Analysis of *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella*, *Klebsiella pneumoniae*, and *Escherichia coli* 0157:H7 in Fresh Hand Picked Blue Crab (*Callinectes sapidus*) Meat

by

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ABSTRACT

This study was conducted to determine the quantitites of *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella*, fecal positive *Klebsiella pneumoniae*, and *Escherichia coli* 0157:H7 in fresh hand picked blue crab (*Callinectes sapidus*) meat. An attempt was made to correlate these selected pathogens and general microbial quality to processors sanitation practices and facility size.

Hand picked crabmeat samples from 12 blue crab processing facilities in the Chesapeake Bay region were collected and analyzed. Twenty samples from each of the different facilities were collected on different processing days. Facilities were chosen based on production levels and Virginia Department of Health inspection scores as an indicator of sanitation practices (excellent $\geq$ 94.5, acceptable < 94.5). All samples were tested between 16 h and 36 h after collection.

Campylobacter jejuni was isolated from 36 (15%) of the

240 samples and *Campylobacter coli* was isolated from 14 (5.8%). Quantitative levels in all case were below limits of detection (<0.30 MPN/g). *Klebsiella pneumoniae* was isolated from a total of 51 (21%) samples. Counts ranged from less then 0.30 to 4.3 MPN/g. Aerobic plate counts ranged from $7.4x10^3$ to $4.6x10^8$ CFU/g with coliform counts ranging from <0.3 to 32.8 MPN/g. Fecal coliform levels were <0.3 to 2.26 MPN/g and *Escherichia coli* from <0.3 to 0.77 MPN/g. *Salmonella* and *Escherichia coli* 0157:H7 were not detected in any of the 240 samples analyzed.

No significant differences ($p<0.05$) between size and inspection scores were observed for general microbial quality, the presence of *Klebsiella pneumoniae*, or *Campylobacter* species.

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INTRODUCTION

In September, 1926 the Food and Drug Administration (FDA) investigated the crabmeat industry of Virginia and Maryland because of a food poisoning outbreak in Chicago linked to crabmeat contaminated with *Salmonella suipestifer* (Slocum, 1955). Since that time, the FDA has continued to initiate regulatory actions against firms producing crabmeat under insanitary conditions. During the last decade, the emergence of "new" pathogens including *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter jejuni* and *Campylobacter coli* has led to increased consumer and governmental interest on the safety and wholesomeness of all food products. Ready-to-eat fresh seafood, because it is highly perishable under the best storage conditions, is continually evaluated for the presence of pathogenic organisms causing, or capable of causing, seafood related foodborne illness (Phillips and Peeler, 1972; Foster et al., 1977; Wentz et al., 1985; Slabyj et al., 1990).

Campylobacter jejuni is the leading cause of gastroenteritis in the world (Gilbert, 1988). In studies where both *Salmonella* and *Campylobacter* species were analyzed, *Campylobacter* cultures out numbered *Salmonella* isolates 10 to 1 in college students and 2 to 1 in a multicenter study (Tauxe et al., 1987). The infectious dose for *C. jejuni* has been estimated at less than 200 organisms

(Doyle, 1985) and this could be one of the reasons for its occurrence as the leading cause of gastroenteritis. Studies showing the presence of thermophilic *Campylobacter* species in seawater indicates the need to evaluate the seafood industry for possible microbiological contamination (Alonso and Alonso, 1993).

Salmonella is also a leading cause of human gastroenteritis. Reported cases of *Salmonella* infections have increased 100 fold from 1942 to 1988 (Chalker and Blaser, 1988). Despite improvements in diagnosis which can account for a definite increases in cases, there is no evidence of a decline in *Salmonella* infections in recent years (Madden, 1994). Instead there may be the overall appearance of an increase in the incidence of *Salmonella* as a result of extremely virulent and infective strains and immunosuppression caused by age, preexisting conditions, and autoimmune disease.

Escherichia coli O157:H7, is the cause of hemorrhagic colitis, which is characterized by large amounts of blood in the stool and can lead to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Riley et al., 1983). The organism is mainly associated with dairy cattle, however it has been the cause of foodborne infection in other food products. *E. coli* O157:H7 has been of great interest in the media in recent years possibly because the highest incident

rate of *E. coli* O157:H7 is in children and because death is associated with the disease. It has been estimated to affect 6.1 children per 100,000 population (Ostroff et al., 1989). Most outbreaks of *E. coli* O157:H7 are related to food consumption, and because of this continued evaluation of *E. coli* O157:H7 in food products is required, especially in ready-to-eat foods.

Klebsiella pneumoniae is an opportunistic human pathogen leading to bacterial infections including pneumonia and urinary tract infections. In recent years, infections caused by *Klebsiella* have increased due to strains with multiple antibiotic resistance. The human gastrointestinal tract is the main reservoir and the hands are the main mode of transmission. The estimated incidence of bacteremia is between 71,000 and 330,000 cases per year in the United States (Wolff and Bennet, 1974; McCabe et al., 1972) and the mortality rate varies between 20 and 50% (Torre et al., 1985). *Klebsiella* species, in relation to *Escherichia coli*, are the second leading cause of bacteremia. Like *Escherichia coli*, the species is known to be present in numerous food products (Roa and Roa, 1983) and can be used to identify spoilage. However, extensive studies on the incidence of *Klebsiella* species in fresh food and their impact on foodborne disease have not been conducted.

The objective of this thesis was to determine the

presence of *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella species*, *Klebsiella pneumoniae*, and *Escherichia coli* O157:H7 in fresh blue crab (*Callinectes sapidus*) meat and to compare quantitative and qualitative levels of these pathogens to processing facility size and general sanitation practices.

SECTION I: LITERATURE REVIEW

A. *Escherichia coli* O157:H7

1. Historical Background

Escherichia coli was first identified by Dr. Theodor Escherich in 1885, and the bacterium was considered to be a harmless commensal of the human intestinal tract (Padhye and Doyle, 1992). However, a few strains of pathogenic *E. coli* do exist and enterohemorrhagic *E. coli* (EHTC), which includes *E. coli* O157:H7 and *E. coli* O26:H11, is the most virulent.

Escherichia coli O157:H7 was first isolated in 1975 from a woman with grossly bloody diarrhea. Then in 1982, *E. coli* O157:H7 was identified as a human foodborne pathogen (Riley et al., 1983). This occurred following two food associated outbreaks of hemorrhagic colitis. The first was in Oregon involving 26 cases and the other involved 21 cases in Michigan (Riley et al., 1983). Since that time it has been well documented as the cause of hemorrhagic colitis which is characterized by large amounts of blood in the stool (Farrag et al., 1992; Madden, 1994).

2. Taxonomy, Nomenclature, and Morphology

Escherichia coli is a gram negative, facultative, nonspore-forming bacillus. The species is classified by serotyping, particularly against the "O" and "H" antigens of various strains. The four sub-groups of diarrheal *E. coli*

are Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Enteropathogenic (EPEC), and Enterohemorragic (EHEC). Enterohemorragic is currently the serotype of greatest concern and is the most virulent of any of the four sub-groups.

E. coli O157:H7 produces most biochemical reactions typical of *E. coli*. However, unlike other *E. coli* serotypes, *E. coli* O157:H7 does not ferment sorbitol and is nonhemoltic on sheep- or rabbit-blood agar. Almost 93% of *E. coli* ferment sorbitol, O157:H7 does not (Honish, 1986). *E. coli* O157:H7 also do not show β-glucuronidase activity like over 90% of other *E. coli* strains (Feng and Hartman, 1982).

3. Growth Characteristics

The temperature of growth of *E. coli* species can range from 2.5 to 45°C depending on the strain. *E. coli* O157:H7, is one of the most hardy of the species and can survive freezing and refrigeration temperatures. Farrag and colleagues (1992) showed that *E. coli* O157:H7 can survive without a decrease in viable counts for 120 hours at 4 or 7°C. Doyle and Schoeni (1984) showed no decrease in *E. coli* O157:H7 after 9 months at -20°C. Doyle and Schoeni also showed that the organism did not grow and could not be isolated at temperatures above 44°C, which is often used in the detection of other *E. coli* strains. This played a very

important role in the early failures of researchers to isolate *E. coli* O157:H7.

The optimum growth temperature of *E. coli* O157:H7 is 37°C, but prolific growth will occur between 30 and 42°C. *E. coli* O157:H7 has generation times of 0.49 hours at 37°C and 0.64 hours at 42°C (Doyle and Schoeni, 1984).

Studies of thermal inactivity in ground beef show *E. coli* O157:H7 is not usually heat resistant. The organism is more sensitive to heat than typical *Salmonella* species with D-values of 270, 45, 24, and 9.6 seconds at 57.2, 60.0, 62.8, and 64.3°C, respectively. *E. coli* O157:H7 does however show a significant heat-shock response after heating to 42°C for 5 minutes (Murano and Pierson, 1992).

4. Pathology

The mechanism for the illness caused by *E. coli* O157:H7 is not fully defined, but several factors have been associated with its virulence. The production of one or more cytotoxins is believed to be the greatest of these factors, and in 1977, Konowalchuck et al., observed the first effects of one or more cytotoxins termed verotoxins (VT) (Padhye and Doyle, 1992). Since that time, the VT of *E. coli* O157:H7 have been determined to be an indistinguishable form of shiga toxin and has been described as a shiga-like toxin (SLT). A second and third verotoxin, named VT1 and VT2, along with variants of VT, which are

antigenically distinct from the previous VT, have also been reported (Padye and Doyle, 1992).

In addition to verotoxins, the adhesion of *E. coli* O157:H7 to intestinal cells may be important in the organism's pathogenicity. Little or no fever is associated with patients infected with *E. coli* O157:H7 which suggests the organism is not invasive and does not enter the circulatory system. This would mean the organism must colonize the intestinal tract producing toxins, which are subsequently active on the colon (Padhye and Doyle, 1992). However, published studies are conflicting on the adherence of *E. coli* O157:H7 to different tissue cell culture and the importance of pili in adherence is unknown.

The three principle illnesses attributed to *E. coli* O157:H7 are hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura. Hemorrhagic colitis is a clinical syndrome which is characterized by abdominal cramps and watery diarrhea, which later becomes extremely bloody. Vomiting also may occur, but little or no fever is generally observed. The incubation period for the onset of disease ranges from 3 to 9 days and the duration of illness ranges 2 to 9 days (Riley et al., 1983).

Hemolytic uremic syndrome (HUS) is the leading cause of acute renal failure in children. The pathogenicity of HUM is associated with toxins damaging endothelial cells,

causing excess clotting and accumulation of waste product in the blood (Cleary, 1988). The disease occurs as a triad of acquired hemolytic anemia, thrombocytopenia, and renal failure, in otherwise healthy individuals. Patients appear seriously ill or jaundiced and hypertensive. Dialysis and blood transfusions are usually required and heart failure, seizures, coma, and hypertensive encephalopathy may develop.

Thrombotic thrombocytopenic purpura involves the central nervous system, but is very similar to HUM. Thrombotic thrombocytopenic purpura consists of microangiopathic hemolytic anemia, thrombocytopenia, fluctuating neurologic signs, fever, and mild azotemia (Padhye and Doyle, 1992). It usually occurs in adults, and patients often develop blood clots in the brain. Death is the most frequent result.

5. Epidemiology

Most *E. coli* O157:H7 infections are related to food consumption. *Escherichia coli* O157:H7 has been linked to the consumption of ground beef, but also has been isolated from raw milk, pork, and poultry (Doyle and Schoeni, 1987). Ground beef was either epidemiologically linked or suspected as the vehicle of transmission in the two outbreaks in Oregon and Michigan in 1982, in Ontario in 1982, in Nebraska in 1984, in Alberta in 1986, in Utah in 1987, in Minnesota in 1988, and recently in several states throughout the

Western United States in 1993. Several other outbreaks have also implicated food vehicles as ham, turkey, cheese, turkey roll sandwiches, milk, and some person to person infection (Padhye and Doyle, 1992).

It is, however, difficult to determine the true incidence of *E. coli* O157:H7 infections, because only eleven states (as of October, 1992) required reporting of the isolation of *E. coli* O157:H7, and only four states require the reporting of Hemolytic Uremic Syndrome (HUS) to the Centers for Disease Control, Atlanta, GA. (MacDonald and Osterholm, 1993).

6. Methods of Isolation

The rapid fluorogenic assay generally used for the determination of *E. coli* species from other coliforms is negative for *E. coli* O157:H7. In the test, the compound 4-methylumbelliferone glucuronide (MUG) is hydrolyzed by glucuronidase enzyme present in most *E. coli* strains, this enzyme is not present in *E. coli* O157:H7. As was mentioned earlier, *E. coli* O157:H7 does not grow at temperatures traditionally used to isolate other *E. coli* strains. For these two reasons, the isolation of *E. coli* O157:H7 was difficult in early years after the discovery of the organism.

Generally, laboratory screening methods have been based on the sorbitol negative reaction of the organism after 24

hours incubation and agglutination in *E. coli* O157:H7 antiserum. Selective media such as MacConkey sorbitol agar, (Difco, Detroit, MI) which contains 1% sorbitol instead of lactose, have been used for the detection of sorbitol-negative *E. coli* O157:H7 colonies in both food and clinical laboratories (March and Ratnam, 1986). The United States Food and Drug Administration uses the previously described isolation technique (Hitchens et al., 1992).

More recently, new, rapid methods have been developed for the isolation of *E. coli* O157:H7 including the use of Antibody coated magnetic beads and a 3M Petrifilm™ test kit-HEC, incorporating a reactive disc blot ELISA for E. coli O157:H7 antigen. In the coated magnetic bead test, *Escherichia coli* O157 specific antibody, coated on magnetic beads, is used to concentrate and remove *E. coli* O157:H7 from mixed cultures and meat samples (Okrend et al., 1992). This procedure allowed for the recovery of 48 to 100% of *E. coli* O157:H7 with the two *E. coli* O157, :H7 and :non-H7, strains competing with one another reducing isolation rates.

The 3M-Petrifilm method combines an immunoblot assay with the USDA culture methods to produce a fast and efficient screening procedure with negative and positive assumptions in 26-28 hours (Okrend et al., 1990). Isolation and confirmation of presumptive positive requires an additional 3 to 4 days. The sample is directly incorporated

into enrichment broth and is incubated for 6 to 8 hours at 37°C. The enrichment broth is then directly added to 3M Petrifilm *E. coli* Count plates and incubated at 42°C for 18 hours and then tested for the presence of O157 antigen by using a reactive disc (contacted with plate surface) which is washed to remove adhered agar from the disc. The disc is then placed at room temperature and if the conjugate is present it attaches to O157 antigens that are bound on the disc, and a gray spot will appear.

B. ***Campylobacter jejuni/coli***

1. Historical Background

Campylobacter was first discovered in 1909 by two veterinarians, J. Macfaydean and S. Stockman. Then in 1919, after isolation of the organism by Theobald Smith investigating infectious abortions of bovines, the first name was proposed, "Vibrio fetus" (Franko, 1987). Twelve years later Jones et al. (1932) described a "Vibrio" they called "Vibrio jejuni", associated with dysentery in calves, and in 1938 a outbreak of acute diarrhea in humans from contaminated milk led to the isolation of the same organism from patient blood cultures (Levy, 1946). In 1963 it was proposed that the genus of "Vibrio" be changed based on different biochemical, serological, and DNA base-pair ratios (G + C mol%) to two separate genuses *Vibrio* and *Campylobacter* (Franco 1987). In 1971, Dekeyser et al. (1972) isolated *Campylobacter jejuni* for the first time from human stool samples, and by 1980 *Campylobacter* was considered a leading cause of gastroenteric disease in humans.

