Methods for Recovery of Campylobacter jejuni from Foods

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ABSTRACT

The triangular relationship between Campylobacter jejuni, foods and disease in humans has been well-documented. Many studies have revealed that C. jejuni causes at least as many cases of human gastroenteritis as does Salmonella sp. Foods are an important vehicle in human infection, and raw milk is most frequently implicated. Other animal products also serve as potential sources of infection. C. jejuni has been found on the carcasses of poultry and other domestic animals throughout the world. The organism is microaerophilic and various methods for establishing appropriate growth conditions, such as the Fortner principle, atmosphere replacement and adding of supplements to encourage growth of C. jejuni, are available. Methods developed for use in clinical laboratories lack the necessary sensitivity and selectivity, and therefore have limited use in detecting small numbers of C. jejuni in foods. In one enrichment method for detecting C. jejuni in foods, washings are filtered and centrifuged, the sediment is placed in the enrichment broth and the suspension is incubated under a constant gas flow at reduced oxygen levels. Following incubation enrichment broth is filtered and plated onto selective media. In another recently developed method, food samples are directly added to an enrichment broth with antibiotics and incubated under a microaerobic atmosphere before selective plating. Butzler’s, Skirrow’s and Campy-BAP selective media use several antibiotics to which C. jejuni is resistant. The plates are supplemented with horse or sheep blood, depending upon the specific formulation. The optimum temperature for growth of C. jejuni, about 42°C, may also be used for selection. It is now possible to recover 0.1 to 1 cell of C. jejuni per 10 to 25 g of food sample from among 10^6 to 10^8 indigenous bacteria. After a characteristic colony is isolated, the key criteria for presumptive identification of C. jejuni by phase-contrast microscopy are darting, corkscrew motion and a comma to spiral shape.

The bacterium Campylobacter jejuni has only recently achieved recognition as an important pathogen of gastroenteritis in humans. C. jejuni is recovered from human diarrheal specimens at a rate that depends on both the awareness of the investigators and the methods employed. This is borne out by following the increase in number of cases of Campylobacter enteritis reported to the communicable Disease surveillance Centre in Great Britain (1) (Fig. 1). Observations confirming C. jejuni as an important agent of gastroenteritis have been well-documented. In one study involving diarrhetic children from Montreal, C. jejuni was isolated from fecal specimens at a rate of 4.3%, Salmonella at 5.1%, Yersinia enterocolitica at 2.8% and Shigella at 1.4% (30). In Sweden, when stool samples from about 2,550 patients with gastrointestinal infections were cultured for bacterial pathogens, Campylobacter was recovered from 277 samples and Salmonella from 183 (48). As the true incidence of Campylobacter infection of humans is becoming understood, the general assessment is that the pathogen is at least as prevalent as Salmonella in patients with gastroenteritis.

Note that the following names have been used in the past for this organism and represent a close estimation of the same bacteria: C. jejuni/coli (54), C. fetus sp. jejuni (41), "related" vibrios (21), and Vibrio fetus (44). A detailed review of the taxonomy and nomenclature for this organism is presented by Doyle (10) in his review of the genus. The name C. jejuni will be used in this paper.

Figure 1. Annual reports of gastrointestinal pathogens over a four-year period. With increasing awareness of Campylobacter sp., the number of reports approach those attributed to Salmonella sp. (1).
FOODBORNE GASTROENTERITIS

The increased awareness of the presence of \textit{C. jejuni} in patients with gastroenteritis has led to attempts to recover the pathogen from incriminated foods. The organism so far has been associated with pork, ground beef, chicken and milk. Unpasteurized milk is the most frequently implicated vehicle of \textit{Campylobacter} infection. \textit{Campylobacter} enteritis was first suspected as being attributed to the consumption of milk in 1946 (24). At two institutions with the same milk supplier, a large outbreak involved 151 individuals. The milk was thought to be the vehicle for two reasons: (a) raw milk could have been shipped by mistake and (b) microscopic examinations of fecal smears from 31 patients yielded almost pure cultures of a vibrio-like microorganism that conformed to characteristics of \textit{C. jejuni}. In addition to this outbreak, numerous other reports have documented the association of \textit{Campylobacter} with unpasteurized milk (3, 34, 36, 50-52). This list is not exhaustive and, indeed, Butzler and Skirrow (7) mention that consumption of milk was implicated in five major \textit{Campylobacter} outbreaks in Great Britain during a 6-month period.

