Campylobacter fetus subsp. jejuni: An Old Pathogen of New Concern

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ABSTRACT
For over 60 years subspecies of Campylobacter fetus (formerly Vibrio fetus) have been recognized as agents responsible for a variety of veterinary diseases. Such diseases range from abortion in cattle and sheep to hepatitis in poultry to dysentery in cattle. In rare instances, they have also been known to cause disease in humans. However, within the last 3 years, with the advent of microbiological methods that can selectively isolate campylobacters from human fecal specimens, C. fetus subsp. jejuni has become a disease-agent of serious concern. Clinical laboratories from throughout the world are now reporting that C. fetus subsp. jejuni is one of the most common bacterial causes of acute gastroenteritis in both children and adults. Its frequency of isolation is comparable to and in many studies exceeds that at which Salmonella is isolated from diarrheal stools of hospitalized patients. Although the source of the organism could not be identified for many of these cases, food and water have been implicated as being important vehicles for transmitting campylobacters to susceptible individuals.

Five years ago subspecies of Campylobacter fetus were rarely associated with human infection. Today C. fetus subsp. jejuni is recognized as one of the most common causes of acute bacterial gastroenteritis in humans. This sudden rise in prominence has generated a number of questions regarding the ecology, disease potential, and cultural characteristics of the responsible organism. Since C. fetus subsp. jejuni has been implicated as an agent of foodborne disease, it is important that individuals involved with production, processing or preparation of food be aware of the foodborne disease potential of this organism. Hence, the purpose of this paper is to review what is currently known about C. fetus subsp. jejuni and other subspecies in the C. fetus group, placing emphasis on those aspects that are relevant to the concerns of individuals interested in the microbiological safety of foods.

HISTORICAL PERSPECTIVE
The disease potential of microaerophilic vibrios (C. fetus subsp.) was first recognized by McFadyean and Stockman (66), who in 1913 reported that such organisms were associated with abortion in cattle and sheep. Their observations were later confirmed by Smith (91), who isolated similar organisms from aborted bovine fetuses. Smith and Taylor (92) subsequently characterized these organisms and named them Vibrio fetus.

Since then microaerophilic vibrios have been associated with a variety of diseases. In 1931, Jones and Little (47,48) associated these organisms with winter dysentery in cattle. To definitively prove that such organisms caused dysentery in cattle, Jones et al. (49) reproduced the disease in healthy cattle after feeding them a pure culture of vibrios that had been isolated from diseased animals. These investigators judged that the jejenum was the first site in the intestinal tract to be infected; hence, they proposed that these vibrios be designated Vibrio jejuni.

In 1944, Doyle (31) isolated a microaerophilic vibrio in apparently pure culture from the mucosa of the colon of hogs with dysentery. After feeding this culture to 8 pigs, 6 developed diarrhea. Doyle therefore proposed that swine dysentery was caused by microaerophilic vibrios which he subsequently named Vibrio coli (32). Doyle’s observations have since been confirmed by some investigators (46,79,104) while others have been unsuccessful in obtaining a diarrheal response in healthy swine challenged with strains of microaerophilic vibrios isolated from pigs with dysentery (1,2,10,26,27,99). More recently Fernie et al. (34) have suggested that a spirochete, Treponema hyodysenteriae, must be present in combination with V. coli for swine to develop dysentery. Currently, the role that microaerophilic vibrios have in causing swine dysentery remains unresolved.

The first putative cases of human infection due to microaerophilic vibrios were reported by Levy in 1946 (64). The infections occurred in two Illinois penal institutions where 357 of 6019 inmates were afflicted with acute gastroenteritis. “Vibrio-like” organisms were observed in 31 of 73 stool specimens that were examined; however, the organisms were not fully characterized before being lost during subculture. Raw milk was suspected as being the source of the outbreak. A local dairy that supplied skim milk to the institutions was...
inspected and was found to have inadequate control over the distribution of its milk. In handling both raw and pasteurized milk, the dairy used identical 10-gal. cans and did not label either to distinguish the milk that had been processed from that which was not. Both raw and pasteurized milk were stored side by side in the same cooler; hence, raw milk may have inadvertently been distributed to the penal institutions in lieu of pasteurized milk.