2. Taxonomy, Nomenclature, and Morphology

The genus *Campylobacter*, meaning "curved rod" in Greek, is a member of the family *Spirillaceae*. *Campylobacter* bacteria are nonsporeforming, gram-negative bacteria with a curved, spiral, or S-shaped morphology. The cells vary in

size from 0.5 to 0.8 μm in length and 0.2 to 0.5 μm in width. Cells are highly motile with a single polar flagellum at one or both ends of the cell allowing a characteristically rapid, darting, corkscrew-like movement. The flagella of *Campylobacter* can be two to three times the length of the bacteria cell (Simbert, 1989).

Campylobacter is a well defined genus with taxonomic problems associated with the species and is hence likely to continue to change (Simbert, 1989). At least 18 species of *Campylobacter* are known, and nine species appear to be important in human disease (Skerman et al., 1980). Of these, catalase positive species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are most commonly isolated from humans with enteric disease (Butlzler et al., 1973; Skirrow, 1977).

3. Growth Characteristics

Campylobacter grow best in a reduced oxygen atmosphere and at higher-than-atmospheric concentration of carbon dioxide. They are sensitive to oxygen tension and are considered microaerophilic. The oxygen required for growth is 3-15% and the carbon dioxide required is 3-5% (Stern and Kazmi, 1989). The optimum atmospheric conditions are 10% oxygen, 15% carbon dioxide, and 85% nitrogen.

Campylobacter growing temperatures range from 25°C to 45°C. Optimum temperatures and specific ranges differ

depending on species. *Campylobacter jejuni* grows from 35°C to 45°C and optimally at 42-43°C. It dies more quickly at 25°C than at 4°C or 30°C, survives up to four weeks under refrigeration and is highly susceptible to freezing. *Campylobacter jejuni* becomes thermally inactive at 46-48°C, and will not survive minimum pasteurization treatments (Franko, 1987; Stern and Kazmi, 1989; Simbert, 1989). It grows at pH 4.9 to 9.9 depending on the types of acids, water activity, and the strain (Oosterom, 1985).

Campylobacter species are chemoorganotrophs and require organic molecules as a source of carbon. The bacteria uses a respiratory metabolism obtaining energy from amino acids or the tricarboxylic acid cycle intermediates. *Campylobacter* cannot ferment or oxidize carbohydrates (Smibert, 1989).

Campylobacter jejuni is oxidase positive, is catalase positive, reduces nitrate to nitrite, produces hydrogen sulfide, generally hydrolyzes hippurate, is resistant to cephalothin and is susceptible to nalidixic acid. These biochemical reactions are the same for *Campylobacter coli*, except that *Campylobacter coli* can not hydrolyze hippurate and since all but a few *Campylobacter jejuni* do hydrolyze hippurate, this test is generally used to distinguish the two species (Simbert, 1989). The production of H_2S by *Campylobacter coli* in iron media is also used to

differentiate the two biotypes. Biochemical characteristics of other important *Campylobacters* in human disease are listed in Table 1.

Table 1: Different Characteristics of Medically Important *Campylobacters* (Barret et al., 1988)

Characteristic	*C. jejuni*	*C. coli*	*C. lari*	*C. fetus spp. fetus*	*C. "cinaedi"*	*C. "fennelliae"*	*C. cyraerophila*	*C. hyointestinalis*	*C. upsaliensis*
Growth 25°C 35-37°C 42°C	 - + +	 - + +	 - + +	 + + D	 - + D	 - + D	 + + -	 D + +	 - + +
Nitrate reduction	+	+	+	+	+	+	-	+	+
Growth 3.5% NaCl	-	-	-	-	-	-	-	-	-
H_2S, lead acetate strip	+	+	+	+	+	+	+	+	+
H_2S, TSI	-	D	-	-	-	-	-	+[f]	-
Catalase	+	+	+	+	+	+	+	+	-
Oxidase	+	+	+	+	+	+	+	+	+
MacConkey agar	+	+	+	+	-	-	-	+	-
Motility (wet mount)	+ (81%)	+	+	+	+	+	+	+	+
Growth in 1% glycine	+	+	+	+	+	+	-	+	+
Glucose utilization	-	-	-	-	-	-	-	-	-
Hippurate hydrolysis	+[c]	-	-	-	-	-	-	-	-
Resistance to nalidixic acid	S[d]	S	R	R	S	S	S	R	S
Resistance to cephalothin	R	R	R	S[e]	S	S	S	S	S

Symbols: +, 90% or more of strains are positive; (-) 90% or more of strains are negative; (D) 11-89% of strains are positive; (R), resistant; (S), susceptible; ("cinaedi") proposed species name.

[c]Hippurate negative strains have been reported.

[d]Nalidixic-resistant *C. jejuni* have been reported.

[e]Cephalothin-resistant *C. fetus subsp. fetus* have been reported.

[f]Small amount of H_2S on fresh (<3 days) TSI slants.

4. Pathology

The mechanism by which *Campylobacter* causes disease is not known. Infection requires adherence of the bacterium to the gut mucosa, perhaps mediated by flagellar adhesions or outer bacterial membrane components. The production of toxins similar to those of *Vibrio cholera*, might be a possible mechanism for pathogenicity (Goossens, 1990). A *Campylobacter jejuni* cytotoxin has been isolated. The toxin produces cytotoxic changes in tissue cells by stimulation of cyclic AMP production. The role of the cytotoxin in pathogenesis is not known, but causes more severe diarrhea in animals given cytotoxin positive strains, unlike those given a cytotoxin negative strains. The presence of the cytotoxin also appears to be related to patients having bloody diarrhea (Pang et al., 1987). Motility and the spiral shape of the organism also play a role in its virulence.

The infective dose for *Campylobacter jejuni* is less than 200 to 100,000 organisms. The infective dose is believed to vary because of the differences in gastric pH of the host and strain differences (Butzler and Oosrerom, 1991). Clinical differentiation of this pathogen from other pathogens is not possible because the signs and symptoms are not very characteristic. Acute enterocolitis is the most common *Campylobacter jejuni* infection. The incubation

period is generally 2 to 5 days and can last as long as 10 days. In many patients, diarrhea is preceded by a febrile period of myalgies, abdominal pain, and malaise. A high temperature with confusion and delirium may also be present and fresh blood may appear in stool by the 3rd day. Vomiting is rare. Bacteremia has been reported in fewer than 1% of patients with *Campylobacter jejuni* infection (Butzler and Skirrow, 1979).

5. Epidemiology

Campylobacter has been isolated from humans with enterocolitis in virtually every country throughout the world (Blaser et al., 1979; Demol and Bosmans, 1978; Pai et al., 1979; Steele and McDermott, 1978; Oosterom, 1980; Yanagisawa, 1980). The estimated number of *Campylobacter* infections in the United States is about 3 million per year and 600,000 per year in Great Britian (Gengeorgis, 1986). Despite this, many important aspects of its prevalence and transmission remain unanswered. The number of reported food and water-borne outbreaks cannot explain all the recorded outbreaks of *Campylobacter* infections. Under reporting, difficulty in investigation, and unexpected routes of transmission have led to poor incomplete data. *Campylobacter* is widely spread among animal reservoirs and the environment, and epidemiology and control of the organism is difficult.

Campylobacter species are commensals of almost all wild and domestic animals' gastrointestinal tracts. They have been isolated from all classes of poultry, cattle, sheep, swine, goats, dogs, cats, and rodents (Franco, 1987). However, reports show that the majority of all *Campylobacter* enteritis is directly related to the handling or consumption of poultry.

Despite being present in the intestinal tract of swine, cattle, and lamb, *Campylobacter* contamination in these foods is rare. In a study by Stern et al. (1985) of retail markets in the United States, *Campylobacter jejuni* and *Campylobacter coli* were isolated from pork chops, ground beef, beef flank, and lamb stew with an incidence of 5.0%, 4.2%, 4.7%, and 8.1% respectively, while isolation rates from chickens were 29.7%. However, transmission of organisms from hamburger meat (Oosterom et al., 1980) and pork (Yanagisawa, 1980) to humans has been reported. *Campylobacter* isolation from chicken is high and may reach 100% (Oosterom, 1985). Strains isolated from humans are mostly associated with poultry serotypes (Oosterom et al., 1980). This has led to the notion that poultry is the highest reservoir of human Campylobacteriosis.

Campylobacter jejuni is a normal commensal in cattle, and a number of different serotypes may be simultaniously present in one heard. Transmission of the organism appears

to be limited to calves via adult cattle and not between adults (Blaser et al., 1983). The occurrence of *Campylobacter* infections in cattle is 57% steers, 40% bulls, 40% heifers, and 22% cows. The highest infection rates occur in gallbladders and the large and small intestines (Franco, 1987). *Campylobacter* contamination of meat generally occurs during slaughter.

Campylobacter in raw milk is associated with fecal contamination during milking. *Campylobacter* mastilitis has also been recorded and the disease has been confirmed experimentally (Lander and Gill, 1980). However, heavy contamination must occur in order to cause large infections from one lot of milk. To prevent milkborne outbreaks, pasteurization or a heat treatment which kills *Campylobacter* is required in all milk sold for human consumption (Blaser et al., 1983).

Recently, shellfish have been associated with *Campylobacter* infections and contaminations. In a study by Griffen et al. (1983) raw clams were the cause of a *Campylobacter* enteritis infection in Washington State. In other studies, Arumugaswamy and Proudford (1987) found 14% of oysters to be contaminated with *Campylobacter jejuni/coli* and Reinhard et al. (1995) found 20% of fresh crabmeat contaminated with the same organisms. Concerns of possible shellfish contamination have been furthered by research

showing shellfish growing waters highly contaminated with *Campylobacter* (Abeyta et al., 1993). However, little or no literature on the presence of *Campylobacter jejuni* or *Campylobacter coli* in shellfish is available, so the epidemiology of many outbreaks remains poorly understood and the role of shellfish contaminations unknown.

Humans have also been reported as reservoirs for *Campylobacter jejuni*. In these cases, those who come in contact with excrement of infected people put themselves at risk. Contamination of food from food handlers is unusual. If cross-contamination does occur, it is best favored under neutral pH (6.5-7.5) and in food which are at refrigeration temperatures (Blaser et al., 1983).

Water is a common source of *Campylobacter*. *Campylobacter* have been isolated from springs, streams, river water, and seawater (Norkrans and Sveden, 1982; Blaser et al., 1979; Franco, 1987; Alonso and Alonso, 1993). Areas heavily polluted with sewage, farm run-off or sludge led to higher isolation rates (Abeyta et al., 1993; Norkrans and Sveden, 1982). Water outbreaks of *Campylobacter* enteritis have traditionally been associated with spring water during outdoor activities or community water contaminated from wild birds (Franko, 1987).

6. Methods of Isolation

Several problems arise in trying to isolate

Campylobacter from foods. Since they are oxygen sensitive, they may occur in very small numbers, and requirements for atmospheric manipulation during enrichment and plating are inconvenient. To overcome this, several methods have been developed, but each exhibits advantages and disadvantages, and a search for the best technique continues.

Karmali and Fleming (1979) developed a technique to overcome atmospheric requirements by using the Fortner principle. By streaking one half of a plate with a rapid growing *Proteus* sp., the oxygen tension of the closed system is reduced, and it is possible to grow *Campylobacter* on the other half of the plate. Another procedure using a closed container flushed with 5% oxygen has been reported successful and superior to those grown in a candle jar system (Luechtefeld et al., 1982). However, the method most commonly used to reach atmospheric conditions required to grow *Campylobacter* is the flushing of an anaerobic jar with an atmospheric mixture of 5% oxygen, 10% carbon dioxide, and 85% nitrogen, with plates and enrichments already inside (Stern, 1982).

A chemical supplement is often used to improve the tolerance of *Campylobacter* to air (George et al., 1978). A mixture of ferrous sulfate, sodium metabisulfate, and sodium pyruvate (FBP) in specific quantities can be added to both enrichment broths and plating agar. These chemicals

increase oxygen tolerance by quenching hydrogen peroxides and superoxides that inherently occur in the culture medium.

Enrichment methods have also been developed to lower indigenous organisms and improve isolation techniques of *Campylobacter* bacteria. Most of these have been developed for clinical laboratories and use antibiotics. However, low levels of *Campylobacter* species in foods and the use of antibiotics have made it difficult and expensive to isolate *Campylobacter* species with these procedures.

Antibiotics increase the selectivity of enrichment broths. *Campylobacter* species are resistant to vancomycin, which is successful in eliminating gram-positive cocci bacteria. *Campylobacter jejuni* is also resistant to polymyxin B, trimethoprim lactate, and cephalosporins. These antibiotics are all added to enrichment broths in varying concentrations to eliminate other organisms. Trimethoprim acts as an inhibitor of *Proteus* sp. Cephalosporins are active against *Enterobacteriaceae, Streptococcus, Serratia, Pseudomonas* species and some *Proteus, Yersinia,* and *Bacteroides* species, and Polymyxin B is inhibitory to *Enterobacteriaceae* and *Pseudomonas* species (Stern, 1982).

Recently, Chan and Mackenzie (1982) showed increasing concentrations of antimicrobial medium in the enrichment broth increased isolation rates of *Campylobacter jejuni* and

Campylobacter coli. Castillo-Ayala (1992), studying the effects of an antimicrobial proposed by J.P. Butzler, showed that double strength vancomycin-trimethoprim-polymyxin B (VTP) and the Butzler antimicrobial, bacitracin-colistin-cephalothin-cycloheximide-novobiocin (BCN), used simultaneously in broths showed increased isolation rates from deboned chickens when compared with traditional antimicrobials by themselves. However, the antimicrobial proposed by Butzler (BCN) is not commonly used in the isolation of *Campylobacter* from foods, and traditional antimicrobials are the norm in most food and clinical laboratories.

Several enrichment media have been proposed for *Campylobacter* isolation. Tanner and Bullin (1977) used alkaline peptone water at pH 8.4 as an enrichment medium. They incubated the medium under microaerophilic conditions, and found the procedure to enable recovery of as few as 1 to 10 cells of *Campylobacter*. This allowed isolation of *Campylobacter* species from samples that were previously found to be negative with direct plating techniques. Blaser et al. (1979) used an enrichment broth with an antimicrobial to isolate *Campylobacter* bacteria. Campy-thrio enrichment broth (thioglycollate broth with 0.16% agar and vancomycin, trimethoprim, polymyxin B, and amphotericin antimicrobial), held under refrigeration for 8 hours, led to a 33% increase

in isolation rates. While this temperature of the medium didn't allow for growth of *Campylobacter* species, the antimicrobial destroyed many of the indigenous microorganisms leading to increased isolation. Similar results of recovery were obtained by Rosef (1981) using the same medium except the antimicrobial, amphotericin, was omitted (Stern, 1982).