Consumption of cake, particularly the icing, was associated with an outbreak of \textit{Campylobacter} enteritis as reported by Blaser et al. (4). In a report from the Netherlands (5), an explosive outbreak of \textit{Campylobacter} enteritis occurred among soldiers on a survival exercise. Of 123 cadets given live chickens to prepare for their evening meals, 89 became ill with symptoms of enteritis within the following week. Fecal samples from 104 of the cadets yielded no \textit{Salmonella} or \textit{Shigella}, but 34 samples yielded \textit{Campylobacter}. The authors speculated that improper heating of the chickens left viable pathogens in the food. Raw hamburger has also been implicated as a source of \textit{Campylobacter} enteritis in a military camp (28). Other reports further implicate \textit{C. jejuni} as the causative agent in foodborne enteritis, and only very small numbers (500 cells) are needed to effect a gastroenteritis response in humans (35).

ASSOCIATION WITH FOODS

Smith and Muldoon (43) were the first to report the incidence of \textit{C. jejuni} from commercially processed poultry. Using comparatively rudimentary methods, they recovered three isolates from 165 poultry meat samples purchased from local retail stores. Subsequently, Simmons and Gibbs reported recovery rates of 48% for processed chickens and 92% for turkeys (38). Studies from New York (13), Denver (25), Sweden (27) and Ohio and Ontario (32) have reported incidences of 22 to 92% of \textit{C. jejuni} on retail market poultry meat. Soaking turkey carcasses overnight in 340 ppm chlorine wash water did not decrease the number of positive carcasses (25).

Like \textit{Salmonella}, \textit{C. jejuni} may also be isolated from red meats, although recovery rates are lower than those for poultry meats. \textit{C. jejuni} has been isolated from lamb carcasses (45) and from eviscerated pork, lamb and beef carcasses (46). Using direct plating onto a selective medium, Stern recovered \textit{Campylobacter} from unwashed carcasses of pig, lamb and beef at rates of 38, 24 and 2%, respectively. Stern suggested that the same abuses in handling and preparation of meats that result in meatborne outbreaks of salmonellosis may also account for \textit{Campylobacter} infections. Hudson and Roberts (17) did not find \textit{C. jejuni} on beef or lamb carcasses but did find it on 59% of the pig carcasses they examined. Turnbull and Rose (53) reported that 1.6% of meat samples from both abattoirs and retail outlets were positive for \textit{Campylobacter}. Investigations by Kaijser and Svedhem (19) indicate that domestic animals are the most probable source of \textit{Campylobacter} involved in human gastroenteritis, and that the organism is capable of surviving for several days in dairy or meat products stored at 4°C. With these reports on the consistent presence of \textit{Campylobacter} in foods come the recognition that the same measures employed for prevention of salmonellosis should also be applied to control gastroenteritis caused by campylobacters.

ISOLATION METHODS

Microaerobic requirements

\textit{C. jejuni} is a strict microaerophile (42), and isolation methods must attend to this requirement. Various methods have been used to create appropriate conditions for growth. Karmali and Fleming (20) developed a method in which the Forner principle is used to isolate \textit{Campylobacter} from stools. A rapidly growing \textit{Proteus} sp. is streaked onto half of a blood agar plate to reduce the oxygen tension in a closed system, thus making growth of \textit{Campylobacter} possible on the other half. A method for obtaining a closed environment containing 5% oxygen is described in the \textit{Anaerobe Laboratory Manual} (16). Luechtefeld et al. (26) found that isolation rates of \textit{Campylobacter} grown in a closed container with 5% oxygen were superior to those grown in a candle jar. In another study comparing the CampyPak II gas generator systems and 5% oxygen in a closed container, no difference in isolation rates of \textit{C. jejuni} was obtained (6). A method we have used for the past two years is exchanging the atmosphere of the container used to culture the organism with 5% O\textsubscript{2}:10% CO\textsubscript{2}:85% N\textsubscript{2}. Blood agar plates containing the samples are inverted and placed in a modified anaerobe jar, the air is evacuated with a standard laboratory vacuum line, and the gas mixture is introduced into the jar until a positive pressure is detected coming out of the jar. The jar is sealed with a clamp and placed into an appropriate incubator.

