] and homogenized
for three minutes in a blender. Inoculum was either used
fresh or stored at -62C until needed. Pre-inoculation
culture of the crude inoculum was negative for Salmonella spp.

Campylobacter sputorum subspecies mucosalis (an 8th
passage isolant obtained from an experimentally infected pig)[b]
was grown in flasks of brucella broth[c] supplemented with 10%
fetal calf serum.[d] Flasks were held under a stream of hydrogen

[a]Sows obtained from North Central Iowa Pork Producers,
Clear Lake, IA.

[b]Isolant number 14-4p.[3]

[c]Difco, Detroit, MI.

[d]Sterile Systems Inc., Logan, Utah.

and carbon dioxide gas (50:50 vol/vol) that had passed over a reduction column. Flasks were incubated at 37C for 24 or 48 hours, and were gently agitated (50 rpm)[a] to increase growth. Colony counts obtained were approximately 1×10^8 colony forming units (CFU) per ml.

Pathologic examinations- Post mortem examinations were conducted on all pigs. Tissues for histopathologic evaluation consisted of duodenum, jejunum, (three levels), ileum (three levels), ileocecal valve, cecum, colon (three levels), ileocecal lymph nodes, liver, spleen, kidney, stomach, lungs, and brain. All tissues were fixed in 10% neutral buffered formalin. Paraffin-embedded sections were stained with hematoxylin and eosin. Intestinal sections were also stained with Warthin-Starry silver stain,[8] and selected sections of intestine were stained with alcian blue.[8]

Intestinal tissues for transmission electron microscopy were obtained adjacent to sections selected for light microscopy. The procedures for tissue preparation for electron microscopy have been described.[2] Ultrathin sections were viewed and photographed with a Hitachi HS-9[b] transmission electron microscope.

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[a]Junior Orbit Shaker, Lab-Line Instruments Inc., Melrose Park, Ill.

[b]Hitachi Scientific Instruments, 328 Eisenhower Lane, Lombard, Ill.

<u>Microbiologic examinations</u>- Tissues were obtained from the ileum, cecum, and colon from all pigs and cultured for CSM[1]. Samples of ileal, cecal, and colonic homogenate, and ileocecal lymph node were obtained from each pig and cultured for <u>Salmonella</u> spp.[1] Procedures used for antisera production to CSM isolants have been described.[1]

<u>Experimental design</u>- Thirty-three 10-week-old pigs were randomly allotted to three groups of eleven pigs each. All pigs were intragastrically inoculated once a week for three weeks (total of four inoculations). Group one pigs were given 200 ml of crude inoculum and 50 ml of sodium bicarbonate.[a] Pigs in group two were given 200 ml of CSM culture (1×10^8 CFU per ml), and 50 ml of sodium bicarbonate. Group three pigs served as controls and were given 100 ml of phosphate buffered saline, 50 ml of brucella broth, and 50 ml of 10% sodium bicarbonate. Feed was removed from all animals 24 hours prior to inoculations. One animal from each group was euthanatized (electrocution) on days 4,7,10,14,18,21, 24,28,31,36, and 38 post initial inoculation (PI).

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[a]Fisher Scientific Company, Fairlawn, New Jersey.

RESULTS

Pigs killed on day four PI from either the group given crude inoculum or the group given CSM cultures had no gross lesions, but they had similar microscopic lesions in the terminal ileum (Tables 1 and 2). Lesions were characterized by multifocal areas of leukocytic exudation into the crypt lumina overlying aggregated lymphoid nodules (ALN; Peyer's patches). There was also infiltration of eosinophils and neutrophils into the surrounding lamina propria (Fig 1), and these inflammatory cells could often be observed between crypt epithelial cells. There were decreased numbers of goblet cells in the affected crypts of histologic sections stained with hematoxylin and eosin (Fig 1). These observations were confirmed by alcian-blue stained sections taken from the same block. Curved bacterial organisms were not observed within the lumina of affected crypts by silver-staining techniques. However, organisms typical of Campylobacter sp. were often observed in the lumina of adjacent crypts which contained no leukocytic exudate. There were no other microscopic lesions in other tissues examined from either pig. The ileal mucosa overlying ALN, and other tissues examined from the control pig had no microscopic lesions (Fig 2), except for occasional foci of leukocytic debris within crypt lumina overlying ALN.

Table 1—Summary of Proliferative Lesions and Presence of Intraepithelial

Campylobacter sp.-like Organisms in Experimental Proliferative Enteritis

| Treatment group | No. of pigs | Proliferative mucosal lesions | | Intracellular Campylobacter sp.-like-organisms (no. of pigs) |
		Gross (no. of pigs)	Microscopic (no. of pigs)	
Crude inoculum	11	1	5	5
C. sputorum subspecies mucosalis	11	1	4	4
Control	11	0	0	0
TOTAL	33	2	9	9

Table 2—Microscopic Lesions in Pigs Between 4 and 38 Days Post Inoculation

Treatment group	Days										
	4	7	10	14	18	21	24	28	31	36	38
Crude inoculum	++	+++	+++	++++	++++	++	++	++	++	+++	++
C. sputorum subspecies mucosalis	++	+++	+++	+++	++++	0	++	++	0	++	0
Control	+	0	+	+	0	0	+	+	0	+	0

+ = leukocytic exudate in crypt lumina.

++ = leukocytic exudate in crypt lumina and inflammation of lamina propria.

+++ = above, plus crypt cell proliferation, and intraepithelial curved organisms.

++++ = above, plus flattened mucosal surface.

0 = no microscopic lesions.