Hunts and Radle (Stern and Line, 1992) introduced an enrichment broth, commonly called *Campylobacter* enrichment broth. The broth contains Nutrient Broth Number 2 with 0.6% yeast extract and supplements of FBP, lysed horse blood, vancomycin, cefoperazone, amphotericin, and trimethoprim lactate. Stern and Line (1992) determined the enrichment to be the most effective in isolating *Campylobacter*. It has been reported that the enrichment technique is capable of the recovery of 0.1 to 1 cell of *Campylobacter jejuni* from one gram of food containing 1,000,000 to 10,000,000 indigenous microorganisms, and the broth is currently recommended by the United States Food and Drug Administration for the isolation of *Campylobacter* species. (Hunt, 1992).

Several selective agar media are commonly used in the isolation of *Campylobacter* bacteria from foods. Most prominent of these are Campy-BAP formulation (Blaser et al., 1979), Campy-cefex formulation (Stern and Line, 1992),

modified CCDA-Preston blood free medium (Hutchinson and Bolton, 1984), the "Butzler" formulation (Dekeyser et al., 1972), and "Skirrows" formulation (Skirrow, 1977). All contain different supplements of antibiotics and animal blood (Table 2).

Comparisons of selective media have shown that CCDA-Preston blood-free medium is best for the isolation of *Campylobacter jejuni*, followed by Campy-cefex and Campy-BAP formulations (Stern and Line, 1992). Butzler medium is more effective than Campy-BAP and Skirrows medium in isolation of *Campylobacter* species, but Campy-BAP medium is best in recovering of *Campylobacter jejuni* from foods when compared with the Skirrow or Butzler formulations (Stern, 1982).

Table 2: Formulation of Different Selective Agars (Bolton et al., 1983)

Campy-Cefex agar		**Modified CCDA-Preston Blood Free Agar**	
Base medium		Base media	
Brucella agar	44 g		
Ferrous Sulfate	0.5 g	Nutrient broth #2	25g
Na Bisulfate	0.2 g	Agar	12g
Na Pyruvate	0.5 g	Distilled water	950 mL
Distilled water	950 mL		
		Suppplements	
Supplements		Polymyxin sulphate	5,000 IU
Na Cefoperazone	33 mg	Ritampicin	10 mg
Na Cycloheximide	200 mg	Inmethoprim	10 mg
Lysed Horse blood	50 mL	Cycloheximide	100 mg
		Lysed horse blood	50 mL
Campy-BAP formulation		**Skirrow's formulation**	
Base medium		Base medium	
Brucella agar	43 g	Blood agar base No. 2	40 g
Distilled water	900 mL		
		Supplements	
Supplements		Polymyxin	2500 IU
Cephalothin	15 mg	Trimethoprim lactate	5 mg
Polymyxin	2500 IU	Vancomycin	10 mg
Trimethoprim lactate	5 mg	Cycloheximide*	100 mg
Vancomycin	10 mg		
Amphotreicin	2 mg		
Whole sheep blood	100 mL		
Butzler formulation			
Base medium			
Blood agar base No. 2	40 g		
Supplements			
Bactracin	2500 IU		
Cephazolin	15 mg		
Colistin	10,000 IU		
Novobiocin	5 mg		
Cycloheximide	50 mg		

*Non-human samples only

C. ***Klebsiella pneumoniae***

1. Historical Background

Klebsiella has been a recognized pulmonary pathogen since its discovery in 1882 by K. Friedlander (Korvick et al., 1991). *Klebsiella pneumoniae* was originally classified as a bacillus and called *Bacillus mucosus capsulatus* which caused Friendlander pneumonia (Ritvo and Martin, 1949). From the 1920s into the 1960s, the organism was shown to be one of the only gram negative bacteria that could cause primary labor pneumonia (Kornblum, 1928; Ritvo and Martin, 1949). Since then, *Klebsiella pneumoniae* has been clinically proven as the cause of urinary tract infections, bacteremia, and bacterial pneumonia (Guentzel, 1986).

2. Taxonomy, Nomenclature, and Morphology

Klebsiella pneumoniae is a gram-negative bacilli, 0.6 to 6.0 μm in length and 0.3 to 1.0 μm in diameter. *Klebsiella* can be arranged singly, in pairs, or short chains, are nonmotile, capsulated, and conform to the general definitions of the family *Enterobacteriaceae*.

Three species of *Klebsiella* were described in the eighth edition of *Bergey's* Manual; *K. pneumoniae, K. ozaenae* and *K. rhinoscleromatis*. However, currently *K. ozaenae* and *K. rhinoscleromatis* are considered a subspecies of *K. pneumoniae* because of DNA reassociation studies showing the three organisms belong in the same DNA related groups. Both

subspecies are metabolically inactive biogroups of *K. pneumoniae*. *K. rhinisclerom*atis is the most metabolically inactive, while *K. ozaenae* strains are variable. *K. pneumoniae* is distinguished from other *Klebsiella* species that phenotypically resemble it by its ability to produce gas from lactose at 44.5°C (Orskov, 1984).

Klebsiella are oportunistic pathogens that can give rise to several types of bacterial infections including pneumonia and urinary tract infections. In recent years, infections caused by *Klebsiella* have increased due to strains with multiple antibiotic resistance. The human gastrointestinal tract is the main reservoir and the hands are the main mode of transmission.

Klebsiella contain both O and K antigens. At least 11 serogroups are differentiated on the basis of their O antigens, but generally serotyping is not practiced in clinical laboratories. Typing occurs based on the K antigens, of which there are at least 80. The K antigen is the characteristic polysaccharide the is morphologically evident and is important in infections caused by the organism (Freeman, 1985).

3. Growth Characteristics

K. pneumoniae has growth characteristics typical of other *Enterobacteriaceae*. The growth temperature of *Klebsiella* species can range from 2.5 to 45°C depending on

the strain. However, *K. pneumoniae* will not grow at a temperature below 10°C (Freeman, 1985). *K. pneumoniae* produce gas at 44.5°C in the fecal coliform test commonly performed on food products.

Klebsiella are facultative anaerobes, with both a respiratory and a fermentative metabolism. Most *Klebsiella* strains can be grown with citrate and glucose as the sole source of carbon, and they are oxidase negative. Fermentation of inositol, hydrolysis of urea, and failure to produce ornithine dehydrolase or H_2S are other distinguishing characteristics. Unlike *E. coli*, *Klebsiella* species can break down adonitol and esculin sugars and this capability can be used in the isolation of the organism.

4. Pathology

K. pneumoniae is found in the alimentary and respiratory tracts of about 5% of normal people. Although the organism occasionally causes urinary tract infections and chronic infections in various organisms, it is the most important cause of severe hemorrhagic pneumonia, accounting for about 2 to 3% of of all cases of acute bacterial pneumonia (Guentzel, 1986). The mortality rate in untreated *Klebsiella* pneumonia is over 50% (Guentzel, 1986), therefore prompt recognition of the infecting organism and early treatment are essential. It is also important to distinguish between pneumoniae due to *K. pneumoniae*, and

pneumonia caused by other organisms because of antibiotic resistance of the organism and treatment desired (Freeman, 1985).

Serotype is also important in *K. pneumoniae* infections. Respiratory tract infections by *K. pneumoniae* predominantly involve capsular types 1 and 2, while urinary tract infections often involve types 8, 9, 10, and 24. Mannose-sensitive pili are important virulence factors of *K. pneumoniae*, serving as adhesions that permit colonization of the mucosal surface in humans (Fader and Davis, 1980). The physiological state of the host is also important in *Klebsiella* infections. Receptor sites on the epithelial cells for the adherence of *Klebsiella* increase. Capsules are also involved in the virulence of *Klebsiella pneumoniae* showing resistant of the organism to phagocytosis and intraphagocytic killing (Freeman, 1985).

5. Epidemiology

Klebsiella species are commonly found in water, soil, occasionally in foods, and to a varying degree in the intestinal tracts of humans and animals. Modes of transfer may be indirect, involving various vehicles, or transfer of the organism may be direct. For example, high numbers of *Klebsiella pneumoniae* have been isolated from hospital foods (particularly fresh salads)(Park and Sanders, 1990), orange juice concentrate (Fuentes et al., 1985), milk (Cousins and

Marlatt, 1990), cheese (Massa et al., 1992), meats (Stiles and Ng, 1981), water, and seafood (Boutin et al., 1986; Singh and Kulshreshtha, 1992). It has also been linked to clinical infections caused by intravenous fluid addition, medical device contamination, and administration of medications (Torre et al., 1985). However, an outbreak (greater than 2 infected) of *K. pneumoniae* associated with food consumption has never been reported in the United States and the Centers for Disease Control in Atlanta, Georgia does not consider *K. pneumoniae* to be a "foodborne pathogen" (CDC, 1995). Despite this, the food industry and various governmental groups (including the FDA, USDA, and CDC) are still investigating the possibility of foods being the vector that leads to *K. pneumoniae* infections, especially when the organism is ingested by those individuals who are immunocompromised.

6. Methods of Isolation

K. pneumoniae isolates are difficult because they have similar biochemical characteristics and are frequently confused with strains of *Enterobacter aerogenes* (Chein and Fung, 1990). Consequently, several different selective medias have been developed for the isolation of *K. pneumoniae*. However, none appear to be simpler or more convenient than any other.

Enrichment of *K. pneumoniae* is generally performed in

lactose containing media at 35 to 37°C as with most other *Enterobacteriaceae*. *K. pneumoniae* are normal inhabitants of the intestinal tract, but they are generally present in much lower numbers when compared to *E. coli*. When enriching for *K. pneumoniae*, it is difficult to selectively streak after 24 h and an incubation of 48 h is generally required (Wong et al., 1985).

Dyes are very important in the selection and differentiation of all bacteria. In studies in 1973 by Fung and Miller it was shown that acriflavine was selective for *K. pneumoniae*. Fung and Niemeic (1977) reported that acriflavine violet red bile agar could be used to differentiate *K pneumoniae*. Other selective media have also been used to isolate *K. pneumoniae* including a methyl violet and double violet agar (Campbell and Roth, 1975; Campbell et al., 1976), a medium with myo-isositol as its sole carbon source, and a MacConkey-inositol-carbenicillin medium (Bagley and Seidler, 1978) on which selectivity is based upon the high resistance of *K. pneumoniae* to carbenicillin. Despite these different selective mediums in the literature, specific governmental recommended methods for the isolation of *K. pneumoniae* in foods are not available. Therefore, the method used in a specific laboratory may be completely different or a variation of previous procedure.

Rapid methods for clinical diagnosis are being sought,

such as those used to detect *Klebsiella pneumoniae* in sputum extracts, so that identification can be accomplished in 1 to 2 hours rather than 1 to 2 days as with conventional microbiological methods (Myrvik and Weiser, 1988). However, this rapid identification has not been applied directly to the food industry and it is unlikely to be used in the near future.

D. ***Salmonella***

1. Historical Background

The period from the late 1800's to the mid 1900's marked a time when *Salmonella* was associated with the cause of typhoid fever and animal infections. In 1885 Dr. Salmon identified *S. choleraesuis* as an important pathogen in swine, *S. bovismorbificans* as the cause of severe infections in cattle, and *S. pullorum* and *S. gallinarum* as pathogens in chickens. Then, in 1888 the first nontyphoid *Salmonella* as a human pathogen was identified. Fifty people in Germany who consumed raw ground beef were affected by the organism (Tauxe, 1991). The organism known today as *Salmonella typhimurium* was isolated from the victim's blood and spleen and from the leftover meat. Since then *Salmonella* typhoid fever has nearly been eliminated in industrialized countries, and *Salmonella* species have been reported more as a gastroenteritis in humans with actual infections ranging from 840,000 to 4,000,000 per year (Chalker and Blaser, 1988).

2. Taxonomy, Nomenclature, and Morphology

The genus *Salmonella* is one of six major divisions of the family *Enterobacteriaceae*. *Salmonella* consist of a large number of serotypes, greater than 2000, which has led to various ways of classifying the genus. *Salmonella* are gram-negative, flagellated, facultatively anaerobic bacilli

containing three major antigens: H, or flagellar antigen; O, or somatic antigen; and Vi antigen, possessed by only a few serotypes. The H antigen occurs in two forms, called phase 1 and phase 2. Phase 1 antigens are shared by only a few organisms and react only with homologous antisera, but phase 2 antigens are shared by many organisms and react with heterologous antisera (Zwadyk, 1992). The O antigen occurs on the outer membrane and is determined by specific sugar sequences on the cell surface. Vi antigen is a superficial antigen overlying the O antigen and is present in only a few types, the most notable being *S. typhi* (Zwadyk, 1992).

3. Growth Characteristics

Salmonellae are typical of other gram-negative bacteria in their ability to grow on a large number of cultured media and produce visible colonies within 24 h at 37°C. The lowest temperature in which *Salmonella* has been reported to grow is 5.3°C for *S. heidelberg* and 6.2°C for *S. typhimurium* (Matches and Liston, 1968). Temperatures around 45°C have been reported by several authors to be the upper limit for growth (Jay, 1992). The optimum pH for growth is about neutral (6.6 to 8.2), with values greater than 9 and less than 4 bactericidal. The minimum pH for growth may be as low as 4.05, but depending on the acid used to lower the pH, the minimum value may be as high as 5.5 (Chung and Goepfert, 1970).

4. Pathology

Surface antigens, factors contributing to invasiveness, endotoxins, cytotoxins, and enterotoxins all contribute to the virulence of *Salmonella* species. The role of each of these factors in the pathogenesis of *Salmonella* infections probably varies with the individual serotype and the host system. *Salmonella* species have the ability to adapt to specific hosts and change virulence. The fact that these organisms have this ability to adapt to a specific host has led to the use of the term facultative intercellular parasites to describe the pathogenesis of *Salmonella*.

Salmonella attach to host receptor cells by O antigenic side chains or, in the case of *S. typhi*, by the presence of Vi antigen. Smooth colonial variants of O-specific side chains are virulent while rough colonial variants deficient in O-specific side chains are avirulent. Studies with human volunteers have also shown that organisms containing Vi antigen are more virulent than those without the antigen (Zwadyk, 1992).

Salmonella are also invasive, penetrating the epithelial lining of the intestinal system's small intestine, and unlike many other invasive organisms, actually penetrating into the subepithelial tissue. *Salmonella* appear to synthesize new protein cells, in the presence of mammalian cells, that are necessary for the

adherence and penetration of the mammalian cells. The oxygen level in which the organism is grown appears to be an environmental cue for the production of these new proteins. If the *Salmonella* organism is grown in an atmosphere of 0 to 1% oxygen, 70% are more adherent and invasive than those bacteria grown in 20% oxygen (Finlay et al., 1989).

Enterotoxins have also been demonstrated in several *Salmonella* species, however, the role of these enterotoxins in disease is not clearly defined. *Salmonella* also produce a cytotoxin and an endotoxin that are distinct from enterotoxins. The cytotoxin appears to be associated with the outer bacterial membrane, which may mean that the toxin is important in cellular invasion and cellular destruction (Ashkenazi et al., 1988). The role of the endotoxin is also not defined. It is presumably responsible for the fever seen in patients with *Salmonella* infections. It is believed this fever is caused either directly or indirectly through the release of endotoxin pyrogens from leukocytes (Zwadyk, 1992).

5. Epidemiology

Salmonella, unlike most other zoonotic infections, does not cause overt disease in animals or birds. Many infected livestock and birds are otherwise healthy carriers of the organism which is naturally cleared in a few weeks. During processing however, cross-contamination often occurs,

particularly with poultry meat. Surveys of broiler meat shows that between 50 and 80% are contaminated with *Salmonella* (Humphrey et al., 1988).

