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

Larry Gene Lomax
Iowa State University

Follow this and additional works at: http://lib.dr.iastate.edu/rtd
Part of the Animal Sciences Commons, and the Veterinary Medicine Commons

Recommended Citation
http://lib.dr.iastate.edu/rtd/6924

This Dissertation is brought to you for free and open access by Iowa State University Digital Repository. It has been accepted for inclusion in Retrospective Theses and Dissertations by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
INFORMATION TO USERS

This was produced from a copy of a document sent to us for microfilming. While the most advanced technological means to photograph and reproduce this document have been used, the quality is heavily dependent upon the quality of the material submitted.

The following explanation of techniques is provided to help you understand markings or notations which may appear on this reproduction.

1. The sign or “target” for pages apparently lacking from the document photographed is “Missing Page(s)”. If it was possible to obtain the missing page(s) or section, they are spliced into the film along with adjacent pages. This may have necessitated cutting through an image and duplicating adjacent pages to assure you of complete continuity.

2. When an image on the film is obliterated with a round black mark it is an indication that the film inspector noticed either blurred copy because of movement during exposure, or duplicate copy. Unless we meant to delete copyrighted materials that should not have been filmed, you will find a good image of the page in the adjacent frame. If copyrighted materials were deleted you will find a target note listing the pages in the adjacent frame.

3. When a map, drawing or chart, etc., is part of the material being photographed the photographer has followed a definite method in “sectioning” the material. It is customary to begin filming at the upper left hand corner of a large sheet and to continue from left to right in equal sections with small overlaps. If necessary, sectioning is continued again—beginning below the first row and continuing on until complete.

4. For any illustrations that cannot be reproduced satisfactorily by xerography, photographic prints can be purchased at additional cost and tipped into your xerographic copy. Requests can be made to our Dissertations Customer Services Department.

5. Some pages in any document may have indistinct print. In all cases we have filmed the best available copy.
LOMAX, LARRY GENE

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

Iowa State University

University Microfilms International 300 N. Zeeb Road, Ann Arbor, MI 48106

Ph.D. 1981
PLEASE NOTE:

In all cases this material has been filmed in the best possible way from the available copy. Problems encountered with this document have been identified here with a check mark.

1. Glossy photographs or pages
2. Colored illustrations, paper or print
3. Photographs with dark background
4. Illustrations are poor copy
5. Pages with black marks, not original copy
6. Print shows through as there is text on both sides of page
7. Indistinct, broken or small print on several pages
8. Print exceeds margin requirements
9. Tightly bound copy with print lost in spine
10. Computer printout pages with indistinct print
11. Page(s) lacking when material received, and not available from school or author.
12. Page(s) seem to be missing in numbering only as text follows.
13. Two pages numbered. Text follows.
14. Curling and wrinkled pages
15. Other
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

Major: Veterinary Pathology

Approved:

Signature was redacted for privacy.

In Charge of Major Work
Signature was redacted for privacy.

For the Major Department
Signature was redacted for privacy.

For the Graduate College

Iowa State University
Ames, Iowa
1981
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>GENERAL INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Preamble</td>
<td>1</td>
</tr>
<tr>
<td>OBJECTIVES</td>
<td>2</td>
</tr>
<tr>
<td>DISSERTATION FORMAT</td>
<td>3</td>
</tr>
<tr>
<td>LITERATURE REVIEW</td>
<td>5</td>
</tr>
<tr>
<td><strong>PORCINE PROLIFERATIVE ENTERITIS (INTESTINAL ADENOMATOSIS):</strong></td>
<td></td>
</tr>
<tr>
<td>CLINICAL FINDINGS AND GROSS LESIONS OBSERVED IN 58 PIGS</td>
<td>23</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>24</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>25</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>28</td>
</tr>
<tr>
<td>RESULTS</td>
<td>32</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>50</td>
</tr>
<tr>
<td>References</td>
<td>53</td>
</tr>
<tr>
<td><strong>THE PATHOLOGY OF NATURALLY OCCURRING PORCINE PROLIFERATIVE ENTERITIS</strong></td>
<td></td>
</tr>
<tr>
<td>SUMMARY</td>
<td>57</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>58</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>59</td>
</tr>
<tr>
<td>RESULTS</td>
<td>62</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>65</td>
</tr>
<tr>
<td>References</td>
<td>88</td>
</tr>
<tr>
<td><strong>REFERENCES</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>94</td>
</tr>
<tr>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>PORCINE PROLIFERATIVE ENTERITIS: THE EXPERIMENTAL DISEASE</td>
<td></td>
</tr>
<tr>
<td>IN CAESAREAN-DERIVED ANDcolostrum-deprived PIGS</td>
<td>99</td>
</tr>
<tr>
<td>SUMMARY</td>
<td>100</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>101</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>104</td>
</tr>
<tr>
<td>RESULTS</td>
<td>111</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>136</td>
</tr>
<tr>
<td>References</td>
<td>139</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>PORCINE PROLIFERATIVE ENTERITIS: THE EXPERIMENTAL DISEASE</td>
<td>142</td>
</tr>
<tr>
<td>IN SPECIFIC-PATHOGEN-FREE PIGS</td>
<td></td>
</tr>
<tr>
<td>SUMMARY</td>
<td>143</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>144</td>
</tr>
<tr>
<td>MATERIALS AND METHODS</td>
<td>145</td>
</tr>
<tr>
<td>RESULTS</td>
<td>148</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>166</td>
</tr>
<tr>
<td>References</td>
<td>171</td>
</tr>
<tr>
<td>GENERAL DISCUSSION AND CONCLUSIONS</td>
<td>175</td>
</tr>
<tr>
<td>ADDITIONAL REFERENCES CITED</td>
<td>185</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>198</td>
</tr>
<tr>
<td>APPENDIX</td>
<td>199</td>
</tr>
</tbody>
</table>
GENERAL INTRODUCTION

Preamble

Campylobacter sputorum subspecies mucosalis (CSM)-associated conditions (intestinal adenomatosis, proliferative 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 proliferative 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 submitted 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 introduction which includes a preamble, objectives of the investigations, 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) 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*. Niacin (nicotinic acid) deficiency has been described as a cause of necrotic enteritis, but only the cecum and colon were involved. Recently, several authors have considered necrotic enteritis a sequel to numerous diseases characterized by enteritis in nursing, growing, and adult swine. These diseases included coccidiosis, rotaviral enteritis, transmissible gastro-enteritis, clostridial enteritis, *colibacillosis*,
salmonellosis, swine dysentery, and trichuriasis. Jennings (1959) 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. The causes included hog cholera virus, Erysipelothrix rhusiopathiae and S. choleraesuis. 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. 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) 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. Metastases of glandular epithelial elements to the ileocecal lymph node were observed in a few of the cases. Rahko and Saloniemi (1972) 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.\textsuperscript{21,22,23,24,25}