Fig 1—Ileum from pig given C. sputorum subspecies mucosalis
culture (4 days PI). There is infiltration of neutrophils
and eosinophils into the lamina propria surrounding crypts
overlying ALN. There is also leukocytic exudate within
crypt lumina and decreased numbers of goblet cells in the
affected crypts. H&E stain.
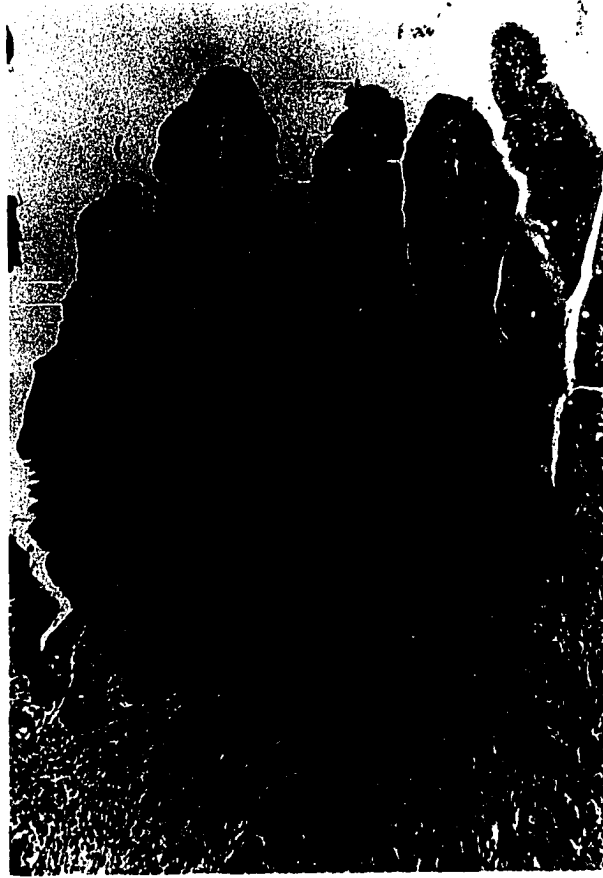
Fig 2—Ileum from control pig with normal mucosa overlying
ALN. Notice the abundant goblet cells in the deep crypt
regions. H&E stain.

The pigs from group one and group two killed on day 7

PI also had no gross lesions. However, they had similar

microscopic lesions in the terminal portions of the ileum

(Tables 1 and 2). There were multifocal areas of prolifera-

tion of immature epithelial cells within crypts overlying

ALN. There was also leukocytic exudation into the crypt

lumina and infiltration of neutrophils, eosinophils, and

macrophages into the adjacent lamina propria (Fig 3). Large

macrophages with abundant pale staining cytoplasm were often

the most abundant inflammatory cell type in the lamina

propria. Goblet cells were absent from affected crypts.

Small numbers of curved Campylobacter sp.-like organisms were

observed by light and electron microscopy in the apical cyto-

plasm of affected crypt epithelial cells (Figs 4 and 5).

In these cells, the organisms were slightly curved to S-

shaped, 1.8 to 2.3 μm long and 0.2 to 0.3 μm in diameter.

The organisms were not enclosed within host cell membranes

(Fig 5). There was no apparent structural damage to the cyto-

plasmic organelles of affected crypt cells which had the struc-

tural appearance of immature epithelial cells. These epi-

thelial cells had low, irregular microvilli, poorly defined

terminal webs, abundant free ribosomes throughout their cyto-

plasm, and prominent secretory granules in their apical cyto-

plasm. Curved organisms were also observed within phagolyso-

somes of leukocytes in crypt lumina (Fig 5) and in close contact
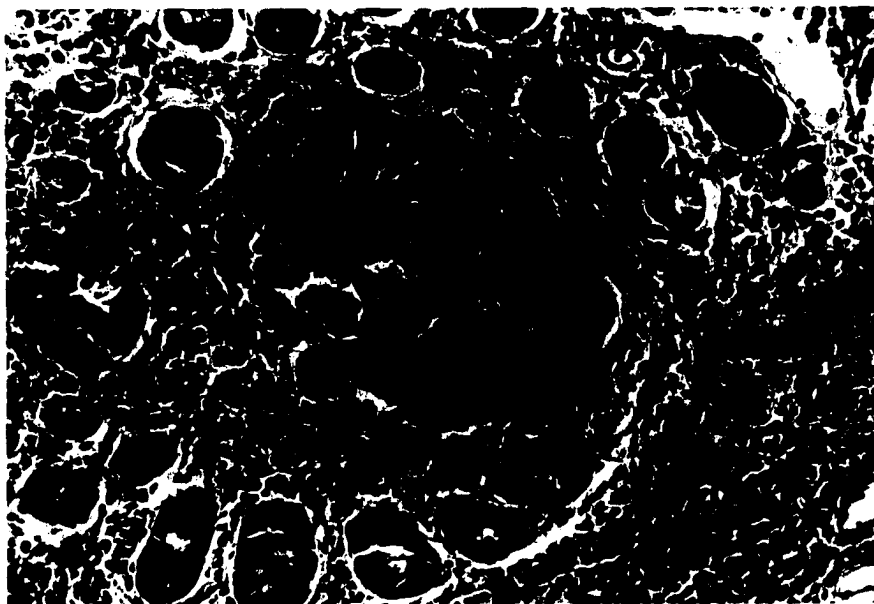
Fig 3—Ileum of pig inoculated with C. sputorum subspecies
mucosalis (7 days PI). There are focal areas of proliferation
of crypt epithelial cells overlying ALN (a). There is also
infiltration of eosinophils, neutrophils, and macrophages
into the surrounding lamina propria, and exudation of leuko-
cytes into the crypt lumina. H&E stain.