Many egg-containing foods, in particular those made in the home with a raw egg, have been identified as being contaminated with *Salmonella* and having been consumed by infected individuals. Human illness from eggborne *Salmonella* species was reported as early as 1960. Ovarian infections with *S. enteritis, S. heidelgberg,* and *S. typhimurium* in chickens was demonstrated as a potential for eggborne infections (Snoeyenbos et al., 1969). However, lately an unpredicted increase in the number of eggborne outbreaks of salmonellosis were reported in both the United States and Britian (Madden, 1990; UKDHSS, 1990).

The largest human salmonellosis outbreak occurred in 1985 in six Midwestern states and involved contaminated milk (Lecus, 1986). There were 16,284 cultures confirmed and an estimated 165,000 affected individuals. The problem was a processing breach at the pasteurizer that resulted in incomplete cleaning and the contamination of already pasteurized milk with unpasteurized milk.

In the early 1980's shrimp imported from several southeast Asian countries had high *Salmonella* species isolation rates. This caused the FDA to require that products shipped from those nations be placed on automatic

detention (Jackson et al., 1989). Other products including ready-to-eat seafood products and unlikely foods such as chocolate and beansprouts have been implicated as vehicles for salmonella infections.

6. Methods of Isolation

The major problem with current microbiological testing for *Salmonella* species in foods is that the official methods for monitoring are very time consuming (4 to 5 days) and adoption of new more rapid methods by government agencies is even slower. The official FDA procedure involves a preenrichment, enrichment, and numerous steps for plating on selective media, followed by biochemical testing and antigen determination (Andrews et al., 1992). Preenrichment in a nonselective broth provides for the uninhibited growth of indigenous bacteria and the resuscitation and proliferation of stressed or injured *Salmonella* to detectable levels (Litchfield, 1973). Enrichment may take place in either selective or nonselective broths, which are superior to direct plating when small numbers of *Salmonella* are present (Galton et al., 1968). Selective and differential plating is the most important part of standard isolation procedures for the isolation of *Salmonella* from foods and the environment (Warburton et al., 1994).

Selective enrichment inhibits the growth of competing nonsalmonellae and facilitates isolation of *Salmonella*

species on differential media (Fagerberg and Avens, 1976). Tetrathionate and selenite cystine broths, selenite brilliant green, and modified Rappaport enrichment mediums have been used in isolating *Salmonella* from foods. In laboratory studies, the Rappaport enrichment medium has been shown to be more sensitive and selective than the other mediums in isolation of *Salmonella* species (Vassiliadis, 1991).

Brilliant Green Sulpha Agar (BGS), Bismuth Sulphite (BIS), Hektoen Enteric Agar (HEK), and Xylose Lysine Desoxycholate Agar (XLD) are used in standard analysis methods by different governmental agencies in the United States, Canada, and Europe (Andrews et al., 1992). EF-18 Agar and Rammbach Agar (RAM) are newly developed agars commonly used in association with those mentioned above for the isolation of *Salmonella* (Todd et al., 1993; Anon, 1993). In a study by D.W. Warburton et al. (1994), it was determined that EF-18 had the highest rate of recovery of *Salmonella* species (100%), followed by HEK (95.3-100%), XLD (88.6%-100%), RAM (79.8-99.2%), BGS (72.2-100%) and BIS (45.3-100%). The study also showed that the largest problem in the isolation of *Salmonella* species was differentiating between them and other bacteria. *C. freundii*, *Pseudomonas* spp., and *E. coli* were some of the more common contaminates.

The absence of *Salmonella* in ready-to-eat food products

should be ascertained before the product goes on sale in the supermarket. This need has led to the development of rapid methods for the analysis of *Salmonella*. Several procedures include the use of a Salmonella-specific bacteriophage, Felix-O1, which increases in numbers when Salmonella species are present (Harish and Martin, 1983). A technique to isolate *Salmonella* using enzyme-labeled antibodies (Krysinski and Heimsch, 1977) and an immunoassay to indicate *Salmonella* species (Thompson, 1991) have also been developed.

References

Abeyta, C., F.G. Deeter, C.A. Kaysner, R.F. Scott, and M.M Wekell. 1993. *Campylobacter jejuni* in a Washington State shellfish growing bed associated with illness. J. Food Prot. 56:323-325.

Alonso, J.L. and M.A. Alonso. 1993. Presence of *Campylobacter* in marine waters of Valencia, Spain. Wat. Res. 27:1559-1562.

Andrews, W.H., V.R. Bruce, G. June, F. Satchell, and P. Sherrod. 1992. *Salmonella*. United States Food and Drug Administration Bacteriological Analytical Manual. Arlington, Virginia.

Anon. 1993. Rambach agar. Int. J. Food Microbiol. 17:243-244.

Arumugaswamy, R.K. and R.W. Proudford. 1987. The occurence of *Campylobacter jejuni* and *Campylobacter coli* in Sydney Rock Oyster (*Crassostrea commercialis*). J. Food. Microbiol. 4:101-104.

Ashkenazi, S., T.G. Cleary, and B.E. Murray. 1988. Quantitative analysis and partial characteristics of cytotoxin production by *Salmonella* strains. Infect. Immun. 56:3089-3093.

Barret, T.J., C.M. Patton, and G.K. Morris. 1988. Differentiatin of *Campylobacter* species using phenotypic characterization. Lab. Med. 19:96-102.

Bagley, S.T. and R.J. Seidler. 1978. Primary *Klebsiella* identification with MacConkey-inositol-carbenicllin agar. Appl. Environ. Microbiol. 36:536-538.

Blaser, M.J., I.D. Berkowitz, F.M. LaForce, J. Cravens, L.B. Reller, and W.L.L. Wang. 1979. *Campylobacter* enteritis: Clinical and epidemiologic features. Ann. Intern. Med. 91:179-185.

Blaser, M.J., D.N. Taylor, and R.A. Feldman. 1983. Epidemiology of *Campylobacter jejuni* infections. In J.P. Butzler (ed.), *Campylobacter* infections in man and animals. CRC Press, Boca Raton, Flordia. pp. 143-161.

Bolton, F.J., D. Coates, P.M. Hinchliffe, and L. Robertson. 1983. Comparision of selective medium for isolation of *Campylobacter jejuni/coli*. J. Clin. Pathol. 36:78-83.

Borczyk, A.A., H. Lior, and B. Ciebin. 1987. False positive identification of *Escherichia coli* O157:H7 in foods. Intl. J. Food Microbiol. 4:347-349.

Boutin, B.K., P.L. Spaulding, and R.M. Twedt. 1986. Evaluation of enterophathogenicity of *Klebsiella pneumoniae* isolated from summer-harvested Louisiana oysters. J. Food Prot. 49(6):442-444.

Butzler, J.P., P. Dekeyser, M. Detrain, and J. Katchaki. 1973. Related *Vibrio* in stools. J. Pediatr. 82:493-495.

Butzler, M.P. and M.D. Skirrow. 1979. *Campylobacter* enteritis. Clin. Gastroenterol. 8:737-765.

Butzler, J.P. and J. Oosterom. 1991. *Campylobacter*: pathogenicity and significance in foods. Int. J. Food Micro. 12:1-8.

Byrd, J.J., H.S. Xu, and R.R. Colwell. 1991. Viable but nonculturable bacteria in drinking water. Applied and Environ. Microbiol. 57(3):875-878.

Campbell, L.M. and I.L. Roth. 1975. Methyl voilet; a selective agent for differentiation of *Klebsiella pneumoniae* from *Enterobacter aerogenes* and other gram-negative bacteria. Appl. Microbiol. 30:258-261.

Campbell, L.M., I.L. Roth, and R.D. Klein. 1976. Evaluation of double agar in the isolation of *Klebsiella pneumoniae* from river water. Appl. Environ. Microbiol. 31:213-215.

Castillo-Ayala, A. 1992. Comparision of selective broths for isolation of *Campylobacter jejuni/coli* from freshly deboned market chicken. J. Food. Prot. 55:333-336.

Centers for Disease Control. 1995. Personal communication. Division of Foodborne Infection. Atlanta, Georgia.

Chan, F.T.H. and M.R. Mackenzie. 1982. Enrichment medium and control systems for the isolation of *Campylobacter fetus* subsp. *jejuni* from stools. J. Clin. Microbiol. 15:12-15.

Chalker, R.B. and M.J. Blaser. 1988. A review of human salmonellosis:III. Magnitude of *Salmonella* infections in the United States. Rev. Infect. Dis. 10:111-124.

Chein, S.P. and D.Y.C. Fung. 1990. Acriflavine violet red bile agar for the isolation and enumeration of *Klebsiella pneumoniae*. Food Microbiol. 7:295-304.

Chung, K.C. and J.M. Goepfert. 1970. Growth of *Salmonella* at low pH. J. Food Sci. 35:326-328.

Cleary, T.G. 1988. Cytotoxin-producing *Escherichia coli* and the hemolytic-uremic syndrome. Pediatr. Clin. North Am. 35:485-501.

Cousins, D.L. and F. Marlatt. 1990. An evaluation of conductance method for the enumeration of *Enterobacteriaceae* in milk. J. Food Prot. 53(7):568-570.

Dekeyser, P., M. Gosssuim-Detrain, J.P. Butzler, and J. Sternon. 1972. Acute enteritis due to related *Vibrio*: First positive stool cultures. J. Infect. Dis. 125:390-392.

Demol, M.P. and E. Bosmans. 1978. *Campylobacter* enteritis in Central Africa. Lancet i:604.

Doyle, M.P. 1985. Foodborne pathogens of recent concern. Ann. Rev. Nutr. 5:25-41.

Doyle, M.P. and D.J. Roman. 1982. Recovery of *Campylobacter jejuni/coli* by selective enrichment. Appl. Environ. Microbiol. 43:1343-1353.

Doyle, M.P. and J.L. Schoeni. 1984. Survival and growth characteristics of *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. 48:855-856.

Doyle, M.P. and J.L. Schoeni. 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. 53:2394-2396.

Faberberg, D.J. and J.S. Avens. 1976. Enrichment and plating methodology for *Salmonella* detection in foods. J. Milk Technol. 39:628-633.

Fader, R.C. and C.P. Davis. 1980. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. Infect. Immun. 30:554-561.

Farrag, S.A., F.E. El-Gazzar, and E.H. Marth. 1992. Behavior of *Escherichia coli* O157:H7 or *Yersinia enterocolitica* at 4°C or 7°C in raw milk inoculated with a commercial culture of lactic acid bacteria. Milchwiss. 47(3):149-152.

Feng, P.C. and P.A. Hartman. 1982. Fluorogenic assays for immediate confirmation of *Escherichia coli*. Appl. Environ. Microbiol. 41:1320-1329.

Finlay, B.B., F. Heffron, and S. Falkow. 1989. Epithelial cell surfaces induce *Salmonella* proteins required for bacterial adherence and invasion. Science 243:940.

Foster, J.F, J.L. Fowler, and J. Dacey. 1977. A microbial survey of various fresh and frozen seafood products. J. Food Prot. 40:300-303.

Franco, D.A. 1987. *Camylobacter* species: Considerations for controlling a foodborne pathogen. J. Food Prot. 51(2):145-153.

Freeman, B.A. 1985. Textbook of Microbiology. W.B. Saunders Company. Philadelphia, Pennsylvania. p.480.

Fuentes, F.A., T.C. Hazen, A.J. Lopez-Torres, and P. Rechani. 1985. *Klebsiella pneumoniae* in orange juice concentrate. Appl. and Environ. Microbiol. 49(6):1527-1529.

Fung, D.Y.C. and R.D. Miller. 1973. Effects of dyes on bacterial growth. Appl. Microbiol. 25:793-799.

Fung, D.Y.C. and M. Niemeic. 1977. Acriflavine violet red bile agar for the isolation of *Klebsiella*. Health Lab. Sci. 14:273-278.

Galton, M.M., G.K. Morris, and W.T. Martin. 1968. Salmonellae in foods and feeds. Review of isolation methods and recommended procedures. U.S. Dep. Health, Education, and Welfare/Publ. Health Serv., Communicable Dis. Ctr., Atlanta, Georgia.

George, H.A., P.S. Hoffman, R.M. Smibert, and N.R. Kreig. 1978. Improvement media for growth and aerotolerance of *Campylobacter fetus*. J. Clin. Microbiol. 8:36-41.

Genigeorgis, C. 1986. The risk of transmittion of zoonotic and human diseases by meat and meat products. In F.J.M. Smulders (ed.). Elimination of pathogenic organisms from meat and poultry. Elsevier Science Publisherss B.V. (Biochemical Division), Amsterdam, The Netherlands.

Gilbert, R.J. 1988. Food-borne Infections and Intoxications: Recent problems and new organisms. British Food J. 90(2):71-73.

Goossens, H. 1990. New insights in isolation, taxonomy, pathogenicity and treatment of *Campylobacter*. Thesis Free University Brussels.

Griffin, M.R., E. Dalley, M.S. Fitzpatrick, and S.H. Austin. 1983. *Campylobacter* gastroenteritis associated with raw clams. J. Med. Soc. N.J. 80:607-609.

Guentzel, M.N. 1986. Escherichia, *Klebsiella, Enterobacter, Serratia, Citrobacter,* and *Proteus*. In S. Baron (ed): Medical Microbiology. Addison-Wesley Publishing Compnay, Inc. Menlo Park, California. pp. 495-506.

Harsh, D.C. and L.D. Martin. 1983. Rapid detection of *Salmonella* sp. by using Felic-01 bacteriophage and high performance liquid chromatography. Appl. Environ. Microbiol. 45:260-263.

Hithcnes, A.D., P. Feng, W.D. Watkins, S.R. Rippey, and L.A. Chandler. 1992. *Escherichia coli* and other coliform bacteria. United States Food and Drug Administration Bacteriological Analytical Manual. Arlington, Virginia.

Honish, A. 1986. Summary of *Escherichia coli* O157:H7 at an Edmonton extended care facility. Alberta Soc. Services Comm. Healthy. 10:363-368.

Humphrey, T.J., G.C. Mead, and B. Rowe. 1988. Poultry meat as a source of human Salmonellosis in England and Wales. Epidem. and Infect. 100:175-184.

Hunt, J.M. 1992. *Campylobacter*. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

Hutchinson, D.N. and F.J. Bolton. 1984. An improved blood-free selective medium for isolation of *Campylobacter jejuni* from fecal specimens. J. Clin. Pathol. 37:956.

Jackson, G.J., C.H. Scarbrough, and F.M. MacKeith. 1989. Report to the government of Indonesia and the United States Agency for International Developement. U.S. Food and Drug Administration. Washington, DC.

Jay, J.M. 1992. Modern Food Microbiology. Van Nostrand Reinhold, New York, New York. pp. 553-579.

Jones, F.S., R.B. Little, and M. Orcutt. 1932. A continuation of the study of aetiology of infectious diarrhea (winter scours) in cattle. J. Am. Vet. Med. Assoc. 81:610.

Karmali, M.A. and P.C. Fleming. 1979. Application of the Fortner principle to isolate *Campylobacter* from stools. J. Clin. Microbiol. 10:245-247.

Kornblum, K. 1928. The roetgen-ray diagnosis of pulmonary infections with the Fiedlander bacillus. Am. J. of Roentgenology and Radium Therapy. 19:513-521.