Biester and Schwarte (1931,\textsuperscript{2} 1939\textsuperscript{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)\textsuperscript{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: \textit{Bacillus anthracis};\textsuperscript{28} \textit{Leptospira icterohemorrhagiae};\textsuperscript{28} \textit{Clostridium perfringens} type \textit{C};\textsuperscript{28} \textit{Escherichia coli};\textsuperscript{29} \textit{Treponema hyodysenteriae};\textsuperscript{28} \textit{Salmonella} spp.; \textit{Isospora} spp.;\textsuperscript{28} Vitamin E deficiency;\textsuperscript{30,31} poisoning with warfarin, arsenic, mercury, sodium fluoride, and copper;\textsuperscript{28} mycotoxins;\textsuperscript{32} torsion of the mesentery;\textsuperscript{33} and gastric ulcers.\textsuperscript{28,30} In addition, an intestinal hemorrhage syndrome of unknown cause has been associated with the feeding of whey to pigs.\textsuperscript{34} This syndrome was also reported
by numerous authors who were unable to associate it with whey feeding or any other cause. 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. 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. The term was subsequently used by other authors throughout the world who published reports which lacked careful histopathologic descriptions. The term was also used by authors who included histopathologic findings in their reports. O'Hara (1972) 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. 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. Rowland and Rowntree (1972) 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\(^4\) (a term apparently synonymous with the term, intestinal adenoma\(^2\)). Other workers described similar conditions, but intestinal adenomatosis did not follow the hemorrhagic syndrome.\(^4\,46\,47\,48\)

A possible relationship between intestinal adenomatosis, necrotic enteritis, regional ileitis, and the hemorrhagic bowel syndrome (later renamed proliferative hemorrhagic enteropathy\(^4\)) was established when Rowland and Lawson (1973\(^4\), \(^5\)) and Rowland et al. (1973,\(^5\) 1976\(^5\)) described the presence of a bacterium within affected epithelial cells of intestinal mucosa from each of these conditions. Lawson and Rowland (1974)\(^5\) and Lawson et al. (1975,\(^5\) 1976,\(^5\) 1979\(^5\)) 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.\(^5\,57\,58\,59\)

In the United States, a condition was observed in feeder pigs which was similar to the four CSM-associated conditions. Bergeland (1975)\(^6\) 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. Roberts et al. (1979) 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) 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. 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). Necroproliferative enteritis primarily affected 16 to 45 kg feeder pigs. 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.
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. The clinical signs of pigs affected with proliferative hemorrhagic enteropathy include pallor, anorexia, normal to subnormal rectal temperatures, 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. 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. 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. Fattening hogs with necrotic enteritis or regional ileitis do not usually have clinical signs. Feeder pigs with necroproliferative enteritis have diarrhea and stools which may contain blood or yellow casts of fibrin and necrotic tissue, and the pigs become emaciated even though they may continue to have a fair appetite.

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. Martinsson et al. (1976) 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.

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. The intestinal mucosa of these pigs is thickened and deeply folded, and occasionally focal raised polypoid areas are present, particularly toward the proximal ileum. 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. 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. These changes are most pronounced at the antimesenteric aspects of the ileum.
where large aggregated lymphoid nodules (ALN; Peyer's patches) are present. In addition, pigs with regional ileitis may have marked thickening of the tunica muscularis. Swine with necroproliferative enteritis have thickening of the ileal mucosa with pseudomembranes and blood in the ileum. 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 proliferations of epithelial cells in the proximal ileum. 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. 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. 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. Hypertrophy of the smooth muscle of the tunica muscularis of the ileum may also be present in swine with regional ileitis. The ileocecal lymph nodes from pigs with any of the CSM-associated conditions may have hyperplasia of lymphoid germinal centers. 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.

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. 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.
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)\(^3\) and Lawson et al. (1975)\(^4\) initially isolated the
organism from cases of intestinal adenomatosis, biochemically
and antigenically characterized it, and named it. Subse-
sequently, CSM was isolated from the intestinal mucosa of pigs
with necrotic enteritis, regional ileitis, proliferative
hemorrhagic enteropathy, and necroproliferative enteritis.\(^5,6,9,72\)
Gunnarsson (1976)\(^8\) 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.\(^5,6,9,74\)

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.\(^75,76\) 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. When grown in culture, CSM ultrastructurally has a single polar flagellum, a trilayered cell wall and a trilaminar cell membrane, and its cytoplasm contains strands of nuclear material and ribosomes.

The genus Campylobacter is composed of Campylobacter fetus, Campylobacter sputorum, and Campylobacter fecalis. Campylobacter fetus has the subspecies fetus, intestinalis, and jejuni. Campylobacter sputorum has the subspecies sputorum, bubulus, and mucosalis. 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. The organisms are motile with a characteristic "corkscrew movement." Campylobacter sputorum and its subspecies are all catalase negative, and strongly positive for hydrogen sulfide production in iron-containing media. Campylobacter sputorum subspecies mucosalis grows on media containing 1.5% sodium chloride and produces yellow colonies.
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. 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. However, pigs affected by these conditions usually do not respond well to treatment. The antibiotics most frequently used were tylosin, sulphadimidine, furazolidone, penicillin, and tetracycline. In vitro, isolants of CSM have been most sensitive to tetracycline, followed in decreasing order by nitrofurantoin, penicillin, chloramphenicol, erythromycin, neomycin, and Polymyxin B. Love and Love (1977) 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) 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. Focal areas of crypt epithelial cell proliferation and intracellular CSM were demonstrated in the intestinal mucosa of the mid-jejunum and terminal ileum of pigs orally inoculated with chalk powder, benzetimidine, and CSM cultures. 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.

Comparative enteric diseases- An enteric disease of the golden hamster (Mesocricetus auratus) is the closest comparative 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", proliferative ileitis, regional enteritis, terminal ileitis, enzootic intestinal adenocarcinoma, transmissible ileal hyperplasia, atypical ileal hyperplasia, and hamster enteritis. The disease is characterized by diarrhea and enteritis, sometimes proliferative enteritis, involving the small intestine of weanling hamsters. In the acute form, there is often diffuse hemorrhagic necrosis of the terminal ileal mucosa without
evidence of mucosal hyperplasia. The proliferative form is characterized by hyperplastic villi which are wider and longer than normal. Hyperplasia is thought to begin in crypt epithelial cells. 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.

