ENTEROPATHOGENICITY OF ARCOBACTER BUTZLERI
IN RABBIT AND PIG ILEAL LOOPS

by

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ABSTRACT

The objectives of this two-part study were: (1) to determine whether strains of *Arcobacter butzleri* independently cause enteritis, and (2) to investigate the enteropathogenicity of *Arcobacter butzleri*. For Part I, six well characterized *A. butzleri* isolates were tested using the gut loop assay in rabbits and pigs. Fluid secreted in loops was measured and cultured for *A. butzleri*. In addition, intestinal segments, liver and splenic tissues were collected, cultured, and examined for histopathology. Of the 60 experimental rabbit loops tested, 13% (8/60) produced fluid. Of those fluid-containing loops, 88% (7/8) had been injected with strain 4056. In young pigs, all experimental strains produced fluid amounts significantly different from the positive enteropathogenic *E. coli* control (P<0.05). Only strain 4056 caused fluid accumulation significantly different from the negative PBS control (P<0.03). There was no significant difference in mean fluid accumulation when experimental strains were compared (P>0.05). The major histological change, seen in rabbit and pig loops with fluid secretions, was an infiltration of leukocytes in the gut wall.

In Part II, strain 4056, the most responsive strain from the first part of the study, was tested in the pig ileal loop assay. No fluid accumulation or pathologic changes were observed in intestinal tissues. Restriction enzyme analysis showed two different band patterns for *A. butzleri* present in tissues.

It was concluded that *A. butzleri*, depending on the strain virulence and host susceptibility, can cause mild enteritis with watery diarrhea.
“I can do everything through Him who gives me strength.”

Philippians 4:13

This work is dedicated to my parents,
Mr. & Mrs. Eddie Carter
and my brothers,
William Anthony & Robert Francis Carter
for
their constant love and continued support in my endeavors.
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I. Introduction

In the 1970's, Ellis and colleagues isolated organisms from bovine and porcine fetuses\textsuperscript{15,16} that were originally classified as "aerotolerant campylobacters" because of their morphologically similar characteristics. Further reports by other investigators associated these aerotolerant campylobacters with abortion, diarrhea, and mastitis in livestock as well as enteritis in humans and nonhuman primates.\textsuperscript{23,24,32,40,41,53,56,66,67,74} After extensive biochemical and DNA analysis in 1992, these aerotolerant campylobacters were reclassified to the separate and distinct genus of \textit{Arcobacter}\textsuperscript{70} with four species being described and designated -- \textit{A. cryaerophilus} (which has been subdivided into hybridization groups 1A and 1B), \textit{A. butzleri}, \textit{A. skirrowii}, and \textit{A. nitrofigilis}. Of the four species identified, \textit{A. cryaerophilus}, \textit{A. butzleri}, and \textit{A. skirrowii} have been linked to clinical illness in humans and animals.\textsuperscript{78} \textit{Arcobacter nitrofigilis} has only been isolated from the roots of a \textit{Spartina alterniflora} plant.

Measures have been taken to develop diagnostic tools for the identification of \textit{Arcobacter},\textsuperscript{22,82} but pathogenicity and virulence studies have been very limited. Investigations have shown that \textit{Arcobacter} infections can induce mastitis.\textsuperscript{40} Several studies have been and are presently being conducted to determine the pathogenicity of \textit{Arcobacter}...
spp. in caesarean derived - colostrum deprived (CDCD) piglets.\textsuperscript{79}

However, little testing has been conducted to determine the role of
\textit{Arcobacter} spp. in human and animal enteritis.

\section*{II. Review of the Literature}

\subsection*{A. Historical Background}

\textit{Campylobacter} species have long been associated with infectious
disease in animals, but only recognized as disease-causing entities in human
beings within the past 20 years.\textsuperscript{65} Described as “resembling a vibron”,
these microorganisms were first discovered in 1909 by the veterinarians
McFadyean and Stockman during a survey of epizootic abortion in ewes.\textsuperscript{44}
In 1913, they also implicated \textit{Campylobacter} as the causative agent of sheep
abortion.\textsuperscript{45} Later, in 1918, Smith and Taylor reported similar spirillum-
like microorganisms in association with bovine abortion, and assigned the
name “\textit{Vibrio fetus}” to this abortifacient.\textsuperscript{63} In 1927, Smith and Orcutt
described the isolation of microaerophilic vibrios from the livers and
spleens of calves with diarrhea.\textsuperscript{62} They noted that these calf diarrhea
strains differed serologically from \textit{Vibrio fetus} and speculated that these
organisms might be linked to bovine enteritis. Investigations conducted in
1931 by Jones, Orcutt, and Little later revealed evidence linking these
organisms to winter dysentery in cattle.\textsuperscript{28} They noted that the calf enteritis
strains, though differing slightly in morphology, resembled *Vibrio fetus* enough to be regarded as a closely related group, and proposed the name “*Vibrio jejuni*” for these organisms. Similar microorganisms, later named “*Vibrio coli*”, were isolated and described as the causative agent of swine dysentery by Doyle in 1944.13

Thirty years after their first description as animal pathogens, these microaerophilic vibrios were linked to human infections. In 1946, Levy isolated these microorganisms during an institutional outbreak of gastroenteritis in which approximately 350 people were affected.39 It was suggested that these organisms were identical to *V. jejuni* described earlier by Jones and colleagues. The following year, Vincent reported the isolation of a microaerophilic vibrio that was consistent with *V. fetus* from the blood cultures of three pregnant women, two of whom aborted, with febrile illness of unknown origin.76 In 1957, King coined the term “related vibrios” which was applied to *V. jejuni* and *V. coli*, as they were shown to be antigenically different from *V. fetus*.35 The samples that King studied had been obtained from blood cultures of infants and young children with diarrhea. Only 12 clinical cases of “related vibrio” infection had been identified, but that was to change when appropriate human fecal culturing techniques were developed in 1972 by Dekeyser.9 By 1973, these organisms were designated *Campylobacter* species following DNA studies
performed by Veron and Chatelain.75

*Arcobacter* species have a similar background to *Campylobacter* species with reference to initial isolation and recognition, original classification, and association with infection. *Arcobacter* were also initially recognized in animal cases. In the late 1970’s, Ellis and colleagues referred to these microorganisms as “spirillum and vibrio-like” when first isolated in semisolid *Leptospira* Ellinghausen McCullough-Johnson-Harris (EMJH) isolation media from the internal organs of bovine fetuses and aborted porcine fetuses.15,16 Initial characterization of these aerotolerant organisms by Neill and coworkers in 1979 based on morphological similarities, DNA base composition, and lack of fermentation capabilities, led to their inclusion in the genus *Campylobacter*.52 However, because these microorganisms could grow under aerobic, microaerophilic, and anaerobic conditions (24 hours at 30°C), they were considered as “aerotolerant campylobacters”. In the early 1980’s, these bacteria were recognized in a bovine mastitis case,40 and isolated from bovine preputial washings.20