Korvick, J.A., A.K. Hackett, V.L. Yu, and R.R. Muder. 1991. *Klebsiella* pneumonia in the modern era. Southern Med. J. 84(2):200-204.

Krysinski, E.P. and R.C. Heimsch. 1977. Use of enzyme-labeled antibiodies to detected *Salmonella* in foods. Appl. Environ. Microbiol. 33:947-950.

Lander, K.P. and K.P.W. Gill. 1980. Experimental infection of the bovine udder with *Campylobacter coli/jejuni*. J. Hyg. (Camb.) 84:421-428.

Lecus, C.W. 1986. A closer look at dairy safety. FDA Consumer 20(3):14-17.

Levy, A.J. 1946. A gastroenteritis outbreak probably due to bovine strain of *Vibrio*. Yale J. Biol. Med. 18:243-259.

Litchfield, J.H. 1973. *Salmonella* and the food industry-methods for isolation, identification, and enumeration. Crit. Rev. Food Technol. 3:415-421.

Luechtefekd, N.W., L.B. Reller, M.J. Blaser, and W-L. L. Wang. 1982. Comparison of atmospheres of incubation for the primary isolation of *Campylobacter fetus* subsp. *jejuni* from animal specimens: 5% oxygen versus candle jar. J. Clin. Microbiol. 15:53-57.

MacDonald, K.L. and M.T. Osterholm. 1993. The emergence of *Escherichia coli* O157:H7 infections in the United States. The changing epidemiology of foodborne disease. J. Am. Med. Assoc. 268:2264-2266.

Madden, J.M. 1994. Concerns regarding the occurrence of *Listeria monocytogenes, Campylobacter jejuni*, and *Escherichia coli* O157:H7 in foods regulated by the U.S. Food and Drug Administration. Dairy, Food and Environ. Sanitat. 53:941-943.

March, S.B. and S. Ratnam, 1986. Sorbitol-MacConkey medium for the detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. 23:869-872.

Massa, S., F. Gardini, M. Sinigaglia, and E. Guerzoni. 1992. *Klebsiella pneumoniae* as a spoilage organism in mozzarella cheese. J. Dairy Sci. 75:1411-1414.

Matches, J.R. and J. Liston. 1968. Low temperature growth of *Salmonella*. J. Food Sci. 33:641-645.

McCabe, W.R., B.E. Kreger, and M. Johns. 1972. Type-specific and reactive antibodies in gram negative bacteremia. N. Engl. J. Med. 287:261-261.

Murano, E.A. and M.D. Pierson. 1992. Effect of heat shock and growth atmosphere on the heat resistance of *Escherichia coli* O157:H7. J. Food Prot. 55:171-175.

Myrvik, Q.N. and R.S. Weiser. 1988. Fundamentals of Medical bacteriology and mycology. Lea and Bebiger, Philadelphia, Pennsylvania pp. 298-299.

Norkrans, G. and A. Svedhem. 1982. Epidemiological aspects of *Campylobacter jejuni* interitis. J. Hygene. 89:163-170.

Okrend, A.J.G., B.E. Rose, and B. Bennett, 1990. A screening method for the isolation of Escherichia coli O157:H7 from ground beef. J. Food Prot. 53:249-252.

Okrend, J.G., B.E. Rose, and C.P. Lattuada. 1992. Isolation of *Escherichia coli* O157:H7 using O157 specific antibody coated magnetic beads, J. Food. Prot. 55:214-217.

Orskov, I. 1984. *Enterobacteriaceae*: Genus V. *Klebsiellla*. In N. Kreig (Ed.): Bergey's Manual of Determinative Bacteriology. 9th ed. Williams and Wilkins Co., Baltimore, Maryland. pp. 461-465.

Oosterom, J., H.J. Beckers, L.M. Van Noorle Jansen, and M. Van Schothorst. 1980. Een expolsie van campylobacter-infectie in een kazrne, waarschijnlijk veroorzaakt door rauwe tartaar. Ned. Tijd. Genees. 124:1631-1634.

Oosterom, J. 1985. Studies on the epidemiology of *Campylobacter jejuni*. Ph.D. Thesis, Erasmus University of Rotterdam, Rotterdam, The Netherlands.

Ostroff, S.M., J.M. Kobayashi, and J.H. Lewis. 1989. Infections with *Escherichia coli* O157:H7 in Washington State. The first year of statewide disease surveillance. J. Am. Med. Assoc. 262:355-359.

Padhye, N.V. and M.P. Doyle. 1992. *Escherichia coli* O157:H7: Epidemiology, pathogeneses, and methods for detection in foods. J. Food Prot. 55:555-565.

Pai, C.H., S. Sorger, L. Lackman, R.E. Sinai, and M.I. Marks. 1979. *Campylobacter* gastroenteritis in children. J. Pediatr. 94:589-591.

Pang, T., P.Y. Wong, D. Puthucheary, K. Sihotang, and W.K. Chang. 1987. In-vitro and in-vivo studies of a cytotoxin from *Campylobacter jejuni*. J. Med. Microbiol. 29:803.

Park, C.E. and G.W. Sanders. 1990. Source of *Klebsiella pneumoniae* in alfalfa and mung bean sprouts and attempts to reduce its occurence. Can. Inst. Food Sci. Technol. J. 23:189-192.

Philips, F.A. and J.T. Peeler. 1972. Bacteriological Survey of the Blue Crab Industry. Appl. Mcrobiol. 24(6):958-966.

Reinhard, R.G., T.J. McAdams, G.J. Flick, R.E. Cronenberghs, R.F. Wittman, and A. Diallo. 1994. Quantitative and qualitative analysis of *Campylobacter jeuni* and *Campylobacter coli* in fresh hand picked blue crab (*Callinectes sapidus*) meat. J. Aqua. Food Produc. Tech. 4(4) in press.

Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Herbert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Eng. J. Med. 308:681-685.

Ritvo, M. and F. Martin. 1949. The clinical and roentgen manifestation of pneumoniae due to *Bacillus mucosus capsulants* (primary Friendlander pneumoniae). AJR. 62:211-222.

Roa, D.V. and K.R.G. Roa. 1983. Incidence of *Klebsiella* in foods and water. J. Food Sci. Tech. 20:265-268.

Singh, B.R. and S.B. Kulshreshtha. 1992. Preliminary examinations on the enterotoxigenicity of isolates of *Klebsiella pneumoniae* from seafood. Intern. J. Food Microbiol. 16:349-352.

Skerman, V.B.D., V. McGowan, and P.H. Sneath. 1980. Approved list of bacterial names. Int. J. Syst. Bacteriol. 30:225.

Skirrow, M.B. 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. 2:9-11.

Slabyj, S.O., S.A. Ismail, B.M. Slabyj, W.A. Halteman, and R.E. Martin. 1990. A simple proceduce to monitor fecal coliforms in seafood processing plants. J. Food Prot. 54:554-556.

Slocum, G.G. 1955. Bacteriology of crabmeat as related to facility sanitation. Ass. Food Drug Officals, U.S. Quart. Bull. 19:43-50.

Smibert, R.A. 1989. *Campylobacter*. In: N.R. Kreig and J.G. Holt (ed.). Bergeys Manual of Systematic Bacteriology. Vol. 1. Williams and Wilkens Co, Baltimore, Maryland.

Snoeyenbos, G., C. Smyser, and H. Van Roekel. 1969. *Salmonella* infections of the ovary and peritoneum of chickens. Avain. Dis. 13:668-670.

Steele, T.W. and S. McDermott. 1978. *Campylobacter* enteritis in South Australia. Med. J. Aust. 2:404.

Stern, N.J. 1982a. Selectivity and sensitivity of three media for the recovery of inoculated *Campylobacter fetus* ssp. *jejuni* from ground beef. J. Food Safety 4:169-175.

Stern, N.J. 1982b. Methods of recovering *Capylobacter jejuni* from foods. J. Food Prot. 45:1332-1337.

Stern, N.J., M.P. Hernandez, L. Blankenship, K.E. Deibel, S. Doores, M.P. Doyle, H. Ng, M.D. Pierson, J.N. Sofos, W.H. Sveum, and D.C. Westhoff. 1985. Prevalence and distribution of *Campylobacter jejuni* and *Campylobacter coli* in retail meats. J. Food Prot. 48:595-599.

Stern, N.J. and S. Kazmi. 1989. *Capylobacter jejuni*. In: M.P. Doyle (ed.). Foodborne Bacterial Pathogens. Marcel Dekker, Inc. New York, New York.

Stern, N.J. and J.E. Line. 1992. Comparison of three methods for recovery of *Campylobacter* spp. from boiler carcasses. J. Food Prot. 55:663-666.

Stiles, M.E. and Lai-king Ng. 1981. *Enterobacteriaceae* associated with meats and meat handling. Applied and Eviron. Microbiol. 41:867-872.

Tanner, E.I. and C.H. Bullin. 1977. *Campylobacter* enteritis. Br. Med. J. 2:579.

Tauxe, R.R.V., D.A. Pegues, and N. Hargrett-Bean. 1987. *Campylobacter* infections: the emerging national pattern. Am. J. Public Health 77:1219-1221.

Tauxe, R.R.V. 1991. *Salmonella*: A postmodern pathogen. J. Food Prot. 54:563-568.

Thompson, B.M. 1981. Curent status of immunofluorescent methodology for salmonellae. J. Food Prot. 44:381-387.

Todd, E.C.D., J.M. MacKenzie, and P.I. Peterkin. 1993. Developement of enzyme-linked antibody hydrophobic grid membrane filter method for the detection of *Salmonella*. Food Microbiol. 10:87-99.

Torre, M.G., J. Romero-Vivas, J.M. Beltran, A. Guerrero, M. Meseguer, and E. Bouza. 1985. *Klebsiella* bacteremia: An analysis of 100 episodes. Rev. Infect. Diseases. 7(2):143-150.

Untited Kindom Agriculture Committee. 1990. *Salmonella* in eggs-a progress report. House of Commons Paper 33.

U.S. Department of Health and Human Services, Annual Summary 1990 Public Health Services, Centers for Disease Control. Water-related Disease outbreaks.

Vassiliadis, P., V. Kalapothaki, and D. Trichopoulos. 1991. Isolation of *Salmonella* from fluid milk with the use of Rappaport-Vassiliadis medium. J. Food Prot. 54:421-423.

Warburton, D.W., B. Bowen, A. Konkle, C. Crawford, S. Durzi, R. Foster, C. Fox, L. Gour, G. Krohn, P. LaCasse, G. Lamontage, S. McDonagh, V. Arling, J. Mackensie, E.C.D. Todd, J. Oggel, R. Plante, S. Swaw, N.P. Tiwari, Y. Trottier, and B.D. Wheeler. 1994. A comparision of six different plating media used in the isolation of *Salmonella*. Inter. J. Food Microbiol. 22:277-289.

Wentz, B.A., A.P. Duran, A. Swartzentruber, A.H. Schwab, F.D. McLure, D. Archer, and R.B. Reead, Jr. 1985. Microbiological quality of crabmeat during processing. J. Food Prot. 48(1):44-49.

Wolff, S.M and J.V. Bennet. 1974. Gram-negative-rod bacteriamia. N. Engl. J. Med. 291:733-4.

Wong, S.H., D.R. Cullimore, and D.L. Bruce. 1995. Selective medium for the isolation and enumeration of *Klebsiella* spp. Appl. and Environ. Microbiol. 49:1022-1024.

Yanagisawa, A. 1980. Large outbreak of Campylobacter enteritis amoung school children. Lancet ii:153.

Zwadyk, P. 1992. Enterobacteriaceae: *Salmonella* and *Shigella*, intestinal pathogens. In: W.K. Jolik, H.P. Willett, D.B. Amos, and C.M. Wilfert (eds): Zinsser Microbiology. Appleton and Lange, Englewoods Cliffs, New Jersey.

Qualitative and Quantitative analysis of *Campylobacter jejuni* and *Campylobacter coli* in Fresh Blue Crab (*Callinectes sapidus*) Meat

(Paper accepted for publication in the Journal of Aquatic Food Products Technolgy)

ABSTRACT

Samples of fresh picked crabmeat from twelve different blue crab processing facilities were analyzed for general microbial quality and the presence of *Campylobacter jejuni* and *Campylobacter coli*. Twenty samples from each plant were analyzed for a total of 240 samples. Aerobic plate counts ranged from $7.4x10^3$ to $4.6x10^8$ CFU/g. Coliform and Fecal coliform counts ranged from <0.3 to 32.8 MPN/g and <0.3 to 2.26 MPN/g, respectively. *Eshcherichia coli* counts ranged from <0.3 to 0.77 MPN/g. *Campylobacter jejuni* was isolated from 36 (15%) samples and *Campylobacter coli* was isolated from 14 (5.8%). There was no statistical correlation between general microbial quality, plant size or inspection score (sanitation) and the presence of *Campylobacter* species ($p<0.05$).

INTRODUCTION

Campylobacter has been the cause of bacterial diarrhea in every country throughout the world and the relationship between its presence in foods and disease is well documented (Franko, 1987). Isolation of *Campylobacter jejuni* from patients with diarrhea has been greater than the isolation of *Salmonella* species (Stern, 1982). Despite this occurence, the *Campylobacter* genus has only recently become widely studied as an important pathogen in human gastroenteritis.

Foods of animal origin are the primary vehicles involved in human infection of *Campylobacter*. *Campylobacter* are commensals of all wild and domestic animals gastrointestinal tracts, and these warm blooded animals and birds continually contaminate the environment with *Campylobacter*. The bacterium has been isolated from fresh and marine waters around the world (Norkrans, 1982; Blaser, 1979; Franco, 1987; Alonso and Alonso, 1993; Knill et al., 1978; Jones et al., 1990), and areas heavily polluted with sewage, farm-runoff, or sludge have led to higher rates of isolation (Abeyta et al., 1993). Shellfish growing waters have also been found contaminated with *Campylobacter jejuni* and *Campylobacter coli* (Abeyta, et al., 1993) and in 1986, the organisms were isolated from 14% of oysters harvested from contaminated beds (Arumugaswamy and Proudford, 1987).

However, *Campylobacter jejuni/coli* as an agent in shellfish associated outbreaks of humans and the presence of *Campylobacters* in shellfish still remains undefined.

MATERIALS AND METHODS

Sample Collection.

From July through August, 1994, hand picked crabmeat samples from 12 blue crab (*Callinectes sapidus*) processing facilities in the Chesapeake Bay region were collected and analyzed. Twenty samples from each of the different facilities were collected on different processing days and analyzed. Facilities were chosen based on production levels (small, medium, large) and Virginia Department of Health inspection scores as an indicator of sanitation practices (excellent $\geq$ 94.5, acceptable < 94.5). Meat samples were collected from the picking tables, packed in an insulated cooler with cold gel-packs, and shipped overnight to Virginia Tech in Blacksburg, Virginia. All samples arrived at the laboratory between 0 and 4°C and were tested between 16 h and 36 h of collection.

Crab samples were prepared and analyzed according to FDA (BAM) procedures (Peeler and Maturin, 1992; Hitchins et al., 1992; Hunt, 1992; Peeler and McClure, 1992). Five randomly selected samples from each plant were tested for aerobic plate count, coliforms, and *Escherichia coli*, while twenty samples were tested for *Campylobacter jejuni/coli*. All serial dilutions were prepared in Butterfield's Phosphate-Buffered distilled water (Solomon, 1992).