Causative agents have not been described. Bacteria, viruses, parasites, and dietary factors all have been implicated but Koch's postulates have never been fulfilled with any of them. 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. Oral administration of n-methyl-N-nitrosourea will induce intestinal adenocarcinomas in hamsters; however, these lesions have no resemblance to the proliferative lesions of hamster enteritis.

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. In one such experiment, Frisk and
Wagner (1977)\textsuperscript{90} observed by electron microscopy two morphologically different bacterial profiles within affected epithelial cells. Intracytoplasmic organisms observed early in the disease were identified as \textit{E. coli} and those observed later in the disease structurally resembled \textit{Campylobacter} spp.\textsuperscript{90}

Barthold et al. (1978)\textsuperscript{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.\textsuperscript{94}

Laboratory mice have a naturally occurring infectious disease called transmissible murine colonic hyperplasia which is characterized by mucosal hyperplasia, and occasionally accompanied by erosion and inflammation of the distal colon.\textsuperscript{95} The condition has been experimentally reproduced with a variant of \textit{Citrobacter freundii} (isolant 4280).\textsuperscript{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.\textsuperscript{97}

Geil and Davis (1968)\textsuperscript{98} reported on 64 cases of spontaneous
ileitis in rats. The condition was characterized by segmental dilatation 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. 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). There was degeneration and loss of surface epithelial cells in the cecum accompanied by crypt epithelial cell hyperplasia and inflammation of the lamina propria and submucosa. Bacteria resembling Vibrio sp. were observed by electron microscopy within the cecal lumen and within the cytoplasm of degenerating epithelial cells.

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). Terminal ileitis was diagnosed by Cross and Smith (1973) 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. Histopathologic findings were epithelial cell proliferation, which had an adenomatous appearance, and inflammation of the lamina propria.

Isolated reports of conditions affecting the ilea of other domestic animals have appeared in the literature.
Idiopathic muscular hypertrophy of the ileum has been observed in horses. The similarity between this condition and terminal ileitis in swine has been reported; however, histologic findings in the ilea of these horses were not described. Regional enterocolitis having some resemblance to regional ileitis in swine has been described in two cocker spaniel dogs.

In man, regional enteritis (Crohn's disease) is a chronic inflammatory disease of unknown cause affecting primarily the terminal ileum. 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, submucosa, and subserosa. 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

Larry G. Lomax, DVM
Robert D. Glock, DVM, PhD
John E. Hogan, BS

This manuscript has been submitted to the
Journal of the American Veterinary Medical Association

From the Department of Veterinary Pathology, Iowa
State University, Ames, IA 50011.
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.\textsuperscript{1} and Rowland and Lawson\textsuperscript{2,3,4} described the intracellular presence of a bacterium in intestinal mucosa epithelial cells from cases of intestinal adenomatosis,\textsuperscript{1,2} proliferative hemorrhagic enteropathy,\textsuperscript{3,4} necrotic enteritis,\textsuperscript{4} and terminal ileitis (regional ileitis).\textsuperscript{4} Lawson and Rowland\textsuperscript{5} and Lawson et al.\textsuperscript{6,7} isolated a species of bacterium from the intestinal mucosa of each of these four conditions and named it \textit{Campylobacter sputorum} subspecies \textit{mucosalis} (CSM). Similar organisms were subsequently isolated from like conditions in Australia\textsuperscript{8} and the Scandinavian countries.\textsuperscript{9}

There are few epidemiologic studies on the four CSM-associated conditions. Intestinal adenomatosis occurs sporadically in post-weaned pigs.\textsuperscript{10} Lawson et al.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{8} However, the incidence
of proliferative hemorrhagic enteropathy usually is much lower.\textsuperscript{12}

The clinical findings of each of the four CSM-associated conditions have been reported by numerous authors.\textsuperscript{3,12,13} Pigs affected with intestinal adenomatosis often have no clinical signs, but emaciation may occur in a few.\textsuperscript{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.\textsuperscript{12} Young weaned pigs affected with regional ileitis usually have weight loss, anemia, and hypoproteinemia.\textsuperscript{13} Market weight hogs with regional ileitis usually have no clinical signs.\textsuperscript{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.\textsuperscript{8,12} Pigs clinically affected with proliferative hemorrhagic enteropathy usually do not respond to treatment.\textsuperscript{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.\textsuperscript{14} Growing pigs with intestinal adenomatosis have been reported to recover without treatment.\textsuperscript{10}

The gross and microscopic lesions of intestinal
adenomatosis, proliferative hemorrhagic enteropathy, necrotic enteritis, and regional ileitis have been described.\textsuperscript{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. Confine­ment production unit will refer to swine raised without outside access in closed, controlled environment houses.

Pathologic studies- Postmortem examinations of 58 animals were conducted. Samples taken for histopathologic evaluation 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 fibrinogen concentration. Blood urea nitrogen, glutamic-oxaloacetic 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 novobiocin, 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.\(^2\) 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.

---

\(^a\)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 Salmonella spp. by inoculation onto brilliant green agar\(^a\) and incubation at 37°C 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 performed on isolants with appropriate biochemical reactions.\(^{18}\)

**Telephone survey**—Ninety-two Iowa veterinarians engaged 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.

\(^a\)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 frequently in conventionally housed feeder pigs than in confinement 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 epizootic on a given farm was difficult to determine but less than 10% of the animals usually had clinical signs. However, 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 conventionally housed feeder pigs. Adult breeding swine in confinement 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

<table>
<thead>
<tr>
<th></th>
<th>Feeder pig (16-45 kg) (no. of farms)</th>
<th>Finishing pig (50-114 kg) (no. of farms)</th>
<th>Adults (no. of farms)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>21</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Confinement</td>
<td>6</td>
<td>1</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>TOTAL</td>
<td>27</td>
<td>1</td>
<td>14</td>
<td>2</td>
</tr>
</tbody>
</table>
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; however, proliferative enteritis often occurred in conventional 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 important affect on the occurrence of disease. Feeder pigs purchased 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 conventional 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 temperatures 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 concentration were decreased and the average plasma protein to fibrinogen ratio was 12.0 (Table 3).