Fig 4—Ileum of pig inoculated with C. sputorum subspecies
mucosalis (7 days PI). Curved organisms (arrow) are in the
apical cytoplasm of crypt epithelial cells overlying ALN
(a). Warthin-Starry stain.
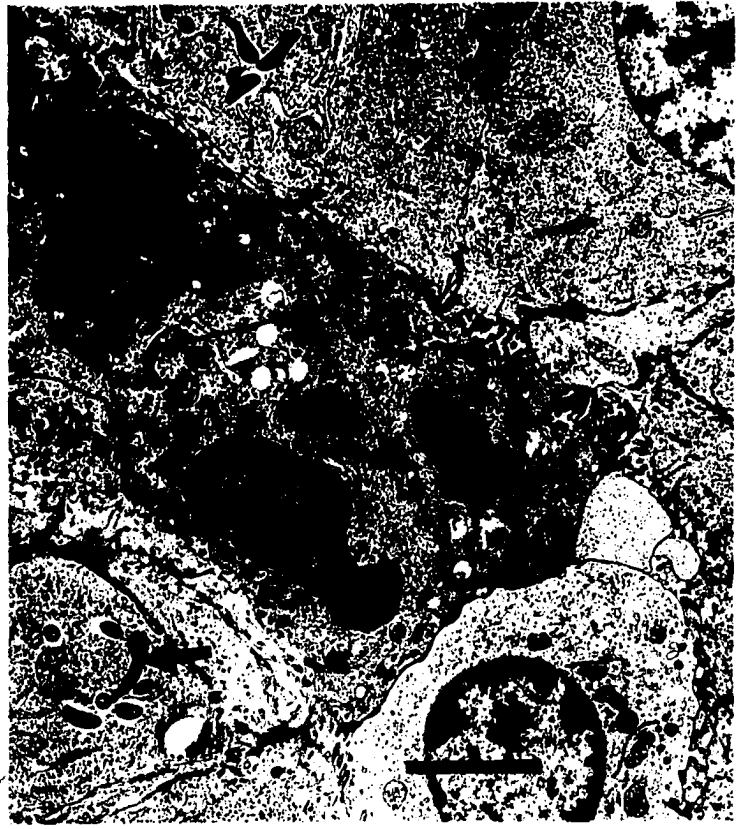
Fig 5—Ileum from pig given C. sputorum subspecies mucosalis
(7 days PI). Curved organisms (small arrow) are within
phagolysosomes of polymorphonuclear leukocytes in crypt lumen
and within the apical cytoplasm of crypt epithelial cells
(large arrow). Bar represents 3.0 μm.

with the microvilli of crypt epithelial cells (Fig 6), but the entry of these organisms into cells could not be demonstrated.

Ileocecal lymph nodes from pigs in groups one and two had mild multifocal suppurative lymphadenitis. There were no other microscopic lesions in either pig. There were no gross or microscopic lesions in the control pig.

Pigs killed from either group one or two on day 10 PI had no gross lesions, but both had focal to diffuse areas of crypt cell proliferation, elongation and widening of crypts, and mild flattening of villi in the ileum overlying ALN (Fig 7). Absorptive epithelial cells were often extruded from villi in ribbons composed of several cells. There was also exudation of leukocytes into the crypt lumina and infiltration of neutrophils, macrophages, and eosinophils into the lamina propria surrounding these crypts. Curved Campylobacter sp.-like organisms were observed in the cytoplasm of affected crypt epithelial cells by utilizing both silver-stained histologic sections and electron microscopy (Tables 1 and 2). The CSM were not within the cytoplasm of mature villus epithelial cells. Both pigs had focal lymphadenitis of their ileocecal lymph nodes. The only microscopic lesions in tissues from the control pig were focal areas of leukocytic debris in crypt lumina overlying ALN in the ileum and distal jejunum.

Fig 6—Ileum from pig given C. sputorum subspecies mucosalis
(7 days PI). Curved organism is in close contact with
microvilli and cell membrane of crypt epithelial cell.
Bar represents .25 µm.

Fig 7—Ileum from pig given C. sputorum subspecies mucosalis
(10 days PI). There is diffuse proliferation of crypt
epithelial cells. Leukocytic exudate is in the crypt lumina
and eosinophils, neutrophils, and macrophages are in the
adjacent lamina propria. H&E stain.

The pig killed on day 14 PI which was given crude inocula had hyperemia of the mucosal surface of the ileum, and thickened mucosal folds overlying ALN. Gross lesions were not present in the pig given CSM cultures. Both pigs had microscopic lesions in the ileum and cecum (Tables 1 and 2), but these lesions were more diffuse in the pig given crude inocula. The lesions were characterized by proliferation of crypt epithelial cells, crypt elongation, shortening of villi, and infiltration of macrophages into the lamina propria. The villi of the affected ileal mucosa in the pig given crude inocula were shorter than those affected villi in the pig given CSM. Warthin-Starry silver-stained histologic sections from both pigs had numerous curved, Campylobacter sp.-like organisms in the apical cytoplasm of affected crypt epithelial cells (Fig 8). Similar organisms were observed in infected cells by using electron microscopy. Campylobacter sputorum subspecies mucosalis was cultured from the ileal mucosa of the pig given crude inocula. Both pigs had focal areas of suppurative lymphadenitis in the ileocecal lymph nodes. The control pig killed at 14 days PI had no gross or microscopic lesions other than occasional areas of leukocytic debris within crypt lumina in the ileum.