*Arcobacter* were not recognized in human illnesses until 1988, in part because the microorganisms required a two-stage isolation protocol, and do not grow on routine *Campylobacter* selective media. The bacteria were first isolated from an Australian patient, who presented with
intermittent diarrhea at an AIDS clinic. Later, these aerotolerant *Campylobacter*-like organisms were reported in other cases of intermittent diarrhea, gastroenteritis, bacteremia, and peritonitis associated with appendicitis.32,33

Like *Campylobacter fetus*, these "aerotolerant campylobacters" had been associated with abortion and infertility15,16,23,24,41,56, and diarrheal illnesses, as with *Campylobacter jejuni*.32,53,66,67 In 1991, the genus *Arcobacter* was proposed for these microorganisms70 and by 1992, three species were linked to clinical illness - *A. cryaerophilus*, *A. skirrowii* and *A. butzleri*.78 A fourth species, *A. nitrofigilis*, has not been implicated in disease (Table 1). Although these changes in identification and classification have taken place, few studies have been conducted on *Arcobacter*. It is anticipated that what has been comprehended about the pathogenicity, epidemiology, and virulence mechanisms of *Campylobacter* spp. will clarify the veterinary and medical significance of *Arcobacter* spp. The following review will highlight *Campylobacter* enteritis, and focus on what is currently known about *Arcobacter*, particularly its association with enteric disease.

B. *Campylobacter* Enteritis

Since the early 1900's, *Campylobacter* organisms have been
identified as a cause of diarrhea in livestock, but they were not recognized as an important cause of human diarrheal illness until the 1970’s, when filtration of fecal samples and selective culture media were introduced.58 During the past decade, campylobacteriosis has been confirmed as a significant and economically important foodborne zoonosis by prevalence studies conducted in both industrialized and developing nations.65

Three members of the genus, *C. jejuni, C. coli, and C. lari,* occur in human, mammalian, and avian hosts as both pathogens and commensals.29 *C. jejuni* has been identified as the principal enteric pathogen. Since campylobacters live as commensals in the intestinal tracts of poultry and livestock, *C. jejuni* infection in man has been shown to be associated with the consumption of meat, meat products, and unpasteurized milk.58

Transmission of *C. jejuni* to susceptible flocks and livestock herds has been shown through exposure to insect vectors, rats, wild birds, and fomites including farming equipment and bedding.58 Transmission of *C. jejuni* to humans has been demonstrated by direct and indirect contact with infected pets and through fecal-oral transmission.59,60 In addition, untreated drinking water has been implicated as the source of *C. jejuni* infection for both humans and animals.65

The most common clinical presentation of *C. jejuni* infection is enterocolitis characterized by mucoid to bloody diarrhea with
dehydration. The severity of enterocolitis following infection is dependent on pathogenicity of the strain and the immune status of the host. Gross intestinal lesions include catarrhal to severe hemorrhagic enteritis. Microscopically, lesions consist of edema of the mucosa in mild cases, progressing to mononuclear infiltration of the submucosa and villous atrophy in the most severe cases.

Diagnosis of enteric campylobacteriosis is based on isolation and identification of the organism. Several selective growth media have been developed for Campylobacter, particularly media formulations by Butzler, Skirrow, and Blaser. Direct microscopic examination of fresh feces can be used to visualize the organisms. Dark-field or phase contrast microscopy is preferred to facilitate observation of the characteristic darting, tumbling motility of the organism. Highly sensitive and specific latex agglutination tests have been developed for confirmation of C. jejuni. In addition, DNA probes for C. jejuni have been developed as a diagnostic tool, but have been used minimally in clinical laboratories.

The mechanisms by which C. jejuni causes disease are not well understood. Three mechanisms have been postulated on the basis of clinical symptoms: (1) adherence to the epithelium and production of enterotoxins, inducing secretory diarrhea; (2) invasion and proliferation within the epithelium, inducing cell destruction and inflammatory response
clinically manifested as dysentery and inflammatory diarrhea with fecal leukocytes; and (3) translocation, in which the organism penetrates the intestinal mucosa and proliferates in the lamina propria and mesenteric lymph nodes, with further extraintestinal infections. Frank dysentery and profuse watery-diarrhea may represent two ends of the clinical spectrum or the variation in symptoms may be the result of different mechanisms predominating for different strains, such as those seen for the various types of *Escherichia coli* enteritis. Since individual virulence factors have not been fully determined for *C. jejuni*, pathogenesis remains obscure.

C. Enteric Disease Associated with *Arcobacter*

Enteritis associated with *Arcobacter* has not been well documented in animals, although *Arcobacter* spp., originally designated *Campylobacter cryaerophil*a, have been isolated from normal and diarrhetic stools of livestock.\(^3\)\(^2\)\(^7\)\(^4\) Investigations in nonhuman primates with diarrhea have implicated *Arcobacter butzleri* in the development of colitis. Richardson and colleagues reported the isolation of 12 aerotolerant *Campylobacter* isolates from macaques.\(^5\)\(^4\) Histological evaluations revealed a mild to moderate active colitis. A follow-up study extended Richardson’s investigation by examining 532 clinical diarrheal specimens from 222 nonhuman primates along with 76 colonic specimens obtained from routine
necropsies. During the eight month study, *A. butzleri* was identified in 14 of the 222 animals. Co-infection with *C. coli* or *C. jejuni* was noted in seven of these 14 macaques. Despite the fact that feces from 76 macaques were cultured at necropsy, *A. butzleri* was not isolated from normal feces. *Arcobacter butzleri* was isolated in two necropsy cases in which chronic active colitis was evident upon histologic examination. In a third case, co-infection with *C. jejuni* was observed.

*Arcobacter* in humans was first isolated in 1988 from a 35-year old male with a history of intermittent diarrhea. Other enteric organisms were recovered from stool samples, but on one occasion, a *Campylobacter cryaerophilus* strain, later identified as *A. butzleri*, was isolated in the absence of other enteric pathogens, and in association with abdominal pain. Lerner reported two individual cases of prolonged, persistent diarrhea, 12 days and three weeks, respectively, accompanied by abdominal cramps. In both cases, patients had previously diagnosed chronic conditions (diabetes and alcoholism), and *A. butzleri* was the only enteric pathogen isolated. After antibiotic therapy, acute symptoms quickly subsided. After termination of antibiotic treatments, *A. butzleri* was no longer cultured from stool samples.

In 1991, Kiehlbauch and coworkers examined several aerotolerant *Campylobacter* strains from human feces, blood and peritoneal fluids, and
from nonhuman primate feces. Three human blood isolates were from patients who had aspirated fecal contaminated fluid and one human fecal isolate came from a patient with acute gastroenteritis. These isolates were all identified as *Arcobacter (Campylobacter) cryaerophilia*. Half of the human fecal isolates used in this study were from a study conducted by Taylor in which atypical *Campylobacter* strains were isolated from the feces of Thai children with diarrhea. Fifteen isolates from 17 children were identified as *A. butzleri*.