Average group values for hemoglobin, packed cell volume, and plasma protein were decreased in 10 adult swine. Fibrinogen 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 indicated neutrophilia, left shift, and lymphopenia. However, 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

<table>
<thead>
<tr>
<th></th>
<th>Chronic or intermittent diarrhea, wasting</th>
<th>Chronic diarrhea with some blood</th>
<th>Hemorrhagic or tarry stools for 1-3 days</th>
<th>Found dead</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeder pig</td>
<td>20</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>27</td>
</tr>
<tr>
<td>Gilt, sow, boar</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Confinement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeder pig</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Finishing pig</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gilt, sow, boar</td>
<td>1</td>
<td>3</td>
<td>16</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>24</td>
<td>14</td>
<td>16</td>
<td>4</td>
<td>58</td>
</tr>
</tbody>
</table>
Table 3—Average Values for Hematologic Parameters in Two Groups of Pigs with Proliferative Enteritis (group ranges are in parentheses)

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

*aSchalm, O. W., 1975.21

`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 adherent 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 bilateral 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)

<table>
<thead>
<tr>
<th></th>
<th>Intestinal wall thickened with pseudomembrane in lumen</th>
<th>Intestinal wall thickened with free or clotted blood in lumen</th>
<th>Intestinal wall thickened with no exudate in lumen</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeder pig</td>
<td>25</td>
<td>3</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Gilt, sow, boar</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Confinement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeder pig</td>
<td>4</td>
<td>2</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Finishing pig</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gilt, sow, boar</td>
<td>6</td>
<td>15</td>
<td>1</td>
<td>22</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td>36</td>
<td>21</td>
<td>1</td>
<td>58</td>
</tr>
</tbody>
</table>
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, Intraepithelial Campylobacter sp.-like organisms, and isolation of Campylobacter sputorum subspecies mucosalis (CSM) (58 pigs from 44 farms)

<table>
<thead>
<tr>
<th></th>
<th>Proliferation of immature epithelial cells</th>
<th>Intracytoplasmic Campylobacter sp.-like organisms</th>
<th>Bacterial isolations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CSM</td>
</tr>
<tr>
<td>Conventional</td>
<td></td>
<td></td>
<td>Salmonella choleraesuis</td>
</tr>
<tr>
<td>Feeder pig</td>
<td>28</td>
<td>28</td>
<td>16</td>
</tr>
<tr>
<td>Gilt, sow, boar</td>
<td>1</td>
<td>1</td>
<td>ND^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Confinement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feeder pig</td>
<td>6</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Finishing pig</td>
<td>1</td>
<td>1</td>
<td>ND</td>
</tr>
<tr>
<td>Gilt, sow, boar</td>
<td>22</td>
<td>22</td>
<td>10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>58</td>
<td>58</td>
<td>30</td>
</tr>
</tbody>
</table>

^aND = 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.

\(^a\)Upjohn Company, Kalamazoo, MI.
Porcine proliferative enteritis is the proposed name for *Campylobacter sputorum* subspecies *mucosalis*-associated conditions in swine in the midwestern United States. The disease has somewhat different pathologic and clinical manifestations in growing and adult swine. The reason for these differences 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 prolonged stress from disease. Workers in Sweden have demonstrated 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 demonstrated by their decreased hemoglobin concentration and packed cell volume values. These pigs are also hypoproteinemic. 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 *Campylobacter* 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. *Salmonella choleraesuis* 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; *Clostridium perfringens* enteritis; *E. coli* enteritis; anthrax; leptospirosis; 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.

References


THE PATHOLOGY OF NATURALLY OCCURRING PORCINE PROLIFERATIVE ENTERITIS

Larry G. Lomax, DVM
Robert D. Glock, DVM, PhD

This manuscript has been submitted to the American Journal of Veterinary Research.

From the Department of Veterinary Pathology,
College of Veterinary Medicine, Iowa State University,
Ames, Iowa 50011. The authors thank Ms. J. A. Fagerland, Ms. M. J. Hennig, and Ms. K. A. Finley for assistance with electron microscopy.
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, and others. 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. Proliferative hemorrhagic enteropathy has mucosal proliferation in the small and large intestine accompanied by superficial mucosal necrosis and hemorrhage into the intestinal lumen. Coagulative necrosis of proliferated mucosa in the ileum and large intestine is characteristic of necrotic enteritis. Regional ileitis has chronic inflammation and ulceration of proliferated mucosa in the terminal ileum. Hypertrophy of smooth muscle of the tunica muscularis has also been described in this condition. Rowland et al. 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.

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. The organisms were irregularly shaped, approximately 2-3 μm long, 0.3 μm in diameter, and had an undulating cell wall. Love and
Love\textsuperscript{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 duodenum, jejunum (three levels), ileum (three levels), ileocecal valve, cecum, colon, ileocecal lymph nodes, liver, spleen, kidney, stomach, lung, cardiac and skeletal muscle, and brain. Tissues collected from slaughterhouse hogs consisted 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 microscopy 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% glutaraldehyde 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. 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. They were viewed and photographed with a Hitachi HS-9 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. Homogenates of ileocecal lymph node were also cultured for CSM. Antisera to CSM isolants were produced, and used in a slide agglutination test as the means for positive CSM identification. Samples of gut homogenate, } 

\[ ^a \text{Ladd Research Industries, Burlington, VT.} \]
\[ ^b \text{Hitachi Scientific Instruments, 328 Eisenhower Lane, Lombard, Ill. 60148.} \]
liver, ileocecal lymph node, and gall bladder were cultured for *Salmonella* spp.
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 characterized 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 inflammatory 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 elongation, dilatation 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 progression 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 formation 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 within 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 dilatation 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 multinucleated 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 metastatic 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 artifact. 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. Campylobacter 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. Immature 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.\textsuperscript{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. 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. 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 intestinal microvilli in order for fibrillar cores of microvilli and tight junctions to undergo degeneration. 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 inside 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. The cavity eventually pinches off from the apical cytoplasm and forms a vacuole around the organism. Salmonella typhimurium may also penetrate through the intercellular junctional complex and become enclosed by the lateral cell membranes of two adjacent epithelial cells. Salmonella enteritidis has been shown to
penetrate similarly in experimentally infected chickens.\textsuperscript{24} Takeuchi\textsuperscript{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.\textsuperscript{25} \textit{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.\textsuperscript{25} This group is represented by \textit{Shigella} spp. organisms. The third group is primarily found in the microvillous border and does not invade, or at least not deeply, into the epithelial cell cytoplasm.\textsuperscript{25} Members of this group include \textit{Cryptosporidia} spp., \textit{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.\textsuperscript{25} This group is represented by \textit{Vibrio cholerae} and certain strains of pathogenic \textit{Escherichia coli}. \textit{Campylobacter sputorum} subspecies \textit{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. 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. 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. 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. 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.\textsuperscript{28} It is believed that crypt cells undergo accelerated mitosis to replace increased cell loss along the villous surface. Excessive loss of mature intestinal epithelium has been documented in several enteric diseases and appears to be a general reparative response of the mucosa to injury.\textsuperscript{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\textsuperscript{14} have suggested that proliferative hemorrhagic enteropathy resembles an immediate hypersensitivity reaction. Ferguson and co-workers\textsuperscript{29-31} and MacDonald and Ferguson\textsuperscript{32} have studied the effects of local delayed hypersensitivity 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.\textsuperscript{33} Interestingly, crypt cell proliferation
preceded villus atrophy by several days. The authors postulated that the effects may be produced by lymphokines which are mitogenic for immature crypt epithelial cells.