A supplement that improves the aerotolerance of \textit{Campylobacter} has been described (12, 14, 15). It was found that specific quantities of ferrous sulfate, sodium metabisulfite and sodium pyruvate (FBP) added to broth and agar increased the oxygen tolerance of \textit{C. jejuni}. It was concluded that these supplementary compounds enhance oxygen tolerance by quenching superoxide anions and hydrogen peroxide that occur spontaneously in the culture medium. FBP has been used to promote growth, and such a supplement may also be useful in an enrichment broth.
TABLE 1. Summary of enrichment procedures used to select for Campylobacter jejuni.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Reference</th>
<th>Selective agents (per liter)</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blaser et al.</td>
<td>2</td>
<td>Vancomycin, 10 mg</td>
<td>33% greater than direct plating</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimethoprim, 5 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polymyxin B, 2,500 IU</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amphotericin, 2 mg</td>
<td></td>
</tr>
<tr>
<td>Chan and Mackenzie</td>
<td>8</td>
<td>a) Same as ref. 2 with 30 mg cephalothin or b) Same as ref. 2, with 50,000 IU polymyxin B</td>
<td>6% greater than direct plating</td>
</tr>
<tr>
<td>Tanner and Bullin</td>
<td>49</td>
<td>Alkaline peptone water (pH 8.4) under reduced atmosphere</td>
<td>Allowed for recovery of 1-10 organisms</td>
</tr>
<tr>
<td>Lander and Gill</td>
<td>22</td>
<td>Vancomycin, 20 mg</td>
<td>Not given</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimethoprim, 5 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polymyxin B, 10,000 IU</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Actidione, 100 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-Fluorouracil, 500 mg</td>
<td></td>
</tr>
<tr>
<td>Park and Stankiewicz</td>
<td>31</td>
<td>Vancomycin, 8 mg</td>
<td>1 cell per 10^5 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimethoprim, 4 mg</td>
<td>10^5 to 10^7 bacteria/g</td>
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<tr>
<td></td>
<td></td>
<td>Cephalothin, 3 mg</td>
<td></td>
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<td></td>
<td></td>
<td>Colistin, 3 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1-2 d under constant flow of reduced atmosphere</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Filtration through filter with 0.65 µm pores</td>
<td></td>
</tr>
<tr>
<td>Doyle and Roman</td>
<td>11</td>
<td>Vancomycin, 15 mg</td>
<td>1 cell per 10^9 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Trimethoprim, 5 mg</td>
<td>among 10^6 to 10^9 bacteria/g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polymyxin B, 20,000 IU</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cyclohexamide, 50 mg</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reduced atmosphere for 18 h</td>
<td></td>
</tr>
</tbody>
</table>

**Enrichment methods**

Several enrichment methods that may be used to select for *C. jejuni* from specimens containing other indigenous flora are described in Table 1. However, most of these methods were developed in clinical laboratories and do not have the sensitivity and selectivity needed for detection of *C. jejuni* in foods. Although fecal specimens from infected individuals usually contain comparatively large numbers of *Campylobacter* (about 10^6 and 10^8/g), these large numbers may not occur in foods. Only 500 cells in a cup of milk have caused an enteric response in a human (35). Direct plating of swabs from inoculated meat has shown that the organism can be recovered from an indigenous flora of about 10^4 cells/cm^2 when present at levels of at least 32 per cm^2 (45). The enrichment methods described below are still in need of comparative studies for sensitivity, selectivity and other practical considerations, such as time, cost and simplicity.