The first case of human infection that was definitively attributed to a microaerophilic vibrio was reported by Vinzent et al. (108) in 1947. These investigators isolated "V. fetus" from two blood cultures from a pregnant woman who was ill with influenza-like symptoms that included fever and headache. After five weeks of illness she prematurely delivered a stillborn infant, and a placenta that was infected with "V. fetus." Approximately 10 years later microaerophilic vibrios were associated with yet another disease, hepatitis in poultry. This disease is characterized by low mortality and high morbidity with affected birds acting listless and producing fewer eggs than healthy birds. Hofstad (44) was the first investigator to associate vibrios with hepatitis in poultry when, in 1956, he reported isolation of a vibrio from the liver of diseased adult chickens. The livers of these chickens were studded with hemorrhages ranging from 2 to 3 mm in size. Two years later Peckham (73) substantiated the fact that a microaerophilic vibrio was the causative agent of this disease by reproducing hepatitis in healthy chickens after challenging them with a vibrio that was originally isolated from the livers of diseased birds. He then proposed that this disease be designated avian vibronic hepatitis.

In 1957, King (55) reported the results of a study that was designed to compare the characteristics of microaerophilic vibrios that were obtained from different sources. She observed that two distinct groups of vibrios were present among several isolates that were obtained from blood cultures from infected humans. The characteristics of one group corresponded with the then existing descriptions of V. fetus. Characteristics of the second group were very similar to those of V. fetus except that their optimum temperature for growth was much higher than that of the other isolates. Hence, King designated this group of organisms as "related" vibrios. Interestingly, she noted that all of the organisms in the group of "related" vibrios were isolated from blood cultures of patients with gastroenteritis and astutely speculated that these organisms were a more common cause of gastroenteritis than was recognized at that time (55,56).

Very little could be done to confirm or disprove King's hypothesis since there was no method available to isolate "related" vibrios from stool specimens of patients with gastroenteritis. However, the first major breakthrough needed to confirm King's theory came in 1972 when Dekeyser et al. (28) developed a procedure to selectively isolate microaerophilic vibrios from stool specimens. This technique involved filtering suspensions of stools through a 0.65-μm membrane filter and inoculating the filtrate onto an agar plate. Since microaerophilic vibrios are quite slender, i.e., 0.2-0.4 μm wide, and most other fecal bacteria are larger than 0.65 μm, vibrios can selectively pass through the filter while most other fecal bacteria are retained. Shortly after this technique was developed, Butzler et al. (18) used it to screen the stools of a large number of patients with diarrhea to assess the occurrence of campylobacters in this specific population of patients. Of 800 children and 100 adults with diarrhea, C. fetus subsp. jejuni was isolated from the stools of 5.2 and 4.0% of each population, respectively. The organism was isolated from only 1.3% of 1000 individuals who did not have diarrhea and served as a control group.

Although these findings were noteworthy, no other reports concerning the enteropathogenic potential of C. fetus subsp. jejuni appeared in the literature until 1977 when Skirrow (85) reported the results of a study that he had done using a new, more direct method to isolate C. fetus. Rather than using the selective filtration technique of Dekeyser that some investigators feel is too cumbersome, Skirrow developed a selective culture medium onto which stool specimens could be directly inoculated. The medium contained antibiotics that inhibited most fecal organisms but allowed growth of campylobacters. Using this technique, Skirrow assayed fecal specimens from 803 patients with diarrhea and 194 patients who did not have diarrhea. C. fetus subsp. jejuni was isolated from 57 (7.1%) of the individuals who had diarrhea and none was isolated from those patients who did not have diarrhea.

Not only did the study reported by Skirrow add credence to the earlier observations made by Butzler et al. (18) and support the hypothesis of King (55,56), but Skirrow's study appeared to generate a substantial amount of interest within the medical community to determine the disease potential of campylobacters in humans. Hospital laboratories from throughout the world began to implement the methodology necessary to isolate campylobacters from stool specimens and, as will be discussed later, many of these laboratories began isolating C. fetus subsp. jejuni from diarrheal stool specimens at rates comparable to those with which salmonellae were isolated.

**TAXONOMY AND NOMENCLATURE**

C. fetus was originally assigned to the genus *Vibrio* by Smith and Taylor (92) who used the morphology of the organism as their primary criterion for classification. They noted that as the organism grew, comma-shaped, vibrio-like cells predominated over spirilloid ones, particularly in young cultures (92,106). Hence they named the organism *V. fetus*.