A pig given CSM cultures had gross lesions in the ileum at 18 days PI. There was mild thickening of the terminal

Fig 8—Ileum of pig given crude inocula (14 days PI). Curved
organisms (arrow) are present in the apical cytoplasm of
crypt epithelial cells. Warthin-Starry stain.

ileal wall and serosal hyperemia. The mucosa was hyperemic
and had prominent mucosal folds, primarily overlying ALN.
The pig given crude inocula did not have gross lesions.
However, both pigs had microscopic lesions in the ileum
which were similar to those observed in the ilea of group
one and group two pigs killed on day 14. In addition, these
two pigs had flattened avillous mucosal surfaces (Tables 1
and 2) which appeared covered by immature epithelial cells.
Crypts often opened directly onto the flattened mucosal
surfaces (Fig 9). Curved _Campylobacter_ sp.-like organisms
were within affected epithelial cell cytoplasm of both pigs.
Both pigs had focal infiltration of neutrophils into the
parenchyma of ileocecal lymph nodes. There were no lesions
in the control pig.

Pigs killed on days 21, 24, 28, 31, and 38 PI from either
group one or two had no gross lesions. However, they had
microscopic lesions in the ileum, distal jejunum, cecum, and
proximal colon (Tables 1 and 2). These lesions were charac-
terized by leukocyte exudation into crypt lumina and infil-
tration of neutrophils, eosinophils, and macrophages into the
adjacent lamina propria. Lesions in the pigs given crude
inocula were more diffuse than those given cultures of CSM.
There was no evidence of crypt cell proliferation or intra-
cellular microorganisms.

The pig from group one killed on day 36 had focal

Fig 9—Ileum of pig given C. sputorum subspecies mucosalis

cultures (18 days PI). There is diffuse crypt cell pro-

liferation and a flattened mucosal surface. Crypts open

directly onto the mucosal surface. Mononuclear cells are

present in the lamina propria. H&E stain.

proliferative lesions in the ileal mucosa. Campylobacter sp.-like organisms were observed in the cytoplasm of affected crypt epithelial cells (Tables 1 and 2). Salmonella choleraesuis was not isolated from the intestine or ileocecal lymph nodes in any pig in the experiment.

The control pigs killed on days 24, 28, and 36 PI had focal areas of leukocyte exudation into crypt lumina without demonstrable inflammation of the adjacent lamina propria (Table 2). There were no proliferative mucosal lesions, and Campylobacter sp.-like organisms were not identified in either crypt lumina or crypt epithelial cells (Table 2).

Control pigs killed on days 21, 31, and 38 had no microscopic lesions in any tissues examined.

DISCUSSION

This experiment indicates that similar gross and microscopic proliferative mucosal lesions are produced by intragastric administration of either crude inoculum or cultures of CSM to 10-week-old specific-pathogen-free pigs.

The initial lesions were inflammatory and centered around the basal crypt regions overlying ALN. These lesions were followed by proliferation of immature crypt epithelial cells and the appearance of <u>Campylobacter</u> sp.-like organisms in their cytoplasm. Progressive crypt elongation and flattening of villi followed. The occurrence of leukocytic exudate in scattered crypts overlying ALN without inflammation of the lamina propria was interpreted as a nonspecific change since control pigs often had similar histologic changes.

The reason for lack of prominent proliferative lesion development in pigs killed beyond the 21st day is unknown. It is possible that host resistance developed and/or there was failure of intestinal luminal colonization by CSM.

There is experimental evidence that CSM invades crypt epithelial cells in the pig intestine and causes or is associated with proliferation of these epithelial cells.[3,9,10] However, the typical field disease has not been consistently duplicated. The closest model to date which resembles the field disease has been the combined effect of CSM and

<u>Salmonella</u> <u>choleraesuis</u>.[3] These two organisms have been iso-
lated from 16-45 kg feeder pigs with the naturally occurring
disease, but <u>S</u>. <u>choleraesuis</u> was isolated infrequently from
these pigs.[1,2] <u>Salmonella</u> <u>choleraesuis</u> has not been isolated
from bred gilts, sows, or boars manifesting intestinal
hemorrhage.[1]

Little is known of the biology of CSM. Lawson et al.
[6,11,12,13] biochemically and antigenically characterized
isolants of the organism obtained from cases of intestinal
adenomatosis, proliferative hemorrhagic enteropathy, necrotic
enteritis, and regional ileitis, and they have demonstrated
antigenic similarity between all of these isolants. How-
ever, cell components or products responsible for the
pathogenicity of CSM are unknown.

<u>Campylobacter</u> <u>fetus</u> and its subspecies <u>fetus</u>, <u>intesti-</u>
<u>nalis</u>, and <u>jejuni</u>, are monotrichus, microaerophilic, cata-
lase positive, gram-negative curved rods.[5] They are asso-
ciated with infertility and abortion in cattle and abortion
in sheep.[5] It has been demonstrated that <u>C</u>. <u>fetus</u> has a
glycoprotein antiphagocytic surface component called
antigen-a.[14] This antigenic component corresponds
structurally and functionally to a microcapsule, and is
easily removed from the cell by mild acid extraction pro-
cedures.[15] Bacterial cells which have this antigen are

refractory to phagocytosis except in the presence of specific antiserum.[15] It is thought that this antiphagocytic component is a major virulence factor of the organism.[14,15] The presence or absence of this component has not been determined for CSM.