Microbiological analysis

Aerobic plate count(APC). Duplicate plates for dilutions 10^{-2} to 10^{-7} were prepared and poured with Standard Methods Agar (SMA). Plates were incubated for 48 h at 35°C (Peeler and Maturin, 1992).

Coliforms (coliform, Fecal coliform, and *Escherichia coli*). A 3-tube most probable number (MPN) method was used to enumerate coliforms and *E. coli* (Hitchins et al., 1992)

Campylobacter. Enrichment. Enrichment medium consisted of Nutrient broth No. 2 (Oxoid), 0.6% yeast extract (Difco), 5% lysed horse blood (Virginia Maryland College of Veterinary Medicine), sodium cefoperazone (30mg/L)(Sigma), Trimethoprim lactate (12.5 mg/L)(Sigma), vancomycin (10 mg/L)(Sigma), cycloheximide (100 mg/L)(Sigma) and 0.4% high concentrate FBP (Hunt, 1992). Enrichment was preincubated under static conditions for a period of 4 h at 37°C and 15 mg/L of cefoperazone was added. Enrichment was then incubated under static conditions at 42°C for another 23 h. Selective plates of CCD agar with CCDA antimicrobial supplement (Oxoid) were streaked and incubated for 48 h at 42°C under microaerophilic conditions (5% oxygen, 10% carbon dioxide, 85%, nitrogen).

Identification. Plates were observed for typical colonies. Wet mounts and gram-stains were preformed and observed under phase-contrast microscope. Typical *Campylobacters*, gram

negative, curved shaped rods, with characteristic corkscrew like motility were tested biochemically. Confirmed biochemical tests included growth at 25°C (-), 35°C (+), 42°C (+), production of oxidase and catalase, nitrate reduction, H_2S production, sensitivity to naladixic acid, and resistance to cephalothin. Differentiation between species was determined by the ability to hydrolyze hippurate and the production of H_2S in triple sugar iron agar.

Quantitative analysis. A three tube MPN method was used. An initial dilution of 1:1 for each sample was prepared by stomaching 50 g of crabmeat with 50 mL of Butterfield's phosphate buffer for 30 seconds. Enrichment medium, selective plating, biochemical tests, temperatures, and conditions were as mentioned above.

Data generated was subjected to analysis of variance and multiple analysis of variance to determine effect of APC, coliforms, *E. coli*, plant size, and sanitation on isolation rates of *Campylobacter jejuni* and *Campylobacter coli* (Zar, 1984).

RESULTS AND CONCLUSIONS

Campylobacter jejuni and *Campylobacter coli* were isolated for the first time from fresh crabmeat. Of the 240 samples analyzed, thermophilic *Campylobacter* were detected in 50 samples. The occurrence of Campylobacters was compared to aerobic plate counts, coliform levels, fecal coliform levels, and *E. coli* counts and was not significant ($P<0.05$). Plant size and inspection scores were also not significant when compared with *Campylobacter* isolation ($P<0.05$).

Thirty six of the isolates were determined to be *Campylobacter jejuni* based on hippurate hydrolysis and failure to produce H_2S when grown on TSI agar. The remaining isolates (14) did not hydrolyze hippurate and produced H_2S when grown on TSI agar. They were classified as *Campylobacter coli*. In all positive samples, quantitative levels of *Campylobacter* were below analysis limits (<0.3 MPN/g).

Little or no literature on the presence of *Campylobacter jejuni/coli* in shellfish is available, and the role of shellfish in human disease is still not defined. Crabmeat could become contaminated with *Campylobacter* in several different ways. The crab may have fed and lived in waters containing *Campylobacter* leading to contamination of the crab's digestive tract and surface. Handling procedures

during processing could then have led to meat contamination.

Humans have also been reported as reservoirs for *Campylobacter jejuni*. In these cases, those who come in contact with excrement of infected people put themselves at risk. Food may also become contaminated by food handlers, however, reports of cross-contamination of food from infected food handlers with *Campylobacter* is unusual (Blaser et al., 1983).

Campylobacters are sensitive to cooking temperatures used in processing crabmeat (Franco, 1987) so cross-contamination usuallly occurs post cooking. *Campylobacter* cross-contaminations after processing have been reported in other foods, and the condition under which contamination is favored, neutral pH (6.5-7.5) and refrigeration temperatures, exist in all crab processing facilities (Blaser et al., 1983).

The incident of *Campylobacter* contamination observed in this study was very low (MPN/g <0.3). It is unlikely that this level of *Campylobacter* would represent a health hazard in humans. However, futher study is needed to postively identify the source of *Campylobacter* contamination in the blue crab industry.

Table 3: General microbial quality and presence of *Campylobacter jejuni/coli* (# pos. of 20 samples) for twelve processing plants

Plant	Size	Inspection score (sanitation)	Mean APC (CFU/g)	Mean Coliform (MPN/g)	Mean Fecal Coliform (MPN/g)	Mean *E. coli* (MPN/g)	*C. coli* (# pos.)	*C. jejuni* (# pos.)
1	L	E	$9.5x10^7$	13.78	<0.30	<0.30	0	1
2	L	E	$2.5x10^4$	1.44	1.44	0.28	4	9
3	L	A	$1.9x10^4$	0.85	0.85	<0.3	0	0
4	L	A	$1.5x10^4$	2.23	2.08	0.77	2	3
5	M	E	$7.8x10^4$	16.72	<0.3	<0.3	1	3
6	M	E	$7.4x10^3$	2.23	2.08	0.34	0	2
7	M	A	$4.6x10^8$	11.28	2.26	0.66	0	0
8	M	A	$2.0x10^7$	2.76	0.93	<0.3	1	0
9	S	E	$1.8x10^6$	20.52	0.39	<0.3	1	5
10	S	E	$1.0x10^4$	<0.3	<0.3	<0.3	0	0
11	S	A	$3.8x10^8$	32.8	0.66	<0.3	5	13
12	S	A	$1.5x10^5$	2.02	0.5	<0.3	0	0

Symbols: (L) large; (M) medium; (S) small; (E) excellent >94.5; (A) acceptable <94.5

REFERENCES

Abeyta, C., F.G. Deeter, C.A. Kaysner, R.F. Scott, and M.M Wekell. 1993. *Campylobacter jejuni* in a Washington State shellfish growing bed associated with illness. J. Food Prot. 56:323-325.

Alonso, J.L. and M.A. Alonso. 1993. Presence of *Campylobacter* in marine waters of Valencia, Spain. Wat. Res. 27:1559-1562.

Arumugaswamy, R.K. and R.W. Proudford. 1987. The occurence of *Campylobacter jejuni* and *Campylobacter coli* in Sydney Rock Oyster (*Crassostrea commercialis*). J. Food. Micro. 4:101-104.

Blaser, M.J., I.D. Berkowitz, F.M. LaForce, J. Cravens, L.B. Reller, and W.L.L. Wang. 1979. *Campylobacter* enteritis: Clinical and epidemiologic features. Ann. Intern. Med. 91:179-185.

Blaser, M.J., D.N. Taylor, and R.A. Feldman. 1983. Epidemiology of *Campylobacter jejuni* Infections. In J.P. Butzler (ed.), *Campylobacter* infections in man and animals. CRC Press, Boca Raton, Florida. pp. 143-161.

Franco, D.A. 1987. *Camylobacter* species: Considerations for controlling a foodborne pathogen. J. Food Prot. 51(2):145-153.

Hitchins, A.D., P. Feng, W.D. Watkins, S.R. Rippey, and L.A. Chandler. 1992. *Escherichia coli* and the coliform bacteria. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

Hunt, J.M. 1992. *Campylobacter*. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

Jones, K., M. Betaieb, and D.R. Terford. 1990. Thermophilic *Campylobacters* in surface water around Lancaster, UK; negative correlation of *Campylobacter* infections in the community. J. Appl. Bact. 69:758-764.

Knill, M., W.G. Suckling, and A.D. Pearson. 1978. Environmental isolation of heat tolerant *Campylobacter* in Southhampton area. Lancet ii:1002-1003.

Norkrans, G. and A. Svedhem. 1982. Epidemiological aspects of *Campylobacter jejuni* interitis. J. Hygene. 89:163-170.

Peeler, J.T. and F.D. McClure. 1992. Most probable number determination. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

Peeler, J.T. and L.J. Maturin. 1992 Aerobic plate count. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

Solomon, H.M. 1992. Media and Reagents. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

Stern, N.J. 1982. Methods of recovering *Campylobacter jejuni* from foods. J. Food Prot. 45:1332-1337.

Zar, J.H. 1984. Biostatistical Analysis, 2 edition. Prentice Hall, Englewood Cliffs, New Jersey.

Analysis of *Campylobacter jejuni, Campylobacter coli, Salmonella, Klebsiella pneumoniae,* and *Escherichia coli* 0157:H7 in Fresh Hand Picked Blue Crab (*Callinectes sapidus*) Meat

(Paper for publication in the Journal of Food Protection)

ABSTRACT

This study was conducted to determine the incidence and occurrence levels of *Campylobacter jejuni, Campylobacter coli, Salmonella,* fecal positive *Klebsiella pneumoniae,* and *Escherichia coli* 0157:H7 in fresh hand picked blue crab (*Callinectes sapidus*) meat. An attempt was made to correlate the presence of these selected pathogens and general microbial quality (aerobic plate counts and presence of coliforms) to processors' sanitation practices and facility size. Finally the general microbial data of the fresh picked blue crab meat was compared to the International Commission on Microbiological Specification for Foods Quality Criteria.

Campylobacter jejuni was isolated from 36 (15%) of the 240 samples and *Campylobacter coli* was isolated from 14 (5.8%). Counts in all case were below limits of detection for quantitative analysis (<0.3 MPN/g). Fecal positive *Klebsiella pneumoniae* was isolated from a total of 51 (21%) of crabmeat samples. Counts ranged from less than 0.30 to

4.3 MPN/g. Aerobic plate counts ranged from $7.4x10^3$ to $4.6x10^8$ CFU/g (geometric) with coliform counts ranging from <0.3 to 32.8 MPN/g. Fecal coliform levels were <0.3 to 2.26 MPN/g and *Escherichia coli* quantitative analysis ranged from <0.3 to 0.77 MPN/g. *Salmonella* and *Escherichia coli* 0157:H7 were not detected in any of the 240 samples analyzed.

Crabmeat produced in plants with high inspection scores were found to have 29% and 22% of the samples positive for fecal positve *Klebsiella pneumoniae* and *Campylobacter* species, respectively. Plants with low inspection scores were found to have 13% of crabmeat contaminated with fecal positive *Klebsiella pneumoniae* and 20% with *Campylobacter* species. Fecal positive *Klebsiella pneumoniae* was found in crabmeat from small (16%), medium (23%), and large (25%) facilities. *Campylobacter jejuni* and *Campylobacter coli* were isolated respectively from 23% and 8% of samples from small facilities, 6% and 3% of samples from medium facilities, and 16% and 8% of samples from large facilities. No significant differences ($p<0.05$) between size and inspection scores were observed for general microbial quality, fecal positve *Klebsiella pneumoniae*, or *Campylobacter* species. A correlation model of plant size and inspection scores based on general microbial and pathogenic organism level was also insignificant ($p<0.05$).

General microbial data for the crabmeat showed 65% of

the hand picked meat from small facilities, 80% of the meat from medium size facilities, and 85% of the crabmeat from large facilities were of good quality. When the plants were broken down by sanitation practices, 80% of the meat from those facilities with excellent practices and 73.4% of the meat from those facilities with acceptable sanitation practices were of good quality.

INTRODUCTION

In September, 1926 the Food and Drug Administration investigated the crabmeat industry because of a food poisoning outbreak in Chicago linked to crabmeat contaminated with *Salmonella suipestifer* (26). Since that time, the FDA has continued to initiate regulatory actions against firms producing crabmeat under insanitary conditions. During the last decade the emergence of "new" pathogens including *Listeria monocytogenes, Escherichia coli* O157:H7, *Campylobacter jejuni* and *Campylobacter coli* has led to increased consumer and governmental interest on the safety and wholesomeness of all food products. Ready-to-eat fresh seafood, because it is highly perishable under the best storage condition, is continually evaluated for the presence of pathogenic organisms causing seafood related foodborne illness (7, 22, 25, 31).

Campylobacter jejuni is the leading cause of gastroenteritis in the world (9). In studies where both *Salmonella* and *Campylobacter* species were cultured, *Campylobacter* out numbered *Salmonella* isolates 10 to 1 in college students and 2 to 1 in a multicenter study (29). The infectious dose for *C. jejuni* has been estimated at less than 200 organisms (6) and this could then be one of the reasons for its occurrence as the leading cause of gastroenteritis. Studies showing the presence of

thermophilic *Campylobacter* species in seawater indicate the need to evaluate the seafood industry for possible microbiological contamination (2).

Salmonella is also a leading cause of human gastroenteritis. Reported cases of *Salmonella* infections have increased 100 fold from 1942 to 1988 (2). Despite improvements in diagnosis which can account for a definite increase in cases, there is no evidence of a decline in *Salmonella* infections in recent years (13). Instead there may be the overall appearance of an increase in the incidence of *Salmonella* as a result of extremely virulent and infective strains and immunosuppression caused by age, preexisting conditions, and autoimmune disease.

Escherichia coli O157:H7, is the cause of hemorrhagic colitis, which is characterized by large amounts of blood in the stool and can lead to hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (23). The organism is mainly associated with dairy cattle, but has been the cause of foodborne infection in other food products. *E. coli* O157:H7 has been of great interest in the media in recent years possibly because the highest incident rate of *E. coli* O157:H7 is amoung children and because death is associated with the disease. It has been estimated to effect 6.1 children per 100,000 population (18). Most outbreaks of *E. coli* O157:H7 are related to food consumption, however day

care centers have also been implemented in the transfer of the organism to uninfected children.

Klebsiella pneumoniae is an opportunistic human pathogen leading to bacterial infections including pneumonia and urinary tract infections. In recent years, infections caused by *Klebsiella* have increased due to strains with multiple antibiotic resistance. The human gastrointestinal tract is the main reservoir and the hands are the main mode of transmission. The estimated incidence of bacteremia is between 71,000 and 330,000 cases per year in the United States (15, 32) and the mortality rate varies between 20 and 50% (30). *Klebsiella* species in relation to *Escherichia coli*, are the second leading cause of bacteremia. Extensive studies on the incidence of *Klebsiella* species in fresh food and their impact on foodborne disease have not been studied. However, like *Escherichia coli*, the species is known to be present in numerous food products (24) and can be used to identify spoilage.

The objective of this research project was to determine the presence of *Campylobacter jejuni*, *Campylobacter coli*, *Salmonella species*, *Klebsiella pneumoniae*, and *Escherichia coli* O157:H7 in fresh blue crab (*Callinectes sapidus*) meat and to compare quantitative and qualitative levels of these pathogens to processing facility size and general sanitation practices.

MATERIAL AND METHODS

Selection of Firms

The firms chosen for participation in this study were selected randomly from all (57) blue crab processing facilities inspected by the Virginia Department of Health. Inspection scores were determined by Virginia Department of Health Inspectors to indicate sanitation practices (excellent $\geq$ 94.5, acceptable < 94.5). Scores were averaged over five inspections to normalize sanitation practices and minimize seasonal variations (Table 4). Facility size was determined by the number of pickers because production quantities are not available from all private firms (Table 5).

