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The objective of this project was to evaluate various culturing and isolation techniques of Campylobacter jejuni and to develop methods to detect the organism in foods. The morphological, cultural and biochemical characteristics of C. jejuni were studied using developed microbiological methods. A variety of media, broths, microaerophilic atmospheres and diluents, now available, were tested for their applicability to detect low numbers of the organism in food samples.

Direct plating, filtration, double incubation enrichment, milk separation enrichment and swabbing methods were used to recover C. jejuni from seeded milk and fowl samples. As few as 16 organisms per ml of milk could be recovered using the double incubation enrichment. Raw milk samples from retail supermarkets and the Oregon State University Dairy Herd were tested for the presence of C. jejuni with the double incubation enrichment. No positive confirmation of the organism was made, although suspect microorganisms were observed microscopically.

The survival of C. jejuni in foods and effect of sanitizers was studied. Raw and underprocessed foods pose the greatest risks as vehicles of Campylobacter infections. If contaminated foods are held at refrigeration temperatures C. jejuni could survive. Properly sanitized dairy equipment poses no apparent health problem and water should have a residual chlorine level of greater than 5 ppm to be safe.

Isolation and Survival of *Campylobacter jejuni* in Foods

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ISOLATION AND SURVIVAL OF CAMPYLOBACTER JEJUNI IN FOODS

INTRODUCTION

Campylobacter jejuni has recently been implicated as one of the leading causes of gastrointestinal illness. As early as 1946, this organism was suspected to be a foodborne pathogen, but it was not until 1973 that C. jejuni was shown to be commonly associated with gastroenteritis (Butzler et al., 1973).

Earlier studies failed to show the organism as an important enteropathogen because C. jejuni is difficult to isolate in the laboratory as a result of its fastidious requirements. It is a thermophile, and a microphile with a long generation time. The development of highly selective culture media for the isolation of C. jejuni has greatly simplified the study of the organism. The identification of Campylobacter when present in large numbers is now a relatively simple procedure. The true incidence of foodborne infections caused by C. jejuni however, remains undetermined because of incomplete reporting of outbreaks, difficulty in culturing the organism and lack of information on susceptible food and control measures (Taylor et al., 1980). The epidemiology of human enteric infection due to C. jejuni is not well understood. The studies to date have shown certain warm-blooded animals, fowl, cattle, swine and sheep, to be a reservior of the organism. Meat, eggs, raw milk and water have been suspected as sources of the organism in some of the outbreaks. It appears, but has not been proven because of the difficulty in isolating the organism, that C. jejuni is present in foods in very low numbers.

The purpose of this project was to evaluate various techniques for the isolation and culturing of C. jejuni and to develop methods to detect the organism in foods. Also of importance to this project was the effect of processing and food handling procedures on growth of the organism.

REVIEW OF LITERATURE

Several hundred foodborne disease outbreaks are investigated each year by the Centers for Disease Control (CDC). In 1978, 481 outbreaks of foodborne diseases involving 10,639 cases were reported to the CDC. The causative agent was not identified in 30-60% of the outbreaks reported in each of the previous five years because of late or incomplete laboratory investigations or because the pathogen could not be identified by available laboratory techniques (CDC, 1981). Campylobacter jejuni is one of the pathogens that cannot readily be detected by current techniques. The extent to which C. jejuni is a foodborne pathogen has not been adequately assessed (CDC, 1981; Doyle, 1980).

C. jejuni rose from obscurity to notoriety during the 1970's. Workers in Australia, Canada, the Netherlands, Sweden, the United Kingdom and the United States have reported the isolation of C. jejuni from 5-14% of the diarrhea cases (WHO, 1980). These isolation rates compare with an isolation rate of 9.3% for the combined enteric pathogens of Salmonella, Shigella, and Yersinia enterocolitica (Pai et al., 1979). Present reports from around the world indicate that C. jejuni may be the single most common bacterial cause of acute gastrointestinal illness in adults and children (Doyle, 1980).

Classification and Nomenclature

The classification of Campylobacter has caused confusion since its recognition as a vibrio-like organism (MacFadyean and Stockman, 1913). Smith and Taylor (1919) classified the microaerophilic, oxidase-positive,

curved, motile, gram-negative organism in the genus Vibrio. The type species was named Vibrio fetus because it was associated with bovine and ovine abortions

Vinzent et al. (1947) recognized the first human infection with the vibrio-like organism and in the following 20 years about 100 cases were reported. King (1962) reported that certain isolates of V. fetus had different biochemical and seriological characteristics and called them "related vibrios". The "related vibrios" had a higher optimum temperature requirement and all were isolated from blood cultures of patients with gastroenteritis. King speculated that the "related vibrios" were a more common cause of gastroenteritis than was recognized at that time.

Sebald and Veron (1963) showed that there were fundamental differences between the microaerophilic vibrio reported by Vinzent, King and others and the true Vibrio. They therefore proposed the formation of a new genus, Campylobacter, for the microaerophilic vibrios.

Differentiation between Campylobacter species was still not possible until techniques for isolating it from fecal samples were developed. Dekeyser et al. (1972) filtered stool specimens through a 0.65 μm millipore filter and cultured the specimen in a 5% oxygen atmosphere on thioglycollate agar with antibiotics. Microaerophilic vibrios were selectively isolated from stools. Skirrow (1977) cultured unfiltered stool specimens on blood agar with antibiotics and cooraborated Dekeyser's findings that certain Campylobacter species were a common cause of diarrhea.

The nomenclature continued to change as more was learned about the microaerophilic, thermophilic, comma-shaped organism associated with

diarrhea. Veron and Chatalain in 1973 identified two separate species, C. jejuni and C. coli. Smibert listed the organism in Bergey's Manual as C. fetus ssp. jejuni (Smibert, 1978). According to Mehlman (1981), Blaser stated the proper name is C. jejuni.

Occurrence

In Animals

C. jejuni is an agent of infectious abortion, enteritis and hepatitis in cattle, sheep, other ungulates and fowl. It is also part of the normal gastrointestinal flora in cattle, sheep, swine, fowl, dogs, cats, rodents and migratory birds (Blaser et al., 1979b).

In Man

C. jejuni is not normally present in the fecal flora of healthy adults; chronic carriers are rare (Blaser et al., 1980b). Possible means of transmission of the organisms to humans are: direct contact with infected animals, ingestion of contaminated food, ingestion of contaminated water, veneral transmission, placental transfer or exposure at delivery by an infected mother, or by person to person transfer via the fecal to oral contacts (Taylor et al., 1980).

Campylobacter enteritis

Symptoms

In humans, C. jejuni causes acute gastroenteritis. Abdominal pain is severe and has been confused with appendicitis, mesenteric adenitis,

intussusception and visceral perforation (Rettig, 1979). Many people have a prodromal febrile period before the onset of diarrhea, blood may also appear in the stools. The illness is self limiting and usually lasts from several days to one week. An individual may shed organisms for two to five weeks. Relapses can occur, particularly if solid food is eaten too soon after the illness (Butzler and Skirrow, 1979).

Incubation Period

Skirrow (1977) estimated that the incubation period ranges from two to eleven days. Blaser et al. (1978) and Karmali and Fleming (1979b) suggest that the incubation period is two to five days.

Mode of Action

The precise mode of action is unknown. Butzler and Skirrow (1979) speculate that the organism is invasive; perhaps the ingested organism passes through the mucosa and then invades the bloodstream similar to Salmonella and Yersinia. Butzler and Skirrow cited experiments conducted at the University Hospital St. Pierre in Brussels which showed that only a few strains produced enterotoxin.

Infective Dose

The precise number of C. jejuni needed to cause illness has not been determined. A volunteer in Australia in 1978 ingested a dose of 10^6 organisms per gm and experienced illness (Steele and McDermott, 1978). Butzler and Skirrow (1979) reported an experiment where severe enteritis occured in two out of three Rhesus monkeys 13 days after being fed a dose

greater than 10^6 organisms per ml; no monkeys became ill if fed a dose of 10^2 to 10^4 organisms per ml. Doyle in 1980 speculated that as few as 10 organisms per ml would cause illness in humans. The CDC in 1981 speculated 100 organisms per ml is sufficient to cause illness (Steensma, 1981).

Age and Temporal Distribution

Campylobacter enteritis affects both adults and children. In areas of poor sanitation, young children are affected more often than adults (Bokkenheuser et al., 1979). Infections are more prevalent in warmer months in England (Skirrow and Butzler, 1979) and in the United States (Blaser et al., 1979b).

Microbiological Characteristics

Morphology

Campylobacter are thin, spirally curved, gram-negative rods, 0.2-0.6 µm wide and 1.5-3.5 µm long. Short 'S'-shaped or gullwing chains are normally seen but occasionally longer filamentous chains may occur. In older cultures (greater than 72 h) cocci often appear. The organisms are motile with a single flagellum at one or both poles of the bacterium (Rettig, 1979). C. jejuni gram stains with difficulty. Wang et al. (1978) recommended a counterstain of 0.06% carbol fuchsin. Powers (1980) reported a counterstain of safranin was adequate.

Biochemical Characteristics

C. jejuni is rather limited in its metabolic activities. It does not ferment or oxidize carbohydrates, instead the organism obtains its energy from amino acids or intermediates of the tricarboxylic acid cycle. It has no lipase activity and does not hydrolyze gelatin, urea, casein, RNA, DNA or esculin. *C. jejuni* can reduce nitrate to nitrite but no further and grows in 1% bile but not in 3.5% sodium chloride (Doyle, 1980).

C. jejuni is usually confirmed by the following biochemical reactions: strongly catalase-positive; weakly oxidase-positive; growth at 42°C; no growth at 25°C; sensitivity to 30 µg nalidixic acid; production of hydrogen sulfide on lead acetate strips; growth in 1% glycine and deep red growth on media containing 0.04% triphenyltetrazolium chloride (Doyle, 1980; Reller and Wang, 1980).

Culturing Conditions

C. jejuni is microaerophilic with a long generation time and consequently it can be quickly overgrown by other bacteria (Wilson and Wang, 1979). Culturing techniques for isolating *Campylobacter* have been designed to minimize this problem as much as possible. The oxygen tension is decreased in the incubating environment and antibiotics are incorporated into media preparation to inhibit other bacteria.

Selective Agars

Dekeyser *et al*. (1972) used thioglycollate agar with 15% defibrinated sheep blood and the antibiotics bacitracin, polymyxin, novobiocin and

actidion. Skirrow (1977) used a blood agar base with 7% lysed horse blood and the antibiotics vancomycin, polymyxin B and trimethoprim. Blaser et al. (1979a) recommended a brucella agar base with 5-10% lysed sheep blood and the antibiotics Skirrow used with the addition of cephalothin and amphotericin. Mehlman and Romero (1981) recommended a medium containing proteose peptone no. 3, yeast extract, agar, soluble starch, potassium phosphate monobasic, potassium phosphate dibasic, casamino acid powder, ammonium sulfate and sodium sulfate for optimum growth.

Selective Broths

Various broths have been used as transport media for samples or for mass culturing C. jejuni. Skirrow (1977) used fluid thioglycollate broth with 0.15% agar and his antibiotics. Wilson and Wang (1979) recommended fluid thioglycollate broth without an indicator. Blaser et al. (1980a) recommended substituting fluid thioglycollate broth with brucella broth and subsequently in 1981 recommended Cary Blair media for transporting specimens.

Discrepencies appear in the literature as to the recommended transport media and length of time before subculturing. Hartigan simply used buffered glycerol saline with transit times of one to eight days (Karmali and Fleming, 1979b). Other laboratories stressed subculturing within one to four hours (Powers, 1980).

Atmospheres

George et al. (1978) reported increasing the aerotolerance of C. jejuni by addition of 0.02% ferrous sulphate, 0.025% sodium metabisulfite,

and 0.05% sodium pyruvate to the agar medium. Candle-extinction jars (about 20% oxygen and 2.5-3.0% carbon dioxide) can be used, but the incubation period must be extended to 72 h as the organisms do not grow as well in this environment (Blaser et al., 1979a). Some laboratories have used disposable carbon dioxide and hydrogen gas generating envelopes (Gas Pak from Boston Biological Laboratories (BBL)) in Gas Pak jars without the catalyst. According to Simmons (1977) however, this arrangement can be potentially explosive. Other laboratories use a chamber which can be exhausted and then flushed with a gas mixture of 5% oxygen, 10% carbon dioxide and 85% nitrogen (Blaser et al., 1977a; Doyle, 1980; Smith and Muldoon, 1974). BBL in 1981 introduced a safe disposable gas generating envelope designed for Campylobacter (Campy Pak II).