In a retrospective study conducted by Kiehlbauch, Tauxe, and Wachsmuth, of 29 human patients from whom *Arcobacter (Campylobacter) butzleri* was isolated, 22 had reported frequently severe or prolonged diarrhea, four others had bacteremia, and from three patients *A. butzleri* was cultured from peritoneal fluid following acute appendicitis. Epidemiological surveys linked exposure to drinking contaminated water during travel. *Arcobacter butzleri* has been isolated from two contaminated water sources --a drinking water reservoir in eastern Germany and in canal waters of metropolitan Bangkok, Thailand.

*Arcobacter butzleri* has also been associated with an outbreak of recurrent abdominal cramps in an Italian nursery school. The ten children that were affected had no diarrhea or fever, but *A. butzleri* was cultured from the feces of all the children. All isolates were the same
serotype and no other enteric pathogens were isolated. Case timing suggested person-to-person transmission.

Meat and meat product consumption may be a potential means of human infection with *Arcobacter*. A recent survey of meat processing plants in Iowa was conducted in which samples of ground pork products and turkey skins were obtained. Arcobacter spp. were isolated from approximately 90% of the samples.

Although *Arcobacter* spp., particularly *A. butzleri*, appear to be linked to diarrheal illness, very few published studies have investigated their enteric effects. One study has indicated that *Arcobacter* spp. can colonize the gut mucosa of caesarean-derived colostrum deprived neonatal piglets. Arcobacter butzleri and *A. cryaerophilus* 1B have been shown to colonize the gut more than *A. skirrowii* and *A. cryaerophilus* 1A, based on fecal shedding and isolation of organisms from tissues.

Potential pathogenic mechanisms of *A. cryaerophilus* have been investigated by Fernández and colleagues. Two strains of *A. cryaerophilus* (7625 and 62C) were shown to invade HEp-2 cells and cell-free supernatants of both strains were found to cause fluid accumulation and increase electrolyte concentrations in the rat ileal loop assay.

Since *Arcobacter butzleri* has been incriminated in cases of both animal and human diarrheal illness, this species was chosen as the focus for
this study. The objectives of this study were: (1) to determine whether strains of *A. butzleri* independently cause enteritis, and (2) to investigate the enteropathogenicity of *A. butzleri*. Six *A. butzleri* isolates were tested using the gut loop assay in rabbits and pigs. The research was performed at the AAALAC-accredited animal facility of the USDA-ARS-National Animal Disease Center in Ames, IA, and was conducted in two parts. Part I, which was completed during the summer of 1994, focused on the enteropathogenicity of six different *A. butzleri* strains. In Part II, which was completed during the summer of 1995, efforts were concentrated on evaluating the effects of the most responsive strain from Part I.

III. **Materials and Methods**

A. Animals

Conventionally reared animals were used for this study. Thirteen New Zealand White rabbits, (nine males/four females, 10 weeks old, average weight 2.1 kg) used in Part I of the study, were obtained from Small Stock Industries, Inc., Pea Ridge, AK. Mixed breed pigs used in parts I and II of the study were provided by H&K Enterprise, Nevada, IA. Pigs were grouped in two sets according to age. Three 7-8 week old males (average weight - 13.6 kg) and six (four males and two females) 3-4 week
old pigs (average weight - 4.8 kg) were used in Part I. In Part II, two 7-8 week old male pigs (average weight - 15.2 kg) and two 3-4 week old male pigs (average weight - 7.65 kg) were used. All animals were tested for presence of *Arcobacter* prior to experimentation as follows. Each animal was rectally swabbed with sterile cotton-tipped applicators, which was placed in a plastic screw-capped test tube containing 20 ml of semisolid *Leptospira* selective medium, Ellinghausen-McCullough-Johnson-Harris Polysorbate-80 (EMJH P-80) with 5-fluorouracil (100 μg/ml). This medium, which does not support the growth of *Campylobacter* under the incubation conditions employed, was originally used to isolate and enrich for *Arcobacter*. After incubation (30°C for 48 hours), samples were examined via dark-field microscopy for the morphology (comma or corkscrew shape) and rapid darting motility characteristic of *Arcobacter* by placing a drop of the media on a slide and scanning 15 to 20 fields at 40X magnification. All animals tested negative for the presence of *Arcobacter* in rectal swab cultures.

B. Strains

The origin of the six well-characterized *Arcobacter butzleri* strains used in the study is shown in Table 2. Strains 3554, 3556, 3569 and 3571 were kindly provided by Charlotte Patton of the Centers for Disease
Control, Atlanta, Georgia. The human strains, 3554 and 3556, were recovered from two individuals with diarrhea in Colorado and Kansas.\textsuperscript{32} Strains 3569 and 3571 were isolated from diarrhetic primates housed at the Yerkes Primate Research Center, Atlanta, Georgia.\textsuperscript{32} The porcine strains, 4056 and 4057, were recovered from an Iowa hog farm with reproductive problems.\textsuperscript{79}

Bacteria from frozen stock cultures were grown on brain heart infusion agar (BHIA) with 10% defibrinated bovine blood (blood agar), and incubated microaerobically (5% O\textsubscript{2}, 10% CO\textsubscript{2}, 85% N\textsubscript{2}) at room temperature for two days. Positive control cultures, \textit{Vibrio cholerae} 395 (used for rabbits) and enteropathogenic \textit{Escherichia coli} 263, serotype O8:K87,K88a,b:H19 (used for pigs), were also grown on BHIA with 10% defibrinated bovine blood and incubated aerobically at room temperature for two days. Both positive control strains are known to cause diarrhea by enterotoxin production and have been experimentally shown to cause fluid accumulation in ileal loops. Two different positive controls were used because \textit{E. coli} 263, which was initially available at the beginning of the study, produces variable fluid accumulation responses in rabbits.\textsuperscript{48}
C. Inoculum Preparation

