CAMPYLOBACTER JEJUNI
INFECTION VERSUS CONTAMINATION
OF TURKEYS AND CHICKENS

BY:

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(ABSTRACT)

This study was conducted to determine the extent in which Campylobacter jejuni colonized live birds would survive evisceration and contaminate the processed carcasses. Birds were infected with a marker strain of Campylobacter jejuni and allowed to grow to market age. Cloacal and fecal samples were analyzed to determine the level of Campylobacter jejuni present in the live bird. Prior to slaughter, birds were selectively subjected to two different temperatures (21 and 32°C) and three different times of feed withdrawal for chickens (3, 6, and 9 hours and turkeys 0, 4, and 8 hours). Birds were then slaughtered and the carcasses were sampled to determine the level of Campylobacter jejuni that survived. Results indicated a difference between chickens and turkeys, especially regarding the infective dose and bacterial survival rates. No significant differences in carcass contamination due to feed withdrawal times at either temperature were noted. The correlation of fecal samples with cloacal samples was significant for year 2 with \( r = .53 \) (\( p = .04 \)). For turkeys, the correlations were not significant. A longitudinal study of turkeys showed that the percentage of birds infected with Campylobacter jejuni peaked when the birds
were 5-7 weeks old. The amount of *Campylobacter* contamination in each turkey peaked when the birds were 5 weeks old and then dropped off quickly.
ACKNOWLEDGEMENTS

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Dr. Noel Krieg helped me to understand the different atmosphere that is needed to grow Campylobacter and he showed me how to make an apparatus to achieve this environment. I wish to thank Dr. Krieg for his time and interest in my research.

Dr. Norman Stern provided information about the newest developments in the understanding of how Campylobacter jejuni grows and how to recover it successfully. I wish to thank Dr. Stern for this help.

Last but not least I would like to thank Mary Jane Thompson for moral support and assistance in putting this manuscript together.
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I. INTRODUCTION

Campylobacter jejuni, a Gram-negative bacterium, is a human pathogen that causes the disease campylobacter enteritis. Better methods of recovery using commercially available selective media and improved gas systems have greatly increased the knowledge about this pathogen and its prevalence in domestic poultry (Shane, 1991A). Campylobacter enteritis (campylobacteriosis) is more common in the United States than salmonellosis and shigellosis combined (Stern and Kazmi, 1989). The pathogen can enter the human food chain on fresh poultry since turkeys and chickens may have Campylobacter jejuni as a part of their natural gastrointestinal flora. The predominant mode of transmission for campylobacteriosis is a fecal/oral route via contaminated food or water. Only a small dose of Campylobacter jejuni can cause illness in humans. Carrier rates for chickens sampled at slaughter and market range from 22-87% (Rollins, 1991). Although, newborn chickens are known to be Campylobacter-free and eggs have been demonstrated impenetrable to the bacteria, the route(s) by which these animals are exposed and become colonized during the grow-out stage of production are not understood. If an effective intervention regimen can be formulated, the result would be a cleaner product at market, and potentially a reduction in campylobacteriosis among consumers. To accomplish this goal, modes of transmission and mechanisms of survival of Campylobacter in the environment need to be determined. The research that is described in this paper sought to describe the relationship between the live bird and the finished product by enumerating the
number of *Campylobacter* in the live bird and the number on the carcass of that same bird after evisceration. Knowing if a correlation exists between the live bird and the raw product that goes to the food chain for human consumption will enable us to improve the quality of poultry as a food.
II. REVIEW OF LITERATURE

A. CAMPYLOBACTER JEJUNI

1. Taxonomy, Nomenclature, and Morphology

Campylobacter jejuni is a member of the family Spirillaceae, the genus Campylobacter, and the species jejuni. The genus name, Campylobacter, is derived from the Latin "Campylo" meaning curved and "bacter" meaning rod. Observed under a phase contrast or dark-field microscope, two-cell chains appear S-shaped or gull-winged. This bacterium is a Gram-negative rod curved into small tightly wound spirals. It requires a low oxygen tension of 3-6% for growth, thus it is classified as microaerophilic. Campylobacter jejuni is a non-sporeforming bacterium which is motile with a characteristic corkscrew-like motion that is caused by a single polar flagellum at one or both ends of the cell. The flagella may be two or three times the length of the bacterial cell (Smibert, 1989).

A Campylobacter colony is small, mucoid, gray and nonhemolytic. A small percentage of strains may appear tan or slightly pink. If the medium is excessively moist, the colonies will appear flattened or spread in all directions (Kaplan and Barnett, 1981). According to Smibert (1989) two types of colonies are formed. One is low, flat, grayish, finely granular and translucent with an irregular edge. It spreads along the direction of the streak and tends to swarm and coalesce. The other colony type is round 1-2 mm diameter, raised, convex, smooth, and glistening with an entire edge. It has a translucent edge and a darker, dirty brownish slightly opaque center.
Campylobacter represents a well-defined genus. Taxonomic problems are at the species level (Smibert, 1989). At least nine species appear to be important in human disease (Stern et al., 1990). There are many differences that can be seen between these species, but they also have many things in common.

2. Growth Characteristics

Campylobacter jejuni grows best under an atmosphere that has reduced oxygen and a higher-than-atmospheric concentration of carbon dioxide (microaerophilic). Stern and Kazmi (1989) stated that the oxygen requirement was 3-15% and the carbon dioxide concentration required was 3-5%. The optimum atmospheric composition for growth of this bacterium is 5% oxygen, 10% carbon dioxide, and 85% nitrogen.

Stern and Kazmi (1989) indicate that the growing temperature for Campylobacter jejuni is between 30°C to 45°C. The optimum growth temperature is 42-43°C. Organisms die quicker at 25°C than at 4°C or 30°C. It does survive well in foods at refrigerator temperature, but is highly susceptible to freezing. Thermal inactivation occurs at 46-48°C. It will not survive the minimum pasteurization treatment legally required for milk and milk products. Also, it will not survive the typical cooking procedure for meat products (Stern and Kazmi, 1989). Luechtefeld et al. (1981) reported that Campylobacter jejuni survives longer at 4°C than at room temperature in turkey cecal samples.
Campylobacter jejuni has a respiratory metabolism. Energy is obtained from amino acids or the tricarboxylic acid cycle intermediates, not carbohydrates. These bacteria are chemoorganotrophs thus requiring organic molecules as a source of carbon for metabolic energy (Smibert, 1989).

When hydrogen peroxide comes in contact with Campylobacter jejuni it produces water and oxygen (catalase positive). Catalase in media stimulates the growth of this bacteria (George et al., 1978). Catalase positive campylobacters are most frequently associated with human disease (Stern et al., 1990). It is oxidase positive and reduces nitrate to nitrite. It does not hydrolyze gelatin, casein, deoxyribonucleic acid or esculin. Most strains hydrolyze hippurate (Banwart, 1989). This bacterium neither ferments nor oxidizes carbohydrates (Thippareddi, 1991). It has DNA with a G + C content between 29 and 36 mol% (Stern and Kazmi, 1989). Campylobacter jejuni grows well at pH 5.5 - 8.0 with the optimum range being 6.5-7.5. No growth occurs at pH 4.9 or lower, therefore, lactic and acetic acids can be used to reduce the numbers of Campylobacter on poultry (Stern et al., 1990). Stern and Kazmi (1989) stated that the rate of inactivation of Campylobacter at pH 3.0-4.5 was temperature dependent. At similar pH values, the organism was most rapidly inactivated at 42°C, at an intermediate rate at 25°C, and most slowly at 4°C. Thippareddi (1991) reports that Campylobacter jejuni growth between pH 4.9 and 9.9 varies with strain, type of acid, temperature, and water activity.
In a cold, dry environment *Campylobacter jejuni* can remain viable for several weeks but it is sensitive to drying under some refrigerated conditions. Organisms survive better at 4°C and with 14% or less relative humidity (Stern and Kazmi, 1989). The organism survives better on carcasses stored in water than those stored in air.

*Campylobacter jejuni* is sensitive to sodium chloride but can be recovered well at 0.5% sodium chloride. It will not survive at 2.0% NaCl at optimum conditions, but will survive for weeks in refrigerated food at 4°C with 6.5% salt (Stern and Kazmi, 1989; Stern et al., 1990).

Different nomenclature is used by various authors to classify the *Campylobacter fetus* group. According to Doyle (1981), Smibert, Vernon and Chatelain, King, and Berg each use different names to refer to the same bacterium. Smibert uses the name *Campylobacter fetus* subsp. *jejuni*. Veron and Chatelain use *Campylobacter jejuni* while King refers to this group as "related" vibrios and Berg uses *Vibrio fetus* Serotype C. Care must be taken to note what nomenclature was used to classify the organism (Park et al., 1984).

Shane (1991A) states that various classification schemes for the genus have been described which clearly designate *Campylobacter jejuni* the predominate organism isolated from avian hosts, as a separate and valid species, not a subspecies of *Campylobacter fetus* as stated in earlier literature.
Lambert et al. (1987) used fatty acid profiles to differentiate between campylobacter species. It was necessary to use the fatty acid results with selected biochemical tests to separate Campylobacter jejuni from Campylobacter coli. The cellular fatty acid compositions were determined using gas-liquid chromatography (GLC). This makes it easier to identify Campylobacter at the species level. Campylobacter is difficult to identify at the species level because they are asaccharolytic and inactive in most biochemical tests and may have atypical or inconclusive biochemical reactions.

Other important characteristics that help to differentiate Campylobacter jejuni from other species is its sensitivity to nalidixic acid, its inability to grow in the presence of 1% glycine, and its inability to produce hydrogen sulfide from Albimi Brucella broth plus cysteine as detected by lead acetate strips (Doyle, 1981). Table 1 shows how it is possible to differentiate between different catalase positive species using biochemical characteristics (Shane, 1991A).

The isolation of Campylobacter jejuni has become easier as various media have been tried and then improved. George et al. (1978) studied improved media for the growth of Campylobacter jejuni. Since Campylobacter is microaerophilic, recovery is more difficult. It was found that the addition of 0.025% each of ferrous sulfate, sodium metabisulfite, and sodium pyruvate enhanced the aerotolerance of the bacteria and improved the recovery rate. When this addition is made the agar is referred to as
FPB agar. Brucella agar supplemented with FPB supported growth at lower levels of oxygen and growth appeared earlier.

Wang et al. (1980) reported that a semisolid brucella medium enriched with 10% sheep blood was used for storage and transport of Campylobacter jejuni and it kept isolates alive about three weeks or longer at 25°C. Nair and coworkers (1984) used an egg-based medium and found it to be superior to the existing Wang transport medium for the preservation of Campylobacter jejuni in the laboratory. The egg medium was supplemented with 0.025% each of ferrous sulfate, sodium metabisulfite and sodium pyruvate. This medium supported viable Campylobacter at 4°C for 90 days without any loss and for 120 days with only a 20% loss of viable Campylobacter. It was not as durable at room temperatures where viability dropped to 20% by 40 days and 0% by 60 days.

**TABLE 1**

**DIFFERENTIATION AMONG CATALASE-POSITIVE CAMPYLOBACTER SPECIES ACCORDING TO BIOCHEMICAL CHARACTERISTICS**

<table>
<thead>
<tr>
<th>SPECIES</th>
<th>GROWTH @25°C</th>
<th>GROWTH @42°C</th>
<th>SENSITIVITY TO NALIDIXIC ACID</th>
<th>HIPPURATE HYDORLYSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fetus</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>-</td>
</tr>
<tr>
<td>C. coli</td>
<td>+</td>
<td>+</td>
<td>S</td>
<td>+</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>-</td>
<td>+</td>
<td>S</td>
<td>+</td>
</tr>
<tr>
<td>C. lardis</td>
<td>-</td>
<td>+</td>
<td>R</td>
<td>-</td>
</tr>
</tbody>
</table>

(Shane, 1991A)
Cary Blair medium with decreased agar was found to be the best of six transport mediums tested by Læchtefeld et al. (1981). This medium is recommended for specimens that must be transported or stored for longer than three days as it prevents drying.

Patton et al. (1981) tested three different selective media for recovery of *Campylobacter jejuni* from fecal samples. The introduction and improvement of selective techniques has made it easier to isolate *Campylobacter* from fecal samples that contain mixed bacterial flora.

Merino et al. (1986) tested seven different media for their isolating qualities. Cefoperazone was the antimicrobial supplement of choice. The presence of blood in the media favored the development of competing fecal flora. The blood-free media allowed for easier detection of suspect colonies and was easier to prepare with less cost.

Karmali et al. (1986) reported that a charcoal-based blood-free selective medium (CSM) was superior for growth of purer isolates of *Campylobacter jejuni* than was a Skirrow-type medium (SKM). CSM produced significantly greater suppression of *Pseudomonas* species and Gram positive organisms than SKM, but was not effective in suppressing yeasts.

Goossens et al. (1986) strongly suggested the need for incorporation in selective media of drugs against not only Gram-negative organisms (cefoperazone) but also Gram-positive organisms (rifampin and vancomycin) and yeasts (amphotericin B
and cycloheximide). The antibiotics polymyxin B and colistin are useful in eliminating *Campylobacter coli* since they are more susceptible than is *Campylobacter jejuni*.

Krieg (1990) recommended the use of the Blaser-Wang supplement. It contains the following five antimicrobial agents: vancomycin, trimethoprim, polymyxin, amphotericin, and cephalothin. Vancomycin, used at 10 mg per liter, inhibits peptidoglycan synthesis and is mainly effective against Gram-positive cocci. Trimethoprim, used at 5 mg per liter, inhibits folic acid metabolism, Gram-positive cocci and Gram-negative rods. Polymyxin B, used at 2500 units per liter, binds to the surface of bacterial membranes and causes membrane damage. This antibiotic affects many kinds of bacteria. Amphotericin B, used at 2 mg per liter, is an antifungal agent. Cephalothin, used at 15 mg per liter, affects peptidoglycan synthesis and is effective against many Gram-positive and Gram-negative organisms. These five agents acting together produce a supplement that provides a selective isolation medium for *Campylobacter jejuni*.