Temperature and pH

Doyle and Roman (1981) studied the effect of temperature and pH on the growth of C. jejuni inoculated into tubes of brucella broth supplemented with 0.1% agar. Aliquots of the broth cultures after treatments were spread on prepoured plates of brucella agar supplemented with 5% defibrinated blood, incubated microaerophilically at 42°C and the colonies enumerated. No growth occurred in broth at temperatures less than 30°C or greater than 47°C, with the optimum temperature range being 42-45°C.

C. jejuni could grow in the pH range of 5.5-8.0, optimum growth occurred at pH 6.5-7.5. The sensitivity of the organism to temperature extremes is pH dependent.

Thermal Inactivation

Doyle and Roman (1981) studied the thermal inactivation of C. jejuni in skim milk. They reported D-values of 7.2-12.8 min at 48°C and 0.74-1.0 min at 55°C. Blankenship and Craven (1981) determined D-values for C. jejuni were higher in chicken meat than in peptone water.

Isolation from Fecal Samples

As the knowledge of isolation techniques improved, the rate of positive stool samples from individuals with campylobacter enteritis has increased. C. jejuni is present in 10^6-10^9 organisms per ml in stool samples of infected patients (Blaser, 1980). Many clinical laboratories now routinely test for C. jejuni from patients with gastroenteritis. The fecal sample is plated onto selective agar, incubated microaerophilically at 42°C and examined after 24-48 h for typical colonies (Sazie, 1980; Youngboy, 1980).

Isolation from Food

The incidence of Campylobacter in food products is not clearly known. This organism is most likely present in small numbers and consequently is difficult to isolate and enumerate. Where C. jejuni has been isolated from food samples, it is felt the organism cannot be accurately quantified because of lack of sensitivity of present methods (Stern, 1981b; Grant et al., 1980). The situation today is similar to the one that prompted Levy in 1946 to write after his investigation of a gastroenteritis outbreak, "Although the facts are at hand, the correlation between a definite source and a causative organism with an epidemic

may not be easily accomplished and reliance must be placed on circumstantial evidence."

Red Meats

Stern (1981a and 1981b) reported the first isolation of C. jejuni from red meats. He reported positive identification of the organism from 2% beef, 24% sheep, 38% swine carcasses and 0% beef, 73% sheep, 87% swine stool samples. Surface swabs of the semimembraneous muscle adjacent to the aitchbone were transferred to a modified Blaser media (VPTK) plate. Stool samples were obtained from the large intestine, transported in brucella broth and swabbed onto VPTK plates. The VPTK plates were incubated microaerophilically at 42°C for 48 h. The brucella broth contained 0.025% (w/v) of ferrous sulfate, sodium thiosulfate and sodium pyruvate to decrease the oxygen tension. Preliminary studies indicated that C. jejuni could be recovered consistently when present at greater than or equal to 32 org/cm^2 meat surface.

Chicken

Smith and Muldoon (1974) found a 2.1% incidence of C. jejuni in 140 samples of chicken. Nutrient broth was used to rinse chicken parts, filtered, spread onto selective media and incubated microaerophilically at 37°C for three to five days.

Simmons and Gibbs (1979) determined the incidence and survival of C. jejuni in chicken processing. The organism was found in 72% of the chickens both at the beginning and end of the processing procedure. The organism survived on 48% of the chicken carcasses after simulated refrig-

erated delivery to the point of sale. A further investigation showed C. jejuni survived storage at -20°C for three weeks in six out of fourteen chicken carcasses. Caecal samples were plated on Skirrow medium and incubated microaerophilically at 43°C for 48 h. Alkaline peptone water enhanced the recovery of the organism in some cases.

The Food and Drug Administration (FDA) and the Canadian Department of Health and Welfare in 1980 conducted a simultaneous study on fresh chickens from retail markets. Fifty-four percent of the chicken carcasses from stores in Southern Ohio and 62% in Ontario, Canada were contaminated. The whole chicken was washed with nutrient broth, the broth centrifuged, and the pellet resuspended in brucella broth. An aliquot of the suspension was streaked onto a modified Skirrow agar plate, incubated microaerohilically at 37°C for three days and examined. The remaining sample was inoculated into brucella broth with antibiotics. The broth was incubated at 37°C for three days and continuously flushed with a microaerophilic atmosphere. After the enrichment period, an aliquot of the culture was streaked onto a selective agar plate, incubated microaerophilically at 37°C for three days and examined (Lovett et al., 1981).

Grant et al. (1980) examined fecal samples from broiler chickens at a live poultry market. Eighty-three percent harbored C. jejuni in their intestinal tract with a mean number of 4.4×10^6 organisms per gm of feces. The sample was mixed with brain heart infusion broth, centrifuged, passed through 8.0 and .65 μm filters, seeded on chocolate agar plates and incubated microaerophilically at 37°C for 48-72 h.

Raw Milk

Raw milk has been associated with many campylobacter enteritis outbreaks, however, the majority of the efforts to isolate C. jejuni from the suspect milk, milk socks or cow feces have failed.

Levy (1946) provided the first evidence of a raw milk associated outbreak of campylobacteriosis, when three hundred and six inmates in an Illinois penal institution became ill. Vibrio-like organisms were cultured from patients blood and fecal samples but positive identification was not made because the organism did not survive the routine isolation techniques. Milk was the only common food used daily and it was suspected as a possible vehicle for the bacteria. After the incoming milk was boiled, there were no new cases reported. The milk was supplied by a dairy that sold both pasteurized and raw milk, which stood side by side in a cooler. Investigators speculated a driver accidentally picked up raw milk cans instead of the pasteurized milk cans. All milk samples analyzed were negative for the vibrio organism. Local farmers did not allow their herds to be investigated.

Taylor et al. (1980) investigated the first three cases of campylobacteriosis reported in Los Angeles county caused by C. jejuni. The three patients involved consumed between 20-42 ounces of raw milk daily from a local dairy. Case control analysis indicated the unpasteurized milk as the most significant risk factor. Limited attempts to culture C. jejuni from the implicated dairy's milk were unsuccessful.

Blaser et al. (1979c) reported an outbreak where three out of five family members were ill with campylobacteriosis. Four members of the

family had drunk raw milk from the family cow; the three patients had consumed one pint a day whereas the fourth and asymptomatic individual had consumed only one glass a day. A sample of milk was negative for C. jejuni but repeated fecal samples from the cow were positive for the following two months. The fecal swabs were inoculated immediately onto Blaser media plates and also into thioglycollate broth with antibiotics. The plates were incubated microaerophilically at 42°C for 48 h. The inoculated broth was held at refrigerator temperatures for eight hours. After refrigeration, the broth samples were also swabbed onto selective agar plates and incubated microaerophilically at 42°C for 48 h.

Robinson et al. (1979) reported two outbreaks of campylobacter enteritis in Northern England. In Cumbria 63 people were ill and all had consumed raw milk from a local farmer. The quantity of milk consumed varied from a few ounces to several pints. There was no correlation between the amount of milk drunk and the severity of the enteritis. Seventy percent of the patients' fecal samples were positive for C. jejuni. Milk and milk socks were also examined for three days following the occurrence of the last case of gastroenteritis. No pathogens were isolated. Rectal swabs were positive for C. jejuni in nine cows from the eighty-five cow herd.

In the second outbreak, C. jejuni was found in the feces from 12 of the 14 patients as well as 4 out of 26 asymptomatic individuals. All had consumed raw milk from a local farm. No Campylobacter was isolated from the bottled milk. The organism was identified in two milk socks examined during and after the outbreak. A liquid nutrient broth with 12% blood and antibiotics was used.

Porter and Reid (1980) reported an outbreak of campylobacteriosis from the consumption of milk from a dairy that had experienced a power failure during a storm. C. jejuni was isolated from 148 persons experiencing illness as well as from 57 asymptomatic individuals. Bulk milk samples and milk socks were obtained from the dairy for one week after the breakdown. All milk samples were negative. However, one of sixteen milk socks was positive. Rectal swabs from the entire herd (160 cows) were negative for the organism. Swabs from the milk socks were plated onto Skirrow media and then the milk socks were placed in a selective liquid medium for four days at 43°C and subcultured daily. Plates were incubated microaerophilically at 43°C.

The CDC, in 1981, reported a campylobacteriosis outbreak in the Wilamette Valley, Oregon. During a two month period, 91 stool isolates of C. jejuni were reported. Fifty-seven percent of the patients had drunk raw milk from the same dairy; 33% of the other household members had also drunk the raw milk developed diarrhea and all other household members who had not drunk the raw milk did not develop diarrhea. CDC reported "No significant association was found between developing Campylobacter diarrhea and exposure to sick pets, live poultry, livestock, raw eggs, raw meat, untreated surface water, other individuals with diarrheal illness outside the household or history of foreign travel in the past two months." Milk samples cultured were negative. Three out of four rectal swabs from the implicated herd were positive for C. jejuni. The culturing techniques were not stated (Terhune et al., 1981).

The CDC, in March, 1981, reported that 104 families in Kansas became ill after drinking raw milk from a commerical dairy. C. jejuni was

isolated from 52% of the stools. During the investigation C. jejuni was cultured from stools of 17 out of 29 (54%) individuals who became ill and 4 out of 8 (50%) well individuals. All 21 people with positive isolates had drunk raw milk. Bulk milk samples from the implicated dairy cultured after the outbreak were negative for C. jejuni. The standard plate count of the bulk milk from the dairy exceeded the recommended count of $1.0x10^5$ organisms per ml. Rectal swabs taken from both well and mastitic cows from the implicated dairy and two other nearby dairies were positive for C. jejuni. The culturing techniques were not stated (Tosh et.al., 1981).

McNaughton et al. (1981) recounted an outbreak that occurred in July 1980 at a camp. Twenty-seven people became ill after drinking raw milk. All stools submitted were positive for C. jejuni. A sample of milk taken from the camp was also positive for the organism. This is the only known published article which claims a positive C. jejuni isolation from a milk sample. Mueller (1981) used selective agar plates consisting of trypticase blood agar base with yeast extract, 5% laked sheep blood and the antibiotics trimethoprim, vancomycin, and polymyxin B. An enrichment medium of motility test medium, 5% laked sheep blood and the above antibiotics with the addition of cephalothin was also used. Milk samples were shaken, three drops spread onto selective plates and 20 ml inoculated into 100 ml of the enrichment medium. If sufficient sample was available, 50 ml of milk were centrifuged, three drops of the cream spread onto the plates and one ml of the cream inoculated into 10 ml of the enrichment medium. The supernatant was poured off, three drops of the deposit was spread

onto the plates and one ml of the deposit inoculated into 10 ml of the enrichment medium. The inoculated enrichment media were incubated microaerophilically at 42°C and subcultured onto the plates after 48, 72 and 96 h. The plates were incubated microaerophilically at 42°C and examined after 48 h.

Other Foods

Filtration techniques have been used to try to isolate C. jejuni from food samples. The Microbiol Disease Laboratory (MDL) in California, suggested a modification of Dekeyser's method for examining feces to examine centrifuged suspension of inoculated food samples. The relatively large pore size of the filter, 0.65 or 0.80 µm, removed suspended food particles and most bacteria greater than 0.2-0.4 µm in diameter. The filtrate, which is thought to contain the organism if present, was spread onto selective agar plates, incubated microaerophilically at 42°C and examined after 24-48 h. No mention was made of the types of foods used in the study or the percent recovery (Powers, 1979).

Isolation from Water

Waterborne Campylobacter was thought to have caused a large gastroenteritis outbreak in Bennington, Vermont in 1978. Campylobacter was isolated from 15 patients examined. The organism, however, was not found in water samples or from animals in the vicinity. Investigators speculated equipment used to repair both the sewer and water lines might have contaminated the water supply (Tiehan and Vogt, 1978). Knill et al. (1978) confirmed water could be a vehicle for transmitting C. jejuni.

C. jejuni was found in 7 out of 34 seawater samples and 37 out of 50 fresh water samples. Blaser *et al.* (1980a) inoculated 10^7 organisms per ml into water. He found the organism did not survive in water if held at 25°C but survived 1-4.5 weeks at 4°C.

MATERIALS AND METHODS

General

There are a variety of media, broths, atmospheres and diluents for isolating and culturing C. jejuni presently recommended by different laboratories. The plate preparation, incubation, enumeration, and culture preparation techniques outlined below are the techniques designed for use in this project. In the section "Determination of Optimum Growth Conditions" the techniques outlined below were followed with the exception of the variable being tested in the particular study. All other studies in this project followed the procedure outlined below unless otherwise stated.