In 1963, Sebald and Veron (83) determined the DNA content of several species of *Vibrio* and found that the mol% G + C content for *V. fetus* averaged 34.3 (33-35%)
while that for classical cholera and halophilic species of Vibrio averaged 47.2 (44.50%). It was evident that V. fetus had a significantly different DNA base-pair ratio from that of the classical species of Vibrio. Furthermore, V. fetus had phenotypic differences from other species of Vibrio which included the inability to ferment or oxidize carbohydrates and a requirement for microaerophilic conditions for growth. Most species of Vibrio can ferment carbohydrates and are facultative anaerobes (88,89). Hence, Sebald and Veron (83) concluded that V. fetus should be removed from the genus Vibrio and be reclassified as Campylobacter, with C. fetus being the type species of the genus.

As described by Smibert in Bergey's Manual of Determinative Bacteriology (88), there are three distinct subspecies in the C. fetus group. These are C. fetus subsp. fetus, C. fetus subsp. intestinalis, and C. fetus subsp. jejuni. Several different terminologies have been used to identify organisms that belong to the C. fetus group; hence, to avoid misinterpreting what subspecies may have been used by an investigator, care must be taken to note what nomenclature was used to classify the organism studied. A comparison of some of the different nomenclatures that have been used to classify the C. fetus group is presented in Table 1. The nomenclature proposed by Smibert is most accepted; however, some European investigators continue to use the nomenclature proposed by Veron and Chatelain.

**MICROBIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS**

There are several characteristics that are inherent for all organisms which belong to the C. fetus group. All are gram-negative and morphologically appear as very slender curved-to-spiral shaped rods that are generally from 0.2-0.4 \( \mu m \) wide and 1.5-3.5 \( \mu m \) long. Single cells have one curve or twist and appear vibroid but may also be S-shaped or gull-shaped (89). Cells of old cultures become coccoid (11,69,103), which appears to be a degenerative form as cultures composed mainly of coccoid forms are nonviable (89). The organisms are motile and move with a characteristic corkscrew-like motion that is accomplished by a single polar flagellum which measures two to three times the length of the cell. Some cells may have a polar flagellum at each end of the bacterium (88,89).

Organisms of the C. fetus group need a microaerophilic atmosphere for growth (88). They require oxygen but at a concentration less than that which is present in air. A concentration of 5% oxygen has been determined to be optimal for growth (53), while the presence of 21% oxygen is bacteriostatic (89). Carbon dioxide is also essential for growth of C. fetus, with a concentration of 10% being optimal (53).

The C. fetus group is rather limited in its metabolic capabilities. None of these organisms is able to ferment or oxidize carbohydrates (54,89). Instead they obtain their energy from amino acids or intermediates of the tricarboxylic acid cycle (54). They do not possess lipase
activity and cannot hydrolyze gelatin, urea, casein, ribonucleic acid, deoxyribonucleic acid or esculin (45,88). However, they are strongly catalase-positive and weakly oxidase-positive. In addition, they are capable of reducing nitrates to nitrites but not further. In a semisolid medium, C. fetus subsp. will grow in the presence of 1% bile but not 3.5% sodium chloride (45).

There are several key characteristics that are used to subspeciate organisms in the C. fetus group. These are summarized in Table 2. Important characteristics used to differentiate subsp. jejuni from the other two subspecies include its ability to grow at 42 C but not at 25 C, its sensitivity to nalidixic acid, and its ability to hydrolyze hippurate. Characteristics used to differentiate subsp. fetus from the others include its inability to produce H$_2$S from Albimi Brucella broth plus cysteine as detected by lead acetate strips and its inability to grow in the presence of 1% glycine.

**ECOLOGY AND DISEASE POTENTIAL**

Each subspecies of C. fetus has characteristic reservoirs and is responsible for specific types of disease. C. fetus subsp. fetus is primarily associated with cattle in which it causes abortion and reproductive problems that are characterized by the failure of heifers and cows to become pregnant (21). Its natural habitat is the mucosa of the glans penis, prepuce, and distal portion of the urethra of carrier bulls. These bulls transmit the organism to the reproductive system of cows during sexual contact, resulting in infection of the uterus, cervix, vagina, cervix, uterus and/or oviducts of the cow (21,75). Fortunately subsp. fetus cannot survive or grow in the digestive system of a host so that ingesting the organism with food would not be a health hazard (89).