In 1977 Skirrow (Doyle, 1982) reported results from a study he had done using a selective culture medium. The medium contained antibiotics that inhibited most fecal organisms, but allowed the growth of *Campylobacter*. This study generated interest in the medical community to determine the disease potential of *Campylobacter* in humans.
Agulla et al. (1987) reported that the use of enrichment media slightly improves isolation rates of *Campylobacter jejuni* when fresh samples are used. Enrichment media may be useful for samples that contain few viable organisms. Gun-Munro et al. (1987) states the optimal recovery of *Campylobacter jejuni* requires the use of more than one selective medium. This is not always possible so it is important to determine which medium performs most satisfactorily when tested under different condition. The study conducted by this author indicated that charcoal selective media (CSM) or modified charcoal, cefazolin, sodium deoxycholate agar (CCDA) were superior to earlier formulations. The recognition of *Campylobacter* was improved with this media and there was a greater suppression of other flora which reduced the time spent on screening procedures. Griffiths (1986) stated that modified charcoal cefoperazone deoxycholate agar (CCDA) greatly reduced the number of contaminating organisms. This is superior to Skirrow's media. It is a help in isolating "thermophilic" *Campylobacter* from human feces and the blood free media is easier and cheaper to use.

Lee et al. (1988) reported that temperature, aging, and bisulfite content of an agar can and does affect the aerotolerance of *Campylobacter jejuni* and thus influences its isolation effectiveness. They reported that the bacteria had a greater degree of aerotolerance at 42°C than at 37°C, since growth occurred at higher levels of oxygen - up to 21% oxygen for those incubated at 42°C. Brucella broth and agar were aged for this experiment to see if that had any effect. The aging of dehydrated
brucella broth greatly affected the aerotolerance and viable counts of *Campylobacter jejuni* and the inhibitory effect of aging became more evident when the temperature was lowered from 42 to 37°C. Addition of 0.01% sodium bisulfite to aged medium restored the ability of the medium to support growth of *Campylobacter* to a level equivalent to or greater than that obtained with the fresh brucella medium. The aging of brucella agar affected the growth of one strain tested but did not affect the other one. When the aged medium was supplemented with 0.01% sodium bisulfite the inhibitory effect of aging was reversed. As shown by Hoffman et al. (1979A), toxic forms of oxygen are generated in brucella agar stored in the air and supplements that enhance aerotolerance of *Campylobacter* appear to act by destroying these toxic forms of oxygen. It was found that 0.05% bisulfite was toxic when used alone, but with 0.05% ferrous sulfate and 0.05%, sodium pyruvate it had a stimulatory effect on growth.

Chou et al. (1983) reported that using a combination of ferrous sulfate, sodium metabisulfite, and sodium pyruvate in the solid medium increased the aerotolerance of *Campylobacter jejuni* and enabled it to grow at elevated oxygen tension. FPB supplementation helps in maintaining viability and characteristic cell morphology during prolonged storage.

Buck et al. (1982) evaluated CampyPak II as a gas generator system for isolation of *Campylobacter jejuni*. The authors found that this system of envelopes which produces microaerophilic conditions necessary for isolating *Campylobacter* was
an excellent alternative method to the Torbal jar technique. The difference between the two systems was the cost, which was seven times more for the CampyPak II.

Table 2 gives a listing of important characteristics of *Campylobacter jejuni*.

**TABLE 2**

**CHARACTERISTICS OF CAMPYLOBACTER JEJUNI**

<table>
<thead>
<tr>
<th>Characteristic</th>
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<tbody>
<tr>
<td>Catalase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Nitrite reduction</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Ferment glucose</td>
<td>-</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Oxidize glucose</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Growth in presence of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% Glycine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5 NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1% Bile</td>
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<tr>
<td>H₂S production:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSI or SIM</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Lead acetate</td>
<td>+</td>
<td></td>
<td>-</td>
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<tr>
<td>H₂ required for growth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indole production</td>
<td>-</td>
<td></td>
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<tr>
<td>Inhibited by:</td>
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<tr>
<td>TMAO (growth)</td>
<td>-</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>+</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Cephalothin</td>
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</table>

(Smibert, 1989; Stern, 1989)
3. Pathogenicity (Disease)

Stern et al. (1988) reported that chicks colonized with *Campylobacter jejuni* showed no obvious diseases during dissection. This suggested that the relationship between the chicken host and the bacterial organism is a benign commensalism, at least during this period of the first six to ten days when the bird would be expected to be most susceptible to infection and disease. The hatching of chicks in an incubator away from parent stock and raising them in brooder houses away from old birds tends to break the cycle of internal and external parasites, as well as that of microorganisms (Foust, 1945).

Research indicated that as few as 500 cells of *Campylobacter jejuni* can produce illness in humans (Stern et al., 1990). Foods of animal origin are primary vehicles in human infection. Public health authorities believe that mishandled poultry is the most prominent vehicle in transmission of this bacterium in the United States (Stern et al., 1990). Campylobacter enteritis has in the last few years been found to cause as much enteric disease in man as *Salmonella* and *Shigella*, and thus, has emerged as an important human intestinal pathogen. It infects people of all ages, but is more frequently found in children. It is found in the intestinal tracts of wild birds, domesticated poultry, dogs, cats, sheep, pigs, goats, and cattle. Transmission is most probably oral (Smibert, 1989).

Isolation of *C. jejuni* is best accomplished from fecal materials or swabs. Selective media is used for the isolation of these organisms. Selective media does not
eliminate all other intestinal organisms but it limits their growth to favor isolation of
*Campylobacter* (Smibert, 1989).

The clinical picture of *Campylobacter jejuni* infection varies from symptomless
excretion to severe disease. It is normally a self-limiting disease but complications
such as cholecystitis, peritonitis, septicemia, and meningitis have been reported. The
small intestine is thought to be the main site of infection but the colon is often
involved. *Campylobacter* may be transmitted by direct contact with contaminated
animals or animal carcasses through ingestion of contaminated food and water,
person-to-person from excreters with certain infections, perinatal and childhood
transmissions. Most *C. jejuni* infections appear to occur sporadically without a clear
determination as to the mode of transmission (Franco, 1988).

Fricker and Park (1989) conducted a two-year study of the distribution of
'thermophilic' *Campylobacter* and found them widely distributed in the following
sources: human beings, sewage, river water, poultry, beef, pork, lamb, offals,
cooked meat and seafood. Of these sources, poultry and offal were the most highly
contaminated.

Bashin (1990) reported that *Campylobacter jejuni* is the single most common
bacterial cause of diarrhea striking between two and six million Americans each year.
Most people recover and never bother to consult a doctor. They never suspected
infection from food they had assumed to be safe. *Campylobacter* sickens its victim
privately and quietly one by one, not in mass outbreaks. Victims rarely connect
**Campylobacter** with the food they eat because it strikes two to five days after the contaminated meal is eaten.

Cunningham (1982) states that *Campylobacter jejuni* is seldom found in healthy persons. These organisms were isolated from 91% of caecal and rectal samples and carcasses of healthy chicks. *Campylobacter* may survive in fresh water for eleven days to four weeks at 4°C and for two days at room temperature.

The two human illnesses attributed to subspecies in the *Campylobacter fetus* group are systemic campylobacteriosis usually caused by *Campylobacter fetus* subspecies *intestinalis* and campylobacter enteritis with 99% of cases caused by *Campylobacter jejuni*. Campylobacter enteritis has an incubation period of 2-11 days. The major clinical manifestations are abdominal pain, diarrhea and fever. Diarrhea may be mild to moderate to profuse with blood in the stool. Other symptoms may include malaise, headache, musculoskeletal pain, rigor and delirium. Vomiting may occur but is not common. The severity of the illness is quite variable, but in most cases, is brief and self-limiting (Doyle, 1981).

Franco (1988) reported that *Campylobacter jejuni* was first isolated from human diarrheal stools in 1971. Campylobacter enteritis is a zoonotic disease with apparently different modes of transmission in industrial and developing countries. Animal origin food transmission is the primary mode of spread in developed countries, while evidence indicates that fecal contamination of food and water and
contact with sick people or animals is the predominate mode of transmission in
developing countries.

Shane (1991A) reports that poultry serves as a primary reservoir host of
thermophilic Campylobacter. Broiler chickens, turkeys and ducks may harbor the
organism as well as many game birds. There is no evidence that transmission occurs
vertically by either transovarian infection or by penetration of the egg shell after
oviposition. The evidence of experiments conclusively demonstrated that
contaminated feed and water will transmit Campylobacter to susceptible contacts.
Insects such as the fly or cockroach may play a role in transmission of
campylobacterosis. Campylobacter can be introduced into poultry houses by non-
confined companion animals, vermin, and footwear contaminated with feces and litter.
Campylobacter spreads rapidly within flocks by horizontal, fecal-oral infection.

Both food and water have been implicated as vehicles responsible for
campylobacter enteritis thus confirming that oral ingestion of the organisms is an
important route for transmission of the disease. In 1977, water was the cause of a
large outbreak in Bennington, VT. Other outbreaks have been attributed to raw milk,
undercooked chicken, and pork. Cooking should kill Campylobacter on chicken and
pork but, infections resulted from foods that had been cross-contaminated with
uncooked meat (Doyle, 1981).

Foster (1986) reports that the disease campylobacteriosis or campylobacter
enteritis is more common than salmonellosis and shigellosis combined. Documented
outbreaks occurred with raw milk, unchlorinated water, insufficiently cooked chicken and inadequately pasteurized milk as the probable causes. The high frequency of campylobacter enteritis combined with the high incidence of extra-intestinal complications such as meningitis, cholecystitis, urinary tract infection and reactive arthritis assures an important place for this disease in public health. The organism's sensitivity to environmental influences makes it easy to control as long as correct measures are taken. The pervasiveness of Campylobacter in animal populations and its probably low infective dose assure continued infections as long as we do unwise things as consuming unpasteurized milk, unchlorinated water and food that is improperly handled.

Archer (1986) states that foodborne diarrheal disease has 24-81+ million cases per year. This translates to a large ($5-$17 billion) economic loss. Mishandling of food by time/temperature abuse, cross-contamination with raw commodities and contaminated by food handlers are the main reasons for the association of diarrheal disease and food. According to Archer most of the foodborne disease problem is caused by mishandling of food in the home.

Campylobacter jejuni is an important enteric pathogen of man that is almost always unsuspected and too often unrecognized (Franco, 1988). The organism is widely distributed in animal reservoirs and in foods of animal origin, thus making the control of this foodborne microbe a formidable undertaking. Most illnesses occur sporadically without a finite determination as to the mode of transmission. Factors
that perpetuate the Campylobacter problem are concentrating of animals in feedlots and brooding houses, spreading of Campylobacter during animal slaughtering and processing, poor food handling and storage practices, environmental contamination from animal wastes and other sources.

B. CHICKENS

1. Anatomy and Growth

The anatomy of a chicken’s digestive system begins with the mouth where two physical processes occur: the act of prehension, which is the act of bringing food into the mouth, and deglutition, which is the act of swallowing. Chickens have no teeth and do not chew, but does produce saliva that lubricates the feed and aids in forming a bolus and swallowing. Swallowed feed goes through the esophagus to the crop. Feed is stored and soaked in the crop. When feed leaves the crop it goes to the proventriculus or glandular stomach which is a thick walled organ immediately in front of the gizzard. In the proventriculus, feed is stored temporarily while copious amounts of digestive juices are secreted and mixed with it. Then the feed passes to the gizzard, a muscular organ which normally contains stones or grit which crush and grind the feed. Feed, upon leaving the gizzard, passes through the small intestine, the ceca, and the large intestine to exit at the cloaca (Foust, 1945, Hill, 1971).

The intestinal tract of the chicken is 4-6 times the length of the body. It is made up of four parts which are the duodenum, ilium, ceca, and rectum. The walls
of the entire length of the intestinal tract is marked by the presence of villi or glands of Lieberkuhn (Hewitt, 1945, Hill, 1971).

Penionzhkevich (1968) stated that the length of the intestinal tract of birds varies according to breed, individual features and feed. The approximate length of the small intestine in chickens is 150 centimeters. Paired ceca open at the transition from the small to the large intestine. The diameter of the ceca is small at the beginning and increase considerably at the middle and main part. Each cecum terminates in a pointed cul-de-sac.

The wall of the intestine consists throughout its length of a serous membrane of connective tissue, a middle muscular layer, and an inner mucous membrane. There are three layers of muscles in the intestinal wall. The mucous membrane is covered with numerous cylindrical, digitate, foliate and spherical villi. These villi and longitudinal folds considerably increase the surface of the mucous membrane, which in its turn greatly facilitates the secretionary and absorptive functioning of the membrane. The villi of chickens are particularly long, being 5 times longer than the thickness of the intestinal wall. These villi often reach to the center of the intestinal lumen. The epithelial cell lining of the surface of the villi contains ampullaceous, mucus-producing cells. Digestive juices are secreted by simple tubular Lieberkuhn glands or intestinal crypts located at the bases of the villi (Penionzhkevich, 1968).

Hillerman (1953) reports differences in the time required for food to pass through the alimentary canal of chickens and turkeys. He investigated feed passage
and how it is affected by age, environmental temperature, antibiotics, egg production, and sex. For chickens, the rate of passage of feed appears to be related to the age of the bird as the study showed that feed passed more rapidly through young birds than old birds. Environmental temperature showed no influence in the rate of passage of feed. Penicillin exerted a slight effect of slowing the passage of feed through the digestive tract. Egg production did not have an effect as feed passage was the same for laying and non-laying hens. Males (cocks) and females (pullets) were very similar with times of 3 hours and 12 minutes for pullets and 3 hours 20 minutes for cocks for the passage of feed. The sex of the animal does not significantly affect the time it takes for feed passage.