All media and reagents are from Difco unless otherwise stated.

Plate Preparation

A selective media designed by Skirrow was used. The plate media (campy) consisted of blood agar base no. 2 (Oxoid), 7% laked horse blood (Oxoid), and the antibiotics; vancomycin, 1 mg/ml; trimethoprim lactate, 5 mg/ml; polymyxin B, 250 units/ml (Oxoid). The agar was sterilized, cooled to 50°C, then the blood and antibiotics were added, mixed thoroughly and poured into petri plates. The poured plates were dried for 2 h under filtered flowing air.

Tenfold serial dilutions of food samples, broth culture and sanitizers were made with Butterfield's phosphate buffer (Speck, 1976). One tenth ml of appropriate dilutions were spread onto prepoured campy plates using a sterile bent glass rod. The plates were allowed to dry for 10 min,

inverted and incubated immediately.

Incubation

Inoculated plates to be enumerated were incubated in a chamber prepared by using a standard laboratory vacuum desiccator, 250 mm diameter. Desiccant decreased the swarming which aided in the enumeration of the organism. Excess desiccant however, dehydrated the organism. Inoculated plates were inverted, chamber exhausted twice and flushed with a gas mixture of 10% oxygen, 5% carbon dioxide and 85% nitrogen (Airco Industrial Gases) and plates incubated at 42°C for 24-48 h..

For the detection of C. jejuni only, the inoculated plates were incubated in an anaerobe jar with the atmosphere generated by disposable envelopes. The activation of Gas Pak and Campy Pak II envelopes required the addition of water, consequently the anaerobe jar contained a moist environment. The colonies colesced in a smear, increasing the difficulty of picking individual colony forming units of the organism. The apparatus is inexpensive and the procedure to generate the atmosphere is simple and fast. Disposable Campy Pak II envelopes in anaerobe jars thus are appropriate when a large number of samples are involved.

Enumeration

Most plates were examined after 48 h of incubation as the colonies were then large enough to be easily enumerated and most were still viable. The colonies were counted using a Quebec Colony Counter with the light off to avoid glare. Final calculations were made in accordance with the rules outlined in the Standard Methods for the Examination of Dairy Products

(SMEDP) (Marth, 1978).

Culture Preparation

The organism was cultured in brucella broth with Skirrow's antibiotics (BBA) and 0.16% agar. Approximately 50 ml of the selective broth were placed into a 250 ml Erlenmeyer flask and inoculated with several loopfuls of a young C. jejuni culture (less than 30 h old). The flask was equipped with a two holed stopper with two 2 1/2" glass tubes connected to 2" pieces of rubber tubing. The flask was flushed with the gas mixture for two to three minutes, the tubing clamped shut and the flask incubated at 42°C until abundant filamentous growth appeared in the broth, normally 24-30 h. The broth culture was then centrifuged 10 min at 2300 rpm. The supernatant was discarded, cells resuspended in sterile phosphate buffer and thoroughly mixed. The cells were centrifuged and washed three more times to be certain any adhering medium was removed. The resulting suspension was adjusted to the appropriate optical density using a Bausch and Lomb Spectronic 20 set at 460 nm. A standard curve for C. jejuni was prepared. An appropriate dilution of the suspension was made with sterile phosphate buffer to achieve the proper inoculum. Exact numbers of the inoculum were determined by the plating method described previously.

C. jejuni

Stock Supply

A slant of C. jejuni was initially obtained from C. Powers of Microbial Diseases Laboratory (MDL) in California. Fresh cultures from

patients with diarrhea were obtained during the course of the project from the Oregon State University Student Health Center Laboratory.

C. jejuni was streaked onto campy plates, incubated microaerophilically at 42°C and subcultured every 48 h for a stock supply. A slant of campy media was streaked weekly with the stock culture, incubated microaerophilically at 42°C for 30-48 h, sealed and refrigerated for an emergency stock supply.

Gram Stain and Motility

Microorganisms were presumptively identified as C. jejuni on the basis of their gram stain and motility. Counterstaining with safranin for 15 min and 0.06% carbol fuchsin for 1 min were compared. Under the conditions of this experiment, the spiral morphology of the organism was more distinct with safranin and this was the method chosen for subsequent gram stains. The motility was checked by viewing a wet mount of the culture under oil immersion with a regular light microscope. C. jejuni has a darting corkscrew motion.

Biochemical Tests

Presumptive C. jejuni were routinely subjected to oxidase and catalase tests. Sterile toothpicks were used to transfer a heavy inoculum of the culture to a Pathotec Cytochrome Oxidase (CO) strip (General Diagnostics) and to a glass slide. The culture on the slide was then suspended in 3% hydrogen peroxide (H_2O_2) solution (Mallinckrodt). Typical positive reactions are a blue spot on the CO strip and bubbling in H_2O_2.

Production of hydrogen sulfide (H_2S) and nalidixic acid resistance

were further confirmatory tests. A slant of triple sugar iron (TSI) agar was inoculated by stabbing the butt and streaking the slant. A lead acetate strip was hung approximately 10 mm above the slant and attached by loosely putting the cap on the tube. The tube was incubated microaerophilically at 42°C for 48-72 h. A positive reaction is red/red on the TSI agar slant and darkening of the bottom of the strip. A loopful of the suspect Campylobacter culture was inoculated into a tube of BBA (5 ml) and incubated microaerophilically at 42°C for 24 h. An inoculum of the culture was transferred by a cotton swab to a campy plate, cross streaking for confluent growth. A 30 µg nalidixic acid disc (BBL) was placed in the center of the swabbed area and the plate incubated micraerophilically at 42°C for 48 h. A zone of no growth around the disc is typical of C. jejuni.

Nitrate reduction was done by inoculating tubes of nitrate broth and incubating them microaerophilically at 42°C for 24 h. After incubation, 0.5 ml of sulfanilic acid solution, 0.5 ml of alpha-napthol solution (Analytab) and a few crystals of creatine were added. A positive reaction is a red color.

Motility was examined for by stabbing a semi-solid motility medium, 0.5% yeast extract, 1% tryptose and 0.4% agar, containing 0.01% triphenyltetrazolium chloride (Sigma). The inoculated tube was incubated microaerophilically at 37°C for three to five days and examined daily for growth and deep red color diffusing from the stab line.

An attempt was made to use a rapid identification test with the organism. API E20 strip (Analytab) tests were performed, using C. jejuni diluted with water and also with 0.85% saline. The inoculated strips were incubated aerobically at 42°C for 24-48 h.

Determination of Optimum Growth Conditions

In each of the studies below, the techniques for plate preparation, incubation, enumeration and culture preparation outlined previously were followed with the exception of the variable being tested. Each part outlines how the particular variable under study was tested.

Selective Agars

Campy and a modified Blaser (brucella) selective plating media were compared. The campy plate is described previously. The brucella plate consisted of brucella agar (BBL), 5% laked sheep blood (Prepared Media Laboratory) and Skirrow's supplemental antibiotics. One ml of C. jejuni, 1.0×10^7 org/ml, was serially diluted 5 fold using 9 ml phosphate buffer blanks and 0.1 ml aliquots of 1:100, 1:1000 and 1:10,000 dilutions were spread onto each media. The plates were incubated microaerophilically at 42°C for 48 h and colonies enumerated. The amount of growth and the cultural characteristics of the organism on each media were noted.

Other organisms can grow on the selective media. Slants of possible competing organisms were supplied by the Corvallis Clinic. Campy plates were streaked with Candida albicans, Proteus mirabilus, Psuedomonas aeruginosa and C. jejuni, incubated microaerophilically at 42°C and observed after 24, 48 and 72 h. The cultural characteristics were noted and each organism subjected to catalase and oxidase tests.

Broths

Fluid thioglycollate broth (TB) (Difco), fluid thioglycollate broth without indicator (TBWI) (Difco) and brucella broth (BB) with 0.16% agar

were compared for their culturing ability of C. jejuni. One ml of C. jejuni, $1.0x10^2$ org/ml, was pipetted into each of three 250 ml sidearm flasks containing 30 ml of one of the broths along with Skirrow's antibiotics. Each flask was flushed with the gas mixture, closed and incubated at 42°C.

In the first study, the optical density of each broth culture was read at regular intervals over a 30 h period using a Bausch and Lomb Spectronic 20 set at 460 nm. In the second study, the optical density was read at regular intervals over a 60 h period, as well as enumeration of the broth culture on campy plates as described previously.

Diluents

Deionized water, 0.1% peptone and Butterfield's phosphate buffer diluents were examined for effect on the survival of the organism during serial dilutions. One ml of C. jejuni, $1.0x10^5$ org/ml, was inoculated into nine ml of each diluent, shaken 25 times and serially diluted 3 fold using 9 ml phosphate buffer blanks. One-tenth ml aliquots of each dilution were spread onto plates. The plates were incubated microaerophilically at 42°C for 48 h and colonies enumerated as described previously.

Growth Curve

A loopful of C. jejuni, subcultured 24-30 h previously, was inoculated into 30 ml of BBA in a sidearm flask. The flask was flushed with the gas mixture, closed and incubated at 42°C. The optical density of the broth was read at regular intervals over a 96 h period using a Bausch and

Lomb Spectronic 20 set at 460 nm, as well as enumeration of the broth culture on campy plates as described previously.

Recovery Methods

Five methods; direct plating, filtration, double incubation enrichment, milk separation enrichment and swabbing, were tested for their effectiveness to recover C. jejuni from inoculated food products. Food samples were purchased at local supermarkets. The techniques for plate preparation, incubation, enumeration and culture preparation described previously were followed unless otherwise stated.

Direct Plating

One ml of C. jejuni, 1.0×10^9 org/ml, was inoculated into 99 ml each of pasteurized, homogenized whole milk and a 1:10 homogenate of ground turkey[1]. Immediately after shaking, 25 times, the seeded food samples were serially diluted 3 fold using 9 ml phosphate buffer blanks and 0.1 ml aliquots of each dilution plated. The plates were incubated microaerophilically at 42°C for 48 h and the colonies enumerated. A wet mount and gram stain checked the purity of the resultant colonies grown on the campy plates. The procedure was repeated using an inocula of 1.0×10^5 and 10^4 org/ml.

Filtration

The filtering technique of Microbial Disease Laboratory (MDL) in

[1]The 1:10 homogenate was prepared by blending 25 g of ground turkey with 225 ml of sterile phosphate buffer for two min in a Waring blender.

California was used with 1:10 homogenate of turkey prepared as described previously. One ml of C. jejuni, $1.5x10^7$ org/ml, was inoculated into 99 ml of the turkey homogenate and shaken 25 times. Twenty ml of the seeded food sample was centrifuged five min at 1000 rpm. Six ml of the supernatant was filtered through a sterile swinney filter containing a 0.65 µm membrane filter. The filtrate (3-5 ml) was serially diluted 4 fold using 9 ml phosphate buffer blanks and 0.1 ml aliquots of the 1:1000, 1:10,000 and 1:100,000 dilutions were plated. The plates were incubated microaerophilically, 42°C for 48 h and colonies enumerated. Wet mount, gram stain and biochemical tests checked the purity of the resultant colonies grown on the campy plates. The procedure was repeated using an inocula of $2.7x10^5$ to $6.5x10^6$ org/ml. The exact number of organisms per ml for each inoculum was enumerated as described previously.

Double Incubation Enrichment

Ninety-nine ml of pasteurized, homogenized whole milk was inoculated with one ml of C. jejuni and shaken 25 times. The level of the initial inoculum (Table I) was determined prior to enrichment by plating an aliquot directly onto campy plates. The seeded milk was mixed with 100 ml of BBA, reshaken, the number of organisms per ml determined, and incubated at the temperature and time for the first incubation period (Table I). After completion of the first incubation period, the number of organisms suriving the incubation was determined and then five ml aliquots of each sample were transferred to five replicate tubes containing 10 ml of BB and incubated for the second incubation period (Table I). At the end of the

Table I. Initial inocula and incubation periods used in Double Incubation Enrichment.