Unlike subsp. fetus, C. fetus subsp. intestinalis is a recognized human pathogen. Known reservoirs for this organism include infected sheep, cattle and humans. To date, no one has reported isolating subsp. intestinalis from the feces or intestinal tract of a normal healthy host, suggesting that organisms which belong to this subspecies are not part of the normal intestinal flora of man or animals. The organism is known to cause abortion in sheep (30,36,81); it sporadically causes abortion in cattle (22) and is the usual cause of systemic campylobacteriosis in humans (77). As its name implies, subsp. intestinalis can survive and multiply within the intestine of a host (89). Its mode of transmission is not venereal but rather by oral ingestion of contaminated material (105).

Like subsp. intestinalis, C. fetus subsp. jejuni is a human pathogen; however, subsp. jejuni is much more commonly associated with human disease than is subsp. intestinalis. This may be partially attributed to the fact that subsp. jejuni occurs more widely in nature than the other subspecies. Besides being present in infected animals, subsp. jejuni is often part of the normal intestinal flora of young swine (70), cattle (17,89), sheep (86), goats (88,89), poultry (37,87) and several species of wild birds (87,111). As these animals mature, smaller numbers of subsp. jejuni are generally present as part of their intestinal flora (89). Pets, including dogs (4,5,42,68) and cats (5,95), have also been identified as sources of subsp. jejuni.

Several studies have recently been done to determine what portion of different animal populations harbor subsp. jejuni as part of their normal intestinal flora. Sampling the intestinal contents of 300 apparently normal pigs, investigators in the Netherlands recovered subsp. jejuni from 182 or 61% of the animals (70). Similar studies have been done with chickens. British investigators have found that the fecal contents of 14 to 91% of the chickens assayed were positive for subsp. jejuni (15,78,84). A study of broiler chickens from a live poultry market in New York City revealed that 38 of 46 chickens (83%) harbored subsp. jejuni in their cecal flora (37). In addition to being a part of the fecal flora of chickens, Smith and Muldoon (90) have demonstrated that subsp. jejuni can also be isolated from the meat of chickens. From 165 samples of chicken meat that were obtained from local retail stores, three strains of C. fetus subsp. jejuni were recovered. Bruce et al. (15) substantiated these findings by isolating subsp. jejuni from 39 of 63 carcasses of chickens.

Domesticated animals are not the only reservoirs for this organism. A study recently done in Colorado revealed that migratory waterfowl commonly shed subsp. jejuni in their feces. For this study, the fecal contents of 445 wild ducks were assayed and of them 156 (35%) were

**TABLE 2. Characteristics of C. fetus subspecies (45)**

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>H$_2$S (TSI)</th>
<th>H$_2$S (acetate strips)</th>
<th>25 C</th>
<th>42 C</th>
<th>Nalidixic acid</th>
<th>Growth in 1% glycine</th>
<th>Hippurate hydrolysis (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>fetus</td>
<td></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>intestinalis</td>
<td>-</td>
<td>+a</td>
<td>+</td>
<td>-</td>
<td>R</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>jejuni</td>
<td>-</td>
<td>+b</td>
<td>-</td>
<td>+</td>
<td>S</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

aNegative test or no growth.

bPositive test or growth.

cResistant.

dSensitive.
positive for *C. fetus* subsp. *jejuni*. Since subsp. *jejuni* may be commonly shed in the feces of such animals, one would wonder if water contaminated with such feces could serve as a vehicle for transmitting the organism. To answer this question Knoll et al. (58) assayed 84 samples of water for the presence of *C. fetus* subsp. *jejuni* and found the organism to be present in 7 of 34 samples of seawater and 37 of 50 samples of fresh water. Blaser et al. (6) subsequently evaluated the ability of subsp. *jejuni* to survive in stream water. They found that at 25 C all of 10^7 organisms/ml died within 4 days; however, organisms present in water maintained at 4 C survived for approximately 1-4.5 weeks. Hence, water can serve as a vehicle for transmitting *C. fetus* subsp. *jejuni* and, as will be discussed later, has been implicated as a medium for conveying campylobacter infections (100).

Diseases produced by *C. fetus* subsp. *jejuni* include abortion in sheep (30,89); hepatitis in poultry (44,73); acute gastroenteritis in humans (18,28,85), monkeys (101,102), cats (5,95) and dogs (5,42,68); urinary infections in humans (25) and dysentery in cattle (47-49). Like subsp. *intestinalis*, subsp. *jejuni* can survive and grow in the intestinal tract of its host and transmission of the organism is by the oral route (89).

**HUMAN DISEASES**

Two distinct human illnesses are attributed to two subspecies within the *C. fetus* group. These diseases are systemic campylobacteriosis and campylobacter enteritis (77).