Decreased motility of multiple passage CSM organisms grown in culture has been noticed when the organisms were examined by phase contrast microscopy. It is not known if decreased motility alters virulence of the organism. The variable results obtained during experimental reproduction studies indicate that multiply-passaged organisms might have decreased virulence. Studies with C. fetus and Vibrio cholerae have indicated that motility comprises an important virulence factor.[5] Motility is accomplished by rotary movement of the flagellar hook, and it also apparently requires the functional integrity of the cell envelope.[5]

The importance, if any, of endotoxin elaboration by CSM in the pathogenesis of porcine proliferative enteritis is un-known. Purified lipopolysaccharide representing the active components of endotoxin in C. fetus subspecies intestinalis has been isolated.[16] This material produced a generalized Schwartz-man reaction in rabbits when injected intravenously.[16]

There was apparent localization of the early prolifera-tive enteritis lesion in the mucosa overlying ileal ALN. The significance of this localization in the pathogenesis of

the disease has not been determined. These lymphoid nodules lie close to the intestinal lumen and are separated from the luminal environment by a thin "specialized" epithelium[17] (dome epithelium) which contains cuboidal microvilli-covered epithelial cells, plasma cells, lymphocytes and M-cells.[18] Penetration of the mucosa by particulate antigens is apparently facilitated at these sites.[17] Experiments have shown that dome epithelium may be involved in the early immunologic response in the intestine of swine,[18] and that aggregated lymphoid nodules (ALN) can participate in thymus dependent (t-cell) immunologic responses to live bacterial organisms.[17]

Listeria monocytogenes is an intracellular parasite.[19] Oral administration of this organism to mice produced consistent infection in intestinal ALN and the generation of cellular immunity (thymus-dependent) which rendered the mice immune to subsequent intragastric infection.[19] The mechanism of protection was thought to be by the generation of sensitized t-cells which induced, at the site of infection, a population of activated macrophages with enhanced bactericidal properties.[19] It is not known if such a protective mechanism exists for pigs given CSM. However, the use of multiple oral inoculations of pigs with CSM might indicate that such a mechanism has to be overcome before proliferative lesions can be produced.

The early presence of neutrophils, eosinophils, and macrophages in the lamina propria and crypt lumina have been demonstrated in this experiment. Pigs inoculated with either crude inocula or cultures of CSM had similar early lesions. The significance of this inflammatory response is unknown. It may be an immunologically mediated mechanism of eliminating bacteria from the crypt lumen.

Exudation of neutrophils into the intestinal lumen has been demonstrated to be a specific antibody-mediated immune response which occurs in the absence of intestinal injury.[20] Proliferation of gram-negative intestinal bacteria has been shown to increase neutrophil emigration into the intestinal lumen.[20] The neutrophils, attracted by a specific antigen, disintegrate within the lumen, release their lysosomal enzymes, and inactivate or destroy the antigen. The response to exposure of the intestinal mucosa to the same antigen a second time could determine the fate of the organism (antigen).[20] If enough secretory IgA is present, the organism could remain in the intestinal lumen and be destroyed.[21] If insufficient immunoglobulins are present, the organism could enter epithelial cells.

Despite the various host defense mechanisms, it is evident that some antigens can persist long enough in the intestine to allow for development of a local cell mediated hypersensitivity reaction.[22] These antigens include bacteria,

parasites, viruses, particulate antigens, and cellular components of host tissue. Ferguson and MacDonald[22] have presented experimental evidence that one of the direct effects of local enteric cell mediated hypersensitivity reactions is stimulation of mitosis of immature crypt epithelial cells, followed in several days by villous atrophy. The mechanism of increased crypt cell mitosis is postulated to be the release of mitogenic factors (lymphokines) from sensitized t-lymphocytes.[22]

It is possible that if C. sputorum subspecies mucosalis can survive host responses and remain either within crypt lumina or crypt epithelial cells, they may induce local enteric cell mediated hypersensitivity reactions. It may be that products of the immune response rather than the organism itself are directly responsible for crypt cell proliferation.

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GENERAL DISCUSSION AND CONCLUSIONS

A clinical and epidemiological investigation of a CSM-associated disease (proliferative enteritis) in midwestern swine was recorded in this study. Investigations of this type have not been previously reported in the United States. The type of farm production units where the disease occurred, and the age groups of pigs affected were defined. There was chronic diarrhea, wasting and hypoproteinemia in the majority of affected feeder pigs. Adult swine had hemorrhagic diarrhea of short duration, did not experience loss of condition, and generally died within one to three days after onset of diarrhea. They were also anemic and hypoproteinemic.

This investigation did not establish the natural mode of disease transmission, although the association of epizootics with the introduction of replacement pigs onto a premise or the movement of swine from one building or unit to another would suggest that infection is by the fecal-oral route. Subclinical disease could not be detected. Cultures of feces and serological methods to detect humoral antibodies against CSM in pigs were not used.

The gross, histopathologic, and ultrastructural lesions of naturally occurring proliferative enteritis in midwestern swine were described in detail. All affected animals regardless of age or type of production unit had

thickened intestinal walls, usually of the ileum and distal jejunum, and enlarged ileocecal lymph nodes. Feeder pigs had luminal exudates composed primarily of fibrin and necrotic cellular debris. Adult pigs often had free or clotted blood in the intestinal lumen. All pigs had proliferation of crypt epithelial cells and inflammation of the lamina propria. Affected crypt epithelial cells consistently contained intracytoplasmic <u>Campylobacter</u> sp.-like organisms. The ultrastructure of these bacteria and the first reported evidence for crypt epithelial cell penetration by these organisms were described. The organisms appeared to enter the crypt cell at the microvillus border, and they did not cause demonstrable pathologic changes in either the microvillus border or the cell membrane of the crypt cell. The organisms also did not appear to be enclosed in a membrane bound vacuole once within crypt cell cytoplasm.