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

Crypt epithelial cells within lymphatics in the submucosa and within the ileocecal lymph node were observed in one pig in this study. Emsbo 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. 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.

References


PORCINE PROLIFERATIVE ENTERITIS:
THE EXPERIMENTAL DISEASE IN CAESAREAN-DERIVED
AND COLOSTRUM-DEPRIVED PIGS

Larry G. Lomax, DVM
Robert D. Glock, DVM, PhD
Delbert L. Harris, DVM, PhD
John E. Hogan, BS

This manuscript has been submitted to the American
Journal of Veterinary Research

From the Department of Veterinary Pathology (Lomax, 
Glock, Hogan), and Veterinary Medical Research Institute 
(Harris), College of Veterinary Medicine, Iowa State 
University, Ames, Iowa 50011.

The authors thank Ms. J. A. Fagerland, Ms. M. J. Hennig, 
and Ms. K. A. Finley for assistance with electron microscopy.
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 Campylobacter 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, eosinophils, 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. 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. The organism apparently preferentially penetrates immature crypt epithelial cells by passing through the microvillous border of these cells.

The disease has clinical and pathologic resemblance to other CSM-associated diseases which have been reported throughout the world. Rowland and Lawson and others 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 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.\textsuperscript{9-12} Focal microscopic proliferative lesions in the mucosa of the small and large intestine have been produced, and intracellular \textit{Campylobacter} sp.-like organisms have been demonstrated in a small percentage of neonatal pigs orally inoculated with either type inoculum.\textsuperscript{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.
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 transported 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<sup>a</sup> 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

<sup>a</sup>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), 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 supplemented with 10% fetal calf serum 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).

---

^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 $1 \times 10^8$ 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 37°C overnight with gentle agitation. Colony counts were approximately $1 \times 10^8$ to $1 \times 10^9$ 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.

$^a$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), ileocecal lymph nodes, liver, spleen, kidney, stomach, lung, cardiac and skeletal muscle, and brain were taken for histopathologic 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.\textsuperscript{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.\textsuperscript{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 \textit{S. choleraesuis}. Swabs of gallbladder mucosa, blood samples, and samples from the liver were collected from all pigs for \textit{S. choleraesuis} isolation by methods previously described.\textsuperscript{1} Samples of jejunal mucosa were collected for indirect fluorescent antibody examination for transmissible gastroenteritis virus.\textsuperscript{a} Cecal contents were collected for

\textsuperscript{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 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 bicarbonate intragastrically.

---

aProcedures performed by the Iowa Veterinary Diagnostic Laboratory, Ames, IA.

bicarbonate. Three pigs (group 2) were given 100 ml \( (1 \times 10^8 \text{ CFU per ml}) \) 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 \( (1 \times 10^8 \text{ CFU per ml}) \) of CSM, 25 ml \( (1 \times 10^8 \text{ CFU per ml}) \) 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 \( (1 \times 10^8 \text{ CFU per ml}) \) of *S. choleraesuis* plus 50 ml of sodium bicarbonate as a single initial inoculation. The two pigs in group four were given 100 ml \( (1 \times 10^8 \text{ CFU per ml}) \) 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

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Group</th>
<th>No. of pigs</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>5</td>
<td>CI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>C&lt;sup&gt;b&lt;/sup&gt;</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
<td>7</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>CSM&lt;sup&gt;c&lt;/sup&gt;</td>
<td>CSM</td>
<td>CSM</td>
<td>CSM</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>III</td>
<td>1</td>
<td>6</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
<td>CI</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>SC&lt;sup&gt;d&lt;/sup&gt;&amp;CSM</td>
<td>CSM</td>
<td>CSM</td>
<td>CSM</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>SC</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2</td>
<td>CSM</td>
<td>CSM</td>
<td>CSM</td>
<td>CSM</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
</tr>
</tbody>
</table>

<sup>a</sup>C<sub>I</sub> = crude inoculum.

<sup>b</sup>C = control group.

<sup>c</sup>CSM = *Campylobacter sputorum* subspecies mucosalis.

<sup>d</sup>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 neutrophilic 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 reticulation 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 immature 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 Campylobacter 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 Campylobacter 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. Microvilli 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. Ileocolcal lymph nodes were slightly enlarged, moist, and pale. There were no gross lesions in other
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 (40-42°C) 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 infiltration of neutrophils and macrophages into the lamina propria and submucosa. Note focal necrosis of lymphocytes in aggregated lymphoid nodules (arrow), and lack of proliferation 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 (41-42.5°C) 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 parameters 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 isolated from the intestine, liver, and ileocecal lymph nodes of both pigs.

The two pigs which survived to termination of the experiment 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. Campylobacter 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)

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment(^a) (no. of pigs)</th>
<th>Proliferative mucosal lesions</th>
<th>Intracellular Campylobacter sp.-like organisms (no. of pigs)</th>
<th>CSM cultured from (no. of pigs)</th>
<th>SC cultured from (no. of pigs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>CI (10)</td>
<td>5</td>
<td>7</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>CI (6)</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>ND(^b)</td>
</tr>
<tr>
<td>II</td>
<td>CSM (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>CI (6)</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>SC+CSM (10)</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>SC (4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>CSM (2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>41</td>
<td>12</td>
<td>18</td>
<td>8</td>
</tr>
</tbody>
</table>

\(^a\)Control groups not included.

\(^b\)ND = not determined.
DISCUSSION

This series of experiments using 10-week-old Caesarean-derived and colostrum-deprived pigs indicates that proliferative 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 virulent, but the two pigs which were also given cultures of CSM and survived to termination of the experiment had proliferative lesions and mucosal necrosis. Pigs given cultures of *S. choleraesuis* alone did not develop proliferative intestinal 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 immature 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 unknown. 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 *Campylobacter* sp.-like organisms in infected immature epithelial cells resembled those previously described for naturally-occurring cases of porcine proliferative enteritis,\textsuperscript{1,2,3} intestinal adenomatosis,\textsuperscript{4,14} proliferative hemorrhagic enteropathy,\textsuperscript{15} regional ileitis,\textsuperscript{5,6} and necrotic enteritis.\textsuperscript{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\textsuperscript{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.