Resistance of *C. jejuni* to several antibiotics is frequently employed in selective enrichment. The organism is most often resistant to vancomycin, which is active against gram-positive cocci. *C. jejuni* is resistant to polymyxin B, trimethoprim lactate and the cephalosporins. Polymyxin B is inhibitory to *Enterobacteriaceae* and *Pseudomonas* sp. while trimethoprim acts against *Proteus* sp. The cephalosporins are active against *Streptococcus faecalis*, *Enterobacter* sp., *Serratia* sp., *Pseudomonas aeruginosa*, some *Proteus* sp., *Y. enterocolitica* and *Bacteroides fragilis*. Amphotericin B and cycloheximide (actidione) are used to inhibit yeasts and fungi.

Blaser et al. (2) described a Campy-thio enrichment broth (thioglycollate broth plus 0.16% agar and 10 mg of vancomycin, 5 mg of trimethoprim, 2500 IU of polymyxin B and 2 mg of amphotericin B per liter) that allowed for 33% greater recovery of *C. jejuni* than did the use of selective medium alone. Stool specimens are inoculated into Campythio and held in the refrigerator for 8 h before plating onto selective agar medium. During this enrichment, the low temperature inhibits the growth of *Campylobacter* but the indigenous bacteria are diminished through the action of the antimicrobials.

A similar enrichment procedure, described by Rosef (37), enhanced the recovery of *Campylobacter* from the gallblad-
der of pigs. The same concentrations of antimicrobials described by Blaser et al. (2) were used, but amphotericin B was omitted. In place of thioglycollate broth and agar, 10 g of peptone, 8 g of "Lab lemco" powder (Oxoid L 29), 1 g of yeast extract, 5 g of NaCl and 16 ml of 0.025% resazurin were used per liter. The gallbladder sample was added to 100 ml of this enrichment medium, and the samples were incubated at 42°C in a microaerobic atmosphere. The enrichment medium was plated after 24 and 48 h of incubation. In this study, C. jejuni was isolated only when enrichment medium was used. Forty-two and 58% of the gallbladders sampled were positive for C. jejuni after 24 and 48 h of enrichment, respectively.

Chan and Mackenzie recently described two enrichment media used to isolate Campylobacter from stools (8). The media contained the same antimicrobials as described by Blaser and co-workers (2) except with (a) 30 mg of cephalothin added per liter or (b) the polymyxin B concentration was increased to 50 IU/ml. A fecal swab was plunged into a small vial containing the enrichment medium, and the sample was incubated overnight at 42°C under a normal atmosphere. The swab was subcultured onto a selective plate. Using this method, the isolation rate of C. jejuni was increased by 6% over direct plating.

Tanner and Bullin (49) used alkaline peptone water (pH 8.4) as an enrichment broth. They incubated samples at 43°C in a reduced atmosphere of 5% O₂:10% CO₂:85% N₂. Tanner and Bullin found that this procedure enabled them to recover as few as 1 to 10 cells of Campylobacter and allowed the organisms to proliferate in the presence of large numbers of Escherichia coli and S. faecalis. With this broth, fecal samples that were negative by direct plating methods were positive for Campylobacter.

Lander and Gill (22) used a combination of enrichment and direct plating to determine whether C. jejuni could infect a bovine udder. The selective components of their enrichment broth consisted of 40 mg of vancomycin, 20 mg of trimethoprim, 10,000 IU of polymyxin B, 100 mg of actidione, and 500 mg of 5-fluorouracil per liter. Fecal samples enriched in 20 ml of this broth at 37°C for 2 d in air were subcultured onto selective agar media. In a similar enrichment medium, which also contained 1.5% ox bile, Oosterom et al. (29) increased recovery sensitivity, enabling detection of 3 to 10 cells of C. jejuni per g of meat.