Bacteriologic methods were used to verify the presence of CSM within affected intestinal mucosa. Isolation attempts were successful in 51 per cent (30 out of 58) of the pigs examined which had gross and microscopic lesions of proliferative enteritis. The explanation for the discrepancy between visualization of <u>Campylobacter</u> sp.-like organisms in silver-stained sections and by using transmission electron microscopic techniques, and the isolation of CSM by bacteriologic methods is unknown. It is possible that many of the

organisms are not viable within tissues or that current culture techniques are not refined enough to consistently isolate the organism.

Indirect fluorescent antibody procedures were not described in this study. They were attempted on frozen sections taken from affected intestinal mucosa, but consistent fluorescence of CSM was not observed. The apical cytoplasm of affected epithelial cells would occasionally fluoresce; however, it was rare to observe labeling of individual bacterial cells. The failure to develop a fluorescent antibody technique is unfortunate since it would have provided a valuable diagnostic aid for the detection of CSM in intestinal mucosa. Consequently, culture methods provided the only basis for positive identification of CSM.

The feasibility of the experimental production of proliferative enteritis in swine was investigated. Three separate experiments were designed and conducted. The use of homogenized mucosal scrapings (crude inoculum) obtained from naturally infected swine proved effective in producing proliferative intestinal mucosal lesions in 10-week-old Caesarean-derived and colostrum-deprived experimental pigs when they were intragastrically inoculated once a week for three weeks. Typical clinical signs and lesions of proliferative enteritis were produced in 50 percent of the

experimental pigs. Campylobacter sputorum subspecies mucosalis and Salmonella choleraesuis were isolated from the intestinal mucosa of these affected pigs. When S. choleraesuis was not isolated from intestinal mucosa of pigs given crude inocula, mild focal proliferative lesions developed.

Further experiments were conducted using inocula composed of cultures of CSM or S. choleraesuis, or the two in combination. Combined cultures produced proliferative enteritis in two of ten experimental pigs. Salmonella choleraesuis given alone produced enteritis but no proliferative lesions. The inoculation of CSM alone into pigs produced proliferative lesions in the ileal and cecal intestinal mucosa. These experiments indicated that inoculation with combined cultures of CSM and S. choleraesuis could produce a disease which more closely resembled that seen in naturally infected feeder pigs, while administration of either crude inocula (apparently not containing S. choleraesuis) or cultures of CSM produced focal to diffuse proliferative lesions, primarily in the ileum. Thus, the ability of CSM to directly or indirectly induce proliferative mucosal lesions without the presence of another known enteric pathogen was demonstrated. The sequential development of proliferative lesions could not be determined in these experiments.

A fourth experiment used 10-week-old specific-pathogen-free pigs and was designed to study sequential lesion

development in pigs intragastrically inoculated with either crude inocula or cultures of CSM. Lesion development was similar in pigs given either type of inocula. The earliest histopathologic change seen at 4 days after inoculation was inflammation of the lamina propria overlying ileal aggregated lymphoid nodules (ALN). By 18 to 21 days after initial inoculation, there was ileal mucosal proliferation. This experiment was the first successful sequential lesion study of a CSM-associated disease to be reported.

The description of sequential lesion development should not be interpreted as being synonymous with elucidation of the pathogenesis of the disease. The factor(s) which determine CSM penetration of intestinal epithelial cells and the development of proliferative mucosal lesions could not be determined in this study. However, it was reasonably well established that CSM is either directly or indirectly involved with proliferation of crypt epithelial cells in the intestinal mucosa.

Intestinal mucosal necrosis, often full thickness, was observed in naturally infected feeder pigs and in 5 of the experimental pigs given crude inocula. A possible cause for the development of these lesions may be that the proliferation of crypt epithelial cells, and the infiltration of inflammatory cells into the lamina propria causes compression of vascular spaces in the lamina propria resulting in

ischemic necrosis of the superficial mucosa. Invasion of bacteria, either pathogenic or nonpathogenic, into the necrotic mucosa could establish a chronic inflammatory process.

The key to the hypothetical process of intestinal mucosal inflammation may be the numbers of CSM that reach the lamina propria at any given time. If small numbers of bacteria enter the lamina propria, macrophages could conceivably phagocytize them and allow for the development of a chronic inflammatory process. If large numbers of bacteria enter the lamina propria, the pahgocytic capacity of macrophages could be overwhelmed, resulting in large amounts of bacterial antigens in the lamina propria. These antigens could be responsible for the release of vasoactive amines and immunoglobulins, and the activation of complement which could incite an acute inflammatory response resulting in damage to vascular endothelium and exudation of fibrin and erythrocytes into the lamina propria and intestinal lumen. This situation could be comparable to that seen in adult pigs.

There is apparent enhanced mitosis of Campylobacter sp. infected crypt epithelial cells. Possible mechanisms for this include: (1) CSM-like organisms produce substances which are mitogenic for crypt epithelial cells; (2) thymus dependent lymphocytes are sensitized to CSM antigens in the crypt lumina, epithelial cells or lamina propria, and these

sensitized T-cells then produce lymphokines which are mito-
genic for crypt epithelial cells; (3) CSM-like organisms produce
substances which cause increased loss of absorptive epithelial
cells which results in loss of chalone inhibition over crypt
epithelial cells. The loss of chalone inhibition could then
allow for an increased rate of crypt cell mitosis. The last
hypothetical mechanism could also account for the villous
atrophy which occurs in proliferative enteritis.

Other hypothetical causes for villous atrophy include:
(1) the rapidly dividing crypt cells fail to mature, while
mature epithelial cells are extruded at a normal or increased
rate. Bacterial products might be responsible for this lack
of crypt cell maturation; (2) the proliferating crypt cells
and the abundant mononuclear cells in the lamina propria
could cause compression of vascular spaces in the lamina
propria resulting in ischemia to absorptive cells and/or their
basement membranes. This damage could result in separation of
epithelial cells from their basement membranes at sites other
than extrusion zones at villus tips.