References


PORCINE PROLIFERATIVE ENTERITIS:
THE EXPERIMENTAL DISEASE
IN SPECIFIC-PATHOGEN-FREE PIGS

Larry G. Lomax, DVM
Robert D. Glock, DVM, PhD
John E. Hogan, BS

This manuscript has been submitted to the
American Journal of Veterinary Research

From the Department of Veterinary Pathology, College
of Veterinary Medicine, Iowa State University, Ames, Iowa
50011.

The authors thank Ms. J. A. Fagerland, Ms. M. J.
Hennig, and Ms. K. A. Finley for assistance with electron
microscopy.
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. 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.

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

Campylobacter sputorum subspecies mucosalis (CSM) is a member of the genus Campylobacter which includes curved, gram-negative, monotrichous rods that have microaerophilic growth requirements. 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 -62°C until needed. Pre-inoculation culture of the crude inoculum was negative for \textit{Salmonella} spp.

\textit{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 37°C for 24 or 48 hours, and were gently agitated (50 rpm)\(^a\) to increase growth. Colony counts obtained were approximately 1x10\(^8\) 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.

\(^a\)Junior Orbit Shaker, Lab-Line Instruments Inc., Melrose Park, Ill.

\(^b\)Hitachi Scientific Instruments, 328 Eisenhower Lane, Lombard, Ill.
Microbiologic examinations- 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 *Salmonella* spp.\(^1\) Procedures used for antisera production to CSM isolants have been described.\(^1\)

Experimental design- 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 (\(1 \times 10^8\) 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).

\(^a\)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

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of pigs</th>
<th>Proliferative mucosal lesions</th>
<th>Intracellular Campylobacter sp.-like-organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Gross (no. of pigs)</td>
<td>Microscopic (no. of pigs)</td>
</tr>
<tr>
<td>Crude inoculum</td>
<td>11</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td><em>C. sputorum</em> subsp. <em>mucosalis</em></td>
<td>11</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Control</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>33</td>
<td>2</td>
<td>9</td>
</tr>
</tbody>
</table>
Table 2—Microscopic Lesions in Pigs Between 4 and 38 Days Post Inoculation

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>4</th>
<th>7</th>
<th>10</th>
<th>14</th>
<th>18</th>
<th>21</th>
<th>24</th>
<th>28</th>
<th>31</th>
<th>36</th>
<th>38</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude inoculum</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>C. sputorum subspecies mucosalis</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>0</td>
<td>++</td>
<td>++</td>
<td>0</td>
<td>++</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
</tbody>
</table>

+ = 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 proliferation 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 cytoplasm 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 cytoplasmic organelles of affected crypt cells which had the structural appearance of immature epithelial cells. These epithelial cells had low, irregular microvilli, poorly defined terminal webs, abundant free ribosomes throughout their cytoplasm, and prominent secretory granules in their apical cytoplasm. Curved organisms were also observed within phagolysosomes 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 leukocytes 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 characterized by leukocyte exudation into crypt lumina and infiltration 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 intracellular 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 proliferation 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 ileocecral 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 Campylobacter 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.\textsuperscript{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
Salmonella choleraesuis. These two organisms have been isolated from 16-45 kg feeder pigs with the naturally occurring disease, but S. choleraesuis was isolated infrequently from these pigs. Salmonella choleraesuis has not been isolated from bred gilts, sows, or boars manifesting intestinal hemorrhage.

Little is known of the biology of CSM. Lawson et al. 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. However, cell components or products responsible for the pathogenicity of CSM are unknown.

Campylobacter fetus and its subspecies fetus, intestinalis, and jejuni, are monotrichus, microaerophilic, catalase positive, gram-negative curved rods. They are associated with infertility and abortion in cattle and abortion in sheep. It has been demonstrated that C. fetus has a glycoprotein antiphagocytic surface component called antigen-a. This antigenic component corresponds structurally and functionally to a microcapsule, and is easily removed from the cell by mild acid extraction procedures. Bacterial cells which have this antigen are
refractory to phagocytosis except in the presence of specific antiserum. It is thought that this antiphagocytic component is a major virulence factor of the organism. 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. Motility is accomplished by rotary movement of the flagellar hook, and it also apparently requires the functional integrity of the cell envelope.

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

There was apparent localization of the early proliferative 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 (dome epithelium) which contains cuboidal microvilli-covered epithelial cells, plasma cells, lymphocytes and M-cells. Penetration of the mucosa by particulate antigens is apparently facilitated at these sites. Experiments have shown that dome epithelium may be involved in the early immunologic response in the intestine of swine, and that aggregated lymphoid nodules (ALN) can participate in thymus dependent (t-cell) immunologic responses to live bacterial organisms.

*Listeria monocytogenes* is an intracellular parasite. 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. 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. 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. Proliferation of gram-negative intestinal bacteria has been shown to increase neutrophil emigration into the intestinal lumen. 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). If enough secretory IgA is present, the organism could remain in the intestinal lumen and be destroyed. 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. These antigens include bacteria,
parasites, viruses, particulate antigens, and cellular components of host tissue. Ferguson and MacDonald\textsuperscript{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.\textsuperscript{22}

It is possible that if \textit{C. sputorum} subspecies \textit{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.

References

1. Lomax LG, Glock RD, Hogan JE: Porcine proliferative enteritis (intestinal adenomatosis): clinical findings and gross lesions observed in 58 pigs. (prepared for submission to \textit{J Am Vet Med Assoc}).

2. Lomax LG, Glock RD: The pathology of naturally occurring porcine proliferative enteritis. (prepared for submission to \textit{Am J Vet Res}).


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 Campylobacter 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 Campylobacter 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 con­
sistent 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 pro­
liferative enteritis in swine was investigated. Three sepa­
rate experiments were designed and conducted. The use of
homogenized mucosal scrapings (crude inoculum) obtained from
naturally infected swine proved effective in producing pro­
liferative 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 pro­
liferative 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 phagocytic 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 mitogenic 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 responsible 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.
ADDITIONAL REFERENCES CITED


ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. Robert D. Glock for his patience, his constructive criticism, and his encouragement throughout my graduate study program. I also wish to thank Dr. Frank K. Ramsey for his openness and willingness to give advice.

The support and assistance of the staff and faculty of the Department of Veterinary Pathology and the Veterinary Medical Research Institute at Iowa State University was greatly appreciated.
APPENDIX
Table A1. Serotypes of *E. coli* isolated from the ilea of pigs with naturally occurring proliferative enteritis

<table>
<thead>
<tr>
<th>Number of pigs</th>
<th>Serotype</th>
<th>O</th>
<th>K</th>
<th>H</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Feeder pig</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>?</td>
<td>30</td>
<td>+</td>
<td>a</td>
</tr>
<tr>
<td>1</td>
<td>?</td>
<td>88</td>
<td>-</td>
<td>b</td>
</tr>
<tr>
<td>1</td>
<td>101</td>
<td>?</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>138</td>
<td>?</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>117</td>
<td>30</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>149</td>
<td>91</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gilt, sow, boar</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>117</td>
<td>91</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>117</td>
<td>103</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>?</td>
<td>103</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2</td>
<td>?</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>?</td>
<td>30</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^{a+}\) = motile.