*Arcobacter butzleri* cultures previously plated on blood agar plates were used to develop biphasic cultures of the strains. Due to the slow growth of *Arcobacter* spp., biphasic cultures were necessary to produce large numbers of the bacteria within a 24-hour time period. Colonies were picked from blood agar plates and inoculated in 10 ml of brain heart infusion broth (BHIB). The inoculated broth was pipetted into a 250 ml tissue culture flask containing 10% defibrinated bovine blood agar. An additional 10 ml of BHI broth was also pipetted into the blood agar based tissue flask. The inoculated biphasic cultures were placed on an Orbital shaker (Bellco Glass, Inc. Vineland, NJ) and incubated at 37°C for 24 hours. Prior to inoculation, a gaseous mixture of 5% O₂, 10% CO₂, and 85% N₂ was added to the flasks to optimize growth conditions. *Vibrio cholerae*, the positive control for rabbits, was amplified as follows. A loopful of the culture from blood agar plates was inoculated into two 10 ml glass test tubes containing 7.5 ml of Mueller-Hinton broth. Inoculated tubes were incubated at 37°C for 24 hours. A loopful of *E. coli*, the positive control for pigs, was also inoculated into two glass test tubes containing Mueller-Hinton broth and incubated (37°C, 30 minutes). After incubation, test tube cultures and the liquid portion of the biphasic cultures were
pipetted into sterile 50 ml Oak Ridge-style centrifuge tubes and pelleted (Sorvall Superspeed Automatic Refrigerated Centrifuge - Newtown, CT) at 9,000 rpm for 30 minutes. The supernatant was decanted and bacterial pellets were resuspended in 20 ml of 0.1 M phosphate-buffered saline (PBS), pH 7.4, and recentrifuged (9,000 rpm for 30-45 minutes). Additional centrifugation was performed if bacterial pellets were not completely formed. After centrifugation, the supernatant was decanted and bacterial pellets were resuspended in 15 ml of 0.1 M PBS, pH 7.4. The suspension was drawn into sterile 3 cc syringes with 20 gauge 1-1/2 inch needles. An aliquot (1 ml) sample was taken for enumerating bacteria present in the inoculum.

D. Inoculum Bacterial Count

Bacterial concentrations for all strains (A. butzleri, V. cholerae, and E. coli) were determined as follows. Mueller-Hinton broth (4.5 ml) was pipetted into 2 dram glass vials. Fourteen vials were used for each sample. One half milliliter of the inoculum was pipetted into the first vial. The vial was vortexed, 0.5 ml was taken from the vial, and placed into the second vial. Ten-fold serial dilutions were repeated in subsequent vials for up to seven dilutions. One hundred microliters was plated on blood agar for the last three dilutions (10⁵, 10⁶, 10⁷) and streaked for colony isolation. Plates
were inverted, placed into a sterile desiccator, and incubated under microaerophilic conditions (room temperature, 48 hours). After incubation, colonies per plate were enumerated. The average of two plate counts for each strain was used to determine colony-forming units per milliliter (CFU/ml).

E. Rabbit Ligated Ileal Loop Model

Rabbits were fasted 24 hours prior to surgery and given water *ad libitum*. The rabbits were preanesthetized with 100 mg Ketaset (Ketamine Hydrochloride - Aveco Co, Inc., Fort Dodge, IA) and 15 mg Rompun (Xylazine - Miles Inc., Shawnee Mission, KS). The right flank area was shaved from the spine to the abdominal midline and the paralumbar fossa to the last rib. The surgical area was cleaned and swabbed with Betadine and Nolvasan. Halothane (Halocarbon Laboratories, River Edge, NJ) was administered via nose cone to induce surgical plane of anesthesia. Rabbits were placed on a heating pad to prevent hypothermia and sterile plastic drapes were used to cover the prepped area. A vertical skin incision approximately 8 cm long, was made in the paralumbar region. The muscle layers were cut to allow entry into the abdominal cavity. The appendix was located and externalized along with the ileal portion of the small intestine. The first ligature of sterile nylon was placed approximately 20 cm from the
tip of the appendix. Extending cranially, the ileum was tied off in eight long segments (experimental loops) approximately 6 to 8 cm long, separated by seven short segments (interloops), which were approximately 2 to 4 cm long. A 1 ml sample was injected slowly into the experimental loop lumens with a 26-gauge needle. After all loops were inoculated, the gut was rinsed with heparinized saline and returned to the abdominal cavity. Muscle layers were closed with 2-0 chromic gut in a simple continuous suture pattern and the skin was closed with 4-0 Vetafil in a Ford-interlocking pattern. Rabbits were returned to cages, given water ad libitum, and monitored every 4 hours post-operatively. Torbugesic (Butorphanol tartrate - Aveco Co, Inc., Fort Dodge, IA), 0.3 mg, was administered intramuscularly for analgesia every 4 hours. After 18 hours, rabbits were euthanized by intracardiac administration of Sleepaway (Sodium Pentobarbital - Fort Dodge Laboratories, Fort Dodge, IA). The abdominal cavities were observed for any gross changes, the small intestines were removed and interloops were examined for leakage.

F. Pig Ligated Ileal Loop Model

The procedure for pig ileal loop assay was similar to that described for the rabbit ligated ileal loop model. Halothane (Halocarbon Laboratories, River Edge, NJ) was administered via nose cone to induce
surgical plane of anesthesia. The left flank was cleaned and swabbed with Betadine and Nolvasan. A vertical incision, approximately 20 cm, was made in the paralumbar region through the skin and musculature. The small intestine was externalized. Ligatures were placed every 6 to 11 cm in length beginning at a site 100 cm distal to the ligament of Trieste (anterior-dorsal attachment. In 7-8 week old pigs, 24 loops were tied in Part I and 10 loops were tied in Part II; whereas, 12 and 10 loops were tied respectively for Parts I and II in the 3-4 week old animals.

After all loops were inoculated, the gut was rinsed with heparinizined saline and returned to the abdominal cavity. The muscle and skin layers were sutured, pigs were returned to cages, given water *ad libitum*, and monitored every 4 hours post-operatively. Torbugesic (Butorphanol tartrate - Aveco Co, Inc., Fort Dodge, IA), 0.3 mg, was administered intramuscularly for analgesia every 4 hours. After 18 hours, pigs were euthanized by intracardiac administration of Sleepaway (Sodium Pentobarbital - Fort Dodge Laboratories, Fort Dodge, IA). The abdominal cavities were observed for any gross changes, the small intestines were removed, and interloops were examined for leakage.

G. Fluid Accumulation Ratio Determination

Accumulated fluid in loops was removed using an 18-gauge needle
and a sterile syringe, and placed in sterile plastic test tubes. The amount of fluid accumulated and the lengths of the loops were measured, and the ratio of the fluid (milliliters) to length (centimeters) was calculated (ml/cm).