Experimental studies demonstrated that one of the earliest
lesions in proliferative enteritis occurred in the ileal mucosa
overlying ALN. These early lesions could cause a partial
functional obstruction of the ileum, thereby delaying emptying
of ileal contents into the large intestine. This delayed

emptying time could then allow CSM to colonize crypt lumina in an ascending manner up the ileum and jejunum.

Why then do experimental animals usually develop proliferative lesions which do not progress to diffuse lesions involving large segments of the intestine? Possible explanations include: (1) infected epithelial cells are extruded from the mucosal surface and are eventually replaced by uninfected epithelial cells; (2) CSM-like organisms do not reach the numbers needed to cause diffuse proliferative lesions; (3) bacterial antigens in crypt lumina or in the lamina propria are eliminated by polymorphonuclear leukocytes and macrophages before a large population of sensitized T-lymphocytes are produced.

These investigations, both of naturally occurring proliferative enteritis and the experimental disease have helped to establish the importance of the condition in swine raising operations in the midwestern United States and the association of CSM with the development of the disease. It is hoped that this work will stimulate further research efforts on the condition.

There are many aspects of the disease where further investigation is needed. The ability to detect subclinical disease by serologic or bacteriologic procedures would greatly facilitate the detection of carrier herds and eliminate the introduction of subclinically infected pigs onto

uninfected premises. These procedures could also be useful in determining whether the disease is prevalent enough to justify vaccine production. Improved culture techniques would hopefully enable isolation of the organism from feces and increase the isolation success from affected intestinal mucosa. Controlled field drug studies are needed in order to test the feasibility of antimicrobial treatment, the efficacy of a given product, and the proper route of administration. An accurate and dependable fluorescent antibody procedure would help to document the presence of CSM in affected intestinal mucosa and facilitate the rapid detection of the organism.

The pathogenesis of the disease is by no means clear. An area of further investigation should be the determination of whether delayed hypersensitivity (thymus dependent) reactions are necessary for the expression of the disease. This could perhaps be investigated in laboratory animals (i.e., mice) provided that CSM could survive and multiply in the intestine of these animals.

The mechanisms of a similar disease, hamster enteritis, could be studied using the hamster as an animal model. Campylobacter spp. organisms have been observed in the cytoplasm of proliferated intestinal epithelial cells from these animals. The adaptability of CSM to hamsters could be investigated. If infection of hamsters with CSM proved feasible,

morphometric and kinetic changes of crypt epithelial cell turnover could be studied.

The biologic behavior of CSM requires investigation because the factors reponsible for the pathogenicity of this organism are unknown. It is not known if the organism possesses virulence factors or, if it does, what the importance of these factors are in the production of proliferative enteritis.

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ACKNOWLEDGMENTS

APPENDIX

Table A1. Serotypes of E. coli isolated from the ilea of pigs with naturally occurring proliferative enteritis

	Number of pigs	Serotype		
		O	K	H
Feeder pig	1	?	30	+[a]
	1	?	88	-[b]
	1	101	?	+
	1	138	?	-
	1	117	30	+
	1	149	91	+
	5	untypable		
Gilt, sow, boar	1	117	91	+
	1	117	103	+
	1	?	103	+
	1	2	?	-
	1	?	30	+
	4	untypable		

[a]+ = motile.

[b]- = nonmotile.

Table A2. Hematologic parameters in pigs given crude inocula, pure cultures of CSM, or uninfected media

Group		N	Hemoglobin (mean)	Packed cell volume (mean)	White blood cell count (mean)	Total protein (mean)	Fibrinogen (mean)
Day 0	1[a]	6	12	35	8,917	5.3	338
	2[b]	3	12	35	12,367	5.4	467
	3[c]	5	12	34	10,250	5.4	375
Day 3	1	6	13	33	13,000	5.5	367
	2	3	13	35	12,400	5.7	225
	3	5	12	32	16,675	5.5	225
Day 5	1	6	10	31	15,900	5.4	300
	2	3	11	32	17,000	5.7	200
	3	5	11	30	15,825	5.5	250
Day 8	1	6	11	31	15,667	5.3	333
	2	3	11	32	15,000	5.8	267
	3	5	11	31	14,375	5.4	225
Day 12	1	6	11	30	17,680	5.4	260
	2	3	12	30	17,967	6.0	300
	3	5	11	31	18,100	5.5	275
Day 15	1	6	11	32	11,075	5.5	275
	2	3	12	34	11,333	5.8	233
	3	5	11	31	15,033	5.4	133
Day 20	1	6	11	33	9,950	5.7	225
	2	3	11	32	15,833	5.9	400
	3	5	11	31	13,867	5.7	167
Day 24	1	6	11	31	16,433	5.6	225
	2	3	12	33	17,102	5.8	333
	3	5	11	32	15,857	5.6	200
Day 28	1	6	11	31	18,473	5.5	233
	2	3	11	31	14,871	5.8	300
	3	5	11	32	16,773	5.6	333
Day 32	1	6	12	33	16,422	5.6	267
	2	3	12	32	19,581	5.6	260
	3	5	11	31	17,023	5.5	200

[a]Pigs given crude inoculum.

[b]Pigs given pure cultures of CSM.

[c]Control pigs.