Sampling

From July through August, 1994, hand picked crabmeat samples from 12 blue crab (*Callinectes sapidus*) processing facilities in Virginia were collected and analyzed for their selected microbial populations. Twenty samples from each of the different facilities were collected on different processing days and analyzed. Meat samples were collected from the picking tables, packed in an insulated cooler with frozen gel-packs, and shipped overnight to Virginia Tech in Blacksburg, Virginia. All samples arrived at the laboratory between 0 and 4°C and were analyzed between 16 h and 36 h of collection.

Microbiological Analysis

An initial dilution of 1:1 for each sample was prepared by stomaching 50 g of crabmeat with 50 mL of Butterfield's phosphate buffer (BFD) for 30 seconds. Subsequent serial dilutions were prepared in Butterfield's phosphate buffer.

Microbiological analysis

Crab samples were prepared and analyzed according to United States Food and Drug Administration (FDA) procedures (3, 10, 11, 20, 21) with the exception of *E. coli* O157:H7. *E. coli* O157:H7 was analyzed using the 3M Test kit-HEC for Hemorrhagic *E. coli* O157:H7 which is accepted by the United States Department of Agriculture (USDA) (16). Fecal coliform positive *Klebsiella pneumoniae* (17) was analyzed following FDA fecal coliform test procedures (10) and was then selectively streaked to EMB agar with adonitol and esculin sugars. Five randomly selected samples from each plant were tested for aerobic plate count, coliforms, and *Escherichia coli*, while twenty samples were tested for the presence of pathogens. All serial dilutions were prepared in Butterfield's Phosphate-Buffered distilled water (27).

Statistical Analysis

Data generated was subjected to analysis of variance and multiple analysis of variance to determine effect of APC, coliforms, *E. coli*, plant size, and sanitation on isolation rates of pathogenic organisms (33).

RESULTS AND DISCUSSION

Standard Plate Count

Microbiological data including arithmetic and logarithmic means for APC, and arithmetic mean for coliform fecal coliform and *E. coli* levels are presented in Table 6.

The standard aerobic plate count determinations for fresh handpicked blue crab meat ranged from $7.4x10^3$ to $4.6x10^8$ (geometric mean) and $6.7x10^3$ to $3.5x10^7$ (log transformation) CFU/g (Table 7). The ICMSF recommends that for fresh crabmeat, standard plate counts having a plate count of less than $1x10^6$ CFU/g are of good quality, products with between $1x10^6$ to $1x10^7$ CFU/g are of acceptable quality and products with standard plate counts in excess of $1x10^7$ CFU/g are of unacceptable quality (12). Small facilities were found to have products with 65% good quality, 20% acceptable quality and 15% unacceptable quality. Medium and large facilities had products of 80% good quality, 10% acceptable quality, and 10% unacceptable quality and 85% good quality, 0% acceptable quality, and 15% unacceptable quality, respectively (Table 8). About 10% unacceptable quality results are consistent with a previous quality study on westcoast market crabmeat (1).

Coliforms

The number of coliforms in fresh hand picked blue crab meat ranged from <0.3 to 32.8 MPN/g (geometric) while fecal

coliforms counts ranged from <0.3 to 2.26 MPN/g (geometric) and *E. coli* from <0.3 to 0.77 MPN/g.

Pathogens

Fecal positve *Klebsiella pneumoniae* was found in 51 (21.3%) of the 240 samples of freshly cooked, hand picked blue crab meat analyzed (Table 9). Levels of fecal positive *K. pneumoniae* in the 51 positive samples ranged from less than 0.3 to 4.2 MPN/g (Table 10). This level of the oportunistic pathogen is low when compared with other food products which have been tested for the presence of *K. pneumoniae* (5, 8, 14, 19, 28). However, in our study only fecal positive *K. pneumoniae* were tested becasue of their ability to colonize the intestinal tract of immunocompromised individuals. These quantitative levels probably do not represent a health hazard, however, because crabmeat is a ready-to-eat product and *K. pneumoniae* is a spoilage organism, industry should continue to make efforts to decrease or eliminate it from their products.

Campylobacter species were isolated from a total of 50 (20.8%) of the 240 samples of crabmeat (Table 9). *Campylobacter jejuni* was isolated from 36 (15.0%) samples and *Campylobacter coli* from 14 (5.8%) samples (Table 9). Quantitative levels of *Campylobacter* species were all below limits of detection (<0.3 MPN/g). Species determinations were made on the ability of *C. jejuni* to hydrolyze hippurate

and a failure to produce H_2S when grown on TSI agar since *C. coli* does not hydrolyze hippurate and produce H_2S when grown on TSI agar.

C. jejuni was isolated from 20 samples from plants with excellent sanitation practices and 16 samples from facilities with acceptable sanitation. *C. coli* was found in 6 and 8 samples of fresh blue crab meat from facilities with excellent and acceptable sanitation practices, respectively (Table 11). While *C. jejuni* was isolated from 18 samples from large facilities, 5 samples from medium facilities and 13 samples from sample processing facilities, and *C. coli* was isolated from 6 samples from large facilities, 2 samples from medium facilities and 6 samples from small processing facilities (Table 11).

Escherichia coli O157:H7 and *Salmonella* species were not isolated from any of the 240 crabmeat samples.

Facility size had no significant effect ($p<0.05$) on the presence of pathogens in fresh hand picked blue crab meat (Table 11) or on overall general microbial quality. Sanitation practices also had no significant effect ($p<0.05$) on the isolation of pathogens or overall general microbial quality.

Table 4: Five Inspection Scores and Blue Crab Processing Facility Overall Sanitation Rating

FACILITY	INSPECTION SCORES						SANITATION SCORES
	1	2	3	4	5	MEAN	
1	95.5	96.0	98.0	97.5	97.5	96.9	E
2	86.5	96.0	98.0	95.5	96.5	94.5	E
3	89.5	91.0	89.0	95.0	92.0	91.3	A
4	99.0	94.0	92.0	88.0	91.0	92.8	A
5	97.0	98.0	97.0	98.0	98.0	97.6	E
6	93.0	95.0	97.0	98.0	96.0	95.8	E
7	96.5	93.5	94.0	97.0	89.0	94.0	A
8	90.0	92.0	98.0	96.5	93.0	93.9	A
9	95.5	91.0	99	100	100	97.1	E
10	100	100	97.5	98.0	98.5	98.8	E
11	96.0	93.0	90.0	95.0	90.0	92.8	A
12	91.5	95.0	96.0	93.0	96.0	94.3	A

Table 5: Number of Pickers at Each Blue Crab Processing Facility and Facility Size Designation

FACILITY	TOTAL NUMBER OF PICKERS	FACILITY SIZE
1	80	L
2	75	L
3	42	L
4	80	L
5	24	M
6	26	M
7	40	M
8	30	M
9	3	S
10	1	S
11	3	S
12	9	S

Table 6: Bacterial counts for general Microbial Quality of fresh handpicked crab samples from 12 processing Facilities.

Plant	Size	Inspection score (sanitation)	Mean APC (CFU/g)	Log Transformation means (CFU/g)	Mean Coliform (MPN/g)	Mean Fecal Coliform (MPN/g)	Mean *E. coli* (MPN/g)
1	L	E	$9.5x10^7$	$5.7x10^6$	13.78	<0.30	<0.30
2	L	E	$2.5x10^4$	$2.1x10^4$	1.44	1.44	0.28
3	L	A	$1.9x10^4$	$2.1x10^4$	0.85	0.85	<0.3
4	L	A	$1.5x10^4$	$1.1x10^4$	2.23	2.08	0.77
5	M	E	$7.8x10^4$	$4.8x10^4$	16.72	<0.3	<0.3
6	M	E	$7.4x10^3$	$6.7x10^3$	2.23	2.08	0.34
7	M	A	$4.6x10^8$	$6.0x10^5$	11.28	2.26	0.66
8	M	A	$2.0x10^7$	$3.3x10^6$	2.76	0.93	<0.3
9	S	E	$1.8x10^6$	$2.6x10^5$	20.52	0.39	<0.3
10	S	E	$1.0x10^4$	$9.8x10^3$	<0.3	<0.3	<0.3
11	S	A	$3.8x10^8$	$3.5x10^7$	32.8	0.66	<0.3
12	S	A	$1.5x10^5$	$3.4x10^4$	2.02	0.5	<0.3

Symbols: (L) large; (M) medium; (S) small; (E) excellent >94.5; (A) acceptable <94.5

Table 7: Summary General Microbial Data of Blue Crab Meat from Twelve Processing Facilities

Number Samples	Mean APC (CFU/g)	Log Transformation APC (CFU/g)	Coliform (MPN/g)	Range Fecal Coliform (MPN/g)	*E. coli* (MPN/g)
60	$6.7x10^3$ - $3.5x10^7$	$7.4x10^3$ - $3.8x10^8$	<0.3 - 32.8	<0.3 - 2.26	<0.3 - 0.77

Table 8: A Comparision of Facility Size and Santitation and Standard Aerobic Plate Counts of Fresh Hand Picked Blue Crab Meat with ICMSF Suggested Criteria.

Facility Size/ Sanitation	Standard Aerobic Plate Counts										I C M F F SUggested Criteria		
	10^3 to 10^4 PWR		10^4 to 10^5 PWR		10^5 to 10^6 PWR		10^6 to 10^7 PWR		> 10^7 PWR		Good Quality	Acceptable Quality	Unaccept. Quality
Small	6	30.0	4	20	3	15.0	4	20.0	3	15.0	65.0 %	20.0 %	15.0 %
Medium	6	30.0	8	40.0	2	10.0	2	10.0	2	10.0	80.0 %	10.0 %	10.0 %
Large	1	10.0	15	75.0	1	10.0	0	0.0	3	15.0	85.0 %	0.0%	15.0 %
Excellent	8	26.6	14	46.7	3	10.0	2	6.7	4	13.3	80.0 %	6.7 %	13.3 %
Acceptable	5	16.7	13	43.3	3	10.0	4	13.3	4	13.3	73.4 %	13.3 %	13.3 %

ICMSF, International Commission on Microbiological Specification for Foods (Kaneko and Colwell, 1978).
Suggested Values: < 1 x 10^6CFU/g=good quality; 1 x 10^6 to < 1 x 10^7CFU/g=acceptable quality; > 1x 10^7CFU/g=Unacceptable quality
PWR = Percentage within count range

Table 9: Pathogenic Bacteria in Fresh Blue Crab Meat from Tweleve Processing Facilities

Plant	*C. jejuni** (# pos/20)	*C. coli** (# pos/20)	*E. coli* 0157:H7	*Klebsiella pneumoniae* (# pos/20)	*K. pneumoniae* (mean MPN/g)	*Salmonella*
1	1	ND	ND	4	<0.30	ND
2	9	4	ND	5	0.36	ND
3	ND	ND	ND	8	<0.30	ND
4	3	2	ND	3	<0.30	ND
5	3	1	ND	3	<0.30	ND
6	2	ND	ND	12	4.2	ND
7	ND	ND	ND	ND	ND	ND
8	ND	1	ND	3	<0.30	ND
9	5	1	ND	5	0.91	ND
10	ND	ND	ND	6	0.91	ND
11	13	5	ND	ND	ND	ND
12	ND	ND	ND	2	0.36	ND

*All Campylobacter counts were below limits of detection (<0.3 MPN/g)
ND: Not Detected

Table 10: Summary of Pathogenic Bacteria in Blue Crab Meat from Tweleve Processing Facilities

Number Samples	*E. coli* 0157:H7 (# pos.)	*Salmanella* (# pos.)	*Klebsiella pneumoniae* (# pos.)	Range counts *K. pneumoniae* (MPN/g)	*C. jejuni* (# pos.)	*C. coli* (# pos.)
240	ND	ND	51	<0.3 - 4.2	36	14

Table 11: Number and Species of *Campylobacter* Isolates broken down by facility size and sanitation practices.

	Facility Sanitation Practices (# pos. samples)		Facility Size (# pos. samples)		
Campylobacter spp.	Excellent	Acceptable	Large	Medium	Small
C. jejuni	20	16	18	5	13
C. coli	6	8	6	2	6

REFERENCES

1. Abeyta, C. 1983. Bacteriological quality of fresh seafood products from Seattle retail markets. J. Food Protect. 46(10):901-909.

2. Alonso, J.L. and M.A. Alonso. 1993. Presence of *Campylobacter* in marine waters of Valencia, Spain. Wat. Res. 27:1559-1562.

3. Andrews, W.H., V.R. Bruce, G. June, F. Satchell, P. Sherrod. 1992. *Salmonella*. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

4. Chalker, R.B. and M.J. Blaser. 1988. A review of human salmonellosis:III. Magnitude of *Salmonella* infections in the United States. Rev. Infect. Dis. 10:111-124.

5. Cousins, D.L. and F. Marlatt. 1990. An evaluation of conductance method for the enumeration of *Enterobacteriaceae* in milk. J. Food Prot. 53(7):568-570.

6. Doyle, M.P. 1985. Foodborne pathogens of recent concern. Ann. Rev. Nutr. 5:25-41.

7. Foster, J.F, J.L. Fowler, and J. Dacey. 1977. A microbial survey of various fresh and frozen seafood products. J. Food Prot. 40:300-303.

8. Fuentes, F.A., T.C. Hazen, A.J. Lopez-Torres, and P. Rechani. *Klebsiella pneumoniae* in orange juice concentrate. Appli. Environ. Microbiol. 49(6):1527-1529.

9. Gilbert, R.J. 1988. Food-borne Infections and Intoxications: Recent problems and new organisms. British Food J. 90(2):71-73.

10. Hitchins, A.D., P. Feng, W.D. Watkins, S.R. Rippey, and L.A. Chandler. 1992. *Escherichia coli* and the coliform bacteria. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

11. Hunt, J.M. 1992. *Campylobacter*. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

12. International Commission on Microbiological Specifications for Foods. 1974. Sampling plans for fish and fishery products, vol. 2, University of Toronto Press, Toronto, Ontario, Canada. pp. 92-104.

13. Madden, J.M. 1994. Concerns regarding the occurrence of *Listeria monocytogenes*, *Campylobacter jejuni*, and *Escherichia coli* O157:H7 in foods regulated by the U.S. Food and Drug Administration. Dairy, Food and Environ. Sanitat. 53:941-943.

14. Massa, S., F. Gardini, M. Sinigaglia, and E. Guerzoni. 1992. *Klebsiella pneumoniae* as a spoilage organism in mozzarella cheese. J. Dairy Sci. 75:1411-1414.

15. McCabe, W.R., B.E. Kreger, and M. Johns. 1972. Type-specific and reactive antibodies in gram negative bacteremia. N. Engl. J. Med. 287:261-261.

16. Okrend, A.J.G., B.E. Rose, and B. Bennet. 1990. A screening method for the isolation of *Escherichia coli* O157:H7 from ground beef. J. Food Prot. 53:249-252.

17. Orskov, I. 1984. *Enterobacteriaceae*: Genus V. *Klebsiellla*. In N. Kreig (Ed.): Bergey's Manual of Determinative Bacteriology. 9th ed. Williams and Wilkins Co., Baltimore, Maryland. pp. 461-465.

18. Ostroff, S.M., J.M. Kobayashi, and J.H. Lewis. 1989. Infections with *Eschrichia coli* O157:H7 in Washington State. The first year of statewide disease survalence. J. Am. Med. Assoc. 262:355-359.