Sample	Initial Inoculum org/ml	First Incubation Period		Second Incubation Period	
		h	°C	h	°C
1	73	4	42	4	42
2	7.3×10^3	4	42	4	42
3	1.2×10^2	8	42	6	42
4	1.2×10^4	8	42	6	42
5	3.9	8	42	6	42
6	3.9×10^3	8	42	6	42
7	92	8	42	6	42
8	9.2×10	8	42	6	42
9	1.3×10^4	8	42	6	42
10	1.3×10^4	12	37	12	37
11	31	24	37	6-8	42
12	3.1×10^3	24	37	6-8	42
13	16	24	37	6-8	42
14	1.6×10^2	24	37	6-8	42
15	1.6×10^3	24	37	6-8	42

second incubation period the final number of surviving organisms was determined. As certain combinations of time and temperature demonstrated enrichment of the organism, that method was repeated using lower numbers of C. jejuni to determine recovery limitations. A wet mount and gram stain checked the purity of the resultant colonies grown on the campy plates.

Milk Separation Enrichment

The enrichment technique of Mueller (1981) was used with samples of raw cow's milk. The procedure was modified by using a selective plate agar (campy-2) consisting of blood agar base no. 2, 7% laked horse blood, Skirrow's antibiotics and the antibiotics amphotericin B, 0.2 mg/ml and cephalothin, 1.5 mg/ml (Oxoid). The enrichment medium was a motility test medium with 1.5% proteose peptone, 0.75% yeast extract, 0.3% agar, 0.25% potassium phosphate monobasic, 0.25% potassium phosphate dibasic, 0.15% ammonium phosphate, 5% laked horse blood and Skirrow's antibiotics.

One ml of C. jejuni, $1.8X10^5$ org/ml, was inoculated into 99 ml of milk and shaken 25 times. The seeded samples were treated as outlined previously[2]. One-half and one-tenth ml aliquots of the unseeded milk were also plated, as negative controls. All inoculated plates were done in duplicate, incubated at 42°C in anaerobe jars with Campy Pak II envelopes, and examined after 48 h. Wet mount, gram stain and biochemical tests checked the purity of the resultant colonies grown on the campy-2 plates. The procedure was repeated using an inoculum of $1.8x10^3$ org/ml.

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[2] cf. p. 13.

Swabbing

Stern's (1981a) swabbing technique for red meats was modified for use with fresh chicken carcasses. One drop (0.05 ml) of 1:10; 1:100 and 1:1,000 dilutions of C. jejuni, $3.1x10^3$ org/ml, was spread over a 10 cm^2 area of chicken carcass using a sterile bent glass rod and allowed to absorb onto the meat surface for 10 min. The inoculated surfaces were swabbed with sterile cotton swabs, premoistened with phosphate buffer, and streaked onto campy plates. An uninoculated 10 cm^2 area of chicken carcass was also swabbed and plated as a negative control. The plates were incubated microaerophilically at 42°C and observed after 48 h. A representative number of suspect C. jejuni were picked and observed microscopically under a wet mount for characteristic morphology and motility.

Milk Sampling

Supermarket

One gallon containers of three brands of raw milk were bought at local supermarkets. Aliquots (0.5 and 0.1 ml) of each sample were spread onto campy plates, incubated microaerophilically at 42°C and examined after 48-72 h. In addition, 5 ml of milk was enriched with 10 ml of TBA and incubated microaerophilically at 42°C for 6 h. Aliquots (0.5 and 0.1 ml) of the incubated milk-broth mixture were spread onto campy plates, incubated microaerophilically at 42°C , and examined after 48-72 h.

Typical suspect colonies were picked and microscopically examined under a wet mount. Organisms with characteristic morphology and motility

were subcultured onto campy plates, and incubated microaerophilically at 42°C for 24-30 h. Wet mount, gram stain and biochemical tests checked the purity of the resultant colonies.

Oregon State University Dairy Center

Milk (225 ml) from 50 cows randomly selected from the OSU Dairy herd was collected into sterile glass bottles. The milk was withdrawn from the weighing bottles attached to the milking apparatus. Rectal swabs, using sterile cotton swabs, were also obtained and placed in screw cap tubes containing 10 ml of BBA with 0.16% agar. Samples were packed in ice and transported to the laboratory. Samples were stored between 0-4°C upon receipt in the laboratory and analyzed within 4 h.

The microbial quality of the milk sample was determined by analyzing for standard plate count (SPC), coliform and C. jejuni. Two tenfold dilutions of each sample were made using 9 ml phosphate buffer blanks. SPC was performed according to published methods in SMEDP using duplicate aliquots (0.1 and 1.0 ml) of 1:100 dilution (Marth, 1978). Coliform was performed according to published methods in SMEDP using duplicate aliquots (1.0 ml) of the undiluted sample and the 1:10 dilution on violet red bile (VRB) agar plates and aliquots of the undiluted sample, the 1:10 and 1:100 dilutions in lauryl tryptose (LST) broth (Marth, 1978).

C. jejuni was analyzed for by using directly plated and enriched milk. Aliquots (0.1 and 0.5 ml) of each sample were directly plated onto campy plates and incubated at 42°C for 48 h in anaerobe jars. One hundred ml of each sample were enriched in flasks containing 100 ml of BBA, shaken and incubated microaerophilically at 37°C for 24 h. After incubation,

10 ml aliquots of each mixture were transferred to 5 replicate tubes containing 10 ml of BBA and incubated microaerophilically at 42°C for 8 h. After the second incubation, a tenfold dilution was made, aliquots (0.1 ml) of the undiluted sample and the 1:10 were spread onto campy plates and incubated.

Each rectal swab was streaked onto campy plates in three directions for confluent growth. The swabs were placed back into the transport media and incubated aerobically at 42°C for 24 h. After incubation each swab was again cross streaked onto campy plates and the plates incubated microaerophilically at 42°C for 48 h.

Survival Studies

Holding Tests

C. jejuni's survival in foods held at temperatures the products customarily encounter was investigated. One ml of C. jejuni, 6.0×10^5 org/ml, was inoculated into flasks containing 99 ml of homogenized, pasteurized whole milk and shaken 25 times. One flask was held at 4°C and the other at 42°C for 72 h. Aliquots (1.0 ml) were taken every 12 h, spread onto campy plates, the plates incubated microaerophilically at 42°C for 48 h, and colonies enumerated. Additional milk samples with lower levels of inocula, 120 to 1.7×10^4 org/ml, were prepared and held at 4°C. Aliquots were spread onto campy plates daily and treated as outlined above until less than 10 viable organisms per ml were obtained.

One ml of C. jejuni, 1.4×10^9 org/ml, was inoculated into three flasks containing 99 ml of 1:10 turkey homogenate and shaken 25 times. Samples were held at 4, 37 and 42°C for 48 h. After the holding period, aliquots (1.0 ml) were spread onto campy plates and incubated microaerophilically at 42°C for 48 h. Additionally, a sample containing C. jejuni, 4.0×10^3 org/ml, was held at 4 and 42°C for 72 h. Aliquots (1.0 ml) were taken every 12 h, spread onto campy plates and incubated as outlined above.

Germicidal Tests

The germicidal effects of Sani-du, DYNE and Ioteat (brand names) on C. jejuni, 1.0×10^4 org/ml, were tested. Stock solutions of 1111 ppm for each compound were prepared and serially diluted 5 fold using nine ml phosphate buffer blanks. One ml inoculum of C. jejuni was added to each tube, shaken 25 times and allowed to react for the contact times and temperatures specified in Table II.

Table II. Temperatures and contact times of sanitizers used.

Sanitizer	Solution Temperature (°C)	Contact Times
Sani-du	42.5	30 sec; 2 min
DYNE	42.5	30 sec; 2 min
Ioteat	22	30 sec

After the reaction time, aliquots (1.0 ml) of the solution were diluted 2 fold using 9 ml phosphate buffer blanks and 0.1 ml aliquots of each dilution spread onto campy plates. The plates were incubated microaerophilically at 42°C for 48 h and colonies enumerated.

The germicidal effects of chlorinated water on C. jejuni, 1.0×10^5 org/ml, was tested. Two stock solutions, 5555 and 1111 ppm, were prepared from Master-X Bleach which contained 5.25% sodium hypochlorite. The stock solution was diluted 5 fold using 9 ml phosphate buffer blanks. One ml inoculum of C. jejuni was added to each tube, shaken 25 times and allowed to react 30 sec and 2 min. After the reaction, aliquots (1.0 ml) of the solution were diluted 2 fold using 9 ml phosphate buffer blanks and aliquots (0.1 ml) of each dilution spread onto campy plates. The plates were incubated microaerophilically at 42°C for 48 h and colonies enumerated.

RESULTS AND DISCUSSION

Campylobacter jejuni is a difficult organism to isolate and culture routinely in the laboratory. Also, it's difficulty to gram stain, detection of motility and inconsistent biochemical reactions make confirmation difficult.

C. jejuni does not reproduce itself rapidly and consequently routine transfers must be carefully regulated or the organism will be lost. On several occasions during the course of this project difficulty was encountered in maintaining a viable culture. Successful subculturing was usually obtained when a heavy inoculum of the organism was transferred every 48 h and incubated microaerophilically at 42°C. In some cases however, up to six days of microaerophilic incubation at 42°C was necessary in order to visibly see growth. Slants of the organism could be successfully stored in the refrigerator (4°C) up to one month.

Other researchers have also commented on the difficulty and slow growth of C. jejuni. Blazovio (1979) recommended blind subculturing is sometimes necessary to detect the organism. Butzler and Skirrow (1979) noted as a rule growth is visible after incubation for one day but when only a few organisms are present, incubation should be continued for another day. Levy (1946) found only after twenty repeated transfers did the organism successfully grow in milk.

Confirmation of the microorganism is usually based on characteristic responses to staining, microscopic observations and biochemical tests. C. jejuni is difficult to gram stain. Both a counterstain of saphranin for 15-30 min and 0.06% carbol fuchsin for 1 min resulted in a nebulous

pink color. The organism's morphology however, was much more distinct with the saphranin counterstain.

Under a light microscope, a culture of C. jejuni appeared as a mixture of comma and 'S' shaped organisms. In older cultures (> 72 h) cocci were prevalent. Wilson and Wang (1979) speculated that these cocci are no longer reproductive. Thus the importance of subculturing C. jejuni before 72 h preventing the formation of spherules and culture die off.

C. jejuni is motile with a corkscrew motion. This tumbling motion is difficult to see under a regular light microscope. Darkfield or phase contrast microscopy are much more sensitive methods to observe the organism's motility (Wilson and Wang, 1979; Blaser et al., 1979b).

Young cultures (24-30 h) and heavy inoculum were necessary for the biochemical tests. A very heavy inoculum of C. jejuni was necessary to evoke even a faint blue spot on the CO strip. The reaction was often delayed up to 15 sec even with a pure culture. The TSI slant frequently showed a yellow tinge near the top of the agar where the culture was not growing. The production of H_2S usually required a long incubation, 40-72 h at 42°C. Growth of C. jejuni occurred only near the bottom of the TTC slant. The motility test was not used as the organism sometimes died off before it reacted with the triphenyl-tetrazolium chloride. Consequent double inoculations made the test too time consuming for routine use. Nitrate reduction to nitrite was not routinely used as it was difficult to distinguish C. jejuni from other organisms with this test. API-E20 strips showed no positive reaction for C. jejuni with the exception of arabinose. Wilson and Wang (1979)

confirmed API and other rapid identification tests for anaerobes and gram-negative organisms are not helpful because C. jejuni does not ferment carbohydrates. It was not determined why the organism did ferment arabinose for the culture diluted with either water or saline solution.

The key distinguishing characteristic of C. jejuni is its spiral morphology. However, because of its resistance to staining, pleomorphism and small size, the morphology of the organism may often be indistinguishable. The movement of C. jejuni is distinctive but is not always easily observed, especially if present in a mixed culture. The biochemical tests for C. jejuni are few and its response to them are often faint and consequently difficult to interpret. Wilson and Wang (1979) aptly summed up the culturing of the organism in the statement, "Under ideal atmosphere and temperature, you'll have luxuriant (well--as luxuriant as campy [sic] ever gets!)...growth".

Determination of Optimum Growth Conditions

Media

The cultural characteristics of C. jejuni depend on the type of media on which it is grown. On campy plates the colonies were of two types. Some colonies were blue-grey to tan with erose edges, dull and spreading. Other colonies were creamy-tan, raised, circular, glistening and 0.5-2 mm in diameter. On brucella plates, all the C. jejuni colonies were tan, slightly convex, circular, glistening and 1-2 mm in diameter. On this media colonies of C. jejuni were not easily distinguished from the other organisms that could also grow on the

selective agar.