2. Gastrointestinal Flora and Growth

Sheldon (1979) reported that various researchers found different flora in the digestive tracts of chickens. The predominate flora of the duodenum and ilium are facultative anaerobes and some obligatory anaerobic species. Obligate anaerobes make up nearly the entire microbial population of the ceca.

Stern et al. (1991) concluded that simultaneous colonization of Campylobacter jejuni and Salmonella typhimurium did occur with one of these co-colonizers not influencing the other. Competitive exclusion is a theory that is based on competing microflora diminishing available colonization sites for other microorganisms to which the chicken is subsequently exposed. Because of the lack of a significant difference
between single species and co-challenged colonization in this study, it can be inferred that Campylobacter and Salmonella do not compete or interfere with each other for the same attachment sites on or in the chicken’s gastrointestinal tract. These two organisms occupy separate niches within the gut, particularly within the ceca. Most strains of salmonella adhere to the epithelium as it occurs in the upper portion of the villi. Invasive strains can colonize as intracellular parasites within epithelial cells. Campylobacter, which has been experimentally proven to be attracted to mucin, is most frequently located within the lumens of mucus-filled crypts. Salmonella spp. may also occasionally exist in this niche, but this is a rare occurrence.

The interaction of Campylobacter jejuni with extracellular matrix components was investigated by Kuusela et al. (1989). They concluded that the Campylobacter has multi-potential capability to interact with several proteins. These microbes may use several adherence mechanisms to provide the best possibility for tissue tropism and invasion.

Stern et al. (1990) stated that antibiotics inhibit chicken cecal colonization by Campylobacter jejuni. The role of anti-campylobacter antibodies is suggested to provide protective immunity against human disease. The data in this report indicate that colonization can be neutralized by antibodies. Also, the data indicated that there is a threshold of challenge level required to colonize Campylobacter jejuni in the chicken’s cecum. If the threshold was not reached, the number of organisms found in the cecal materials was either low or undetectable. Once this threshold was crossed,
the organisms appeared to multiply and occupy the niche for at least one week. By identifying and immunizing chickens against pertinent colonization factor antigens, it should be possible to diminish colonization by the organism.

The exact age at which a bird reared under normal conditions becomes a donor of fully protective bacteria is not known, but it appears to be at about 3-5 weeks, when the bacterial population reaches its optimum (Stavric, 1987). This process is accelerated if newly hatched chicks are treated with suspensions or cultures of gut content from donor birds. These undefined treatments have been restricted to experimental use since safety and efficiency must be considered. Since the potential for spreading pathogens exists, attempts have been made to identify the components of protective microflora with the aim of developing treatments with a known bacterial composition. This would be very difficult because it is not known which isolates are protective, there is no adequate selective medium, and all cecal flora have not been identified.

Nurmi (1988) describes a method of preventing enteropathogens in poultry. This method is called competitive exclusion (CE) or the Nurmi concept. Based on the fact that normal adult intestinal flora excludes Salmonella, Campylobacter, and E. coli, older birds are resistant to infection because the flora is well-established, but newly hatched chicks may be infected by a single cell. When chicks were hatched by setting hens they were probably rapidly populated by the autochthonous gut microflora of the adult, but now hatcheries replace setting hens and the sanitary environment has
no autonomous microflora to introduce. These birds are then placed in clean, sanitized rearing barns that have fresh litter too. Young birds have no resistance against the enteropathogens and thus can easily be colonized. With this competitive exclusion in mind, the introduction of an intestinal microflora of adult birds to newly hatched chicks makes them immediately resistant to infective doses of the enteropathogens. This intestinal microflora can be administered directly to the crop, by adding it to drinking water or feed, and by aerosols. It is introduced as a suspension of fecal droppings, cecal material, or anaerobic culture. The source of the microflora is usually from the same species but chicken microflora protects turkeys and vice versa. This method reduces the number of Campylobacter in the intestines of an infected bird by two to three log_{10} but does not completely rid the bird of the bacteria.

Stern and Meinersmann (1989) stated that there are many factors that shape the niches of the gastro-intestinal tract. These include low pH of the stomach, peristaltic clearing, villous sweep, toxic bile acids, phagocytes, oxygen tension, mucin trapping, competitive exclusion, immune exclusion, secretory antibodies and cell-mediated nutrient availability. It is not known how Campylobacter jejuni overcomes these factors but it involves adaptations to grow within the mucin layer. The spiral shape of Campylobacter is similar to organisms that normally grow in the mucin layer. The flagella and perhaps the chemotactic response to components of mucin are integral parts of the ability of the organism to live in mucus. Other capabilities affecting
colonization include survival in phagocytes, adherence to enterocytes, invasiveness and production of enterotoxins.

Chicks can be readily colonized by an oral dose of 3500 CFU of *Campylobacter jejuni* (Stern et al., 1988). Chicks can be equally well colonized by *Campylobacter* through the first three days after hatch. Different isolates of *Campylobacter jejuni* had different colonization potentials with some not colonizing until after repeated passage through the chicks intestinal tract while others readily colonized the chick. Competitive exclusion cultures administered to newly hatched chicks did not diminish cecal colonization of *C. jejuni* but they did provide protection for chicks challenged with colonizing *Salmonella typhimurium*. This finding is at variance with the findings of Nurmi (Nurmi, 1988). Stern et al. (1991) gives two explanations for the inconsistencies between the studies. The first explanation is that critical bacteria having anti-campylobacter competitive exclusion activity used in the Nurmi study may have been lost during storage. Alternatively, the sensitivity of the assay was greater in Stern's study. Other plausible explanations for the ineffectiveness of competitive exclusion with *Campylobacter* would be that it occupies a different ecological niche in the intestines. Its niche is the mucin layer which covers the intestinal tract. The organism remains free swimming, propelling itself against the intestinal flow using flagella. L-fucose found on epithelial tissue or within the mucin gels induces a chemotactic response in *Campylobacter jejuni*. The addition of L-fucose inhibits attachment. If *Campylobacter jejuni* does not adhere to the
intestinal tract, the presence of a competitive exclusion culture would not physically inhibit colonization by the organism at the surface of the epithelium. If *Campylobacter* is drawn to the mucin gel, it is not susceptible to the same competitive exclusion influence on salmonella.

Myszewski and Stern (1990) discussed the influence of *Campylobacter jejuni* cecal colonization on immunoglobulin response in chickens. The response in chickens varied throughout the eight week trial period from the high at hatch to a low at two weeks and then a steady increase until the experiment was terminated at eight weeks. This is very different from the immunological response in humans which develops rapidly after disease onset and generally peaks ten days after onset and then declines relatively quickly. This difference was explained by the lack of pathological manifestations expressed in the chicken exposed to *Campylobacter jejuni* gastrointestinal tract colonization. Since this organism does not appear to be a pathogenic organism for chickens, there is no advantage for the host to mount a substantive immunoglobulin immune response. Resistance to *Campylobacter jejuni* colonization in immune animals may be explained by the antagonistic action of the serum Immunoglobulin A (sIg A). Bacteria are prevented by sIg A from adhering to intestinal epithelial cells. Immune mucus preparations, when analyzed by enzyme-linked immunosorbent assay (ELISA), were found to contain a rich supply of anti-*Campylobacter* Ig A. When these specific antibodies were removed, the immune mucus preparations were no longer capable of blocking *Campylobacter* epithelial cell
binding. Prevention of colonization of chicks by *Campylobacter jejuni* might be achieved if means to increase the anti-*Campylobacter* sIg A response are established and directed against pertinent colonization factors.

Myzewski and Stern (1991) reported that phagocytosis by peritoneal macrophages from *Campylobacter jejuni* colonized chickens and from uncolonized control chickens almost destroyed the organisms within the experimental period of six hours. *Campylobacter jejuni* cells in human peripheral blood monocytes and mouse peritoneal macrophages survive up to seven days intracellularly. Differences in these reports may have been caused by the virulence potentials of the *Campylobacter* used in the studies. The isolates for this study had been maintained for several years IN VITRO. Attenuated bacteria tend to be more readily phagocytized and killed than virulent organisms. Although the chicken peritoneal macrophages were able to ingest and kill *Campylobacter jejuni* effectively in the IN VITRO assay, clearance from the gastrointestinal tract colonization by the organism was never achieved, and the chickens remained colonized until they were killed. These observations suggest that colonization by *Campylobacter* in the chicken host does not depend upon the ability of the organism to evade phagocytosis. Rather, it appears that other parameters influence the initiation and maintenance of gastrointestinal tract colonization.

*Campylobacter jejuni* broiler colonization may be age-related with young birds *Campylobacter* free and the prevalence increasing with flock age (Shanker et al., 1988). Minimal colonization inoculum was determined for 2-3 day old chicks to be
0.2 ml. and for 2 week old chicks to be 0.5 ml. This inoculum was administered orally to some chicks and cloacally to some chicks. The effect of the different route of challenge was apparent with the oral challenge being \(10^4\) CFU and the cloacal challenge being less than \(10^2\) CFU. The oral challenge required at least 2 log\(_{10}\) higher inoculum (more bacteria) than the cloacal challenge probably because of adverse conditions over a longer period of time and over a longer distance. The bacteria live best in the ceca and this is very close to the cloaca and a long distance from the crop. The presence of endogenous gut flora did not affect colonization. There was no significant difference in the number colonized at 2 weeks compared to 2-3 days. The data also suggest that *Campylobacter jejuni* transmission may result from cloacal contaminations. In the chicken’s intestinal tract, *Campylobacter* colonizes the ceca. The low colonization inoculum for cloacal challenge may be explained by the short distance between the ceca and the cloaca.

Engvall et al. (1986) reported on the colonization of broilers with *Campylobacter jejuni*. While eight of sixteen flocks surveyed showed *Campylobacter* infection, *C. jejuni* was not isolated until week three from either birds or environmental samples. The time of detection varied from 3 to 5 weeks, since either too few samples were taken to detect *Campylobacter* during the early stages of spread or *Campylobacter* was introduced late and spread explosively in the house. A contributory factor in such an explosive spread could be airborne spreading. *Campylobacter* was present in the air in some houses with *Campylobacter*.
contaminated broilers. Waterers and droppings from the floor seem to be good indicators of the presence of *C. jejuni* in the broiler flocks. Newly hatched chickens were *Campylobacter* negative. The source of infection might have been drinking water from nearby lakes. Possibly the source of contamination should be looked for in the environment outside or close to the house such as rodents or insects. Another possible way is through cross-contamination by staff or equipment. Virginiamycin, a growth promoting antibiotic, was added to the feed at 10 ppm but this did not affect the ability of *C. jejuni* to colonize broilers.

Histological studies of intestinal tracts of chickens have demonstrated the existence of bacteria in intimate contact with the epithelial surface (Fuller and Coates, 1983). In the cecum, there is a complex mixed population of organisms trapped in the mucous layer. The epithelial cells of the intestinal mucosa are markedly affected by the presence of microorganisms. Those birds with microorganisms have a wider brush border and the total microvillus is greater with increased crypt depth, greater mitotic activity, faster migration and a more rapid turnover of epithelial cells.

A number of factors contribute to the colonization and continued presence of bacteria within the digestive tract of animals (Juvens et al., 1991). These factors include gastric acidity, bile salts, peristalsis, digestive enzymes, immune response and indigenous microorganisms and the antibacterial compounds which they produce.

According to Juvens and co-workers, attempts to control the microbiological flora in the chicken gastrointestinal tract must take into account all the other factors in
order to maintain the desirable organisms and to eliminate human enteropathogens. The native intestinal lactic acid bacteria (LAB), including *Lactobacillus* spp. and *Pediococcus* spp., are candidates because of their ability to colonize the gastrointestinal tract, to produce bacteriocins, hydrogen peroxide, reuterin and certain organic acids, and to deconjugate bile acids and salts to yield a more inhibitory-free bile acid.

*Lactobacillus* spp. are normal inhabitants of the intestinal tract, especially of poultry. An association between lactobacilli and the epithelial lining of the chicken crop is established within a few days and persists throughout the life of the chicken. *Lactobacilli* also colonize the small intestines and adhere to the columnar epithelial cells. *Pediococcus* spp. have been found in the ceca of newly hatched chicks. *Lactobacilli* are the only group of organisms reported as generally present in numbers exceeding 10,000 per gram of the intestinal content in the small intestine of chickens 2-6 weeks of age (Juven et al., 1991).

*Lactobacilli* have been extensively studied for the production of bacteriocins, which are compounds produced by bacteria that have a biologically active protein moiety and a bactericidal action. Bacteriocins synthesized by *Lactobacilli* have been reported to produce an inhibitory activity restricted to Lactobacillaceae. *Pediococci* species produce bacteriocins that exhibit a relatively broad spectrum of antimicrobial activity. One strain of *Pediococci* isolated from a meat product was found to produce
a bacteriocin that inhibits Gram-negative bacteria associated with foods (Juven et al., 1991).

Publications on the subject of antagonistic flora influencing intestinal colonization of *Campylobacter jejuni* have been conflicting. Consequently, the role of an undefined native gut microflora in controlling colonization by *Campylobacter jejuni* has yet to be resolved and awaits further clarification. Enteric strains of lactobacilli and pediococci proliferate under both microaerophilic and anaerobic conditions, and therefore, are able to grow in reduced oxygen concentrations found in various portions of the chicken's gastrointestinal tract. These organisms could serve to antagonize the microaerophilic *Campylobacter jejuni* in those niches of the gastrointestinal tract in which low dissolved oxygen tension exists (Juven et al., 1991).