H. Isolation and Identification of Bacteria

Fluid samples, liver and splenic tissue samples were harvested from both rabbits and pigs to culture and isolate Arcobacter. Samples were placed in individual vials containing 20 ml of semisolid Leptospira selective medium EMJH P-80 and incubated at 30°C for 48 hours. Cultures were then viewed via dark-field microscopy for typical Arcobacter morphology and motility. In addition, polymerase chain reaction (PCR) and restriction enzyme analysis (REA) were used to detect and confirm Arcobacter sp. and Arcobacter butzleri respectively, in Part II. Procedures for PCR were performed as described by Harmon.22 One hundred and fifty microliters of the P-80 medium samples were placed in PCR reaction tubes and heated for 15 minutes at 110°C (Lab-Line Multi-Blok Heater, Melrose Park, IL). The samples were then centrifuged for one minute at 12,400 rpm (Fisher Scientific Micro-Centrifuge Model 235C). Serving as the DNA template, five µl of each sample were amplified. Amplification was performed in a 50 µl volume containing approximately 5.0 ng template DNA, 50 pmole each of primers Arco I and Arco II, 1.25 U of Taq DNA
polymerase (Boehringer Mannheim Biochemicals, Indianapolis, IN), 200 μmol⁻¹ of dATP, dCTP, dTTP, and dGTP, 10 mmol⁻¹ Tris-HCl, 50 mmol⁻¹ KCl, and 1.5 mmol⁻¹ MgCl₂. The reaction mixture was overlaid with sterile mineral oil (Sigma, St. Louis, MO), and amplified in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). The samples were subjected to an initial denaturation step (94°C for four minutes), followed by 25 amplification cycles. Each cycle consisted of one minute at 94°C (denaturation), one minute at 58°C for primers Arco I and Arco II (primer annealing), and one minute at 72°C (primer extension). A primer extension step (72°C for seven minutes) followed the final amplification cycle. PCR reaction products were detected by electrophoretic separation (60 V, 1.5 hours) on a 1.5% agarose gel (Seakem ME agarose, FMC Bioproducts, Rockland, ME) in a horizontal gel bed with TBE (90 mmol⁻¹ Tris, 2 mmol⁻¹ EDTA, 90 mmol⁻¹ boric acid, pH 8.5) as the running buffer. DNA molecular weight marker IX (ØX174/Hae III, Boehringer Mannheim Biochemicals) was included for base pair size comparison. The gel was then stained with ethidium bromide, visualized with shortwave UV light, and photographed.

Bacterial DNA for restriction enzyme analysis (REA) was extracted as follows. P-80 tissue samples found positive for Arcobacter based on
PCR were filtered onto blood agar plates, streaked for colony isolation, and incubated microaerobically at room temperature for two days. *Arcobacter*-characteristic colonies, white and pinpoint, were picked and streaked on blood agar plates for amplification (five plates per sample). The plates were incubated (24°C, two days) and bacterial cells were harvested. Harvesting was accomplished by pipetting 5 ml of sterile PBS, pH 7.4, onto each plate and gently raking colonies from the agar. The bacterial suspension was pipetted from plates (25 ml for each sample), placed in sterile Oak-Ridge style centrifuge tubes, and pelleted at 9,000 rpm (30 minutes, 4°C). The supernatant was decanted and bacterial pellets were resuspended with 600 µl of TE Sucrose (25%) in Beckman ultracentrifuge tubes. Samples were placed in a -20°C freezer overnight. Samples were thawed and transferred into polyallomar ultracentrifuge tubes. Lysozyme (Sigma Chemical Co., St. Louis, MO) was prepared, 60 mg to 3 ml of TE Sucrose, and added (130 µl) to each bacterial cell suspension. Samples were placed in ice for 15 minutes for cell wall lysis. One hundred and thirty microliters of EDTA, pH 8.0, was added to each sample, followed by 70 µl of 10% sarcosine, and 10 µl of proteinase K. The tubes were covered with aluminum foil and incubated overnight at 65°C. After incubation, each ultracentrifuge tube was filled with cesium chloride, air bubbles were removed, and tubes sealed. Samples were then centrifuged at 65,000 rpm.
for 3-4 hours. Gradients were tapped into 5 ml snap cap polystyrene tubes. Samples were dialyzed extensively against TE buffer with three changes over two days.

REA was conducted as described by Wesley et al. DNA (2 μg) for each sample was digested (3-4 hours, 37°C) with PvuII in a buffered 20-μl reaction mixture. After digestion, 5 μl of tracking dye (0.1% bromophenol blue, 20% Ficoll type 400) was added to each sample, and DNA fragments were separated on 1.0% agarose (16 hours, 60 V) in a horizontal gel. At the completion of electrophoresis, the gel was stained with ethidium bromide and photographed.

I. Histopathology

Experimental ileal loops were harvested from both rabbits and pigs for histological examination. In addition, liver and splenic tissues were taken to evaluate possible pathological changes associated with Arcobacter. Tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned, and mounted on microscopic slides. Tissue sections were stained with both hematoxylin/eosin (H&E) and Warthin-Starry (silver) stains. H&E stain was used as the primary stain to observe pathology. Silver stain was used to visualize organisms in the tissues.
J. Splenic Tissue Bacterial Enumeration

This procedure was performed in Part II of the research. Splenic tissues (2.4 g to 4.2 g) were minced and placed in preweighed, 50 mL sterile conical centrifuge tubes (Blue Max, Becton Dickson, Lincoln Park, NJ). The tubes were reweighed and PBS was added to dilute ten times the weight of the splenic tissue (i.e., if splenic tissue weighed 2.4 g, then 24 mL of PBS was added). The splenic suspension was homogenized using a tissumizer (Tekmar Co., Cincinnati, OH). In separate 5 mL plastic test tubes, 1.8 mL of PBS was placed. A total of 8 small PBS tubes were set up for ten-fold serial dilutions for each splenic suspension. The splenic suspension (200 μL) was pipetted into the first tube. The tube was vortexed, and 200 μL was taken from the tube and aliquoted into the second vial. This was repeated in subsequent vials for four dilutions (10^1, 10^2, 10^3, 10^4). One hundred microliters was plated on modified CIN (celfulodin-irgasan-novobiocin) and Campy CVA (cefoperazone-vancomycin-amphotericin B) selective Arcobacter media.7 Plates were incubated (room temperature, 48 hours) and colonies enumerated.

K. Statistical Analysis

Fluid accumulation ratios were calculated and averaged for each Arcobacter strain, as well as for the positive and negative controls. Results
are presented as mean values. Mean values for experimental strains are compared to mean values for positive and negative controls. A two-way Analysis of Variance for an incomplete block design was used to examine strain differences in fluid accumulation for the gut loop assays. Least-squares means were calculated for each strain and compared using the Least Significant Difference method (LSD). Results were considered statistically significant at P<0.05.

IV. Results
A. Fluid Accumulation - Part I

In rabbits, the ileal-loop assay was performed in two sets of six rabbits each. A total of eight loops was tied in each rabbit. Six loops were inoculated with experimental strains, one with 1 ml of sterile PBS (negative control) and one with a 1 ml suspension of V. cholerae cells (positive control).

One rabbit was eliminated (one found dead, one euthanized due to moribund condition) from each set. Fluid, red tinged and thick, was noted in only the V. cholerae positive control loops for all the rabbits including the two that were eliminated from the study. Of the remaining ten rabbits, only four had A. butzleri experimental loops with fluid (clear to yellowish, slightly viscous). A total of eight (13%) experimental loops out of the 60
loops tied were found containing fluid (Table 3). Of the eight fluid-containing loops, seven (88%) contained strain 4056. The average fluid accumulation for strain 4056 was found to be statistically significant when compared to that of the V. cholerae positive control and the PBS negative control (P<0.01). This strain was then inoculated (six experimental loops) into rabbit #296 using the same experimental technique and no fluid accumulation was observed (Table 3).