\(^{b-}\) = nonmotile.
<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Hemoglobin (mean)</th>
<th>Packed cell volume (mean)</th>
<th>White blood cell count (mean)</th>
<th>Total protein (mean)</th>
<th>Fibrinogen (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1</td>
<td>6</td>
<td>12</td>
<td>35</td>
<td>8,917</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>35</td>
<td>12,367</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>12</td>
<td>34</td>
<td>10,250</td>
<td>5.4</td>
</tr>
<tr>
<td>Day 3</td>
<td>1</td>
<td>6</td>
<td>13</td>
<td>33</td>
<td>13,000</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>13</td>
<td>35</td>
<td>12,400</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>12</td>
<td>32</td>
<td>16,675</td>
<td>5.5</td>
</tr>
<tr>
<td>Day 5</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>31</td>
<td>15,900</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>11</td>
<td>32</td>
<td>17,000</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>30</td>
<td>15,825</td>
<td>5.5</td>
</tr>
<tr>
<td>Day 8</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>31</td>
<td>15,667</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>11</td>
<td>32</td>
<td>15,000</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>31</td>
<td>14,375</td>
<td>5.4</td>
</tr>
<tr>
<td>Day 12</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>30</td>
<td>17,680</td>
<td>5.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>30</td>
<td>17,967</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>31</td>
<td>18,100</td>
<td>5.5</td>
</tr>
<tr>
<td>Day 15</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>32</td>
<td>11,075</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>34</td>
<td>11,333</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>31</td>
<td>15,033</td>
<td>5.4</td>
</tr>
<tr>
<td>Day 20</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>33</td>
<td>9,950</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>11</td>
<td>32</td>
<td>15,833</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>31</td>
<td>13,867</td>
<td>5.7</td>
</tr>
<tr>
<td>Day 24</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>31</td>
<td>16,433</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>33</td>
<td>17,102</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>32</td>
<td>15,857</td>
<td>5.6</td>
</tr>
<tr>
<td>Day 28</td>
<td>1</td>
<td>6</td>
<td>11</td>
<td>31</td>
<td>18,473</td>
<td>5.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>11</td>
<td>31</td>
<td>14,871</td>
<td>5.8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>32</td>
<td>16,773</td>
<td>5.6</td>
</tr>
<tr>
<td>Day 32</td>
<td>1</td>
<td>6</td>
<td>12</td>
<td>33</td>
<td>16,422</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>32</td>
<td>19,581</td>
<td>5.6</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>5</td>
<td>11</td>
<td>31</td>
<td>17,023</td>
<td>5.5</td>
</tr>
</tbody>
</table>

\(^a^\) Pigs given crude inoculum.

\(^b^\) Pigs given pure cultures of CSM.

\(^c^\) Control pigs.
<table>
<thead>
<tr>
<th>Band neutrophils (mean)</th>
<th>Segmented neutrophils (mean)</th>
<th>Lymphocytes (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>969</td>
<td>4,228</td>
<td>3,003</td>
</tr>
<tr>
<td>551</td>
<td>4,018</td>
<td>6,659</td>
</tr>
<tr>
<td>510</td>
<td>3,365</td>
<td>5,285</td>
</tr>
<tr>
<td>1,692</td>
<td>4,921</td>
<td>5,272</td>
</tr>
<tr>
<td>1,404</td>
<td>2,097</td>
<td>6,536</td>
</tr>
<tr>
<td>1,230</td>
<td>3,484</td>
<td>10,192</td>
</tr>
<tr>
<td>2,147</td>
<td>7,491</td>
<td>4,963</td>
</tr>
<tr>
<td>2,094</td>
<td>6,504</td>
<td>6,559</td>
</tr>
<tr>
<td>1,744</td>
<td>6,556</td>
<td>5,301</td>
</tr>
<tr>
<td>1,912</td>
<td>6,215</td>
<td>5,987</td>
</tr>
<tr>
<td>1,291</td>
<td>3,405</td>
<td>9,288</td>
</tr>
<tr>
<td>2,175</td>
<td>5,459</td>
<td>5,614</td>
</tr>
<tr>
<td>2,452</td>
<td>7,690</td>
<td>5,827</td>
</tr>
<tr>
<td>2,974</td>
<td>5,696</td>
<td>7,613</td>
</tr>
<tr>
<td>2,426</td>
<td>6,179</td>
<td>7,545</td>
</tr>
<tr>
<td>1,049</td>
<td>5,193</td>
<td>4,188</td>
</tr>
<tr>
<td>1,829</td>
<td>2,503</td>
<td>5,353</td>
</tr>
<tr>
<td>1,986</td>
<td>5,983</td>
<td>4,897</td>
</tr>
<tr>
<td>1,159</td>
<td>2,252</td>
<td>5,330</td>
</tr>
<tr>
<td>1,246</td>
<td>3,297</td>
<td>9,947</td>
</tr>
<tr>
<td>1,568</td>
<td>4,375</td>
<td>6,429</td>
</tr>
<tr>
<td>1,240</td>
<td>3,680</td>
<td>10,563</td>
</tr>
<tr>
<td>2,085</td>
<td>5,831</td>
<td>9,311</td>
</tr>
<tr>
<td>1,820</td>
<td>3,903</td>
<td>9,927</td>
</tr>
<tr>
<td>2,923</td>
<td>8,730</td>
<td>6,587</td>
</tr>
<tr>
<td>1,780</td>
<td>9,427</td>
<td>3,041</td>
</tr>
<tr>
<td>1,320</td>
<td>6,998</td>
<td>8,311</td>
</tr>
<tr>
<td>1,142</td>
<td>11,748</td>
<td>3,981</td>
</tr>
<tr>
<td>2,831</td>
<td>12,432</td>
<td>4,076</td>
</tr>
<tr>
<td>1,851</td>
<td>10,808</td>
<td>3,780</td>
</tr>
</tbody>
</table>
Table A3. Hematologic parameters of pigs given crude inoculum, SC, CSM, or uninfected media