Other antagonistic compounds include organic acids such as acetic and lactic, volatile fatty acids, hydrogen peroxide, deconjugated bile acids and reuterin (a broad-spectrum antimicrobial substance). Organic acids will inhibit growth of many bacteria, including pathogenic Gram-negative organisms. While pH is a factor, lower pH values also potentiate the activity of these acids, since the undissociated forms are the most bactericidal. The antagonistic effect of volatile fatty acids has not been shown for *Campylobacter jejuni*. Hydrogen peroxide is one of the primary metabolites that may be produced by lactic acid bacteria and may contribute to antagonistic activity. This compound is cytotoxic because of its capacity to generate
reactive cytotoxic oxygen species such as the hydroxyl radical which is a powerful oxidant. Hydrogen peroxide is generally found in the upper portions of the gastrointestinal tract because it takes a certain concentration of oxygen to produce hydrogen peroxide in quantities large enough to be effective. Very limited antagonism due to hydrogen peroxide is found in the ceca because the oxygen level is insufficient to produce a large amount of hydrogen peroxide. Deconjugated bile may be important in ecological control of microorganisms in the intestinal tract.

Deconjugated biles are more inhibitory to bacteria than conjugated ones and can be produced by Lactobacillus spp. colonizing the intestinal tract. A newly discovered, broad-spectrum antimicrobial substance, termed reuterin is produced by strains of Lactobacillus. It has been found to be active against Gram-negative bacteria and is a common resident of the gastrointestinal tract of poultry (Juven et al., 1991).

*Lactobacilli* can be effective in controlling undesirable bacteria in the intestinal tract of humans and animals (Juven et al., 1991). The production of antagonistic substances by intestinal *Lactobacilli* may enhance the competitive ability of these bacteria to become residents of the intestinal tract. Several species of *Lactobacillus* appear to be important in maintaining a proper balance among microorganisms in the intestinal tract. Other than the effect of their inhibitory metabolites, lactobacilli also may out-compete other bacteria for nutrients, occupy the same gastrointestinal track sites as gut colonizers and make these sites unavailable to other microorganisms. The effect of bacterial antagonism can be caused by the depletion of nutrients which leads
to competitive inhibition. A non-specific way of protecting chicks against colonization by enteric pathogens is achieved by adhesion of non-pathogenic bacterial cultures to the specific receptor sites of these pathogens in the gut epithelium.

Bacteriocins, bacteriocin-like compounds, reuterin, and/or hydrogen peroxide may play a major role in regulation of bacterial populations and the control of enteric pathogens in the gastrointestinal tract. Studies of these micro-ecosystems is difficult and it is thought that these compounds exert a beneficial influence but this has yet to be clearly demonstrated (Juvens et al., 1991).

Probiotics are preparations containing microorganisms and microbial metabolites that are fed to animals of commercial interest to improve their health and productivity (Juvens et al., 1991). Antibiotics are not included since probiotics have been defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Probiotics have beneficial effects because they are digestible nutrients or digestive enzymes; they produce antibacterial substances antagonistic to harmful bacteria; and they are live bacteria that metabolize in vitro to provide nutrients, enzymes, or antibacterial substances. A good probiotic should be a normal inhabitant of the gastrointestinal tract of healthy birds, must be able to survive the conditions of the intestinal tract as it passes through on the way to the intestine and ceca, and must be a colonizer. To be a viable probiotic organism it has to reach the colonization site, be able to attach to gut epithelium, and grow. Certain strains of *Lactobacillus* and *Pediococcus* species are probiotic
candidates because they are non-pathogenic, are part of the normal chicken gastrointestinal flora and thus capable of colonizing the gut, and are known to produce antagonistic substances active against a broad spectrum of bacterial species.

C. TURKEY

1. Anatomy and Growth

The gross structures of the turkey’s digestive tract, including appendages are essentially the same as those of a chicken (Malewitz and Calhoun, 1958). The beak and structures of the oral cavity are similar to those of the chicken. The oral cavity leads to the esophagus which has a mucosal surface marked by openings of numerous esophageal glands. A definite line separates the esophagus from the proventriculus. Six groups of longitudinally arranged openings are present at this point. Papillae containing centrally located deep gland openings appear in the mucosa of the proventriculus. The papillae end at the juncture between the proventriculus and gizzard where the horny layer of the gizzard appears. The gizzard is very muscular with two pairs of muscles. At the juncture between the gizzard and duodenum, the mucosal surface changes from the thick horny layer of the gizzard to an area with irregularly arranged plicae. Villi characterizes the mucosal surface of the duodenum, the remaining small intestine, cecum, and rectum. Folds with numerous lymph follicles constitute the bursa cloacae.
The mucosae of the various organs in the digestive tract differed according to Malewitz and Calhoun (1958) but all were fairly similar except the gizzard. The mucosa of the esophagus and crop is thick, stratified squamous epithelium with the crop slightly thicker than the esophagus. At the junction between the gizzard and duodenum the horny layer of the gizzard and the glands characteristic of the gizzard are replaced by tubular mucous glands. Villi and crypts of Lieberkühn are present in the duodenum. The wall of the intestinal tract from the pylorus to the cloaca is marked by the presence of villi and glands of Lieberkühn. The small intestine is studded with barely macroscopic absorptive structures possessing both rich lymphatic and venous capillaries (Hewitt, 1945). A distinction between the duodenum, jejunum and ileum is difficult to observe in the avian intestine. Goblet cells and simple columnar epithelium with a cuticular border line the entire small intestine. Villi, which branch several times, can be observed throughout. Branched crypts of Lieberkühn open between the villi. The microscopic anatomy of the small intestine is similar throughout with a decrease in height and branching less prominent in the lower two thirds of the small intestine. The large intestine is similar to the small intestine with villi in the ceca between which are the crypts of Lieberkühn. Villi are long at the proximal end of the small intestine, shorter in the mid-portion, and leaf-like at the distal end. The rectum contains villi and abundant lymphoid tissue and lumpy nodes. Mucosa with longitudinal folds made of lymph follicles are located in
the cloaca. The digestive tract of the turkey has similar parts as the chicken but the living requirements for each is different.

2. **Gastrointestinal Flora and Growth**

Little or no information is available concerning the source and mode of *Campylobacter jejuni* infection in poultry. Acuff et al. (1982) found no *Campylobacter* in turkey eggs or newly hatched turkey pouls but when 19 day old turkeys were tested 76% were positive for *Campylobacter*. The exact source of *Campylobacter* was not found in this study.

In herbivores, the feces contain large amounts of food residues and are therefore bulky (Sturkie, 1970). Most of the undigested food material is made up of crude fibers that are only partially digested. This accounts for the relatively poor digestibility of food by herbivores, as only a portion of the dietary cellulose is digested. Other food materials, for example starch and protein, (which are protected by the cellulose), escape attack by the digestive secretions and microorganisms. The tract of a newborn animal is relatively devoid of microorganisms; however, these are rapidly acquired after hatching, and a characteristic population soon develops. In herbivores, the breakdown of foodstuffs in the digestive tract by growth and metabolism of microorganisms is a characteristic feature of digestion, and the absorbed fermentation products constitute an important source of energy to the animal.
Turkeys have increased proteolytic activity of clarified chyme in hydrochloric acid which indicates the presence of certain elements which inhibit proteolysis (Penionzhkevich, 1968). Eating and the process of digestion produce considerable changes in secretionary and motor activity of the bird’s stomach. The stomach of the turkey reacts to various types of feed according to properties of the feed. Gradual increase of proteolytic activity during digestion proves that peptic gland secretions of the turkey stomach are stimulated by humoral factors. This observation is of practical importance to planning an appropriate schedule for the bird. Periodic alterations of the feeding schedule, such as, designated contrast feeding stimulates the stomach.

Penionzhkevich (1968) further states that accumulations of microflora are found in the ceca. Only part of the chyme passes through the ceca. The cecal opening admits only liquid chyme and the material leaving the ceca is semi-liquid, pungent, and usually a dark yellow or brown mass. Excretion of feces from the ceca occurs once for each 9-10 injections of food material from the small intestines. Large amounts of water and dissolved nitrogenous substances are absorbed in the ceca. Feces from the ceca are more dehydrated than those from the small intestine.
D. PROCESSING PRACTICES

1. Effect of Feed Withdrawal

Chickens deprived of water developed a pronounced nephrosis while those
deprived of both feed and water developed a less pronounced nephrosis (Bierer et al.,
1980). Papa and Dickens (1989) used broiler chickens to test feed withdrawal times
of 4, 8, 12, and 16 hours prior to slaughter. The results indicate significant,
progressive emptying of all segments of the lower gut with increasing time. When
analyzed against time of feed withdrawal, the amount of gut contents decreased with
the increased time off feed. This study using a subjectively scored evaluation failed
to detect the production of a more watery excreta with increasing time of withdrawal,
but the method of evaluation may have influenced the results.

The increased presence of cecal material in the colon for the treatment of 4
hours of feed withdrawal may have a practical significance (Papa and Dickens, 1989).
Human enteropathogens such as Salmonella, Campylobacter, and Listeria are known
to localize in the ceca of chickens and cecal defecation occurring in the coop represent
an important source of contamination of birds, carcasses, and processing equipment.
Catching and cooping birds for transport without allowing sufficient withdrawal time
for the voiding of cloacal feces and partial emptying of the ceca would seem to
enhance the possibility for microbial contamination.

Birds that have been stressed for any length of time do not have a normal
intestinal lining. The intestinal lining of these birds is vulnerable to disease producing
organisms or substances. A few hours off feed or water, extreme temperature, poor sanitation, crowding of birds, over medication are all stresses that can affect the bird and make it more susceptible to disease (Risher, 1963).

Studies reviewed by Hudspeth (1978) showed the most practical time off feed was 6 to 8 hours to produce the least change in final yield. Wabeck (1972) indicated that broilers should be off feed and water for 8 to 10 hours prior to slaughter to reduce fecal contamination.

Hulet (1992) showed that temperature significantly decreased the live weight, carcass weight, and chill weight of chickens heated to 32°C. The cold yield and percent uptake of water was greater for these heated chickens. These results suggested that ambient temperature is more beneficial to chickens than the higher temperature.

Turkeys had significantly higher live weights, carcass weights, chill weights, hot yield, and cold yield at 21°C than 32°C. Uptake and percentage uptake were not different for the two temperatures. The turkeys at the 21°C produced a greater yield than those at 32°C (Hulet, 1992).

Feed withdrawal for chicks of 3, 6, and 9 hours resulted in no significant differences in live weight, carcass weight, or chill weight but hot yield and cold yield were significantly greater for 3 hours of feed withdrawal. For turkey the feed withdrawal of 0, 4, and 8 hours produced no significant differences except in hot
yield and cold yield which were significantly less for 0 hours and uptake which was less for 8 hours withdrawal (Hulet, 1992).

The handling of the live bird prior to processing may have a considerable influence on the microbiological condition of the finished product (Barnes and Mead, 1976). Birds are often starved or taken off feed before slaughter to empty the intestinal tract, thus limiting the number of intestinal organisms which could contaminate carcasses during processing. Transportation can also add to the level of contamination if cages are not properly cleaned between successive batches of birds. The stress of transport often increases the rate of excretion and on the multiple cage transports this leads to cross contamination as the birds get the fecal material on their feet and feathers. In wet or snowy weather, birds tended to be more highly contaminated.

Bilgili (1988) states that length of feed withdrawal has a significant effect on the strength of the gastrointestinal tract. Birds with shorter feed withdrawal times have a greater likelihood of gastrointestinal breakage during processing of broilers.

2. **Effects of Temperature as Stress**

Hamm (1969) described the effect of temperature on poultry performance. There is a range of temperature that is most desirable for good health and production of birds. It is called the thermal neutral zone and defined as the environmental temperature range in which temperature can fluctuate and the bird can maintain its
body temperature with little change in internal heat production. This temperature at one time was thought to be 64-75°F or 17-24°C, but these values are being questioned because factors such as acclimatization, ration, breed, and strain all influence the optimum temperature. Better balanced ration causes the thermal neutral temperature to increase. It is concluded that as dietary formulations for the birds become more precise, a general increase in ideal environmental temperature levels may occur with subsequent improvements in feed utilization. Hot weather rations must be more nutritional. Heat stressed birds were found to become over-stimulated, they could not survive without water, and they could be rendered temporarily sterile. Temperature is not the only factor because the effects of temperature are mediated or exaggerated by relative humidity, air movements, acclimatization, and rations. Birds are over-stimulated to eat more at the cold temperatures (winter) and less at the hot temperatures (summer), thus temperature has an influence on the energy intake. Wind can exacerbate the effect of cold temperature and if the bird is also wet there is even more of a chilling effect. Relative humidity exaggerates temperature on the hot side especially above 90°F or 32°C. Birds produce a lot of heat for their size. Heat loss or dissipation can occur via radiation, convection, water evaporation, and excretion. At 72°F (22°C) air temperature only 7-10% of the heat loss is via evaporation, but as the temperature of the environment warms up, radiation and convection losses become smaller and evaporation becomes more important. At 90°F (32°C) about 40% of heat loss is by evaporation. At 105°F (41°C) or the bird’s
body temperature practically all heat loss must come from evaporation. A chicken or turkey does not sweat. All the heat losses at 105°C must come via respiration. As dry air moves across the moist lung surface, water is vaporized. This requires heat energy from the bird. This loss of heat energy provides cooling. If the air is very moist, less cooling occurs so the effect of relative humidity can be observed.

Broilers can be acclimated by exposure to elevated environmental temperatures although it has not been determined how long such acclimation lasts (Arjona et al., 1988). Fasting increases survival time of broilers at extremely hot temperatures since it decreases body heat production.