Twenty-four loops were tied in the 7-8 week old pigs. For each animal, two loops were injected with 1 ml of sterile PBS, pH 7.0 (negative control) and two loops were injected with a 1 ml suspension of E. coli cells (positive control). Two strains were used in each animal (ten loops per strain). There was no measurable fluid accumulation for any of the A. butzleri strains (Table 4). Only the E. coli positive control loops produced fluid.

For the 3-4 week old pigs, 12 loops were tied in each pig. Two loops were used as positive and negative controls, and two strains were used to inoculate the remaining ten loops (five loops per strain). Clear to yellowish, slightly viscous fluid was noted in A. butzleri experimental loops for four of the six pigs (Table 5). As shown in Table 6, all experimental strains produced fluid amounts significantly different from the E. coli positive control (P<0.01 and 0.04). Only strain 4056 produced fluid that
was significantly different from the negative PBS control (P<0.03). There was no significant difference in mean fluid accumulation when experimental strains were compared (P>0.05). Based on the average fluid accumulation, strain 4056 produced the most significant amount of fluid in loops of all the experimental strains.

B. Bacterial Isolation and Identification - Part I

Nine of 13 (69%) rabbits’ spleens and all fluid and intestinal samples were positive for *Arcobacter* based on dark-field microscopy (Table 3). Rabbit liver samples were culture-negative for *Arcobacter*. All three (100%) splenic samples from 7-8 week old pigs were positive for *Arcobacter* (Table 4). Of the six 3-4 week old pigs, only one (17%) of the six spleens and all fluid samples were positive (Table 5). *Arcobacter* was not cultured from any pig liver samples.

C. Histopathology - Part I

Tissue sections of experimental rabbit ileal loops that contained fluid showed some pathologic changes when compared to negative control loop samples. Numerous heterophils were present in the lamina propria and submucosa of the ileum. Villous blunting, mucosal edema and an increase in intraepithelial lymphocytes within the villi were also noted.
Campylobacter-like organisms could not be visualized in intestinal tissues prepared with both hematoxylin/eosin and silver staining. Liver and spleen samples revealed no pathological changes and organisms were not visualized in Arcobacter-positive splenic tissues. Tissues obtained from 7-8 week old pigs, including experimental loop specimens, revealed no pathology microscopically. In the 3-4 week old pig group, mild to moderate villous blunting in experimental loops was observed (Figures 3-5) when compared to the negative PBS control (Figure 1) and the positive E. coli control (Figure 2). The major histologic change noted in experimental ileal loops, when compared to control loops (Figures 6 & 7), was an increased infiltration of leukocytes (lymphocytes, neutrophils, and plasma cells) in the lamina propria, submucosa, serosa, and the crypts of the ileum (Figures 8-10).

D. Fluid Accumulation - Part II

No fluid was produced by strain 4056 in any ileal loops for 7-8 week old pigs and 3-4 week old pigs. Fluid accumulation was observed in only E. coli positive control loops for both groups of pigs (Table 7).

E. Bacterial Isolation/Identification - Part II

Arcobacter was confirmed in intestinal segments and splenic tissues
for the two 3-4 week old pigs by dark-field microscopy and PCR (Figure 11). Restriction enzyme analysis showed two different band patterns for *A. butzleri* in tissues of 3-4 week old pigs (Figure 12). The DNA pattern of the inoculum (lane 4056) was different from the DNA profiles of *Arcobacter butzleri* recovered from the ileal loops and splenic tissues. Ileal loops from the two 7-8 week old pigs were cultured and found to be positive for *Arcobacter* by dark-field microscopy and PCR. Splenic tissues for these pigs were cultured, but *Arcobacter* was not isolated. *Arcobacter* was also not isolated from any of the pigs’ liver samples.

F. Histopathology - Part II

No histological changes were noted in any tissues (intestine, liver, spleen) for the four pigs inoculated with strain 4056.

G. Bacterial Enumeration of Splenic Tissue

Ten-fold serial dilutions of culture - positive splenic tissue suspensions were prepared, but bacterial colony numbers were not sufficient to be enumerated.
V. Discussion

The major purpose of this two-part study was to determine whether *Arcobacter butzleri*, when experimentally introduced into the gut, could cause enteritis. Factors used to assess enteritis in the two animal species were fluid accumulation and histopathology.

In the first part of the study, four (31%) out of thirteen rabbits responded with fluid accumulation. No fluid was observed in any loops of the 7-8 week old pigs, but all (100%) six 3-4 week old pigs produced measurable fluid responses. Fluid accumulation was not evident for any pigs used in the second part of the study. Although experimental conditions were the same in all cases (husbandry, surgical protocol, necropsy time, etc.), responses were not uniform probably due to inherent individual differences within animal groups. These individual differences, such as genetic variation or gut microflora interaction, can directly affect susceptibility.

The rabbit ileal loop assay, a standard test system for evaluating enteric pathogens, has shown variable fluid accumulation responses when used to evaluate pathogenesis of *C. jejuni*. Oral challenge studies have shown that rabbits develop an immune response and naturally clear *C. jejuni*; therefore, colonization resistance to *A. butzleri* may also be apparent. Gut microflora interaction has also been implicated in affecting
susceptibility. It has been shown that older animals have a more stable gut microflora that may inhibit enteric pathogens, and when exposed to the latter, are less likely to show clinical disease than younger animals. Pathogenic studies of *C. jejuni* have shown that animals become less susceptible to subsequent *C. jejuni* infections. In addition, adult animals have more leukocytes present within the gut that readily phagocytize potential pathogens. For these reasons, susceptibility to and colonization by *A. butzleri* would explain the fluid responses observed in the 3-4 week old pig group when compared to the 7-8 week old pig group.

Strain virulence differences have produced variable fluid responses in ileal loop assays for a number of enteric pathogens. The different mean fluid accumulation ratios in this study seem to imply virulence variations in *A. butzleri* strains as well. In the first part of the study, loops in the 10 week old rabbits were not as responsive as loops in the 3-4 week old pigs. Fluid accumulation, when observed, was mainly associated with strain 4056. In the 3-4 week old pigs, all six *A. butzleri* strains induced fluid secretion into the loop when compared with the PBS negative control, but to a lesser extent than that induced by *E. coli* in the positive control loops. There was no significant difference in fluid accumulation when *A. butzleri* strains were compared (P>0.05). However, the highest mean fluid accumulation ratio and total number of responsive loops were again
observed to be associated with strain 4056. Lack of fluid secretion in the second part of the study may be attributed to several variables, including reduced virulence of the strain. When the second part of the study was performed, difficulty in growing adequate bacterial cell numbers was experienced. Multiple biphasic cultures of strain 4056 were set up and broths of those cultures were combined to achieve cell numbers of \(10^8\). Therefore, the age of the stock culture may have affected the viability, virulence, and pathogenicity of the strain.