Fortunately, systemic campylobacteriosis is not a common illness for humans. From 1947, which was the year that the first proven human case of systemic campylobacteriosis was reported, through early 1979 less than 150 cases of nonenteric human infection due to *C. fetus* subsp. have been documented (13,77). *C. fetus* subsp. *intestinalis* is primarily responsible for this syndrome, having been isolated from most patients afflicted with this illness. *C. fetus* subsp. *jejuni* was responsible for the other incidents.

The most common clinical manifestation of systemic campylobacteriosis is bacteremia without localized infection (12,39,50,56); however, several cases have been reported in which the organism localized and produced infections in specific tissues of the host. Examples of such localized infections include endocarditis (20,53,63,65), meningitis (23,33,38,40), septic arthritis (57,59), thrombophlebitis (50,56,107), salpingitis (14) and abscesses of the lung (62) and chest wall (61). Symptoms commonly expressed by individuals afflicted with systemic campylobacteriosis include fever, malaise, headache, confusion, lethargy and abdominal pain (77). Diarrhea occurs in only about 35% of the cases, which may explain why subsp. *intestinalis* is seldom isolated from diarrheal stool specimens. When subsp. *intestinalis* is identified as the causative agent of human infection, it is usually isolated from the patient’s blood.

Systemic campylobacteriosis is primarily associated with adults with most cases having occurred in individuals between 35 and 70 years of age. Most of these individuals had one or more underlying major medical conditions such as alcoholism or cirrhosis, diabetes mellitus, rheumatic heart disease, leukemia or tuberculosis before infection. Interestingly, there appears to be a predilection towards males as most cases (69% of 102 infections) have occurred in males (77). Consumption of foods, including raw beef (20), raw beef liver (93) and raw milk (110), has been associated with three individual incidents of systemic campylobacteriosis.

In contrast to the infrequent incidence of systemic campylobacteriosis, campylobacter enteritis appears to be a prominent human disease. Within the last 3 years, reports from Belgium (18), England (15,24.72,85,96), Scotland (98), Africa (13,29), Australia (19,94), Canada (71) and the United States (7,82) have documented that Campylobacter is associated with 4.2 to 34% of the cases of acute gastroenteritis occurring in children and/or adults. Results of such studies are summarized in Table 3. Interestingly, many of these studies have revealed that *C. fetus* is the most frequently isolated bacterial pathogen associated with gastroenteritis, with salmonellae being a close second. *C. fetus* subsp. *jejuni* is responsible for more than 99% of the cases of campylobacter enteritis. Only on rare occasions has subsp. *intestinalis* been identified as a cause of enteritis.

Based on circumstantial evidence, Skirrow (85) has estimated that the incubation period of campylobacter enteritis ranges from 2 to 11 days. Other investigators (4,51,76) suggest that a typical incubation period is 2 to 5 days.

The major clinical manifestations are abdominal pain, diarrhea and fever (77). Diarrhea usually occurs at the onset of illness or may develop within a few days after the onset of abdominal pain and fever. Typically, the diarrhea is mild to moderate but may be profuse, watery and frequent. After 1 to 3 days of diarrhea, blood may also appear in the stools. Other symptoms that may be expressed include malaise, headache, musculoskeletal pain, rigors and delirium (4,51,52,85). Although vomiting may occur, it is not common and is normally observed in less than 30% of the cases. The severity of the illness is quite variable but in most cases it is brief and self-limited (51,77). At least one report indicates that males are more commonly afflicted than are females with a male to female ratio of 3:2 (52).

Both food and water have been implicated as vehicles responsible for campylobacter enteritis, thus confirming that oral ingestion of organisms is an important route for transmission of the disease. Contaminated drinking water was responsible for a large outbreak that occurred in Bennington, Vermont in June, 1978 (100,109). More than 2,000 of the town’s 10,000 residents were afflicted with acute gastroenteritis. The town’s water supply was determined to be the source of the organism. The outbreak occurred during a time when the town was constructing new facilities for its water supply. During
TABLE 3. Rates at which C. fetus subsp. jejuni has been isolated from stools of patients with and without diarrhea.