Body temperature of the broilers increased throughout heat exposure, but birds that had been acclimated had significantly lower body temperatures than the control birds. There was less mortality of birds in the acclimated group suggesting that acclimation helps the bird stabilize body temperature above normal (May et al., 1987).
E. **CAMPYLOBACTER** AND FOOD ANIMAL PRODUCTION AND PROCESSING

1. **Beef**

Stern et al. (1984) reported that fresh beef samples tested positive for *Campylobacter jejuni* in 4.7% of the lot. They reported that frozen samples only had 1.7% positive. The highest level of contamination was found on the beef liver.

Incidence of *Campylobacter* is low in ground beef and addition of blood did not aid in recovery (Stern et al., 1985B). Apparently, some component of blood is beneficial in the isolation of *Campylobacter jejuni*, but it is present in ground beef and does not need to be added for recovery of this organism from beef.

Stern et al. (1985A) studied *Campylobacter jejuni* inoculated cubes of beef as reported by eight cooperating laboratories. Previous investigators (Doyle, 1982) recovered levels of 1-4 cells per gram of inoculated hamburger in 100% of the instances. This study recorded a 62% incidence of .7 cells per gram and an 82% incidence of 7 cells per gram. Lack of consistency could be attributed to the fact that organisms were inactivated during transport, competing microflora may have masked the presence of *Campylobacter*, or different levels of competence in recovery capabilities existed.

In a retail market survey, the presence of *Campylobacter jejuni* and *Campylobacter coli* was found in 3.6% of ground beef and 4.7% of beef flank samples. *Campylobacter* is found more often in the summer and fall. The prevalence
of *Campylobacter* on the red meat samples averaged 5.5% for June and September and fell to 3.8% for December and March (Stern, 1985A).

Limited information is available on the prevalence and distribution of *C. jejuni* on beef carcasses (Kwiatek et al., 1990). In this study .9% of the beef carcasses had *Campylobacter*. These results agree with others who found low incidence of *Campylobacter jejuni* on the beef.

Freezing of inoculated ground beef reduced numbers of *Campylobacter jejuni* by approximately one log\(_{10}\) cycle but did not reduce the cooking time required for elimination of all organisms from one centimeter thick hamburgers (Gill and Harris, 1984). More cooking time was required for ground beef that had been frozen. Hamberger cooked enough to kill all *Campylobacter jejuni* still appeared raw. No *Campylobacter* was isolated from fifty samples of retail ground beef. The minimum level for detection was two grams. These results contrast with previous claims that *Campylobacter jejuni* is a common contaminant of ground meat. Since very minimal cooking would rapidly eliminate *Campylobacter* in ground beef it appears unlikely to be a common vector for human campylobacter enteritis. Direct evidence of a quantitatively significant *C. jejuni* contamination seems necessary for convincing involvement of a red meat dish in an outbreak of the disease even when, as with hamburger, it could be consumed half raw.
2. **Pork**

According to Stern et al. (1984) 8.6% of fresh pork samples were positive for *Campylobacter jejuni* and no frozen samples were positive. Pork livers had the most positives of any pork sampled.

*Campylobacter* is found in porcine intestinal contents at $10^4$ cells per gram (Oosterom et al., 1983A). It was found that 60-70% of pigs harbor *Campylobacter* in their intestinal tract. In pig slaughtering, scalding is done at 60°C for 15 minutes, which is most likely to kill all *Campylobacter* on the skin. Some organisms may remain alive in deeper hair follicles. Contaminated pig carcasses were exposed to normally occurring cooling conditions in a slaughter house. Initial contamination varied but after three hours of cooling no *C. jejuni* could be isolated by direct plating methods but *Campylobacter* was recovered after enrichment in all experiments. Air ventilation during cooling resulted in a hundred fold reduction of *Campylobacter* in 24 hours, whereas cooling without ventilation did not appreciably reduce *Campylobacter* contamination. Drying caused by forced ventilation in the cooling rooms was an important factor. *Campylobacter jejuni* was not isolated from 300 two gram samples of minced pork in the laboratory. The lack of contamination could be explained by reduction of *Campylobacter jejuni* numbers during cooling. Inability of *Campylobacter jejuni* to survive on dry surfaces may be important with respect to cleaning and disinfecting pig slaughterhouses.
Kwiatek et al. (1990) found *Campylobacter* in 2.9% of pork carcasses tested. Higher rates have been reported by others but these are low probably due to prolonged exposure to scalding temperatures of 60°C for 1.5 - 4 minutes. These conditions are relatively stringent and this would account for the low percentage of contaminated carcasses.

*Campylobacter* was recovered from 5% of the pork chops tested and 4.2% of the pork sausage tested (Stern et al., 1985A). Greatest numbers of *Campylobacter* were isolated from pork samples in the summer and fall. Pork is exposed to seasonal variations and might be expected to show more influence.

Bracewell et al. (1985) reported on the incidence of *Campylobacter jejuni/coli* on pork carcasses in Georgia. The results indicated that contamination by *Campylobacter* was lower than had been reported for other areas. This difference could be the result of different herd characteristics, geographical location, or the slaughter method. Chilling reduced *Campylobacter jejuni/coli* to below detectible levels. This study suggested pork carcasses processed conventionally are probably not a problem in food-associated campylobacterosis.

3. **Chicken**

*Campylobacter* has been isolated from inoculated and uninoculated chickens (Stern et al., 1985B). These chickens were then subjected to freezing and thawing. This had a detrimental effect on *Campylobacter* but the study was not certain how
many were killed and how many were only injured. Enrichment may allow the cells to repair and/or proliferate and provide a more sensitive technique for observing the effect of freezing on Campylobacter jejuni. Blood supplementation of enrichment broths was needed for increased selectivity in recovering Campylobacter jejuni from chickens. Treating the chickens with dilute acids such as lactic or acetic had little effect on Campylobacter at 5°C but proved detrimental at 50°C with the lactic acid being more detrimental.

After defeathering and eviscerating the chicken carcasses, Campylobacter species are present in large numbers on the surface of 80.3% of all samples taken (Kwiatek et al., 1990). Even intestinal contents of healthy chickens may contain 10^6 - 10^8 colony forming units per gram (CFU/g) of Campylobacter. Because such large numbers of Campylobacter are released from intestinal contents during the defeathering and eviscerating operations they are present in large numbers on the poultry carcasses. Scalding times in poultry processing vary from 120-170 seconds at temperatures ranging from 58-60°C. These time/temperature relationships are inadequate to eliminate all Campylobacter contaminating the poultry carcasses and recontamination occurs again during the defeathering process further serving to contaminate the carcass. Feather pickers and chilling tanks are areas of major carcass contamination and these occur after the scalding operation. This could explain the high levels reported. This study concluded that poultry carcasses appear to be prominent reservoirs of Campylobacter species in Poland. Decontamination of the
poultry carcasses or diminishing the presence of the organisms in live birds would be of assistance in protecting public health.

Decimal reduction times increased with a decrease in temperature from 60°C to 52°C (Oosterom et al., 1983A). D-values for 60°C are 0.18-0.39 minutes and for 52°C there is a wide variance in D-values of 1.96-10.82 minutes. This shows that a temperature of scald of 58-60°C was not enough to eliminate all Campylobacter contamination.

Drying of poultry during air cooling is not as useful in the reduction of Campylobacter as it was for pork for several reasons. The cooling time for poultry is much shorter, approximately one hour versus 16-24 hours for the pig. The most important consideration is the shape and surface of the skin and the cavities of the poultry carcass which may protect Campylobacter from the influences of drying (Oosterom et al., 1983A).

Freezing results in reduction of C. jejuni contamination but does not eliminate it according to Oosterom et al. (1983A). Storage experiments showed that Campylobacter jejuni can survive on cooled or frozen chicken products in such numbers and for such periods of time that after storage these products may be hazards to public health. This also takes into consideration that the minimum infective dose in man may be as low as four organisms per gram of meat for Campylobacter. Sometimes in cooling and freezing, Campylobacter contamination is reduced but factors causing this have not been identified.
Stern et al. (1985A) found that 29.7% of the retail chicken sampled contained *Campylobacter*. This was much higher than that reported for beef, pork, or lamb. Chickens were not analyzed for seasonal variation because current poultry husbandry practices do not provide for much seasonal variation in rearing. Similar ambient temperatures are held year round, similar feeds are administered, and relatively little contact with the outside environment is experienced by the birds. Consequently, it is probable that less seasonal variations will occur with regard to *Campylobacter* prevalence on poultry products. Gill and Harris (1984) obtained chilled and frozen carcasses from retail outlets and found 70% of the chilled carcasses were contaminated by *Campylobacter jejuni* in numbers which could exceed $10^5$ per half carcass. Less than 20% of the frozen carcasses were contaminated. Cooking for 20 minutes in a conventional oven eliminated *Campylobacter jejuni* from 70% of the carcasses no matter what their treatment prior to cooking. When the cooked carcasses were examined, there were areas where wings or legs were closely pressed to the body and the underlying meat was raw. Surviving contaminants were associated with uncooked skin and stripped frames as these areas afforded transient protection from heat destruction. In checking for recontamination, it appears that heavy contamination of raw carcasses coupled with complete disregard of elementary hygiene are necessary for substantial recontamination to occur. Development of carcass processing procedures that ensure *Campylobacter* contamination does not
exceed $10^3$ per carcass may be a practicable approach to substantially reducing the risk of *Campylobacter* infection from poultry consumption.

Not one of the nation’s 8000 meat inspectors has ever rejected a chicken for containing *Campylobacter jejuni* (Bashin, 1990). The inspection program doesn’t routinely test for microbial contamination. It was not designed to do so. Poultry is inspected for disease and deformity but not bacterial contamination according to the 1957 Poultry Inspection Act.

Isolation of *Campylobacter jejuni* was much greater from fresh (12.1%) tissue than frozen tissue (2.3%) (Stern et al., 1984). This study also found a higher prevalence of *Campylobacter jejuni* in liver samples than in other tissues. Avian livers analyzed had 21.3% positive for *Campylobacter* when fresh and 7.5% positive when frozen.

Blankenship et al. (1983) reported that the swab sampling method would be useful for determining the incidence of *Campylobacter jejuni* among freshly processed poultry carcasses. They suggested the use of brucella broth supplemented with 10% polyvinyl pyrodidine as a suitable medium for transport of swab samples in the frozen state.

Certain foods such as meat, fish, and eggs are known to protect bacteria against injury at low temperatures and *Campylobacter* has been isolated from poultry which had been frozen at -20°C for three weeks. It did not survive well in turkey fecal samples held at -21°C or -70°F for 24 hours (Luechtelfeld et al., 1981).
Campylobacter jejuni did not survive at pH 5 or pH 9, but increased in numbers at pH 6.7 and 8 (Christopher et al., 1982). These tests were done in brucella broth. Although the effect of pH on the survival of Campylobacter will depend on the characteristics of the individual food, this study suggests that Campylobacter will survive poorly in acid or acidified foods or in foods in which a lactic acid fermentation is used (cheese, fermented sausage).

Humphreys and Lanning (1987) suggested that pH could influence the contamination of scald tank water. Modern systems of poultry processing emphasize speed. If the scald tank being alkaline at pH 9 would reduce cross-contamination, then speed could be maintained. It was found that using alkaline scald water helped stop contamination during scalding, but it did little to improve the microbiological quality of carcasses from birds already contaminated when they arrive at the processing plant. It appears the real solution is either to ensure that Campylobacter is not allowed to colonize the live bird or is destroyed on the finished carcass by irradiation.

Lambert and Maxcy (1984) reported that results with Campylobacter jejuni indicate that it is quite sensitive to radiation and generally less resistant than most salmonellae. The radiation resistance was influenced by temperature, menstruum, and age. Campylobacter was more resistant to radiation at subfreezing temperatures and survived better in brain heart infusion (BHI) than in ground meat. The organism is most resistant to irradiation in the stationary phase of growth. Evidence indicated that
application of low doses of gamma radiation would drastically reduce the transmission of *Campylobacter*, and thereby, decrease the public health hazard associated with this organism.

Oosterom et al. (1983B) investigated chicken processing plants and found that contamination with *Campylobacter jejuni* can exist on birds, equipment, hands of processing-line workers, and in air samples from the processing facility. It was found that the intestinal tracts of incoming birds were frequently contaminated with *Campylobacter*. Thus, it is probable that contamination enters the plant with the chicken and is of fecal origin. This contamination with *Campylobacter* in processing plants ultimately results in contaminated end products. *Campylobacter jejuni*-contaminated poultry products are undoubtedly serious hazards for public health especially if these products are improperly handled. Hazards for processing line workers also exists within the processing plants themselves, as demonstrated by the presence of *Campylobacter jejuni* in the air and on the hands of workers. Contamination on hands generally decreased when washed and cooled carcasses were handled. The greatest risks were in the areas of defeathering and evisceration. Contamination is almost exclusively of intestinal origin and if this contamination is not sufficiently reduced during processing it results in contamination of poultry end-products. Both the process and the end-products may be hazardous to public health.

The entire slaughter-processing environment has the potential to perpetuate contamination, because of the close contact of the tissues in the slaughterhouse with
animal and poultry feces (Franco, 1988). The cycle of actual or potential contamination does not always end at the processing establishment. Meat and poultry can be contaminated at retail outlets if poor hygiene exists or insufficient attention is given to the sanitizing and cleanliness of equipment such as knives, cutting blocks, and slicing machines.

Jones et al. (1991) conducted a survey and collected samples of Campylobacter from breeder-multiplier houses, broiler houses, feed mills, hatcheries, processing plants, retail carcasses, and insects and mice trapped at each of the above locations. They concluded that Campylobacter was not transmitted by either feed or eggs, but it was present in the breeder-multiplier environments. Contamination in broilers apparently originated from some unknown source(s) in the broiler house. Campylobacter jejuni could be isolated from birds as they entered the processing plant, after chilling, and even those sampled at retail outlets. The insects collected were checked only for internal contamination and none was found. The mice trapped at each location tested negative for Campylobacter, which differed from previous studies. While cross-contamination appeared to occur within processing plants, field control methods for Campylobacter would appear to be required to control this pathogen in broilers.