In an article on the prevalence of C. jejuni in fresh eviscerated whole market chickens, Park et al. (32) described a method that included an enrichment step before selective plating. Store bought chickens were washed in 250 ml of nutrient broth, and the broth was filtered through cheesecloth. The filtrate was centrifuged and the bacteria in the sediment were resuspended in 5 ml of brucella broth. After part of the suspension was streaked directly onto selective agar media, the remainder was transferred to 100 ml of enrichment broth. The enrichment broth contained 8 mg of vancomycin, 4 mg of trimethoprim, 3 mg of cephalothin, 3 mg of colistin, and 30 ml of calf serum per liter of brucella broth. Since the original publication, this broth has been modified to exclude TRIS buffer, thereby reducing the pH to 7.2 (Park, personal communication). Flasks containing the suspended sample and enrichment broth were incubated at 42°C for 1 to 2 d under a constant flow of 5 to 7 ml of 5% O₂:10% CO₂:85% N₂ per minute. After incubation, 5 ml of enrichment broth were filtered through a 0.65-μm membrane filter, and the filtrate was plated onto two selective media. This enrichment system was capable of recovering 1 cell of C. jejuni per 10 g in the presence of 10⁴ to 10⁹ indigenous organisms per gram of chicken (31). Direct plating procedures yielded a 32% recovery rate, whereas the enrichment method of Park et al. yielded a 62% recovery rate for C. jejuni from store bought chickens (32).

Most recently an enrichment technique has been reported that is capable of recovering 0.1 to 1 cell of C. jejuni per g of food containing as many as 10⁶ to 10⁹ indigenous bacteria (11). The effectiveness of this method has been confirmed by others. Ten or 25 g of food are suspended in 90 or 100 ml, respectively, of an enrichment broth consisting of 7% lysed horse blood, 3 g of sodium succinate, 0.1 g of cysteine hydrochloride, 15 mg of vancomycin, 5 mg of trimethoprim, 20,000 IU of polymyxin B, and 50 mg of cycloheximide per liter of brucella broth. The inoculated broth is held in a flask, and the atmosphere is evacuated and replaced three times with a gas mixture of 5% O₂:10% CO₂:85% N₂. The flask is incubated at 42°C for 16 to 18 h at 100 gyations per minute. After incubation, the enrichment broth is plated onto selective agar plates, and these plates are incubated at 42°C for 48 h under microaerobic conditions. This method is sensitive, comparatively simple and requires less time to recover C. jejuni from foods other than reported methods.

Selective plating

Three selective agar media have emerged as most popular among the plating media developed for isolation of C. jejuni from stools of patients with gastroenteritis. What is known as the "Butzler" formulation evolved from the original work of Dakeyser et al. (9) in Butzler's laboratory. Their isolation technique involved suspending stools in broth, allowing the suspension to settle for 1 h and centrifuging the resultant supernatant fluid at 1,500 x g for 5 min. Four milliliters of the resulting supernatant fluid were filtered through a 0.65-μm Millipore filter. The final 0.3 ml of this filtrate was surface-plated onto selective agar. Their selective medium contained 15% defibrinated sheep blood, 25,000 IU of bacitracin, 10,000 IU of polymyxin B, 5 mg of novobiocin, and 50 mg of actidione per liter of fluid thioglycollate agar medium. The inoculated plates were held at 37°C for 3 d under a reduced atmosphere. This original selective medium enabled workers to isolate C. jejuni from the stools of sick individuals.

This original medium has been modified several times. Recently, Patton et al. (33) described a modified Butzler's formula (23) comprised of 29.8 g of fluid thioglycollate medium, 30 g of agar, 1,000 ml of distilled water, 100 ml of defibrinated sheep blood, 25,000 IU of bacitracin, 5 mg of novobiocin, 50 mg of actidione, 15 mg of cephalothin and 40,000 U of colistin per liter. Of three selective media com-
pared for efficiency in primary isolation of *C. jejuni* from rectal swabs of animals, recovery rates with the modified Butzler formulation were significantly higher than those obtained with Skirrow’s (39) and Butzler’s original formulation (23).

Skirrow’s formulation (39) consists of 5 to 7% lysed horse blood, 10 mg of vancomycin, 2,500 IU of polymyxin B and 5 mg of trimethoprim per liter of either blood agar base no. 2 or brucella agar. Using this selective medium, under a reduced atmosphere at 43°C, Skirrow isolated *C. jejuni* from 7.1% of 803 patients with diarrhea. No filtering is needed before plating with this selective formulation. Blaser et al. (2) modified Skirrow’s formula to produce Campy-BAP, the third of the commonly used selective media for isolating *Campylobacter*. To Skirrow’s formulation, 2 mg of amphotericin B and 15 mg of cephalothin were added per liter. Cephalothin reduced the normal enteric flora contaminants and amphotericin B inhibited the growth of *Candida albicans*.