Other researchers also report C. jejuni forming colonies similar to the two types seen on the campy plates. Smibert (1978) reported a change from smooth to rough cut glass appearance when subcultured repeatedly. Wilson and Wang (1979) reported a grey color, Blaser et al. (1979a) reported pinpoint and glossy colonies, Hallet et al. (1977) reported a viscous and buff color colony. One must be familiar with the cultural variability of the organism in order to discern and pick suspect C. jejuni.

Campy media is a better growth media than brucella. More growth, $3.9x10^7$ org/ml, was obtained with the campy plates vs. $2.4x10^7$ org/ml for brucella plates from duplicate inocula of C. jejuni. The campy media contains blood agar base no. 2 with proteose peptone. Mehlman and Romero (1981) have reported proteose peptone is a good nitrogen source and recommend it for optimum growth. Whereas, brucella agar contains polypeptone. Mehlman and Romero reported peptone to be a poor nitrogen source.

The ability of competing organisms to grow on campy agar was studied. The colony characteristics and distinguishing biochemical properties are listed in Table III.

Table III. Colony and biochemical characteristics of competing microorganisms on campy plates.

Organism	Colony Characteristics	Oxidase	Catalase
C. jejuni	blue-grey to tan, erose	+	+
Candida albicans	white, large, circular	+	-
Proteus mirabilus	cream bead	+	-
Psuedomonas aeruginosa	tan, erose	+	+

Candida albicans and Proteus mirabilus were easily distinguishable from C. jejuni because of different colony types. Pseudomonas aeruginosa had similar type colonies and biochemical reactions as C. jejuni and the two organisms were difficult to distinguish on campy agar. Microscopically, Pseudomonads are only slightly curved and larger rods than C. jejuni. Smibert (1978) had also found selective media aided isolation of Campylobacter, but it did not completely eliminate competing organisms.

Broths

Figure 1 illustrates the growth of C. jejuni in brucella broth (BB) and in fluid thioglycollate broth (TB). The growth of the organism, as indicated by the optical density, was higher in BB than in TB. At the end of 45 h of incubation, an aliquot of each of the broth cultures was taken and the colonies enumerated as described previously. In BB there were 1.1×10^9 cfu/ml and in TB 9.0×10^8 cfu/ml. Mehlman and Romero (1981) reported fluid thioglycollate broth gave sparse and inconsistent growth of C. jejuni and demonstrated that sodium thioglycollate was inhibitory to the organism.

Diluents

The effect of different diluents on the growth of C. jejuni is shown in Table IV.

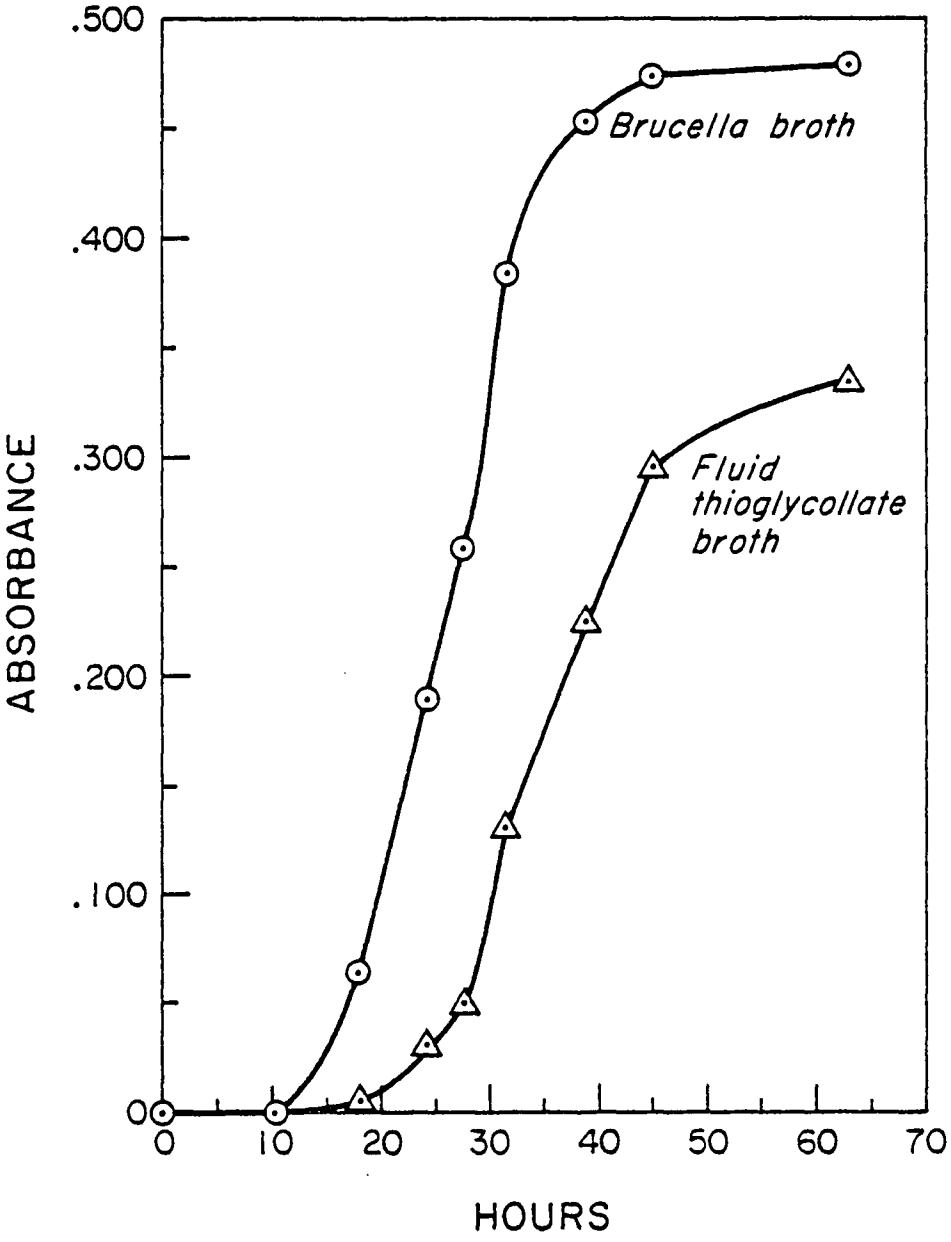

Figure 1. Growth of C. jejuni in brucella broth and fluid thioglycollate broth at 42°C.

Table IV. Effect of different diluents on the growth of C. jejuni.

Diluent	after 48 h, 42°C incubation cfu/ml
1% peptone	5.9×10^4
sterile water	1.0×10^5
Butterfield's Phosphate Buffer	1.0×10^5

A slightly higher number of organisms survived when sterile water or phosphate buffer was the diluent. Phosphate buffer was the diluent chosen for subsequent experiments. Phosphate buffer is recommended for diluting dairy product samples (Marth, 1978). Mehlman and Romero (1981) reported potassium phosphate dibasic is a requirement for the growth of C. jejuni.

Scientists have not yet agreed upon a single procedure for the isolation and culturing of C. jejuni. Each laboratory prefers a different media and equipment. The above experiments were necessary to determine the general procedure to be used in the study. Campy plates were used because of the distinctive colony types for C. jejuni, brucella broth was selected as it is not inhibitory to C. jejuni and its pH of 6.6 is within the optimum pH range for the organism. Phosphate buffer was the diluent used for dilution of all food samples.

Growth Curve

The growth of C. jejuni in BB incubated microaerophilically at 42°C is illustrated in Figure 2. Under the conditions used in this

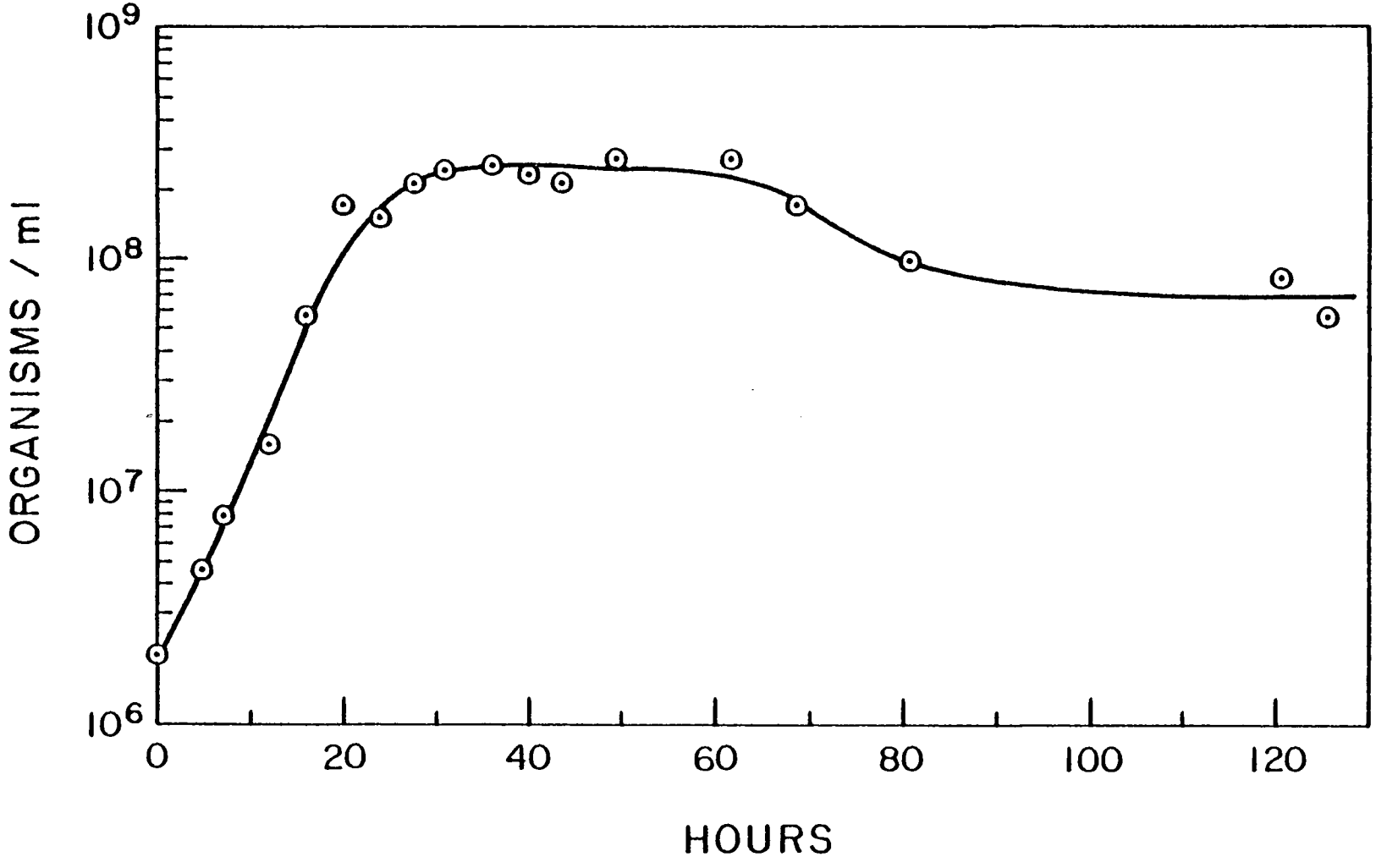

Figure 2. Growth curve of C. jejuni at 42°C.

laboratory, the lag phase was short, lasting 4-6 h; the exponential phase ending at 30 h; the stationary phase ending approximately after 70-72 h and then die-off occurred. Doyle and Romen (1981) reported similar growth curves for C. jejuni incubated at 42 and 45°C. At lower temperatures, 35 and 37°C, growth was slower and the exponential phase lasted until 48-60 h after inoculation.

C. jejuni has a long generation time. Under the optimum conditions of this study, the generation time was 3 h. Other organisms often studied have much shorter generation times, for example E. coli has T_G of 20 min. It is important to incubate the organism long enough to have large and easy to pick colonies but not to extend the incubation period beyond the stationary phase.

Recovery Methods

Direct Plating

The recovery of C. jejuni when inoculated into food products, thoroughly mixed and directly plated onto campy plates is shown in Table V.

Table V. Recovery of C. jejuni by direct plating from inoculated food samples.