Flies may play a linking role in the epidemiology of Campylobacter infection (Rosef and Kapperud, 1983). Seventy-four of one hundred forty-six flies collected from a chicken farm were positive for bacteria and thirteen percent of those isolates
were positive for *Campylobacter jejuni*. The fly may function as a temporary mechanical vector or the pathogen concerned may survive for a longer period of time within the fly’s body with no adverse effects on the carrier host. If the organism is in the fly’s body, this possibly provides an opportunity for multiplication of the pathogen. No attempts were made to discriminate between superficial contamination and infection of the alimentary tract. A study (Ullmann and Kischkel, 1981) found that *Campylobacter* survived 7 days on desiccated swabs which indicates that *C. jejuni* may remain viable for several days on the body surface of the fly or in the flies’ feces even when they have become desiccated. Although this by no means proves that the bacteria isolated from flies originated from the poultry, it is strongly suggestive and would help to explain the transmission of *Campylobacter* to uninfected sites.

Poultry products are different from other animal products in that the skin of poultry is not normally removed during processing (Izat et al., 1988). The outer surface of the bird comes in contact with equipment surfaces, workers’ hands, trimming gloves, and knives producing the potential for carcass to carcass cross-contamination. There is a great concern in poultry processing facilities for microbiological controls and product safety. The study by Izat and co-workers found that *Campylobacter jejuni* is present throughout broiler processing operations, and that levels are reduced whenever water is used. Picking, evisceration, and operations involved with harvesting giblets were associated with increased *Campylobacter jejuni* levels on the skin surface. *Campylobacter* was detected at very low levels on the
final product. Equipment surfaces proved to be likely sources of contamination and recontamination of poultry products.

Baker et al. (1987), reporting on the prevalence of Campylobacter jejuni in poultry meat in New York, found that the major area of contamination in the processing plant was the evisceration stage where the potential exists for transferring intestinal microflora. This study showed a three to four fold increase in carcass contamination after evisceration.

Campylobacter jejuni was isolated from the vent cavity in 48% of the fresh chickens, 100% of uneviscerated chickens, and only 4% of frozen chickens in a retail store in the south of England (Hood et al., 1988). The counts on the birds ranged from $1.5 \times 10^6$ per fresh chicken and $2.4 \times 10^7$ per chicken when uneviscerated. The contamination was distributed generally over the whole carcass. Hood also suggests that the rising incidence of campylobacter enteritis could be a reflection of the increasing proportion of fresh chickens consumed over the time period tested.

Shane (1991A) reported that it is unrealistic to attempt elimination of Campylobacter infections during the growing period, but it is possible to ameliorate processing plant contamination by disinfecting transport coops and by withholding feed for at least 8 hours prior to flock depletion. Post processing decontamination of carcasses and portions with chemical solutions will reduce the level of Campylobacter. A 0.5% acetic or lactic acid rinse effectively reduces levels of assumed to be viable organisms under controlled laboratory conditions. Other studies
showed that 120 ppm chlorine, warm succinic acid and 0.5% glutaraldehyde all reduced *Campylobacter* contamination. Gamma radiation of poultry meat at subradicidation (pasteurization) levels of 1-5 kilogram (kGy) using a cobalt-60 source will eliminate *Campylobacter* without inducing any undesirable organoleptic or biochemical changes in the product.

*Campylobacter jejuni* has been isolated from commercially processed poultry (Cunningham, 1982). Organisms may survive commercial processing procedures including chlorination as well as storage for 2-5 days at 3°C and 1-2 months at about -20°C. Cunningham also found that *Campylobacter* strains isolated from patients, poultry products, and poultry slaughterhouses were indistinguishable.

4. **Turkey**

*Campylobacter jejuni* is very sensitive to dehydration and was unable to survive when dried in the presence of turkey feces on filter paper (Genigeorgis, 1986). The conditions in the slaughterhouse are moist thus more suitable for *Campylobacter* survival. A high prevalence of *Campylobacter* is found in the feather picker drip water which emphasizes the extensive contribution of the defeathering machines to cross-contamination. Increased prevalence of *Campylobacter* in the feather picker drip water may be due to additional cross-contamination of fecal origin as a result of pressure exerted upon the carcass from the rubber fingers of the
defeathering machine. Water recycled in the plant can cause contamination problems as well as the unnecessary movements of plant personnel.

The design of a modern processing plant is such that the later stages in processing are separated from earlier stages in order to minimize contamination of the processed carcass (Barnes et al., 1976). It is particularly important to isolate the killing area since there is considerable airborne contamination from organisms carried on feet and feathers. Almost every stage in the process can have some effect on carcass contamination and ultimately on the microbiological condition of the bird. In the United Kingdom turkeys, are 'hard' scalded between 58-63°C to facilitate the removal of the large feathers. Changes in the skin caused by hard scalding may encourage the growth of spoilage bacteria; however, there is an advantage since many bacteria are destroyed in the scald tank at the higher temperatures. Organisms that remain viable on the scalded carcass are firmly adhered to the skin and are not easily removed during subsequent washing stages. Experiments have shown that a single contaminated carcass can cross-contaminate more than 200 other carcasses passing through the picking machines. The extent of fecal contamination during evisceration appears to be variable since there are many opportunities for cross-contamination. Passing the carcass through an efficient spray washer decreases contamination about ten-fold, but rarely more. If the carcass is not chilled below ambient temperature rapidly the microorganisms present can multiply, doubling almost every hour or more rapidly if the temperature is high. Operating mechanical immersion chillers without
adequate controls results in increased carcass contamination rather than decreased, and
cross contamination becomes a problem. Handling of the product during the post-
chilling steps provides further opportunities for contamination and bacterial
contamination can increase significantly if carcasses are not dealt with promptly
and/or the muscle temperature is allowed to rise above 10°C.

Research by Wesley and Stadelman (1985) confirmed previous reports that
poultry products are highly contaminated with Campylobacter jejuni. No apparent
differences were found in isolation rates or estimated numbers of Campylobacter
between carbon dioxide packaged carcasses and conventional ice-packed birds.
Freezing carcasses caused the isolation rate to drop dramatically, indicating that a
freeze-thaw cycle may effectively reduce the number of Campylobacter on poultry
carcasses.

Kwiatek et al. (1990) reported that 3% of the turkey carcasses examined were
contaminated with Campylobacter jejuni. This is much lower than other reports by
Moran, Luetchtelfeld and Wang according to Kwiatek. This discrepancy may be due
to differences in colonization rates of the live birds, differences in sample location on
the process line, or differences of the feeding regimen of the animals.

The contamination rate of individual carcasses with thermophilic
Campylobacter is greater than contamination by Salmonella. Modified rearing
practices may influence elimination of the Campylobacter from poultry flocks
(Lammering et al., 1988). Turkeys were more frequently contaminated with
*Campylobacter jejuni* than either hens or broiler chickens. *Campylobacter* was absent from some lots of poultry. This indicates that modified rearing practices may eliminate the organism from poultry flocks.

Live turkeys had a median count of approximately 4,700 organisms per square centimeter, whereas the final product had a median count of approximately 44,000 per square centimeter of skin (Walker and Ayres, 1959). The number of organisms found decreased in the scald tank, probably attributable to the temperature of the water and the fact that there was a large volume of fresh water added as scalding progressed. Counts went up after picking which would be expected as organisms from feet, feces and feathers probably are disseminated over the skin surfaces by the flailing rubber fingers of the picking machine and as a result of handling by workers. In the chill tank, especially during aeration, organisms are washed from the surfaces and tend to be evenly distributed over all the birds. This method of chilling can easily cause contamination to be spread from birds carrying high loads of organisms to those birds harboring few or no organisms. The surface of the bird has more total bacteria than the cavity but the cavity has more coliforms. The total bacterial loads tended to be higher on turkeys than chickens but only slightly.

A study by Acuff et al. (1986) found that minimal cooking procedures destroy *Campylobacter jejuni* on turkey but the possibility of cross contamination of other foods that are not heat treated can be potentially dangerous.
F. CONCLUSIONS AND STATEMENT OF PROBLEM

_Campylobacter jejuni_ is a Gram-negative bacteria that grows microaerophically in small mucoid colonies. The growth of _Campylobacter jejuni_ occurs best at 42°C and a neutral pH. This bacteria is catalase and oxidase positive and neither ferments nor hydrolyzes carbohydrates (Smibert, 1989). _C. jejuni_ is hard to differentiate when at the species level, but improved techniques and tests have made it much easier to isolate. The addition of .25% each of ferrous sulfate, sodium metabisulfite, and sodium pyruvate (FPB) greatly increase aerotolerance and allows for better isolation (Krieg, 1990). Addition of Blaser-Wang supplement provides a better selective medium for isolation of _Campylobacter jejuni_. CampyPak II works well to produce microaerophilic conditions.

_Campylobacter jejuni_ is not harmful to chickens or turkeys but a very few cells can produce human infection (Stern, 1990). Campylobacter enteritis in humans can vary from no symptoms to severe disease (Doyle, 1981). _Campylobacter_ is found most often in poultry (Fricker, 1989). _Campylobacter_ may be transmitted by direct contact with contaminated animals or animal carcasses, through ingestion of contaminated food and water, person-to-person from excreters with active infections, perinatal and childhood transmission (Franco, 1988). _Campylobacter_ is widely distributed and is spread during concentration of animals in brooding houses, slaughtering, and processing.
The intestinal tract of birds varies according to breed, individual features, and feed (Penionzhkevich, 1968). The flora in the gastro-intestinal tract differs from bird to bird and also within the various areas of the tract (Sheldon, 1979). The digestive tract of the turkey is very similar to the chicken with the major difference being in overall size (Malewitz et al., 1958). Microflora accumulates in the ceca where the feces are dehydrated (Penionzhkevich, 1968). *Campylobacter* is most frequently found in the lumens of mucus-filled crypts (Stern et al., 1991). The newly hatched bird has no microflora and can easily be colonized by the first bacteria it encounters (Nurmi, 1988). Nurmi also suggests that one way to avoid pathogens like *Campylobacter* may be to competitively exclude it with other harmless bacteria.

Chickens can be colonized by an oral dose of 3500 CFU of *Campylobacter jejuni* within the first three days after hatch. The site of attachment for the *Campylobacter* is the mucin layer of the intestines (Stern et al., 1988).

Juven et al. (1991) suggested that probiotics used as microbial feed supplements could improve the intestinal microbial balance. Certain strains of *Lactobacillus* and *Pediococcus* species are good candidates for probiotics because they are non-pathogenic and part of the normal chicken gastro-intestinal flora.

Birds withdrawn from feed the longest had the greatest excrement during slaughter. The birds withdrawn from feed the shortest time had the largest amounts of cecal material which had the greatest concentration of bacteria. This would enhance the possibility for microbial contamination (Papa et al., 1989).
A very low incidence of *Campylobacter jejuni* was found on beef. This contamination could easily be eliminated by cooking (Stern et al., 1984, Stern et al., 1985, and Kwiatek et al, 1990). Although present pork contamination is greatly reduced by scalding and subsequent drying as the carcass cools (Oosterom et al., 1983A).

Kwiatek et al. (1983) reported that 80.3% of chicken carcasses were contaminated with *Campylobacter jejuni*. Processing of chickens lowers the overall bacterial count, but much contamination remains. *Campylobacter jejuni* is in the intestinal tracts of many birds that come into the processing plant. This often results in a contaminated end product that could be a serious public health hazard (Oosterom et al., 1983B).

Chickens and turkeys are different from other animal products since the skin of poultry is not removed during processing. Bacteria can live on the skin and be found on the final product (Izat et al., 1988). Almost every stage in the processing can have some effect on carcass contamination as bacteria are in the air and water. Washing and chilling decreases contamination but the immersion can also cross-contaminate (Barnes et al., 1976).

Nurmi (1988) states the importance of foodborne infections and intoxication is considerable and the problems caused for public health by various bacteria are increasing. The prevention of these infections requires large-scale research and measures including the whole food chain, from production to consumption. A most
important factor is to keep the amount of pathogens causing food infections as low as possible in the animals, especially in their intestines. Competitive exclusion is the most modern and promising method in preventing and reducing enteropathogens in the intestines and it is also economically feasible. The competitive exclusion method has been developed for poultry. Additives may have a greater impact in the prevention of pathogens in food processing.

*Campylobacter jejuni* is associated most often with poultry. It should therefore be helpful to poultry producers and processors to know if the amount of *Campylobacter* in live birds has any correlation to the levels of *Campylobacter* on the carcass. The following objectives were designed to determine this information.

**OBJECTIVES:**

1) Determine whether there is a statistically significant correlation between the numbers of *Campylobacter jejuni* in the intestinal tract of the live bird and the number of *Campylobacter jejuni* on the carcass of eviscerated poultry.

2) Determine if temperature stress and feed withdrawal will affect the degree of *Campylobacter jejuni* contamination on the finished product.
III. MATERIALS AND METHODS

A. CULTURE

The culture of *Campylobacter jejuni* for this study was obtained from the Richard B. Russell Agricultural Research Center (U.S.D.A.- A.R.S.) in Athens, Georgia. It is strain P.S. 969 which is resistant to 15 mg tetracycline/liter and 50 mg kanamycin/liter. These resistance factors are plasmid-mediated. Stock cultures were maintained in brucella broth supplemented with ferrous sulfate, sodium metabisulfite, and sodium pyruvate. The culture was transferred monthly, incubated at 42°C for 48 hours, and then kept at 4°C. It was tested for purity by Gram-staining and microscopic examination.