<table>
<thead>
<tr>
<th>Location</th>
<th>Type of patient</th>
<th>No. of patients</th>
<th>No. of positive cultures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brussels (18)</td>
<td>Hospitalized children with diarrhea</td>
<td>800</td>
<td>41 (5.1%)</td>
</tr>
<tr>
<td></td>
<td>Hospitalized adults with diarrhea</td>
<td>100</td>
<td>44 (4.0%)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic, all ages</td>
<td>1000</td>
<td>13 (1.3%)</td>
</tr>
<tr>
<td>Worcester, England (85)</td>
<td>Children and adults with diarrhea</td>
<td>803</td>
<td>57 (7.1%)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic controls</td>
<td>194</td>
<td>0</td>
</tr>
<tr>
<td>Manchester, England (24)</td>
<td>Sporadic cases of diarrhea</td>
<td>182</td>
<td>14 (7.7%)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic controls</td>
<td>60</td>
<td>11 (1.6%)</td>
</tr>
<tr>
<td>Surrey, England (96)</td>
<td>Patients with diarrhea</td>
<td>330</td>
<td>19 (5.8%)</td>
</tr>
<tr>
<td></td>
<td>Patients without diarrhea</td>
<td>120</td>
<td>10 (8.3%)</td>
</tr>
<tr>
<td>Edinburgh, Scotland (98)</td>
<td>Patients with diarrhea</td>
<td>196</td>
<td>17 (8.7%)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic controls</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Hereford, England (15)</td>
<td>Patients with acute gastroenteritis</td>
<td>280</td>
<td>39 (13.9%)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic controls</td>
<td>156</td>
<td>10 (6.4%)</td>
</tr>
<tr>
<td>Rwanda, Africa (29)</td>
<td>Patients with diarrhea</td>
<td>204</td>
<td>22 (10.8%)</td>
</tr>
<tr>
<td></td>
<td>Patients without diarrhea</td>
<td>58</td>
<td>0</td>
</tr>
<tr>
<td>Denver (7)</td>
<td>Patients with diarrhea</td>
<td>532</td>
<td>27 (5.1%)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic controls</td>
<td>81</td>
<td>0</td>
</tr>
<tr>
<td>South Australia (94)</td>
<td>Patients with infectious diarrhea</td>
<td>224</td>
<td>13 (5.8%)</td>
</tr>
<tr>
<td></td>
<td>Asymptomatic controls</td>
<td>530</td>
<td>0</td>
</tr>
<tr>
<td>Australia (19)</td>
<td>Patients with diarrhea, all ages</td>
<td>NR</td>
<td>55 (13.9%)</td>
</tr>
<tr>
<td>Southampton, England (72)</td>
<td>Adults and children with acute gastroenteritis</td>
<td>860</td>
<td>36 (4.2%)</td>
</tr>
<tr>
<td>Montreal (71)</td>
<td>Children with diarrhea</td>
<td>1004</td>
<td>43 (4.3%)</td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>176</td>
<td>0</td>
</tr>
<tr>
<td>Iowa (82)</td>
<td>Patients with diarrhea</td>
<td>238</td>
<td>11 (4.6%)</td>
</tr>
<tr>
<td>Soweto, South Africa (I3)</td>
<td>Children with diarrhea</td>
<td>50</td>
<td>17 (34.0%)</td>
</tr>
<tr>
<td></td>
<td>Children without diarrhea</td>
<td>48</td>
<td>6 (12.5%)</td>
</tr>
</tbody>
</table>

aNR—not reported.
bOne isolate was C. fetus subsp. intestinalis.

despite this time, unchlorinated surface water was used to supplement the town's main supply of water when the water pressure was too low. The source from which the water was contaminated was never definitively identified; however, it was speculated that campylobacters may have entered the water supply through fecal material shed from wild or domesticated animals or by equipment that was used to make repairs on the town water supply. Apparently the town of Bennington used the same equipment to repair its sewage system as was used to maintain the system for supplying drinking water. Health officials have speculated that cross-contamination from this equipment may have been a factor in spreading the disease.

Besides this major outbreak, several other recent incidents of campylobacter enteritis have been associated with ingestion of raw milk, undercooked chicken and pork. Robinson et al. (80) reported two outbreaks in England that were traced to consumption of unpasteurized milk. A total of 77 people were involved. Examination of rectal swabs from the cattle concerned and milk socks yielded strains of thermophilic campylobacter that were indistinguishable from those strains isolated from the patients. In California, three cases of campylobacter enteritis were associated with consumption of commercially available certified raw milk (97). In yet another incident, three members of a family in Colorado developed gastroenteritis following ingestion of raw milk from their cow. Stool cultures from all three patients and the cow yielded C. fetus subsp. jejuni (8).