Food	No. cfu/ml inoculated	No. cfu/ml recovered	% recovery
Pasteurized homogenized whole milk	3.9×10^2	3.0×10^2	77
	1.7×10^4	1.1×10^4	65
	1.5×10^7	1.4×10^7	93
Turkey homogenate	1.2×10^3	4.0×10^3	333
	3.4×10^3	2.7×10^3	79
	1.4×10^7	1.5×10^8	107

Sixty to ninety percent of the C. jejuni inoculated into milk samples was recovered. The decrease in the number of viable organisms in milk when directly plated could have resulted from injuries caused by handling. A method to help recover stressed bacteria is needed. Often 80 to 330% of the C. jejuni inoculated into turkey samples was recovered. The increase in growth of C. jejuni on the spread plates of turkey homogenate was probably due to nutrients present in turkey that are conducive to growth of C. jejuni. Also the meat tissue may have protected the bacteria and prevented as much injury to the bacteria as occurred in the milk samples during handling. The turkey homogenate spread plates however, were difficult to examine and the suspect C. jejuni hard to pick as the debris obscured the colonies.

Filtering

The results of filtering inoculated turkey homogenate samples through a swinney filter are shown in Table VI.

Table VI. The effect of filtering on recovery of C. jejuni from inoculated turkey meat.

Organisms before filtering cfu/ml	Organisms after filtering cfu/ml	% Recovery
2.7×10^3	1.5×10^3	12
3.1×10^3	2.6×10^3	84
3.2×10^3	1.7×10^3	53
3.7×10^3	1.8×10^3	47
5.1×10^3	3.5×10^3	69
7.2×10^3	4.3×10^3	60
2.5×10^4	9.5×10^3	38
6.5×10^4	3.0×10^4	43
1.5×10^5	8.9×10^4	59

A range of 12-84% (mean of 52%) of the organisms were recovered from inoculated turkey homogenate after filtration. The majority of the meat tissue was removed, making detection and enumeration of C. jejuni easier. However, difficulty in filtering the suspension through the swinney filter was encountered. Often the filter membrane was torn and the sample was contaminated. Fluid milk samples were also difficult to filter as the average diameter of the milk fat globule is 3.4-4.5 µm (Walstra and Mulder, 1974), and much too large to pass through the 0.65 µm filter which is necessary to separate C. jejuni from other microorganisms. Butzler and Skirrow (1979) found filtration too tedious for routine use and it was less sensitive than selective agar methods.

Double Incubation Enrichment

The results of enriched inoculated milk samples in BBA using double incubation treatment are shown in Table VII. When both incubation periods were 4 h at 42°C, the number of org/ml of C. jejuni recovered after the second incubation was nearly the same as the initial inoculum (Samples 1 and 2). The first incubation period was then increased to 8 h to allow the organism to reach its exponential growth phase before transfer (Samples 3 and 4). There was a one log cycle increase in the number of organisms per ml recovered after the second incubation. However, this data could not be repeated (Samples 5 through 8).

A lower temperature (37°C) was tested to determine if injured organisms would recover better at the lower temperature (Sample 9) as compared to the higher temperature (42°C) (Sample 10). When the organism was incubated at 37°C for 24 hrs, transferred and then incubated

Table VII. Effect of double incubation enrichment on recovery of C. jejuni from inoculated milk.

Sample[a] Number	Initial Inoculum (cfu/ml)	First Incubation Time h	First Incubation Temp. °C	First Incubation No. of organisms (cfu/ml)	Second Incubation Time h	Second Incubation Temp. °C	Second Incubation No. of organisms (cfu/ml)	Change ±
1	7.3×10^3	4	42	6.9×10^3	4	42	6.5×10^3	-
2	7.3×10			6.6×10			8.0×10	+
3	1.2×10^4	8	42	4.9×10^4	6	42	1.5×10^5	+
4	1.2×10^2			3.3×10^2			$>3.3 \times 10^2$	+
7	9.2×10^3	8	42	1.8×10^2	6	42	9.0×10	-
8	9.2×10			8.0			N.G.	-
9	1.3×10^4	12	37	5.1×10^4	12	37	2.8×10^5	+
10	1.3×10^4	8	42	2.4×10^4	6	42	1.1×10^4	-
11	3.1×10^3	24	37	1.8×10^5	6-8	42	$>1.8 \times 10^5$	+
12	3.1×10			1.5×10^3			1.6×10^4	+
13	1.6×10^3	24	37	7.1×10^3	6-8	42	$>7.1 \times 10^3$	+
14	1.6×10^2			4.5×10^2			$>4.5 \times 10^2$	+
15	1.6×10			$>3.0 \times 10$			$>3.0 \times 10$	+

[a]Sample No. 5 and 6 were overgrown by another microorganism.

at 42°C for 6-8 hrs there was a several log cycle increase. The increase of C. jejuni at 37°C demonstrated the injured bacteria were able to make metabolic repairs at the lower temperature and consequently keep multiplying. The 37-42°C sequence was repeated (Samples 11 and 12). Verification of the enrichment using the 37-42°C sequence prompted a repeated trial with a lower initial inoculum (Samples 13 through 15). There was a slight increase in the number of organisms but most importantly, low levels of the organism (16 cfu/ml) could be recovered. The double temperature, double incubation was subsequently chosen to analyze raw milk samples for C. jejuni.

Milk Separation Enrichment

The results of enriched inoculated milk samples using a milk separation treatment are shown in Table VIII. C. jejuni was recovered upon direct plating near the level of the initial inoculum for only the cream fraction. The organism was also recovered after enrichment of the cream after 48 h and of the milk after 72 h of incubation. The recovery of C. jejuni from the cream fraction might indicate the organism concentrates in the cream layer during centrifugation because of its small size and is protected by the cream. This theory is supported by the absence of the organism in the deposit fraction. The milk fat globule could also protect the organism during handling, thus avoiding stress to the cells. Growth in the whole milk after 72 h but not at 48 h implies the bacteria were perhaps injured but then made metabolic repairs and were able to multiply. This data could be repeated.

Table VIII. Effect of milk separation enrichment on recovery of C. jejuni from inoculated milk.

Initial Inoculum (cfu/ml)	Milk Fraction	Direct Plating (cfu/ml)	After incubation: 42°C 48h	72h	96h
1.8×10^3	whole	6.0×10^2	-	+	+
	cream	1.4×10^3	+	+	contaminated
	deposit	4.2×10^2	-	-	-
1.8×10^2	whole	1.3×10^2	0	-	-
	cream	1.8×10^2	+	+	+
	deposit	1.0×10^2	0	-	-

+ = typical C. jejuni

- = atypical colonies

Swabbing

The results of swabbing inoculated pieces of chicken are shown in Table IX.

Table IX. Recovery of C. jejuni by swabbing from inoculated chicken pieces.

Estimated initial inoculum-(cfu/ml)	Number of organisms recovered-(cfu/ml)	% Recovery
160	81	50
16	27	168
1.6	16	1000

Recovery of C. jejuni from inoculated chicken pieces proved to be difficult because of high numbers of indigenous bacteria which also grew on the campy plates and complicated the enumeration of C. jejuni. Microscopic examinations and biochemical tests confirmed growth was mixed cultures.

A more effective method to isolate C. jejuni from chicken would be one which incorporated a nutrient rinsing followed by a centrifugation, as now proposed by the Food and Drug Administration (Lovett et al., 1981). Centrifugation allows the organism to be concentrated in one of the layers and thus separated out more easily. The swabbing technique was not used further in the study.

Direct plating of food samples is adequate to detect C. jejuni when large numbers of organisms are present, however, direct plating is not

sensitive enough for low numbers of the organism. For food samples, where the number of organisms is likely to be low, a concentrating or enrichment technique is necessary. Filtering large samples of food through a 0.65 μm membrane to concentrate the organism is too difficult and time consuming. The double incubation and milk separation enrichments were effective for culturing low numbers of bacteria. The recommended procedure for milk samples used the spread plates and enrichment media prescribed in the milk separation enrichment. The enrichment medium includes components required for the optimum growth as determined by Mehlman and Romero (1981). Milk samples are centrifuged and the whole milk, cream and deposit layers are directly plated. The three fractions are then incubated as prescribed in the double incubation enrichment; first at 37°C for 24 h, transferred and then incubated at 42°C for 48 h.

Milk Sampling

Supermarkets

One of three raw milk samples purchased from local supermarkets revealed an organism with the typical cultural, microscopic and biochemical characteristics of C. jejuni. However, the culture was mixed and upon further subculturing the suspect C. jejuni was lost. The same sample, when enriched, did not reveal any suspect C. jejuni. The enrichment treatment used had an incubation period of less than 8 h. Possibly if a longer incubation period had been used, C. jejuni would have been isolated.

Oregon State University Dairy Herd

The results of the microbial quality of milk samples from the OSU Dairy Herd are shown in Table X.

Forty-six out of 50 had a Standard Plate Count (SPC) less than 20,000 cfu/ml, the Oregon standard for Grade A Raw Milk. Forty-eight out of 50 had SPC less than 80,000 cfu/ml, the Oregon standard for Grade A Raw Milk to be Pasteurized. SPC is an indicator of sanitiation and is sometimes used as a gross indicator of mastitis (Tosh *et al.*, 1981). The milk sampled from the OSU dairy herd was of good microbial quality from healthy cows.

Thirty-one out of 50 had a coliform count of < 10 MPN/ml. Thirty-two out of 50 samples had fecal coliform $\leq$ 10 MPN/ml and 39 out of 50 samples had *E. coli* $\leq$ 10 MPN/ml. There are no coliform standards for Grade A Raw Milk in Oregon. Pasteurized milk must not contain more than 10 MPN/ml. The coliform counts may include nonenteric members, thus fecal coliform counts may be a better indicator of fecal contamination. Identification of *E. coli* is also considered an indicator of fecal contamination. Although *E. coli* is not the perfect indicator, it is the best indicator available at this time (Marth, 1976). The levels of *E. coli* identified indicate very little fecal contamination of the milk samples.

No positive confirmation of *C. jejuni* in the raw milk was made. Nine out of the 50 samples however, showed typical darting motion microscopically in suspect cultures picked from campy plates. Subsequent gram staining of these suspect colonies revealed mixed cultures.

Table X. Standard plate count, coliform, fecal coliform and E. coli counts of raw milk sampled from OSU Dairy Herd.

Sample Number	SPC cfu/ml	Coliform MPN/ml	Fecal Coliform MPN/ml	E. coli cfu/ml	Suspect C. jejuni
1	< 3000	2.1	0.7	0.4	
2	"	<0.3	<0.3	<0.3	
3	"	0.7	0.7	0.4	
4	"	<0.3	<0.3	<0.3	
5	"	0.9	0.9	0.4	*
6	"	0.9	9.3	<0.3	*
7	"	9.3	0.4	<0.3	
8	"	0.4	0.3	<0.3	
9	"	<0.3	4.3	4.3	
10	"	>240	>240	>240	
11	"	0.4	0.4	0.4	
12	"	46	46	4.3	
13	"	<0.3	<0.3	<0.3	
14	"	<0.3	<0.3	<0.3	
15	"	0.9	0.4	0.4	
16	"	110	-	0.7	*
17	"	24	24	24	
18	"	15	15	15	
19	"	110	2	23	
20	"	46	46	<0.3	*
21	"	46	46	4.3	
22	"	24	24	24	*
23	"	0.7	0.7	0.4	
24	"	0.3	0.3	0.3	
25	"	110	110	2.3	
26	"	7.5	7.5	0.3	
27	4100	<0.3	<0.3	4.0	
28	4100	46	-	4.3	*
29	4400	0.9	0.9	24	

Sample Number	SPC cfu/ml	Coliform MPN/ml	Fecal Coliform MPN/ml	*E. coli* cfu/ml	Suspect *C. jejuni*
30	4400	0.3	0.3	<0.3	
31	4500	1.5	1.5	0.3	
32	4500	9.3	9.3	9.3	
33	4900	2.3	0.4	<0.3	
34	5000	>240	-	150	*
35	5200	46	-	46	
36	5400	2.3	0.4	0.4	
37	5700	0.4	<0.3	<0.3	
38	6200	24	-	2.3	*
39	6300	0.4	<0.3	<0.3	
40	6300	0.3	0.3	0.3	
41	7000	110	-	46	
42	7400	4.3	4.3	4.3	
43	8500	110	110	110	
44	9500	24	24	24	*
45	12,000	9.3	-	4.3	
46	14,000	110	110	46	
47	44,000	>240	>240	21	
48	58,000	4.3	4.3	4.3	
49	110,000	24	-	1.5	
50	130,000	<0.3	<0.3	<0.3	

*Suspect *C. jejuni* corkscrew motion observed microscopically.