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

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).
<table>
<thead>
<tr>
<th>Fibrinogen (mean)</th>
<th>Band neutrophils (mean)</th>
<th>Segmented neutrophils (mean)</th>
<th>Lymphocytes (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>333</td>
<td>1,494</td>
<td>8,992</td>
<td>10,538</td>
</tr>
<tr>
<td>260</td>
<td>2,557</td>
<td>6,109</td>
<td>10,099</td>
</tr>
<tr>
<td>155</td>
<td>1,548</td>
<td>7,478</td>
<td>11,301</td>
</tr>
<tr>
<td>200</td>
<td>3,791</td>
<td>8,299</td>
<td>8,869</td>
</tr>
<tr>
<td>300</td>
<td>3,744</td>
<td>8,997</td>
<td>10,599</td>
</tr>
<tr>
<td>267</td>
<td>3,376</td>
<td>5,913</td>
<td>8,458</td>
</tr>
<tr>
<td>380</td>
<td>13,319*</td>
<td>6,531</td>
<td>5,611</td>
</tr>
<tr>
<td>475</td>
<td>12,844*</td>
<td>8,576</td>
<td>4,978</td>
</tr>
<tr>
<td>400</td>
<td>4,898</td>
<td>7,321</td>
<td>6,174</td>
</tr>
<tr>
<td>200</td>
<td>3,622</td>
<td>8,420</td>
<td>6,728</td>
</tr>
<tr>
<td>267</td>
<td>8,768</td>
<td>5,008</td>
<td>8,406</td>
</tr>
<tr>
<td>400</td>
<td>14,501*</td>
<td>674*</td>
<td>5,218</td>
</tr>
<tr>
<td>150</td>
<td>14,759*</td>
<td>2,870*</td>
<td>5,597</td>
</tr>
<tr>
<td>250</td>
<td>8,813</td>
<td>4,038</td>
<td>8,361</td>
</tr>
<tr>
<td>200</td>
<td>8,936</td>
<td>5,209</td>
<td>7,108</td>
</tr>
<tr>
<td>340</td>
<td>6,473</td>
<td>7,200</td>
<td>9,705</td>
</tr>
<tr>
<td>300</td>
<td>12,655*</td>
<td>5,977</td>
<td>10,130</td>
</tr>
<tr>
<td>250</td>
<td>23,870*</td>
<td>4,763</td>
<td>7,520</td>
</tr>
<tr>
<td>350</td>
<td>8,838</td>
<td>5,142</td>
<td>7,112</td>
</tr>
<tr>
<td>150</td>
<td>2,748</td>
<td>5,442</td>
<td>8,765</td>
</tr>
<tr>
<td>340</td>
<td>5,303</td>
<td>6,222</td>
<td>7,747</td>
</tr>
<tr>
<td>180</td>
<td>5,542</td>
<td>8,418</td>
<td>11,233</td>
</tr>
<tr>
<td>300</td>
<td>6,235</td>
<td>8,667</td>
<td>4,937</td>
</tr>
<tr>
<td>250</td>
<td>7,340</td>
<td>10,913</td>
<td>6,939</td>
</tr>
<tr>
<td>150</td>
<td>4,697</td>
<td>9,219</td>
<td>6,211</td>
</tr>
<tr>
<td>Group</td>
<td>N</td>
<td>Hemoglobin (mean)</td>
<td>Packed cell volume (mean)</td>
</tr>
<tr>
<td>-------</td>
<td>---</td>
<td>------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><strong>Day 15</strong></td>
<td>1</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td><strong>Day 20</strong></td>
<td>1</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td><strong>Day 24</strong></td>
<td>1</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td><strong>Day 28</strong></td>
<td>1</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td><strong>Day 32</strong></td>
<td>1</td>
<td>6</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>Fibrinogen (mean)</td>
<td>Band neutrophils (mean)</td>
<td>Segmented neutrophils (mean)</td>
<td>Lymphocytes (mean)</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------</td>
<td>-----------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>220</td>
<td>6,494</td>
<td>8,337</td>
<td>6,379</td>
</tr>
<tr>
<td>240</td>
<td>6,407</td>
<td>8,527</td>
<td>11,204</td>
</tr>
<tr>
<td>100</td>
<td>3,711</td>
<td>12,559</td>
<td>12,151</td>
</tr>
<tr>
<td>400</td>
<td>3,386</td>
<td>15,400</td>
<td>10,552</td>
</tr>
<tr>
<td>150</td>
<td>5,597</td>
<td>6,524</td>
<td>7,701</td>
</tr>
<tr>
<td>380</td>
<td>3,697</td>
<td>8,205</td>
<td>8,398</td>
</tr>
<tr>
<td>200</td>
<td>4,287</td>
<td>6,400</td>
<td>4,731</td>
</tr>
<tr>
<td>250</td>
<td>2,341</td>
<td>8,823</td>
<td>6,525</td>
</tr>
<tr>
<td>300</td>
<td>1,237</td>
<td>15,885</td>
<td>5,295</td>
</tr>
<tr>
<td>300</td>
<td>2,375</td>
<td>8,530</td>
<td>7,390</td>
</tr>
<tr>
<td>400</td>
<td>6,353</td>
<td>9,815</td>
<td>9,302</td>
</tr>
<tr>
<td>325</td>
<td>2,109</td>
<td>4,182</td>
<td>4,363</td>
</tr>
<tr>
<td>300</td>
<td>1,871</td>
<td>6,591</td>
<td>5,432</td>
</tr>
<tr>
<td>300</td>
<td>3,366</td>
<td>9,692</td>
<td>4,950</td>
</tr>
<tr>
<td>250</td>
<td>2,256</td>
<td>3,992</td>
<td>7,408</td>
</tr>
<tr>
<td>225</td>
<td>6,131</td>
<td>6,499</td>
<td>8,123</td>
</tr>
<tr>
<td>200</td>
<td>2,805</td>
<td>4,680</td>
<td>7,985</td>
</tr>
<tr>
<td>250</td>
<td>3,123</td>
<td>5,416</td>
<td>7,318</td>
</tr>
<tr>
<td>200</td>
<td>2,685</td>
<td>3,401</td>
<td>10,561</td>
</tr>
<tr>
<td>250</td>
<td>1,333</td>
<td>3,252</td>
<td>9,242</td>
</tr>
<tr>
<td>250</td>
<td>4,431</td>
<td>7,352</td>
<td>8,071</td>
</tr>
<tr>
<td>300</td>
<td>6,176</td>
<td>10,036</td>
<td>3,088</td>
</tr>
<tr>
<td>200</td>
<td>3,018</td>
<td>7,452</td>
<td>5,327</td>
</tr>
<tr>
<td>300</td>
<td>3,908</td>
<td>4,660</td>
<td>7,668</td>
</tr>
<tr>
<td>300</td>
<td>3,350</td>
<td>4,351</td>
<td>6,778</td>
</tr>
</tbody>
</table>