Campylobacter enteritis has also been associated with consumption of chicken. In Iowa, five individuals involved in two separate incidents developed gastroenteritis after consuming undercooked barbecued chicken (82). C. fetus subsp. jejuni was isolated from the stools of all five patients. Although none of the suspect chickens was available for culture, C. fetus subsp. jejuni was isolated from dressed, refrigerated chickens that were obtained from the distributor who supplied the chickens involved in one of the incidents. Chicken was also implicated as being indirectly responsible for an outbreak that involved 5 of 29 guests at a wedding reception (43). Although campylobacter was isolated from the skin but not from the meat of uncooked chicken obtained from the wholesaler who supplied the chicken for the reception, investigators concluded that cooking should kill campylobacters so that the chicken itself was not the final vehicle for infection. This was supported by the fact that one individual who became ill did not eat any chicken. It was noted that before being cooked, pieces of chicken were skinned on a working surface upon which other cooked meats were later prepared. Hence, it was concluded that the infections resulted from consumption of cooked foods that had been cross-

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contaminated with undercooked chicken.

An interesting episode reported by Peel and McIntosh (74) suggests that luncheon meat prepared from pork was also a vehicle of campylobacter enteritis. A nurse had eaten this meat and the following day developed diarrhea, abdominal pain, vomiting and fever. Her sister had been offered some of the same meat but did not like it after having tasted it so gave her share to their dog. Four days after eating the luncheon meat the dog became ill and collapsed and died after 36 h. Campylobacter sp. was isolated from the feces of both the nurse and the dog. Pork was also suggested as the source of a large outbreak of campylobacter enteritis that recently occurred in Japan (112). Of 2500 school children who were given a lunch of bread, pasteurized cow’s milk, oranges and vinegared pork with vegetables, 800 developed enteritis. Although the origin of what was believed to be food poisoning was not determined, the vinegared pork was thought to be the most likely source.

**PROCEEDURES FOR ISOLATING C. FETUS**

As a consequence of the association that has been made between *C. fetus* subsp. *jejuni* and enteritis in humans, recent efforts toward developing methodology for isolating campylobacters have primarily been focused on recovering *C. fetus* subsp. *jejuni* from stool specimens (7,28,60,85). Two distinct procedures have been developed to selectively isolate subsp. *jejuni* from stools. The first involves the use of a medium that contains antibiotics that are inhibitory to most organisms associated with feces but not to *C. fetus* subsp. *jejuni*. A few drops of stool suspension are directly streaked onto this medium, and the resulting growth should be primarily *C. fetus* subsp. *jejuni*. Skirrow (85) was first to develop and use such a medium. It consists of blood agar plus vancomycin, polymyxin B and trimethoprim. This medium was subsequently modified by Blaser et al. (7), who added amphotericin B and cephalexin to more effectively inhibit growth of normal enteric flora without affecting subsp. *jejuni*. Based on the same principle, a similar type of selective antibiotic medium was also developed by Lauwers et al. (60). However, their selection of antibiotics was different and included bacitracin, novobiocin, actidione, colistin and cephalexin. Although cephalexin is not inhibitory to most strains of subsp. *jejuni*, it is restrictive to growth of subsp. *intestinalis*. Hence media containing cephalexin should not be used to isolate subsp. *intestinalis*.

A second approach which may be taken to isolate subspecies of *C. fetus* from stools is based on filtration. As was mentioned earlier, this technique was originally developed by Dekeyser et al. (29) and involves filtering an extract from a stool specimen through a 0.65-µm filter. The slender, 0.2-0.4-µm campylobacters pass through the filter while most other enteric microorganisms are retained. The stool specimen is prepared for filtration by suspending it in a liquid medium, centrifuging this suspension at 600 × g for 10 min to remove large particles, and collecting the supernatant fluid. The supernatant fluid is subsequently passed through three membrane filters, i.e., 8.0, 1.2, and 0.65 µm, and 4 to 6 drops of the filtrate are seeded onto blood or chocolate agar plates (37).