In a recent study, selective plates of Skirrow’s medium (8) modified with 15 mg of cephalothin per liter, Campy-BAP (3) and Butzler’s (23) agar were tested for their sensitivity and selectivity in the recovery of inoculated *C. jejuni* from ground beef (47). Campy-BAP medium was the most sensitive and Butzler’s medium the most selective. Breakthrough contaminants on these plates were noted and discussed. Stern (47) suggested that a combination of both Campy-BAP and Butzler’s media would be most useful in the recovery of *C. jejuni* from food sources.

**Identification**

Plates should be examined at 24, 48 and 72 h for typical colonies of *C. jejuni*. If none is apparent, the plates should be returned to the incubator and microaerobic conditions should be re-established. Typical colonies of *C. jejuni* on selective blood agar plates will be non-hemolytic flat or slightly raised, have an irregular edge or round, and appear gray to pink or tan mucoid (Fig. 2). If the humidity is high within the incubation chamber or the plates are wet, the organism tends to spread in a characteristic water droplet-like fashion. After 48 h of incubation, the colonies are generally 1 to 2 mm in diameter.

Characteristic colonies should be transferred to a wet-mount slide and observed with a phase-contrast microscope. Details of taxonomic criteria may be found in *Bergey’s Manual of Determinative Bacteriology* (41). The most important criterion to consider in identifying *C. jejuni* is its rapid corkscrew, to-and-fro motion. The organism is narrow (0.2 to 0.5 μm) and appears in comma, S, gull or spiral shapes (Fig. 3). If an isolate has the characteristic shapes and motion, one should further test for lack of growth under microaerobic conditions at 25°C. Further, *C. jejuni* does not grow under aerobic conditions, is resistant to discs of cephalothin (30 μg), and is often susceptible to discs of nalidixic acid (30 μg) with some notable exceptions (40). The organism is capable of growth in 1% glycine, produces both oxidase and catalase, and reduces nitrate to nitrite without further reduction. *C. jejuni* does not grow in 3.5% sodium chloride nor does it ferment glucose. The organism does not produce H₂S in triple sugar iron agar but does pro-
duce H₂S as detected by the presence of a lead acetate strip over a medium containing 0.02% cysteine-HCl. An excellent basal medium for assessing these characteristics is brucella broth containing 0.16% agar (16).

It has been suggested that isolates conforming to the above criteria may further be divided into two separate species, C. jejuni and C. coli (40). C. coli grows at 30.5°C, resists 2, 3, 5-triphenyltetrazolium chloride and does not hydrolyze sodium hippurate (18). This species may be discriminated from C. jejuni, which produces the opposite results as C. coli does for these three tests. In our hands, the hippurate test is important to food microbiologists since both C. jejuni and C. coli are responsible for human infections.

An abbreviated flow chart is given as a procedure useful in isolating C. jejuni from foods (Fig. 4). Some of these methods are new and still need confirmation by other laboratories. By using this protocol, workers in my laboratory have routinely isolated C. jejuni from food sources. The recovery method appears to be no more difficult than the well-established procedures currently in use for other foodborne pathogens. Rapid presumptive tests for use by the food industry and in public health laboratories still needs to be developed.

**Figure 4. Flow chart suggesting procedures for the isolation of Campylobacter jejuni from foods.**

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### References


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5. Incidents caused by rancid milk powder (unpublished)

The incidents which were presumably caused by rancid milk powder involved single cases only. Infants with reconstituted milk powder developed gastrointestinal symptoms. Bacteriological examination of the milk powders revealed that these were not contaminated; on the other hand all the products involved were rancid. It is presumed that the rancid milk powders were responsible for the gastrointestinal syndromes in the infants. Although rancidity is not an accepted cause of diarrhea in these cases the suspicion is strong. Sources: Food Inspection Services, Enschede and Haarlem; also published by S. A. Bouwer-Hertzberger, Food-transmitted disease of microbial origin. Thesis, Utrecht, 1982.

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REFERENCES