Subculturing of the mixed cultures resulted in pure cultures but C. jejuni could not be confirmed from any of the colonies. The rectal swab samples were all negative for C. jejuni.

For both samples from cow No. 1398 suspect C. jejuni with corkscrew motility were observed on campy plates. The standard plate counts, 6.2×10^3 and 9.5×10^3 cfu/ml, were higher than the majority of the other samples. Both samples had a coliform count of 24 MPN/ml. The samples, which had been refrigerated since their receipt in the laboratory, were subjected to repeated enrichment, plated and the colonies enumerated as described previously. Suspect colonies again revealed typical darting motion in mixed culture when examined microscopically. However, no pure cultures of C. jejuni could be isolated.

Even though there was no positive confirmation of C. jejuni in the raw milk samples, the organism was suspect in 9 of the 50 samples. Perhaps the recovery methods were not sensitive enough to positively identify the organism.

The cultural variability of C. jejuni alone may have been responsible for the inability to positively identify the organism. Doyle (1980), reported a strain of C. jejuni which produces a colony with a dark center and translucent edges. Some colonies of the above description were observed on campy plates but not picked as that particular type of colony had not been seen in the preliminary studies of C. jejuni.

When the organism is present in low numbers its isolation is diffi-

cult. Other researchers report being unable to isolate suspect organisms after observing them microscopically. Levy (1946) saw vibrio-like organisms in 13 blood samples from patients with gastroenteritis but none grew on blood agar. Smibert (1965) frequently observed vibrio-like organisms in fecal samples but could not isolate them on the selective agar plates incubated microaerophilically. Butzler et al. (1973) reported being unable to isolate C. jejuni when in association with other organisms even though adequate media was used. The fastidious nature of the organism is a continual hindrance to isolating it.

C. jejuni has been isolated from fecal samples. Karmali and Fleming (1979a) reported rectal swabs were not substitutes for stool samples. Powers (1980) found feces samples yielded a higher number of positive cultures than rectal swabs. Stern (1981b) found only a 2% incidence of C. jejuni in fecal samples from cows and speculated the organism might be in isolated pockets in the feces. Taking large fecal samples would have been a better approach than rectal swabs.

Raw milk has been shown to carry microbial pathogens which may cause salmonellosis, brucellosis, tuberculosis, Q fever, typhoid fever, shigellosis and streptococcal disease (Taylor et al., 1980). It seems feasible raw milk may carry C. jejuni also. In all milk outbreaks associated with Campylobacter, the suspect milk has been either unpasteurized or improperly pasteurized (Robinson et al., 1979).

There are two theories regarding the association between C. jejuni and cows milk. Robinson et al. (1979) asserts C. jejuni in milk results from fecal contamination. Lander and Gill (1980) state C. jejuni comes from an infection of the udder and is in milk indirectly.

If the presence of C. jejuni is only fecal contamination, then raw milk drinkers would be safe from campylobacteriosis as long as proper sanitation procedures were followed. If, however, C, jejuni is present because of udder infections, some sort of monitoring program of individual cows must be designed and implemented. If a certain level of organisms per ml are needed to cause illness, as is implied in the Colorado outbreak where the family members who drank less raw milk remained well (Robinson et al., 1979) or in the Kansas outbreak by the asymptomatic individuals who excreted C. jejuni (Tosh et al., 1981), the mere presence of C. jejuni would not be cause for alarm. Instead only when a large number of organisms were present, would the implicated milk have to be disposed of.

The raw milk situation can only be adequately assessed by continuously surveying samples from individual cows in a herd using an enrichment technique for C. jejuni. Information on a possible link between mastitis and C. jejuni could be obtained and the situation reevaluated.

Survival Studies

Effect of Temperature

The survival of C. jejuni inoculated into pasteurized homogenized whole milk and held at constant temperatures is illustrated in Figures 3 and 4. C. jejuni gradually died off when held at 4°C and rapidly died off after slight initial multiplication when held at 42°C (Figure 3). The survival of C. jejuni in milk was again tested using lower initial inocula of C. jejuni and holding the samples only at 4°C (Figure 4). At

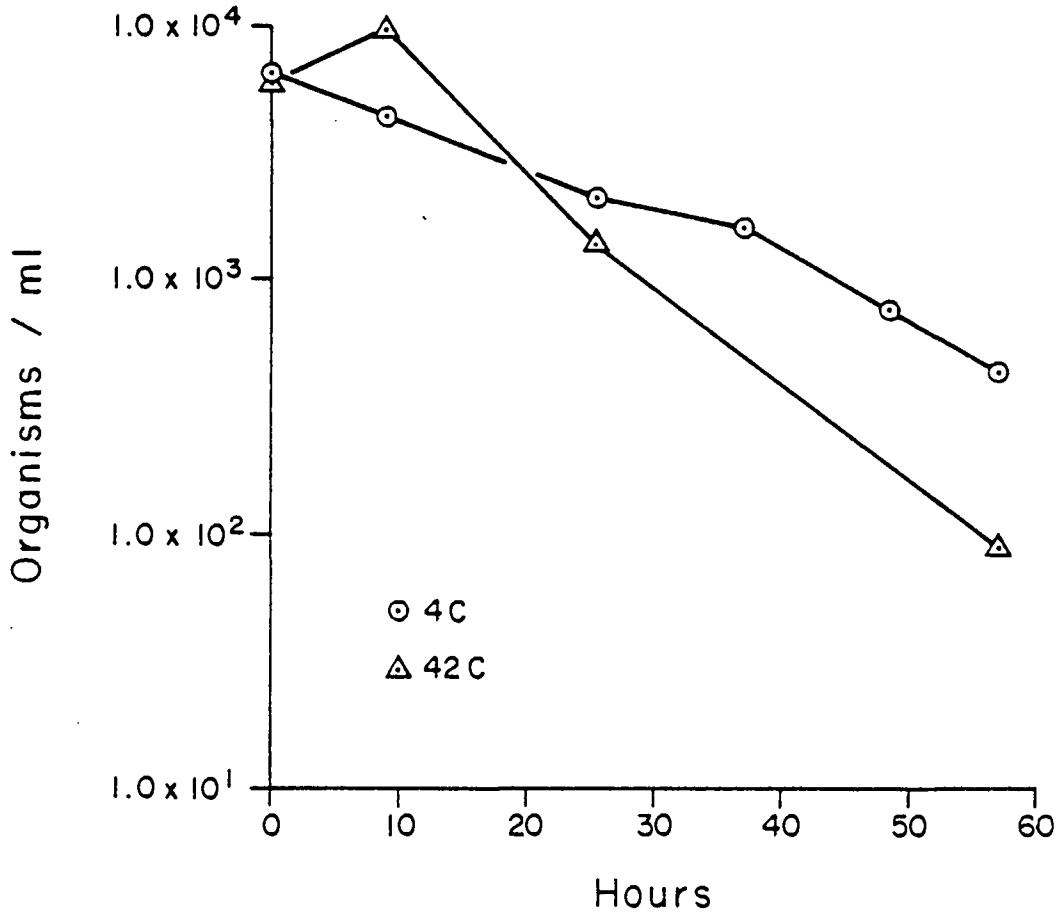

Figure 3. Survival of C. jejuni in pasteurized, homogenized whole milk held at 4 and 42°C.

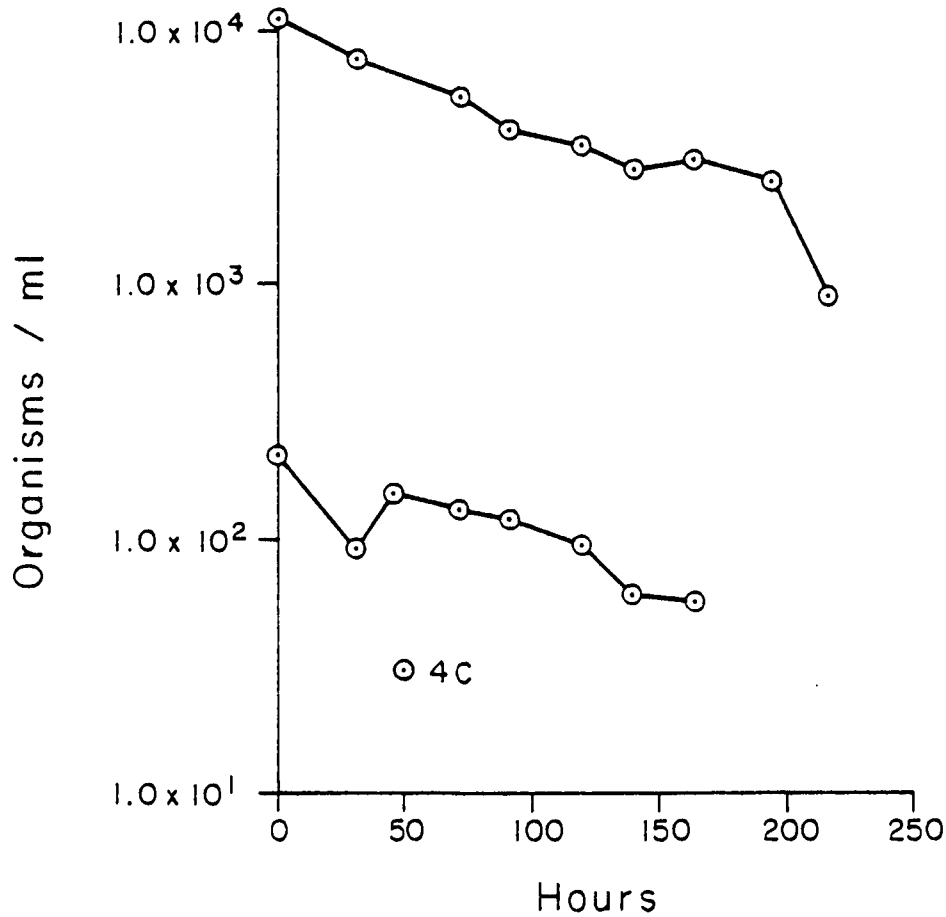

Figure 4. Survival of C. jejuni in pasteurized, homogenized milk held at 4°C.

both 10,000 and 200 org/ml there was less than a one $\log_{10}$ cycle decrease in seven days. Robinson et al. (1979) reported C. jejuni survived 164 days in milk kept at 4°C, the level of inoculum however, was not stated. As C. jejuni can survive under the conditions milk is usually held and it often is not further processed before consumption, its presence in milk is a definite health hazard.

The survival and growth of C. jejuni inoculated into a 1:10 turkey homogenate and held at constant temperatures is illustrated in Figures 5 and 6. C. jejuni slowly died off when held at 4°C, however, it multiplied when held at 37 or 42°C (Figure 5). The ability of C. jejuni to multiply at higher temperatures in turkey but not in the milk samples most likely results from the turkey tissue and muscle providing nutrients to the organism as well as protecting the organism from injury during handling. The faster growth of the organism in the inoculated sample at 37°C vs. 42°C supports the theory that injured organisms cannot withstand the higher temperature even though 42°C is usually the optimum temperature for growth. At 37°C, stressed organisms can make metabolic repairs and multiply. Figure 6 illustrates that C. jejuni, if unchecked, could easily continue to multiply. Other scientists report similar data. Blankenship and Craven (1981) found the survival of C. jejuni inoculated onto the surface of chicken meat varied with temperature during 17 days of incubation. The organism grew for four days followed by minimal die-off at 37°C, substantial steady die-off at 23°C and moderate steady die-off at 4°C. Grant et al. (1980) found C. jejuni survived 96 h in five out of six chicken guts kept at 4°C. The ability of the organism to survive at normal refrigerator temperature implies many consumers handling