Since subspecies of *C. fetus* are microaerophilic and grow best in an atmosphere which contains 5% oxygen and 10% carbon dioxide (53,89), all agar plates should be incubated in such an environment. This may be accomplished by placing the plates in an anaerobe jar and replacing the atmosphere with a mixture of air containing 5% oxygen, 10% carbon dioxide and 85% nitrogen. Alternatively, such an atmosphere can be achieved by evacuating two thirds of the air from the anaerobe jar and replacing the evacuated air with a mixture of carbon dioxide and nitrogen. For recovery of *C. fetus* subsp. *jejuni*, jars containing plates should be incubated at 42°C for 24-48h as this is the bacterium’s optimal temperature for growth. Investigators interested in isolating subsp. *intestinalis* should incubate jars at 37°C as this organism will not normally grow at 42°C.

On primary isolation, subsp. *jejuni* may form two types of colonies. One is flat, grayish and translucent with an irregular edge. It spreads along the direction of the streak and tends to swarm and coalesce. The other is round, 1-2 mm in diameter, raised, convex, entire, smooth and glistening. It has a brownish center with a translucent edge. On blood agar, both types of colonies are nonhemolytic (89).

It should be realized that when *C. fetus* subsp. *jejuni* is present in feces, it is usually present in very large numbers. Grant et al. (37) determined that the number of subsp. *jejuni* present in the cecal contents of five apparently normal, freshly slaughtered chickens ranged from 5.6 × 10^4 to 1.2 × 10^7/g of rectal content with an average number of 4.4 × 10^6/g. Diarrheal stool specimens from humans infected with subsp. *jejuni* generally contain even larger numbers. Blaser et al. (9) assayed the stools of four patients with acute gastroenteritis and determined that the number of colony-forming units of subsp. *jejuni* per gram of stool ranged from 8.4 × 10^6 to 1.1 × 10^9. Campylobacters are not likely to be present in such large numbers in foods. Hence, before methodology that has been developed for isolating *C. fetus* subsp. *jejuni* from stools is applied to foods, studies are needed to determine how effective these methods are in recovering low numbers of subsp. *jejuni* in the presence of the indigenous flora of different foods. These studies may reveal that to effectively recover low numbers and injured cells of subsp. *jejuni* from foods, it is necessary to develop and use a pre-enrichment procedure before samples are plated onto a selective medium.

**CONCLUDING REMARKS**

Although *C. fetus* subsp. *jejuni* has been a recognized pathogen for many years, its prevalence as an agent of human disease has only recently been realized. To date, relatively little is known about subsp. *jejuni* as it relates to foods. We know what types of foods have been associated with individual cases of campylobacteriosis.
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but we don’t know what the incidence is of C. fetus in our food supply in general. We know very little about what effect various food processes or methods of food preservation have on the ability of campylobacters to survive or possibly grow in foods. We don’t know what treatments can effectively inactivate campylobacters when they are present in foods. We don’t know if all strains of C. fetus subsp. jejuni are equally pathogenic or how many organisms must be ingested to make an individual sick. Hence there are many questions that need to be answered and much to be learned about C. fetus subsp. jejuni before its significance as a foodborne pathogen can be fully and accurately assessed.

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REFERENCES


Karmali, M.A., and King, E.


King, E.

Kutner, L. J., and W. D. Arnold.


Loeb, H., McFadyean, J., and Mohanty, Nayar, G.


Campylobacter jejuni. n. sp.) associated with intestinal disorders of cows and calves. J. Exp. Med. 53:853-863.

Diarrhea (winter scours) in cattle. J. Exp. Med. 53:835-843.


suspension/recovery media. However, data do suggest that the mechanism of action of potassium sorbate and sodium benzoate to synergistically contribute to heat inactivation may be different from that associated with preventing germination and/or outgrowth of conidia. Also, data suggest that the mechanism of action of the two preservatives may differ. In addition to reports indicating that potassium sorbate (sorbic acid) inhibits certain enzyme activities in microbial cells (14, 15, 16), the chemical has also been shown to cause a decrease in the ATP content of conidia of _A. parasiticus_ (11).

In the present study, the increased sensitivity of molds to low concentrations of preservatives during heat treatment may be a result of increased permeability due to expansion of the cell wall and cytoplasmic membrane at elevated temperature. That is, the preservatives might more easily enter cells as the temperature is increased simply due to a weakening of the physical barriers inherent in the cell wall and membrane. Tween 80 may also have influenced the susceptibility of conidia to thermal inactivation. Ease of transport or entry of sorbic and benzoic acids into cells of molds at various temperatures may be influenced by differences in their structural configuration, thus also contributing to the relative effectiveness of the two preservatives at various temperatures.

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REFERENCES


Doyle, con't. from p. 488