19. Park, C.E. and G.W. Sanders. 1990. Source of *Klebsiella pneumoniae* in alfalfa and mung bean sprouts and attempts to reduce its occurence. Can. Inst. Food Sci. Technol. J. 23:189-192.

20. Peeler, J.T. and F.D. McClure. 1992. Most probable number determination. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

21. Peeler, J.T. and L.J. Maturin. 1992. Aerobic plate count. In L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

22. Philips, F.A. and J.T. Peeler. 1972. Bacteriological survey of the Blue Crab industry. Appl. Mcrobiol. 24(6):958-966.

23. Riley, L.W., R.S. Remis, S.D. Helgerson, H.B. McGee, J.G. Wells, B.R. Davis, R.J. Herbert, E.S. Olcott, L.M. Johnson, N.T. Hargrett, P.A. Blake, and M.L. Cohen. 1983. Hemorrhagic colitis associated with a rare Escherichia coli serotype. N. Eng. J. Med. 308:681-685.

24. Roa, D.V. and K.R.G. Roa. 1983. Incidence of *Klebsiella* in foods and water. J. Food Sci. Tech. 20:265-268.

25. Slabyj, S.O., S.A. Ismail, B.M. Slabyj, W.A. Halteman, and R.E. Martin. 1990. A simple proceduce to monitor fecal coliforms in seafood processing plants. J. Food Prot. 54:554-556.

26. Slocum, G.G. 1955. Bacteriology of crabmeat as related to facility sanitation. Ass. Food Drug Officals, U.S. Quart. Bull. 19:43-50.

27. Solomon, H.M. 1992. Media and Reagents. In: L.A. Tomlinson (ed.). Food and Drug Administration Bacteriological Analytical Manual, 7th edition. AOAC International, Arlington, Virginia.

28. Stiles, M.E. and Lai-king Ng. 1981. *Enterobacteriaceae* associated with meats and meat handling. Appl. Eviron. Microbiol. 41:867-872.

29. Tauxe, R.R.V. 1991. *Salmonella*: A postmodern pathogen. J. Food Prot. 54:563-568.

30. Torre, M.G., J. Romero-Vivas, J.M. Beltran, A. Guerrero, M. Meseguer, and E. Bouza. 1985. Klebsiella bacteremia: An analysis of 100 episodes. Rev. Infect. Diseases. 7(2):143-150.

31. Wentz, B.A., A.P. Duran, A. Swartzentruber, A.H. Schwab, F.D. McLure, D. Archer, and R.B. Reead, Jr. 1985. Microbiological quality of crabmeat during processing. J. Food Prot. 48(1):44-49.

32. Wolff, S.M and J.V. Bennet. 1974. Gram-negative-rod bacteriamia. N. Engl. J. Med. 291:733-4.

33. Zar, J.H. 1984. Biostatistical Analysis, 2 edition. Prentice Hall, Englewood Cliffs, New Jersey.

SUMMARY

The incidence of *Campylobacter* species in freshly cooked and hand picked blue crab (*Callinectes sapidus*) meat was determined to be approximately 21%. *C. jejuni* was isolated from 36 (15.0%) of 240 samples while *C. coli* was isolated from 14 (5.8%) samples. The quantitative levels at which *Campylobacter* species were found were below limits of detection for analysis (<0.3 MPN/g).

Fecal positvie *Klebsiella pneumoniae* was also isolated from fresh hand picked blue crabmeat. Of the 240 samples tested, *K. pneumoniae* was isolated a total of 51 times or approximately 21% of the time. Quantitative levels were very low, between less than 0.3 and 4.2 MPN/g. This level represents much lower numbers than those for fecal positive *K. pneumoniae* in other food products (5, 8, 14, 19, 28).

Escherichia coli O157:H7 was not isolated from any of 240 samples tested. *Salmonella* species were also not isolated from any of the fresh crabmeat samples.

An attempt was made to compare pathogenic levels and general microbial quality of fresh crabmeat to processing facility size and sanitation practices, however it was not significant ($p<0.05$) when an analysis of variance and multiple analysis of variance was determined.

The overall general quality of fresh blue crab meat was compared with the International Committee Microbiological

Specifications for Foods Quality Standards. Overall the quality of the meat was high. The standard aerobic plate count determinations for fresh handpicked blue crab meat ranged from $7.4x10^3$ to $3.8x10^8$ (geometric mean) and $6.7x10^3$ to $3.5x10^7$ (log transformation) CFU/g (table 7). The ICMSF recommends that for fresh crabmeat, standard plate counts having a plate count of less than $1x10^6$ CFU/g are of good quality, products with between $1x10^6$ to $1x10^7$ CFU/g are of acceptable quality and products with standard plate counts in excess of $1x10^7$ CFU/g are unacceptable quality (12). Small facilities were found to have products with 65% good quality, 20% acceptable quality and 15% unacceptable. Medium and large facilities had products of 80% good quality, 10% acceptable quality, and 10% unacceptable quality and 85% good quality, 0% acceptable quality, and 15% unacceptable quality, respectively. About 10% unacceptable quality results are consistent with a previous quality study of crabmeat (1).

While Virginia Department of Health Inspection scores did not correlate to the presence or quantities of fecal positive *K. pneumoniae* and *C. jejuni/coli*, the current quality of fresh crabmeat is an indicator of the success the Division of Shellfish Sanitation is having in regulating and requiring the production of a wholesome and safe product. The use of the current inspection sheets (appendix 1) has

prevented foodborne illness and the safety history of fresh crabmeat is an indicator of this fact. The inspection sheets are not able to relate plant sanitation to specific pathogens, but this does not indicate a need for change. The large number of crabmeat samples tested and both the few positive pathogens and the quantities of pathogens support the use of a general inspection score in determining the safety of processed crabmeat.

The value of using Virginia Department of Health inspection scores is also indicated by the inability to isolate *Salmonella* species and *Escherichia coli* O157:H7 from any of the crabmeat samples analyzed. *Salmonella* has been a problem for the seafood industry in the past and has previously been isolated from fresh hand picked blue crabmeat (26). Not detecting *Salmonella* indicates the crabmeat industries accomplishments in improving and implementing effective sanitation programs. *Escherichia coli* O157:H7 was not expected to be isolated from fresh crabmeat. While this study does not eliminate the need for continued sanitation practices in the industry to avoid *E. coli* O157:H7, it does demonstrate that no level of end-product testing could accurately reflect sanitation practices and the possibility of some pathogen contamination.

Finally, the isolation of *Campylobacter* species from

crabmeat is not a reason for great concern in the seafood industry. *Campylobacter* species do not grow at refrigeration temperatures and the levels of the organisms isolated in this study were well below the human infective dose. Since, *C. jejuni* and *C. coli* are destroyed by the thermal process, contamination must have occurred post cooking. It should be a goal for the industry, to identify the sources of *Campylobacter* species contamination and reduce the incidence.

VIRGINIA DEPARTMENT OF HEALTH
DIVISION OF SHELLFISH SANITATION
CRUSTACEA PLANT INSPECTION FORM

Plant Name: Plant Certification Number: Date:

Articles: 1 - Picking and Packing of Crustacea Meat, 2 - Pasteurization of Crabmeat
3 - Mechanically Processed Crustacea Meat 4 - Steamed Crustacea Operations
5 - Custom Crustacea Operations, 6 - Repacking Crustacea Meat

		MANUAL REFERENCE	*
PLANT & GROUNDS	1. Plant not subject to flooding	1,2,3,4,5,6	C
	2. Processing operations separated by partitions, space or time	1,3,6,	K
	3. Storage/lunch facilities for employees, used	1,3,5,6	O
	4. Plant premises constructed, clean, refuse containers, stored equipment, litter, drainage	1,3,5,6	O
PLANT INTERIOR	5. Floors: impervious, adequate/proper drainage, maintained, clean	1,3,5,6	O
	6. Walls, ceilings, attached equip.: constructed, smooth, lt. colored, clean, good repair	1,3,5,6	O
VECTORS	7. Insects, rodents, vermin and other animals excluded, controlled	1,3,5,6	K
UTILITIES	8. Lighting adequate, fixtures shielded	1,3,5,6	O
	9. Heating, cooling and ventilation adequate	1,3,5,6	O
WATER	10. Water supply: safe source, protected from contamination	1,2,3,5,6	C
	11. Adequate quantity, temperature and pressure of water	1,2,3,5,6	O
PLUMBING	12. Plumbing: meets code, adequate, functional, and maintained	1,2,3,5,6	K
	13. Protection against backflow, backsiphonage, cross connections	1,2,3,5,6	C
	14. Toilets: construction, location, repair, clean, adequate number, self-closing doors, paper	1,3,6	K
	15. Handwashing: number, location, repair, clean, soap, sanitizing solution, sanitary towels, waste receptacles, handwashing signs posted	1,3,5,6	K
SEWAGE	16. Sewage disposal system: properly installed, maintained, meets code, adequate	1,2,5,6	C
CHEMICALS	17. Poisonous/toxic materials: properly used, stored, separated, labeled	1,3,5,6	K
EQUIPMENT & UTENSILS	18. Food contact surfaces: properly constructed and located, identified, clean, maintained, protected from contamination	1,3,4,5,6	K
	19. Non food contact surfaces: properly constructed and located, maintained, clean	1,2,3,4,5,6	O
	20. Pressure cookers properly vented, temperature and pressure gauges provided and calibrated, vent drain or exhaust properly terminated	1,5	K
	21. Refrigeration units adequate, temperature measuring devices	1,2,3,5,6	C
CLEANING & SANITIZING	22. Facilities: properly constructed and used, detergents, brushes, three compartment sinks, test kits, approved sanitizers provided	1,3,5,6	O
	23. Food and non-food contact surfaces cleaned and/or sanitized, within time limits, effective	1,3,4,5,6	K
CRUSTACEA HANDLING & STORAGE	24. Uncooked crustacea: properly stored, refrigerated within time limits	1,5	C
	25. Crustacea properly cooked	1,5	K
	26. Cooked crustacea properly air cooled, refrigerated within time limits	1,2,4,5,6	K
	27. Cooked crustacea properly stored, handled, protected	1,5	K
	28. Processed crustacea delivered to packing room within 3 hours	1,5	K
	29. Cooked crustacea meat protected from contamination	1,2,3,4,5,6	K
	30. Dip cans not used	1,3,5	K
	31. Single service containers: clean, sanitized, stored properly, room and equipment requirements met	1,2,3,5,6	O
	32. Single service containers: approved, properly labeled	1,2,3,5,6	O
	33. Packing into containers with authorized certificate number	1,2,3,5,6	C
	34. Packed fresh product stored, shipped between 32°- 40°F	1,3,5,6	K
	35. Packed frozen product stored, shipped at 0° or less	1,3,5,6	K
	36. Chits or checks not used, overages not returned	1,5	K
	37. Packed product protected from contamination	1,2,3,5,6	K
	38. Ice: adequate, approved source, sanitary, properly protected	1,2,3,5,6	O
PERSONNEL	39. Hands washed/sanitized, good hygienic practices	1,2,3,4,5,6	K
	40. Clean outer garments. Gloves, finger cots, and other coverings impermeable, sanitized as necessary and properly stored, hair restraints	1,2,4,5,6	O
	41. Personnel with infections restricted	1,4,5,6	K
	42. Unauthorized persons prohibited	1,3,5,6	O
WASTE	43. Crab scrap waste: proper disposal, promptly removed, located	1,5	O
	44. Water disposal: meets code, adequate, installed	1,2,3,4,5,6	O
SUPERVISION	45. Supervision: responsible person designated, effective	1,2,3,4,5,6	K
RECORDS	46. Records: complete and maintained	1,2,3,4,5,6	K
MECHANIZED OPERATION	47. Crustacea bodies or claws protected from contamination, proper processing procedure	3	K
	48. Containers for mechanically picked crustacea adequate size, number, identification of containers	3	K
	49. Crustacea claws or bodies refrigerated to 40°F within time limits, processed within 48 hours	3	K
PASTEURIZED CRUSTACEA	50. Crustacea meat pasteurized in accordance with process controls within 48 hours, chilled to 36°F or less within 2 hours, maintained at 36°F or below, construction and operation of pasteurization equipment	2	C
REPACKING	51. Single, approved source	2	C
	52. Single repacking operation: duration of less than 30 min., product temperature not to exceed 50°F	2	K
	53. Emptied containers not reused	2	K

CODES: (C) Critical Item = 4 pts (K) Key Item = 2 pts (O) Other = 0.5 pt
Any critical item violation results in automatic failure

SCORE

VIRGINIA DEPARTMENT OF HEALTH
DIVISION OF SHELLFISH SANITATION

INSPECTION REPORT - CRUSTACEA PROCESSING PLANT

Pre-certification [ ] Certification [ ] Routine [ ]

Follow-up [ ] FDA-DSS Co-inspection [ ] Check Rating [ ]

Date: _____ Time Begin: _____ Time End: _____

Plant Name: _____ Certification Number: _____

Plant Location: _____

Plant Rep. Signature: _____ Title: _____

Inspector Signature: _____

ITEM NO.	REMARKS	DEALER AGREES TO CORRECT BY	CORRECTION DATE

HPD-2B

VITAE

PERSONAL:

Born: July 5, 1971 in Dalton, Georgia
Father: William Gordon Reinhard Jr.
Mother: Patricia Bragg Reinhard
Sister: Ann Prescot Reinhard

EDUCATION:

Master of Science in Food Science and Technology. August 1995. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Bachelor of Science in Food Science and Technology. May 1994. Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

High School Diploma. June 1990. Findlay High Scool. Findlay, Ohio.

PUBLICATIONS

Reinhard, R.G., T.J. McAdams, G.J. Flick, R.F. Wittman, R.E. Croonenberghs, and A.A. Diallo. 1995. "Qualitative and Quantitative analysis of *Campylobacter jejuni* and *Campylobacter coli* in Fresh Blue Crab (*Callinectes sapidus*) Meat". **J. Aquatic Food Prod. Tech.** In Press

PRESENTATIONS AT PROFESSIONAL MEETINGS

International Association of Milk, Food, and Environmental Sanitarians. IAMFES. Pittsburgh, Pennsylvania. July 1995.
●Presentation of Paper "A Comparison of Plant Size and Sanitation Practices to the Presence of *Escherichia coli* 0157:H7, *Klebsiella pneumonia, Campylobacter,* and *Salmonella* in Fresh Blue Crab (*Callinectes sapidus*) Meat". Developing Scientist Competition.

Institute of Food Technologists, Anaheim, California. June 1995.
●Presentation of Paper "Effect of Plant Size and Santitation on Bacteriological Quality of Fresh Blue Crab (*Callinectes sapidus*). Graduate paper competition.

Lipid Symposium. Virginia Polytechnic Institute and State University. Department of Human Nutrition and Foods. Blacksburg, Virginia. April 1995.
●Presentation of Paper "The Effects of Genetic Engineering of Plant Seed and the Effect on the Rate of Seed Oil Autoxidation".

Carolinia-Virginia Institute of Food Technologists. Blacksburg, Virginia. April 1995.
●Presentation of Paper "Qualitaive Analysis of Select Pathogens in Hand Picked Fresh Blue Crab (*Callinectes sapidus*) Meat.

PROFESSIONAL AFFILIATION:

Member of the **Institute of Food Technologists.**

Member of the **International Association of Milk, Food, and Environmental Sanitarians. IAMFES.**