B. DESIGN OF EXPERIMENT

Research was conducted using chickens and turkeys as test animals. The chicks and poults were obtained on day of hatch from the Rocco Hatchery, Harrisonburg, Virginia. The design of this experiment involved live animals, two different holding temperatures of 70°F (21°C) and 90°F (32°C); and three different feed withdrawal times of 3, 6, 9 hours for chickens and 0, 4, 8 hours for turkeys (Table 3). This study was performed on birds used as part of other research to evaluate temperature, cooping times, feed withdrawal, and sex on carcass yields. Cooping time and sex were not variables in the research detailed here.
### TABLE 3

**EXPERIMENTAL DESIGN**

**TEMPERATURE 1  32°C (90°F)**

<table>
<thead>
<tr>
<th>Feed Withdrawal</th>
<th>Short Cooping 6 hours</th>
<th>Long Cooping 10 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Withdrawal Short 3 hr or 0 hr</td>
<td>Group 1 5 Birds</td>
<td>Group 2 5 Birds</td>
</tr>
<tr>
<td>Feed Withdrawal Medium 6 hr or 4 hr</td>
<td>Group 3 5 Birds</td>
<td>Group 4 5 Birds</td>
</tr>
<tr>
<td>Feed Withdrawal Long 9 hr or 8 hr</td>
<td>Group 5 5 Birds</td>
<td>Group 6 5 Birds</td>
</tr>
</tbody>
</table>

**TEMPERATURE 2  21°C (70°F)**

<table>
<thead>
<tr>
<th>Feed Withdrawal</th>
<th>Short Cooping 6 hours</th>
<th>Long Cooping 10 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed Withdrawal Short 3 hr or 0 hr</td>
<td>Group 1 5 Birds</td>
<td>Group 2 5 Birds</td>
</tr>
<tr>
<td>Feed Withdrawal Medium 6 hr or 4 hr</td>
<td>Group 3 5 Birds</td>
<td>Group 4 5 Birds</td>
</tr>
<tr>
<td>Feed Withdrawal Long 9 hr or 8 hr</td>
<td>Group 5 5 Birds</td>
<td>Group 6 5 Birds</td>
</tr>
</tbody>
</table>
Campylobacter jejuni was inoculated into male birds only. Only birds from the long cooping time were evaluated for Campylobacter jejuni. Five birds were in each feed withdrawal group, each bird was swabbed, and Campylobacter counts were averaged for each group. The experiment was replicated three times the first year for chickens and turkeys. The second year it was replicated three times for chickens and two times for turkeys.

C. INOCULATION

Birds were inoculated when they were one day old. The inoculum for the birds was prepared by isolating Campylobacter jejuni on brucella agar, supplemented with 0.25% ferrous sulfate, 0.25% sodium metabisulfite, and 0.25% sodium pyruvate. This is referred to as FPB agar (Kreig, 1991). The agar additionally had 15 mg tetracycline per liter, 50 mg kanamycin per liter, and two vials of Blaser-Wang supplement per liter. Filter sterilized antibiotics were added to the agar after it had been autoclaved and cooled to 55°C. The Blaser-Wang supplement (Oxoid Ltd.) contained 10 mg per liter of vancomycin, 2,500 IU per liter of polymyxin B, 5 mg per liter trimethoprim, 2 mg per liter of amphotericin B, and 15 mg per liter of cephalothin. Bacteria on these plates were incubated for 48 hours at 42°C in BBL GasPak jars in which the atmosphere was adjusted. Several colonies were taken from the isolation plate and streaked on another brucella agar (FPB) plate and incubated for 48 hours. At the end of 48 hours, 2 ml of sterile 0.1% peptone water was added.
aseptically to the plate and a sterile cotton swab was used to wash the lawn of bacteria from the plate in 98 ml of 0.1% sterile peptone water. This inoculum was thoroughly mixed and sampled to enumerate Campylobacter. The inoculum was then gavaged into the crop of every male chick or poult. Each bird was given 0.5 ml.

The first year, the birds were only gavaged to inoculate them. The second year the birds were all gavaged, and in addition, Campylobacter was added to the bird's water for five days. The inoculum for water was prepared in the same manner as the gavage inoculum with 100 ml of the inoculum added to one gallon of water. Tests of the water were made to determine survival time of the Campylobacter in tap water. Campylobacter jejuni counts were made on the contaminated water every hour for eight hours and then at two to four hour intervals up to 24 hours.

D. GAS SYSTEM

The gas system used for the first year was Campy-Pak Plus (Becton Dickinson Company, Cockeysville, MD). This is a disposable hydrogen + carbon dioxide generator envelope which creates an environment suitable for microaerophiles. A palladium catalyst is attached to the envelope. The envelope is opened and water is added to dissolve the sodium borohydride tablet and the sodium bicarbonate plus citric acid tablet. This generates hydrogen which unites with oxygen to form water and carbon dioxide so that the atmosphere in the BBL Gas Pak 100 jars or anaerobe jars has reduced oxygen and increased carbon dioxide.
The gas system used the second year consisted of a Precision Scientific Model 25 Vacuum Pump, vinyl tubing, a Fisher Scientific Manometer, and compressed carbon dioxide and nitrogen gas (Airco). Using the vacuum pump, air was drawn out of the anaerobe jar to 21 inches of mercury on the manometer. Then, carbon dioxide was added to a manometer reading of 19.5 inches and nitrogen was used to equalize the pressure to atmospheric pressure (0 inches of mercury). This gives a gas mixture of 85% nitrogen, 6% oxygen, and 9% carbon dioxide (Krieg, 1991). The gas mixture was monitored using a Fisher-Hamilton Gas Partitioner - Model 29 to verify the correct levels of each gas.

E. GROWTH AND SAMPLING OF BIRDS

Inoculated chicks were grown for five weeks and the turkey pouls were allowed to grow for 14 weeks under optimum conditions. Birds had access to feed and water at all times during this growth period. One week prior to slaughter the 60 birds were divided into two separate pens. One pen of 30 birds was kept at a temperature of 21°C (70°F). The other pen of 30 birds was kept at 32°C (90°F).

Chickens and turkeys had similar schedules for feed withdrawal prior to cooping. Nine hours prior to cooping feed was withdrawn from Group 5 and 6. At 6 hours prior to cooping group No. 3 and 4 had feed withdrawn and at 3 hours prior to cooping Group No. 1 and 2 had feed withdrawn. The process was the same for turkeys except the times when feed was withdrawn were 0 hours, 4 hours, and 8
hours prior to cooping. Ten hours before slaughter all birds in Group No. 2, 4, and 6 (Table 3) the long cooping groups, were cooped and at 6 hours before slaughter the other three Groups No. 1, 3, and 5 the short cooping groups were cooped.

One week prior to slaughter; chickens - 5 weeks, turkeys - 14 weeks, three groups (Treatments 2, 3, and 4) of five birds each were swabbed cloacally using a sterile cotton swab that was immediately placed in 9 ml of 0.1% sterile peptone water which was kept on ice. Birds were all males and had been cooped for 10 hours. The three groups differed in lengths of feed withdrawal. Each bird’s sample was vortexed and 0.1 ml of serially diluted sample was plated using duplicate plates for each dilution. They were plated on FPB agar which was supplemented as previously mentioned with tetracycline, kanamycin and Blaser-Wang supplement. Plates were placed in anaerobe jars, the gas level was adjusted, and the anaerobe jars were placed in the incubator at 42°C for 48 hours. After incubation, a Campylobacter count was made and recorded.

One fecal sample was also taken from each feed withdrawal group of five birds one week prior to slaughter. This sample was gathered fresh a2nd placed in a Stomacher bag and placed on ice for transport to the lab. The sample was weighed and 99 ml. of 0.1% sterile peptone water was added. This was placed in the Stomacher Lab Blender Model #400 and allowed to mix for three minutes. It was then serially diluted, plated on the supplemented FPB agar, and incubated for 48 hours. The Campylobacter jejuni colonies were counted and recorded.
One week later, birds were slaughtered, plucked, eviscerated, and chilled overnight in an ice water bath. Each bird was hung by its feet, stunned, and slaughtered by cutting the throat with a knife and allowing time for exsanguination. Birds were water scalded in a Honeywell Gas Drum Scalder for 45 seconds at 62°C. They were plucked using a barrel picker manufactured by Leroy Somers, Inc. which continually washed the bird with water for the 60 second tumble. After defeathering each bird was eviscerated by hand, rinsed by being sprayed with water and placed in an icy water bath where they remained overnight (16-18 hours). Each group of birds was chilled separately. Samples of skin were taken from each bird. Forty square centimeters of skin was taken from each bird, ten square centimeters of skin from each of four locations. Samples came from the breast, the lower back, and from under each wing. A template was used to determine the size of each sample and the skin was cut using a sharp sterile knife and rubber gloves so as not to add any contamination. Skin samples were placed in stomacher bags which were placed on ice for transport back to the laboratory. In the laboratory, 99 ml of 0.1% sterile peptone water was added to each sample and mixed for three minutes in the stomacher. Plates were placed in anaerobic jars, the atmosphere was adjusted, and each jar was incubated at 42°C for 48 hours. Campylobacter counts were then made.
F. LONGITUDINAL STUDY OF TURKEYS

A study was made using 25 poulets that were gavaged when they were one day old. Each poult was given 0.5 ml of inoculum prepared as previously described and Campylobacter jejuni was added to the drinking water for five days after hatch. These turkeys were allowed to grow with access to feed and water at all times. Individual cloacas were swabbed when the birds were three weeks old and every other week until they were 13 weeks old. Swabs were placed in 9 ml of 0.1% sterile peptone water which was kept on ice. Each sample was vortexed and 0.1 ml of serially diluted sample was plated using duplicate plates for each dilution. Plating was on FPB agar which also contained Blaser-Wang supplement, tetracycline, and kanamycin. Plates were incubated in anaerobe jars with the gas level properly adjusted for 48 hours at 42°C. After incubation, a Campylobacter count was made and recorded.

G. STATISTICAL ANALYSIS

Campylobacter jejuni counts for each bird were put into scientific notation and converted to logarithmic numbers. An average was found for each group of five birds for the cloacal samples and the carcass samples. These averages then were analyzed by ANOVA for correlations between log_{10} colony forming units (CFU) of Campylobacter from cloacal samples and from carcass samples; cloacal samples and fecal samples; and fecal samples and carcass samples.
IV. RESULTS AND DISCUSSION

A. INOCULUM

_Campylobacter jejuni_ inoculum prepared for each replication of birds was found to contain _Campylobacter_ in the range of $10^5$ - $10^7$ per 0.1ml. The dose given each bird was 0.5 ml. of inoculum.

The water inoculum contained _Campylobacter_ at $10^5$ - $10^6$ and those bacteria were viable for at least 24 hours. See Table 4. Water was changed and fresh inoculum was added every 24 hours for five days after hatch.

B. CHICKEN BACTERIAL COUNTS

_Campylobacter jejuni_ was found at high levels in the chicken fecal and cloacal samples. _Campylobacter_ was found at a much lower level in the carcass samples. The results of three replications during year 1 are live _Campylobacter_ counts which ranged from $1.7 \times 10^6$ to $1.9 \times 10^8$ colony forming units (CFU)/swab and carcass _Campylobacter_ count which ranged from $1.5 \times 10^3$ to $3.0 \times 10^4$ CFU/cubic centimeter. The live _Campylobacter_ count was higher than the carcass _Campylobacter_ count as would be expected since the process of evisceration will decrease the numbers of bacteria as will the difference between a cloacal swab and a skin sample. The fecal counts were higher than the live counts. The fecal counts ranged from $1.2 \times 10^7$ to $7.3 \times 10^9$ CFU/gram.
**TABLE 4**

**BACTERIAL VIABILITY IN WATER**

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Begin Hour One</td>
<td>3.3 x 10⁶ Peptone</td>
</tr>
<tr>
<td></td>
<td>1.6 x 10⁶ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>3.0 x 10⁶ Tap Water B</td>
</tr>
<tr>
<td>Hour Two</td>
<td>7.2 x 10⁵ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>4.0 x 10⁵ Tap Water B</td>
</tr>
<tr>
<td>Hour Three</td>
<td>4.8 x 10⁵ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>4.0 x 10⁵ Tap Water B</td>
</tr>
<tr>
<td>Hour Four</td>
<td>2.1 x 10⁵ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>1.3 x 10⁵ Tap Water B</td>
</tr>
<tr>
<td>Hour Five</td>
<td>1.7 x 10⁵ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>4.2 x 10⁵ Tap Water B</td>
</tr>
<tr>
<td>Hour Six</td>
<td>1.7 x 10⁵ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>6.9 x 10⁵ Tap Water B</td>
</tr>
<tr>
<td>Hour Seven</td>
<td>1.5 x 10⁵ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>5.1 x 10⁵ Tap Water B</td>
</tr>
<tr>
<td>Hour Twelve</td>
<td>3.0 x 10⁴ Tap Water A</td>
</tr>
<tr>
<td></td>
<td>2.1 x 10⁵ Tap Water B</td>
</tr>
</tbody>
</table>

The results from the three replications completed during year 2 are live *Campylobacter* counts which ranged from 0.5 x 10⁵ to 1.4 x 10⁶ CFU/swab, carcass *Campylobacter* counts which were less than the live count ranging from 1.3 x 10⁴ to
32.4 x 10² CFU/cm², and fecal counts which were the highest ranging from 9.3 x 10⁴ to 2.9 x 10⁸ CFU/gram.

The overall range for chickens live counts was from 0.5 x 10² to 1.0 x 10⁸ CFU/swab (Figure 1). This wide range for the counts reflects a large difference in the populations of Campylobacter recoverable from chickens. The first year, the plates were crowded with many different bacteria, but the second year was much better as the plates contained Campylobacter with very few other bacteria. This could be attributed to the improved techniques used in year two which include the use of the addition of ferrous sulfate, sodium pyruvate, sodium metabisulfite, and the Blaser-Wang supplement. These additions helped the Campylobacter to grow better and kept other bacteria from growing thus allowing the count of Campylobacter to be made with greater ease and certainty. The overall carcass count ranged from 1.3 x 10¹ to 3 x 10⁵. The overall fecal count was 9.3 x 10⁴ to 7.3 x 10⁹.