Band neutrophils (mean)	Segmented neutrophils (mean)	Lymphocytes (mean)
969	4,228	3,003
551	4,018	6,659
510	3,365	5,285
1,692	4,921	5,272
1,404	2,097	6,536
1,230	3,484	10,192
2,147	7,491	4,963
2,094	6,504	6,559
1,744	6,556	5,301
1,912	6,215	5,987
1,291	3,405	9,288
2,175	5,459	5,614
2,452	7,690	5,827
2,974	5,696	7,613
2,426	6,179	7,545
1,049	5,193	4,188
1,829	2,503	5,353
1,988	5,983	4,897
1,159	2,252	5,330
1,246	3,297	9,947
1,568	4,375	6,429
1,240	3,680	10,563
2,085	5,831	9,311
1,820	3,903	9,927
2,923	8,730	6,587
1,780	9,427	3,041
1,320	6,998	8,311
1,142	11,748	3,981
2,831	12,432	4,076
1,851	10,808	3,780

Table A3. Hematologic parameters of pigs given crude inoculum, SC, CSM, or uninfected media

Group		N	Hemoglobin (mean)	Packed cell volume (mean)	White blood cell count (mean)	Total protein (mean)
Day 0	1[a]	6	11	30	21,533	6.3
	2[b]	10	10	28	19,930	6.2
	3[c]	4	11	28	18,050	5.9
	4[d]	2	9	25	21,050	5.9
	5[e]	2	10	28	27,600	6.1
Day 3	1	6	10	30	18,617	5.7
	2	10	10	28	29,550*	5.7
	3	4	10	30	28,800*	5.7
	4	2	9	26	13,340	5.7
	5	2	10	31	12,675	5.4
Day 5	1	6	10	30	21,733	5.6
	2	9	11	30	28,650	6.5
	3	2	10	29	28,900	6.6
	4	2	9	28	22,080	5.8
	5	2	10	30	22,050	5.5
Day 8	1	6	10	29	23,940	5.8
	2	6	9	27	29,367	5.6
	3	2	12	34	3,6300	6.3
	4	2	8	27	21,800	5.8
	5	2	11	32	18,250	5.6
Day 12	1	6	11	30	20,300	6.1
	2	4	10	30	26,260	5.4
	3	2	10	31	26,100	5.7
	4	2	10	29	26,150	5.7
	5	2	11	31	21,350	5.7

[a]Pigs given crude inoculum.

[b]Pigs given SC and CSM.

[c]Pigs given SC.

[d]Pigs given CSM.

[e]Control pigs.

*Means are significantly different (p<.05).

Fibrinogen (mean)	Band neutrophils (mean)	Segmented neutrophils (mean)	Lymphocytes (mean)
333	1,494	8,992	10,538
260	2,557	6,109	10,099
155	1,548	7,478	11,301
200	3,791	8,299	8,869
300	3,744	8,997	10,599
267	3,376	5,913	8,458
380	13,319*	6,531	5,611
475	12,844*	8,576	4,978
400	4,898	7,321	6,174
200	3,622	8,420	6,728
267	8,768	5,008	8,406
400	14,501*	674*	5,218
150	14,759*	2,870*	5,597
250	8,813	4,038	8,361
200	8,936	5,209	7,108
340	6,473	7,200	9,705
300	12,655*	5,977	10,130
250	23,870*	4,763	7,520
350	8,838	5,142	7,112
150	2,748	5,442	8,765
340	5,303	6,222	7,747
180	5,542	8,418	11,233
300	6,235	8,667	4,937
250	7,340	10,913	6,939
150	4,697	9,219	6,211

Table A3 (Continued)

Group		N	Hemoglobin (mean)	Packed cell volume (mean)	White blood cell count (mean)	Total protein (mean)
Day 15	1	6	10	29	26,000	5.7
	2	2	9	28	27,400	5.5
	3	2	11	29	31,900	5.8
	4	1	10	29	32,100	6.0
	5	2	11	33	20,550	5.5
Day 20	1	6	11	30	22,480	6.4
	2	2	11	33	16,200	5.7
	3	2	11	32	18,462	5.5
	4	1	10	28	25,300	5.6
	5	2	10	31	23,250	5.6
Day 24	1	6	10	30	25,750	6.4
	2	2	12	34	12,225	5.6
	3	2	11	30	14,787	5.7
	4	1	11	29	19,800	5.7
	5	2	11	31	13,850	5.5
Day 28	1	6	8	28	21,650	6.3
	2	2	12	37	16,150	6.0
	3	2	11	33	16,375	6.1
	4	1	12	35	17,900	6.6
	5	2	12	34	16,000	5.9
Day 32	1	6	12	35	23,800	7.2
	2	2	9	28	19,300	4,8*
	3	2	11	30	17,428	6.2
	4	1	10	32	18,400	6.0
	5	2	13	36	14,550	6.4

Fibrinogen (mean)	Band neutrophils (mean)	Segmented neutrophils (mean)	Lymphocytes (mean)
220	6,494	8,337	6,379
240	6,407	8,527	11,204
100	3,711	12,559	12,151
400	3,386	15,400	10,552
150	5,597	6,524	7,701
380	3,697	8,205	8,398
200	4,287	6,400	4,731
250	2,341	8,823	6,525
300	1,237	15,885	5,295
300	2,375	8,530	7,390
400	6,353	9,815	9,302
325	2,109	4,182	4,363
300	1,871	6,591	5,432
300	3,366	9,692	4,950
250	2,256	3,992	7,408
225	6,131	6,499	8,123
200	2,805	4,680	7,985
250	3,123	5,416	7,318
200	2,685	3,401	10,561
250	1,333	3,252	9,242
250	4,431	7,352	8,071
300	6,176	10,036	3,088
200	3,018	7,452	5,327
300	3,908	4,660	7,668
300	3,350	4,351	6,778