Restriction enzyme analysis of *Arcobacter butzleri* cultured from tissues obtained from the 3-4 week old pigs in part II revealed two different DNA patterns. The different patterns may indicate previous exposure to another *A. butzleri* strain which could reduce susceptibility by competitive exclusion. Another explanation of the different DNA patterns may be that upon enriching for bacterial isolation and cultivating colonies for DNA analysis, the genetic makeup of the bacteria may have been altered leading to a loss of virulence properties.

Histopathologic changes were only observed in loops containing fluid for both rabbits and 3-4 week old pigs. When compared to negative PBS control loops, the *A. butzleri* strains caused increased leukocytic infiltration and mild villus blunting, but to a lesser extent when compared to *V. cholerae* or *E. coli* positive control loops. Mucosal hemorrhage was
not apparent with *A. butzleri* strains as with *V. cholerae*. These pathological changes with the fluid secretion, indicative of watery diarrhea, suggest that *A. butzleri* can cause mild inflammatory enteritis.

Isolation of *A. butzleri* in splenic tissues from both rabbits and pigs suggests that this organism has invasive properties. Dissemination to the spleen involves penetration of the intestinal mucosa and bacteremia. It has been shown that there are elements of active intestinal fluid secretion in *Campylobacter* diarrhea that result from acute intestinal inflammation following tissue invasion. This may be similar for *A. butzleri*, but further studies are warranted. An *in vitro* trial study has shown that one field isolate (4056) of *A. butzleri* to be invasive in INT407 cells which is relatively significant given the lesser invasive results of present *Campylobacter* isolates tested in this system.

In conclusion, *A. butzleri*, as the sole pathogen, can cause mild enteritis with watery diarrhea depending on the strain virulence and perhaps age-dependent host susceptibility. Further study is needed to examine the pathophysiological mechanisms of *A. butzleri*. Research should be directed toward: (1) isolation/identification of the most pathogenic strain(s), (2) assessment of the minimum effective dose of bacteria needed to induce enteritis, (3) studies of cell invasiveness, and (4) elucidation of the interaction of *Arcobacter* with resident gut microflora.
Table 1. Bacterial species of the genus *Arcobacter* and their associations*

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<tr>
<th>Species</th>
<th>Main hosts</th>
<th>Association</th>
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</thead>
<tbody>
<tr>
<td><em>A. nitrofigilis</em></td>
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<td>Roots of salt marsh plants; no known animal host</td>
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<tr>
<td><em>A. cryaerophilus</em></td>
<td>cattle, pig, sheep</td>
<td>Aborted bovine, porcine, ovine, and equine fetuses; bovine mastitis; children with diarrhea in developing countries</td>
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<tr>
<td><em>A. butzleri</em></td>
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<td>aborted porcine fetuses; domestic animals with diarrhea; children with diarrhea &amp; abdominal pain syndrome</td>
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<tr>
<td><em>A. skirrowii</em></td>
<td>cattle, sheep, pig</td>
<td>Preputial fluids of bulls; aborted bovine, porcine, ovine fetuses; domestic animals with diarrhea</td>
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* adapted from Skirrow (59).
Table 2. Strains of *Arcobacter butzleri*.

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- <sup>a</sup> Referenced in this study as listed with NADC
- <sup>b</sup> Original classification of strain
- <sup>c</sup> Stool samples (S); rectal swabs (RS)
- <sup>d</sup> Area where strain was originally isolated
- <sup>e</sup> CDC strains from Kiehlbauch et al. (32); NADC strains from Wesley et al. (79)
### Table 3. Summary of Results for the Rabbit Ileal Loop Assays - Part I.

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<tr>
<th>Animal Number</th>
<th>Inoculum (Strains)</th>
<th>Bact. Count in Inoculum ((10^8))</th>
<th>No. Loops with Fluid</th>
<th>Total Fluid Accum. (ml/cm)</th>
<th>Arcobacter (+) Tissues</th>
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* - Died during study

a - 395 (V. cholerae) positive control
Table 3 continued. Summary of Results for the Rabbit Ileal Loop Assays - Part I.

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<td>3556</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>ileum, spleen</td>
</tr>
<tr>
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<td>3571</td>
<td>6.8</td>
<td>0</td>
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<td>395</td>
<td>7.1</td>
<td>1</td>
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</tr>
<tr>
<td>294*</td>
<td>3556</td>
<td>4.0</td>
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<td>6.8</td>
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<td>0</td>
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<td>4057</td>
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<td></td>
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<tr>
<td></td>
<td>395</td>
<td>7.1</td>
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</tr>
<tr>
<td>296</td>
<td>4056</td>
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<td>ileum, spleen</td>
</tr>
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Table 4. Summary of Results for the 7-8 week old Pig Ileal Loop Assays - Part I.

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<th>Animal Number</th>
<th>Inoculum (Strains)</th>
<th>Bact. Count in Inoculum (10^8)</th>
<th>No. Loops with Fluid</th>
<th>Total Fluid Accum. (ml/cm)</th>
<th>Arcobacter (+) Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>1127</td>
<td>3569</td>
<td>4.3</td>
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<td>ileum, spleen</td>
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<tr>
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<td>4057</td>
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</tr>
<tr>
<td></td>
<td>263(^a)</td>
<td>1.0</td>
<td>2</td>
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</tr>
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<td>263</td>
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\(^a\) - 263 (E. coli) positive control
Table 5. Summary of Results for the 3-4 week old Pig Ileal Loop Assays - Part I.

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<th>Animal Number</th>
<th>Inoculum (Strains)</th>
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<th>No. Loops with Fluid</th>
<th>Total Fluid Accum. (ml/cm)</th>
<th>Arcobacter (+) Tissues</th>
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<td>4056</td>
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<td>4</td>
<td>2.4</td>
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<td></td>
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<td>1.2</td>
<td>1</td>
<td>1.7</td>
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<td>0.3</td>
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</tr>
<tr>
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<td>5</td>
<td>2.5</td>
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<tr>
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<td>6.0</td>
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</tr>
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<td>1</td>
<td>1.2</td>
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</tr>
<tr>
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<td>PBS</td>
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<td>ileum, spleen</td>
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<td>3571</td>
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<td>5</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
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<td>5</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
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<tr>
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<td>1.9</td>
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<td>5</td>
<td>1.8</td>
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</tr>
<tr>
<td></td>
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<td>0.5</td>
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<tr>
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<td>ileum</td>
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<td>3569</td>
<td>5.0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td></td>
<td>263</td>
<td>0.4</td>
<td>1</td>
<td>2.0</td>
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</tr>
</tbody>
</table>