During Year 1 of the experiment the chickens had a high level of contamination when live as can be seen in the fecal and cloacal counts. The carcass counts were much less and not all birds were contaminated. For some birds, the evisceration process reduced the bacterial count to levels that were undetected (10⁴ year 1 and 10⁸ year 2) or not present. The highest counts were for the fecal samples. More Campylobacter were present in these samples but these samples were taken from only one bird per group. The live count from cloacal swabs was less than the fecal swabs and is an average of five birds. There was a significant correlation
Figure 1. The range of $\log_{10}$ colony forming units (CFU) found in samples taken from cloacas, feces, and carcasses of market age chickens for each year of the study.
between the live count from cloacal swabs and the fecal samples for year 2 and year 1 was close to statistical significance (r = .45 year 1 and r = .53 year 2). The carcass count averages were lower because some birds with no contamination were included.

C. TURKEY BACTERIAL COUNTS

The first year had three replications. The live or cloacal counts ranged from not detectable to $5.2 \times 10^6$ CFU/swab. The carcass counts were not detected. These counts showed that *Campylobacter* level was lower on the carcass than in the live birds. Fewer *Campylobacter* on the carcasses was probably due to the evisceration process which kills some of the bacteria. The fecal counts ranged from not detected to $3.5 \times 10^9$ CFU/cubic centimeter.

The second year had two replications. The live or cloacal count ranged from $5 \times 10^0$ to $2.0 \times 10^3$. Carcass counts ranged from $1.3 \times 10^1$ to $3.9 \times 10^2$. The carcass count is less than the live count but there is a slight overlap. This could be caused by the contamination of the rinse water by one bird with high numbers of *Campylobacter* which would then be on all birds in that group. Not all birds contained significant numbers of *Campylobacter jejuni* prior to slaughter but when checked after evisceration and storage in the contaminated rinse water more birds showed some contamination. The overall range of *Campylobacter* for turkeys was $5 \times 10^0$ to $1.4 \times 10^6$ CFU/swab for the live count (Figure 2). The carcass count ranged from $1.3 \times 10^1$ to $2.5 \times 10^3$ CFU/cubic centimeter. The fecal count ranged from
1. No CFU of *Campylobacter jejuni* were detected at $10^{-3}$ on the carcass Year 1.

Figure 2. The range of $\log_{10}$ colony forming units (CFU) found in samples taken from cloacas, feces, and carcasses of market age turkeys for each year of the study.
2.5 x 10^1 to 3.5 x 10^9 CFU/gram. This large range indicates that some birds had no contamination and some had a very high level of contamination with Campylobacter. The first year other bacteria crowded out the Campylobacter growth. The second year more Campylobacter grew and were identified with lower dilutions, the FPB supplement, and the Blaser-Wang supplement.

The turkey plates had many different kinds of bacteria and Campylobacter was confirmed in only a few of the cloacal or fecal samples (Figure 3). The detection threshold was higher the first year so very low levels of Campylobacter were not detected and the numbers reported reflect this. The fecal samples were plated at a higher level than the cloacal samples. In order to read the fecal plates they were diluted and plated at 10^4 - 10^8. The number appears to be greater for year 1 but the dilution level of plating was so high that when Campylobacter was not detected the reported count was greater than 10^4. The "not detected" for year 1 was higher than the average for year 2. The first year no FPB or Blaser-Wang Supplements were used and the level of other bacteria was high enough to make it difficult to detect the Campylobacter. Year 2 for turkeys continued to show very low numbers of Campylobacter but it was detected more than the first year.
Figure 3. The percentage of turkey cloacal and carcass samples that produced positive growth of C. jejuni for each year of the study.
D. TEMPERATURE EFFECTS

The chickens that were subjected to the hot temperature of 32°C or 90°F had a live cloacal count of Log 5.7 CFU/swab overall. The chickens subjected to temperatures of 21°C or 70°F had a live Campylobacter count of Log 5.7 CFU/swab. If these counts are broken down according to year it would be as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Temperature</th>
<th>Log Count ± Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 1</td>
<td>32°C or 90°F</td>
<td>7.0 ± .58</td>
</tr>
<tr>
<td></td>
<td>21°C or 70°F</td>
<td>6.5 ± .47</td>
</tr>
<tr>
<td>Year 2</td>
<td>32°C or 90°F</td>
<td>4.3 ± .93</td>
</tr>
<tr>
<td></td>
<td>21°C or 70°F</td>
<td>4.4 ± .68</td>
</tr>
</tbody>
</table>

The turkeys that were subjected to the hot temperature of 32°C had a live cloacal count of Log 2.6 CFU/swab overall. Those turkeys at the cooler temperature of 21°C had a live cloacal count of Log 2.6 CFU/swab overall. If these counts are broken down according to year it would be as follows:

<table>
<thead>
<tr>
<th>Year</th>
<th>Temperature</th>
<th>Log Count ± Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year 1</td>
<td>32°C or 90°F</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>21°C or 70°F</td>
<td>3.6 ± 1.31</td>
</tr>
<tr>
<td>Year 2</td>
<td>32°C or 90°F</td>
<td>1.9 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>21°C or 70°F</td>
<td>1.1 ± 0.36</td>
</tr>
</tbody>
</table>
When the influence of temperature was assessed, no significant difference was found between the Campylobacter levels at the two temperatures (Figure 4 and Figure 5). These graphs show that with only small variations the 32°C (90°F) and 21°C (70°F) temperatures resulted in similar Campylobacter jejuni counts. Temperature did not affect the growth of Campylobacter.

E. FEED WITHDRAWAL EFFECTS

Chickens that had feed withdrawn 3 hours prior to slaughter had a live cloacal count of Log 2.8 CFU/swab, whereas those with feed withdrawn 6 hours prior to slaughter had a count of Log 2.9 CFU/swab, and those with feed withdrawn 9 hours prior to slaughter had a count of Log 2.7 CFU/swab. These are the average of all replications performed.

Turkeys that had feed withdrawn 0 hours prior to slaughter had a live cloacal count of Log 2.6 CFU/swab, while those with feed withdrawn at 4 hours prior to slaughter had a count of Log 2.7 CFU/swab, and those with feed withdrawn at 8 hours prior to slaughter had a count of Log 2.6 CFU/swab. These are the average of all replications done.

The difference between the Campylobacter counts for each feed withdrawal group is very small and no effect was seen. Withdrawing of feed did not affect growth of the bacteria as seen in this experiment (Figure 6 and Figure 7).
Figure 4. The $\log_{10}$ CFU of *C. jejuni* found on chicken carcasses at each tested temperature.

Figure 5. The $\log_{10}$ CFU of *C. jejuni* found on turkey carcasses at each tested temperature.
Figure 6. The log_{10} CFU of *C. jejuni* found on chicken carcasses sorted by feed withdrawal.

Figure 7. The log_{10} CFU of *C. jejuni* found on turkey carcasses sorted by feed withdrawal.
F. **LONGITUDINAL STUDY**

This study began with 25 birds and ended with 20 birds. Four birds died during weeks two and three. At this time the mortality rate was high in other pens of birds within the same house who had not been inoculated with *Campylobacter*. One bird died during week eight. This bird had maintained stable levels of *Campylobacter* from week two to death. The level of *Campylobacter* in each bird rose quickly as did the number of birds infected. By week five, 100% of the turkeys showed infection with *Campylobacter jejuni* and the average number of bacteria per bird is 5000 per 0.1 milliliter. Week 5 was the peak for the number of *Campylobacter*. As the turkeys approached 7 weeks, the actual average number of bacteria declined and continued to decline to week 13 when the average contamination is log 0.9 or less than 10 per 0.1 milliliter. The number of birds infected peaks during weeks 5 and 7 when 100% are positive but drops off to only 50% positive at week 13 (Figure 8). These results help to explain the low levels of bacteria present when turkeys were swabbed just prior to slaughter at 14 weeks.

G. **STATISTICAL CORRELATIONS**

The correlation between the live cloacal count and the carcass count for chickens can be broken down into the two years. For year 1 $r = -0.34$ and for year 2 $r = -0.19$. This showed a negative correlation. Neither year was significant. The correlation between the live cloacal count and the fecal count for chickens is positive.
Figure 8. The $\log_{10}$ CFU of \textit{C. jejuni} found in turkey cloacal samples taken every other week from week 3 to week 13 of age and the percentage of turkey cloacal samples with \textit{C. jejuni} growth at each of the times sampled.
The $r = 0.43$ for year 1 and 0.53 for year 2. Year 2 is significant ($p = .04$) and year 1 is close, but not significant ($p = .08$).

The correlation for the turkeys between the live cloacal count and the carcass counts was $r = -0.36$ ($p = .26$). This is similar to the first year for the chickens. The correlation between the live cloacal counts and the fecal counts was $r = 0.49$ ($p = .13$). This also was similar to the chicken data.

These results indicate that the amount of *Campylobacter* in test animals is not affected by either the temperature stress or the feed withdrawal stress. The amount of *Campylobacter* found in the live bird is not a good indication of the level of contamination found on the carcass of birds. The amount of *Campylobacter* in the fecal droppings is an indication of the level of infection of the birds.
V. SUMMARY AND CONCLUSIONS

Birds were inoculated with *Campylobacter jejuni* which became a part of the microflora of both chickens and turkeys. This confirms reports by Shane (1991B) that broiler and turkey flocks can be contaminated with *Campylobacter jejuni*. Although the *Campylobacter* was part of the microflora of each type of bird, there were differences in the number of bacteria that could be recovered. The amount of *Campylobacter jejuni* found in the live chicken varied over a large range. A study by Stern et al. (1990) states that chickens can be colonized and remain colonized for at least eight weeks and the data from our study confirms this finding. The *Campylobacter* count in the fecal samples varied as did the *Campylobacter* count on the carcasses of the chickens. When the fecal count was correlated with the cloacal count it was found to be \( r = 0.53 \) for the first year and \( r = 0.43 \) for the second year. When live count was correlated with the carcass count the correlations was \( r = -0.19 \) for the first year and \( r = -0.34 \) for the second year. These correlations indicate that the level of contamination of the live bird is not a good indication of the contamination of the carcass. The possible reason for this poor correlation is that while many living birds did not contain *Campylobacter* those that did could contaminate the rinse water which would contaminate birds that were not originally contaminated with *Campylobacter jejuni*.

The turkeys had a lower overall *Campylobacter* count on the cloacal, fecal, and carcass swabs than did the chickens. Acuff et al. (1982) reported that 76% of
young turkeys 19 days of age are contaminated with *Campylobacter jejuni* but that study did not give results for older turkeys. Our data confirms that of Acuff and adds information about the growth and colonization for older birds. Our longitudional study provides information on the long term survival of *Campylobacter jejuni* in turkeys. *C. jejuni* can infect 100% of the 5 to 7 week old turkeys but only 50% are still infected when the birds are 13 weeks old. The correlation of cloacal to fecal samples for turkeys was $r = 0.49$. The correlation of the cloacal counts to carcass counts was $r = -0.36$.

This study looked at the effect of temperature and feed withdrawal on *Campylobacter* growth and survival in turkeys and chickens. The temperature stress did not have an affect on *Campylobacter* counts for either chickens or turkeys. The temperature at which the birds were kept did not make a difference on the number of *Campylobacter jejuni* found in the birds. The feed withdrawal stress did not affect the *Campylobacter jejuni* counts for either chickens or turkeys. The number of colony forming units for *Campylobacter* were within one tenth of a point for the different feed withdrawal groups.

More *Campylobacter* in our study was recovered from chickens than from turkeys. This difference can possibly be explained by the fact that the age of the birds when they were evaluated was different. Chickens were evaluated for *Campylobacter* when they were five weeks old and turkeys were evaluated when they were fourteen weeks old. The results of the longitudinal study indicate that in turkeys
Campylobacter jejuni grows well for eight to ten weeks and then begins to decrease in number. The chickens were evaluated when Campylobacter growth was high and turkeys were evaluated when growth was low.

The comparison of cloacal and carcass Campylobacter counts showed that they were independent variables and not influenced by each other. There is not a correlation between the number of Campylobacter in the live bird and how many Campylobacter will be on the carcass. The stresses of temperature and feed withdrawal had no effect on carcass contamination. The level of Campylobacter jejuni in turkeys varied from a high of \( \log_{10} 3.63 \) at week 5 to a low of \( \log_{10} 0.385 \) at week 13.

The conclusions reached are:

- There is not a correlation between the level of Campylobacter jejuni in the live bird and the level of contamination on the carcass;

- The stresses of temperature and feed withdrawal do not affect the level of Campylobacter jejuni that is found on the carcasses of the birds;

- The level of Campylobacter jejuni in the turkeys varied over their lifetime reaching a peak at week 5-6 and dropping off after that to 50% by week 13.
VI. REFERENCES


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VITA

Genevieve Friedman was born December 28, 1941 in Ronceverte, West Virginia, the daughter of Harvey Black Wilson (deceased 1970) and Edith Rodgers Wilson. She graduated from Lewisburg High School in 1959 and received a Bachelor of Science in Education from Radford College in 1967.

Genevieve is the mother of four children: Frederick, Foster, Frank, and Christie. She taught in the public school for 17 years: 14 years in Buena Vista, Virginia teaching fifth grade, 1 year in Lantana, Florida teaching seventh and eighth grade Social Studies, and 2 years in Renick, West Virginia teaching seventh, eighth, and ninth grade Social Studies and Science.

The author began work toward a Masters degree in Food Science and Technology at Virginia Polytechnic Institute and State University in August 1990.

While a student she was a member of the Institute of Food Technologists and the Carolina-Virginia section of this organization. She is also a member of the Muscle Foods and Microbiology groups.

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