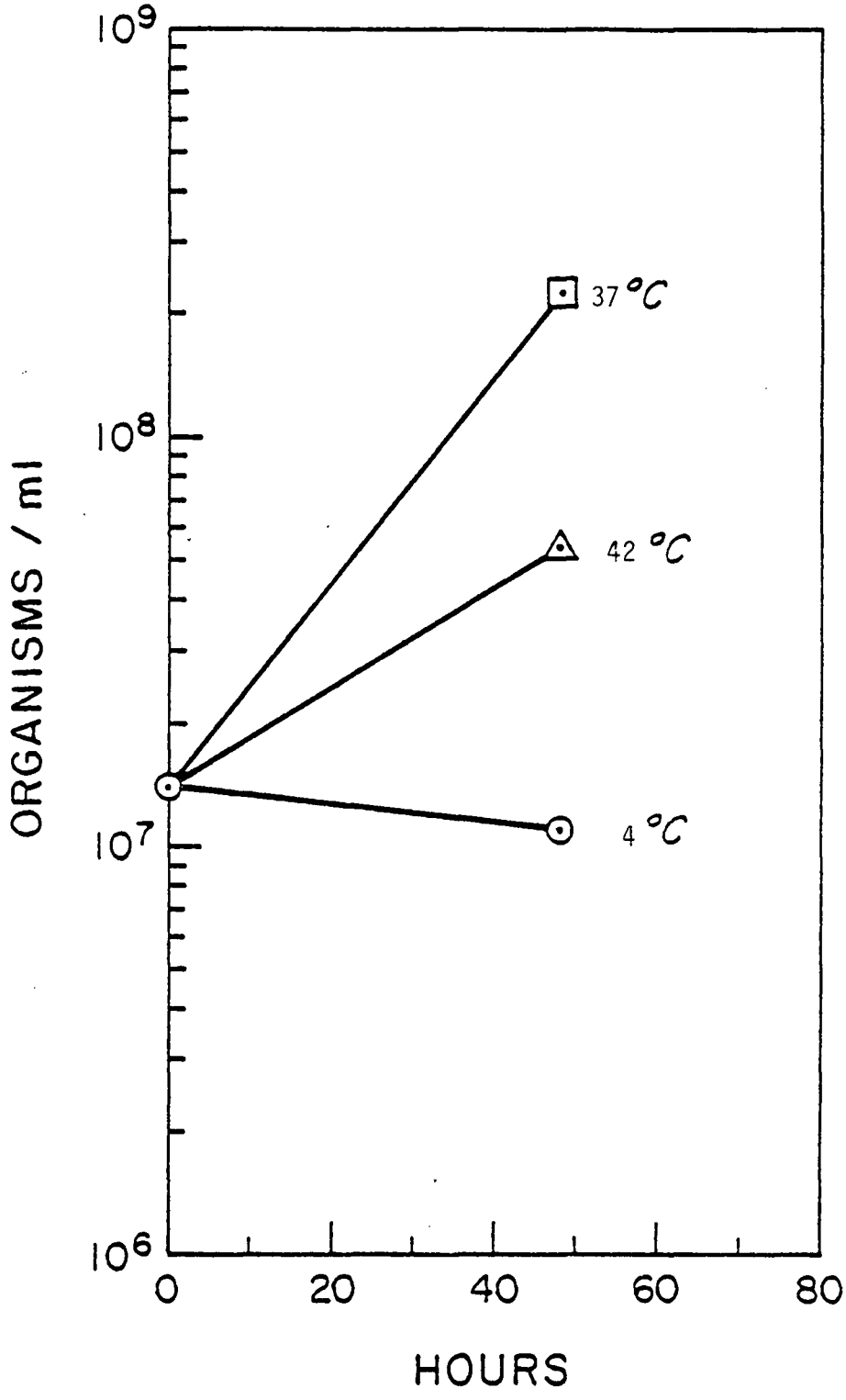

Figure 5. Survival of C. jejuni in turkey homogenate (1:10) held at 4, 37 and 42°C.

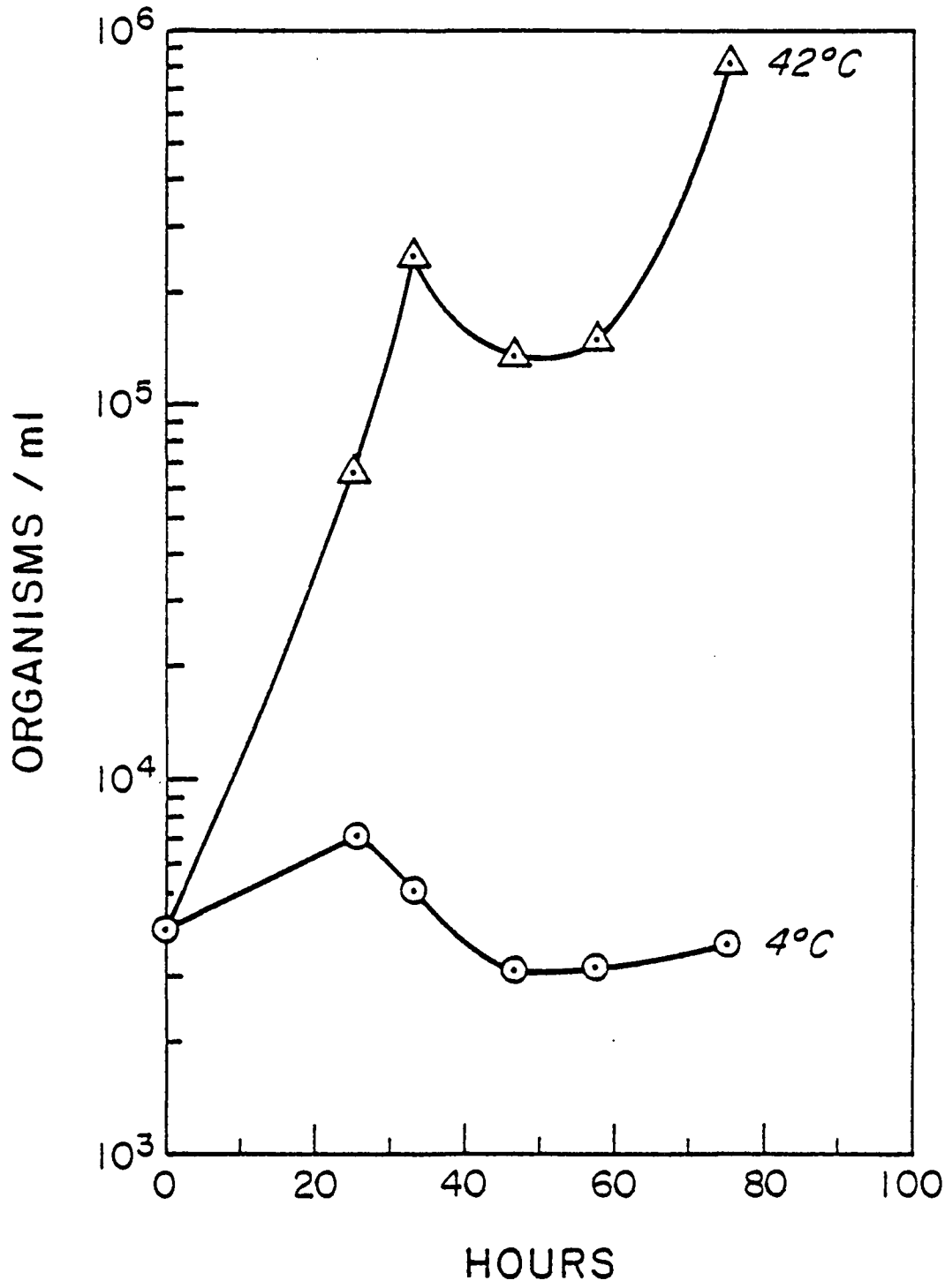

Figure 6. Survival of C. jejuni in turkey homogenate (1:10) held at 4 and 42°C.

raw meat could be exposed to the organism.

Germicidal Studies

The results of the germicidal effect of sanitizers against C. jejuni are given in Tables XI, XII and XIII. Sani-du is a chlorine sanitizer and is normally used at 100 ppm with a 2 min contact time on dairy equipment surfaces. Sani-du was effective against the organism at 100 ppm even for a 30 sec. contact time (Table XI).

Table XI. Germicidal effect of Sani-du at 42.5°C against C. jejuni.

	Concentration					
Contact time	1000 ppm	100 ppm	10 ppm	1 ppm	100 ppb	10 ppb
30 sec	+	+	-	-	-	-
2 min	+	+	+	±	-	-

+ = effective

- = not effective

Dyne is an iodophor. Iodophors are effective against most microorganisms at 25 ppm with a 2 min contact time on all dairy equipment surfaces (Campbell and Marshall, 1975). Dyne was effective against the organism at 10 ppm for both 30 sec and 2 min contact times (Table XII).

Table XII. Germicidal effect of Dyne at 42.5°C against C. jejuni.

Contact time	Concentration						
	1000 ppm	200 ppm	100 ppm	10 ppm	1 ppm	100 ppb	10 ppb
3 sec	+	+	+	+	-	-	-
2 min	+	+	+	+	-	-	-

+ = effective

- = not effective

Ioteat is used at full strength, 100 ppm, to dip the cow's teats in after milking. Ioteat was effective even at 100 ppb (Table XIII).

Table XIII. Germicidal effect of Ioteat at 22°C against C. jejuni.

Contact time	Concentration				
	100 ppm	10 ppm	1 ppm	100 ppb	10 ppb
30 sec	+	+	+	+	-

+ = effective

- = not effective

The results of the germicidal effects of chlorinated water against C. jejuni are given in Table XIV.

Table XIV. Germicidal effect of chlorinated water at 22°C against C. jejuni.

Contact time	Concentration						
	1000 ppm	100 ppm	10 ppm	5 ppm	1 ppm	100 ppb	10 ppb
30 sec	+	+	+	±	-	-	-
2 min	+	+	+	±	-	-	-

+ = effective

- = not effective

The breakpoint for chlorinated water being effective against C. jejuni was 5 ppm. The results of the survival of C. jejuni exposed to 5 ppm chlorine are given in Table XV.

Table XV. Survival of C. jejuni exposed to 5 ppm chlorine at 22°C.

Exposure Time	Initial inoculum cfu/ml	Number recovered cfu/ml	% Survival
30 sec	2.4×10^4	1.7×10^4	30
	9.8×10^5	1.9×10^5	81
2 min	2.4×10^4	1.3×10^4	46
	9.8×10^5	3.8×10^5	61

For both 30 sec and 2 min exposure of C. jejuni to 5 ppm chlorine, an average of more than 50% of the organisms initially inoculated were recovered. The water supply for the city of Corvallis is chlorinated to leave a residue of 1 ppm (Master, 1981). One ppm is not adequate to destroy C. jejuni. Tables and conveyor belts in food processing plants

are often subjected to frequent or continuous rinsing with 5 ppm chlorinated water (Potter, 1978) which is also inadequate to kill C. jejuni.

It is in raw or improperly processed foods that Campylobacter will be a hazard. The survival of C. jejuni at 4°C shows refrigeration is not a recommended means to control C. jejuni. Doyle and Roman (1981) reported the D-value of C. jejuni at 50°C was 3.5-5.4 min and at 55°C it was 0 .75-1.0 min. Present standards for pasteurization are 30 min at 62.8°C or 16 sec at 71.7°C. Milk would be completely free from C. jejuni if it were properly pasteurized. Cooked meats stored above 45°C would not be a health hazard.

Properly sanitized dairy equipment would also pose no health hazard. C. jejuni contamination of water however, could be a health hazard. Higher levels of residual chlorine than are normally present in drinking and food rinsing water are needed if C. jejuni is to be destroyed.

CONCLUSIONS

Campylobacter jejuni is a difficult organism to work with because of its variability. Careful study of the microscopic characteristics and biochemical reactions are necessary in order to recognize the organism when present. The microaerophilic atmosphere must be carefully controlled as exposure to too much oxygen is bacteriostatic. The organism's optimum temperature for growth is 42-45°C. During handling however, the organism appears to be stressed, thus it cannot survive at 42°C but is able to make metabolic repairs at 37°C and subsequently multiply. Incubation for 24-48 h is usually required in order to visibly detect *C. jejuni* because of the organism's long generation time. Within 72 h of incubation however, the organism must be subcultured in order to avoid culture die-off.

Once the needs of the organism are recognized and catered to, it appears isolation of *C. jejuni* would not be difficult as evidenced by the relative ease many clinical laboratories now detect the organism in feces. The difficulty lies in detecting low numbers of *C. jejuni* in food samples. The procedure recommended as a result of this study is to centrifuge the sample to concentrate the organism and separate it from competing organisms. An enrichment-incubation treatment helps the organism multiply.

C. jejuni was not positively identified in raw milk samples studied, however, several suspect samples were obtained. *C. jejuni* can survive refrigerator temperatures and consequently refrigerated raw or underprocessed foods containing the organism are potential health hazards.

Foods with tissue, muscle, or fat decrease the extent of injury to C. jejuni during handling or temperature exposure, thus increasing the risk of causing an infection. In water chlorinated less than 5 ppm a potential public health hazard also exists.

C. jejuni is destroyed at temperatures used for pasteurization and its sensitivity to air and heat can be advantageously used to control its presence in foods. Commonly used sanitizers also effectively destroy the organism.

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