\textsuperscript{a} - 263 (E. coli) positive control  
\textsuperscript{b} - PBS (sterile phosphate-buffered saline) negative control
Table 6. Statistical Analysis of Fluid Accumulation Results in 3-4 week old Piglets

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample Size</th>
<th>Fluid Accum. Mean</th>
<th>Comparison</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>3556</td>
<td>n=10</td>
<td>0.27</td>
<td>3556 vs 4056</td>
<td>0.10</td>
</tr>
<tr>
<td>4056</td>
<td>n=10</td>
<td>0.57</td>
<td>3556 vs PBS</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3556 vs 263</td>
<td>0.01*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4056 vs PBS</td>
<td>0.03*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4056 vs 263</td>
<td>0.04*</td>
</tr>
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<td>n=10</td>
<td>0.21</td>
<td>3571 vs 4057</td>
<td>0.78</td>
</tr>
<tr>
<td>4057</td>
<td>n=10</td>
<td>0.26</td>
<td>3571 vs PBS</td>
<td>0.56</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>3571 vs 263</td>
<td>0.01*</td>
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<td></td>
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<td>4057 vs 263</td>
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</tr>
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<td>0.66</td>
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<td>3569 vs 263</td>
<td>0.01*</td>
</tr>
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<td>PBSa</td>
<td>n=6</td>
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<td>PBS vs 263</td>
<td>0.01*</td>
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<tr>
<td>263b</td>
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* - significant difference
a - negative control
b - positive control
Table 7. Summary of Results for the Pig Ileal Loop Assays - Part II.

<table>
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<tr>
<th>Animal Number</th>
<th>Inoculum (Strains)</th>
<th>Bact. Count in Inoculum (10^8)</th>
<th>No. Loops with Fluid</th>
<th>Total Fluid Accum. (ml/cm)</th>
<th>A. coci (+) Tissues</th>
</tr>
</thead>
<tbody>
<tr>
<td>(3-4 week old pigs)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>4056</td>
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<td>0</td>
<td>0</td>
<td>ileum, spleen</td>
</tr>
<tr>
<td>263&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>1.1</td>
<td>2</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>1740</td>
<td>4056</td>
<td>1.3</td>
<td>0</td>
<td>0</td>
<td>ileum, spleen</td>
</tr>
<tr>
<td>263</td>
<td></td>
<td>1.1</td>
<td>2</td>
<td>2.9</td>
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</tr>
<tr>
<td>(7-8 week old pigs)</td>
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<tr>
<td>1741</td>
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<td>0</td>
<td>ileum</td>
</tr>
<tr>
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<td>1.1</td>
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<td>6.7</td>
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<tr>
<td>1742</td>
<td>4056</td>
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<td>0</td>
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<tr>
<td>263</td>
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<td>1.1</td>
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</tbody>
</table>

<sup>a</sup> - 263 (E. coli) positive control
Figure 1. Villus Architecture - PBS Negative Control Pig
Ileal Loop. Normal histological appearance. (20X magnification)
Figure 2. Villus Architecture - *E. coli* Positive Control Pig Ileal Loop. Villus atrophy and severe villus blunting. (20X magnification)
Figure 3. Villus architecture - *A. butzleri* Stain 3556 Experimental Pig Ileal Loop. Mild blunting shown at arrows. (20X magnification)
Figure 4. Villus Architecture - *A. butzleri* Strain 3571 Experimental Pig Ileal Loop. Moderate villus blunting evident by the clumping of crypts. (20X magnification)
Figure 5. Villus Architecture - *A. butzleri* Strain 4056 Experimental Pig Ileal Loop. Moderate villus blunting shown at arrows. (20X magnification)
Figure 6. Pig Ileal Wall, Lamina Propria to Serosa - PBS Negative Control Loop. Normal histological appearance. LP = lamina propria, S = serosa (50X magnification)
Figure 7. Pig Ileal Wall, Lamina Propria to Serosa - *E. coli* Positive Control Loop. Infiltration of lymphocytes (L) in the lamina propria and neutrophils (PMS) within the submucosa. (50X magnification)
Figure 8. Pig Ileal Wall, Lamina Propria to Serosa - A. butzleri Strain 3556 Experimental Loop. Edema and leukocytes present within the lamina propria. Leukocytes also present in submucosa and serosa. Arrow points to extravasation of leukocytes. (50X magnification)
Figure 9. Pig Ileal Wall, Lamina Propria to Serosa - A. butzleri Strain 3571 Experimental Loop. Infiltration of leukocytes, predominantly lymphocytes (L), throughout lamina propria, muscular layers, submucosa, and serosa. Edema (E) also present in submucosa. (50X magnification)
Figure 10. Pig Ileal Wall, Lamina Propria to Serosa - A. butzleri Strain 4056 Experimental Loop. Infiltration of leukocytes throughout ileal wall. L = lymphocytes, PMNs = neutrophils, MP = mixed population of leukocytes. (50X magnification)
Figure 11. PCR analysis of *Arcobacter* cultured from pig tissue samples. Positive P-80 tissue samples yielded PCR products of about 1223 bp when amplified with Arco I and Arco II primers. Rectal swabs samples that were *Arcobacter*-negative via dark field microscopy were also negative for PCR.
Figure 12. REA of *A. butzleri* cultured from pig tissue samples.

At the level of about 5,459 bp, band patterns differ as shown by arrow.

Band patterns of *A. butzleri* cultured from loops 2 and 3 for Pig 1740 appear to be similar to the inoculum Strain 4056.
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*Infect. Immun.* (accepted)

Recovery of *Arcobacter* from pigs in Iowa. (submitted)


VITA

Eddy Rachelle Carter was born on September 12, 1967 in Jackson, Mississippi. Eddy graduated from Provine High School in 1985 and began formal college education the same year. In 1989, she received her bachelor of science degree in Animal and Poultry Sciences from Tuskegee University. Following the completion of her undergraduate studies, Eddy was admitted to the Tuskegee University School of Veterinary Medicine. During the second year of veterinary school, Eddy developed an interest in laboratory animal medicine. She became a Junior Assistant Health Officer in the United States Public Health Service during the summer interim following the second year of veterinary school. As a veterinary assistant in the Genetics Resources section of the National Center for Research Resources, Bethesda, Maryland. Eddy gained advanced training in genetic definition and clinical care of germ-free transgenic animals as well as assisted in monitoring the functions of the animal facility.

Following the completion of her third year in veterinary school, Eddy returned to the Public Health Service. Also working as a veterinary assistant, she entered a summer training program in laboratory animal
medicine at the National Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia.

On May 9, 1993, Eddy received her Doctor of Veterinary Medicine degree. Following graduation, she worked temporarily as a veterinary scientist in the *Campylobacter/Listeria* section researching *Arcobacter* spp. for the National Animal Disease Center in Ames, Iowa. Eddy began the residency in laboratory animal medicine at the Virginia - Maryland Regional College of Veterinary Medicine on September 1, 1993.