WHAT THE SANITARIAN SHOULD KNOW ABOUT *CLOSTRIDIUM PERFRINGENS* FOODBORNE ILLNESS

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(Received for publication August 7, 1969)

ABSTRACT

This paper is a follow-up of two articles, which were previously published in this Journal, concerned with what the sanitarian should know about staphylococci and salmonellae. The nature of *Clostridium perfringens*, including factors that support or limit its growth, is discussed. This organism is widely distributed in the intestinal contents of man and animals, in sewage, and in soil. From these sources foods frequently become contaminated with this organism. Meats and meat products prepared in food-service establishments are frequently involved in outbreaks. To prove that *C. perfringens* is responsible for outbreaks, this organism should be recovered in large numbers from both the patients' stools and the incriminated food, and isolates from both should be correlated serologically. Of the various methods of controlling foodborne diseases—only inhibition of growth is practical for controlling outbreaks of *C. perfringens* foodborne illness. Appropriate control features are delineated.

NATURE OF THE ORGANISM

Clostridium perfringens (*C. welchii*) is a nonmotile, encapsulated, short and thick bacillus with blunt ends. It occurs singly, in pairs, and, less frequently, in short chains. Filamentous cells are sometimes produced. Young cultures are gram-positive, but old cultures may appear gram-negative. Subterminal ovoid spores are produced. Vegetative cells sporulate readily in the intestines, but rarely in cooked meat.

Strains of *C. perfringens* are divided into five toxicological types (A to E) on the basis of four major toxins (*alpha, beta, epsilon, iota*) which may be produced *(58)*. Only types A and C have caused human gastroenteritis. Type A strains also cause gas gangrene. Type C (formerly known as type F) has caused outbreaks of necrotic enteritis in Germany *(73)* and in New Guinea *(43)*.

The only major toxin produced by *C. perfringens* type A is *alpha* toxin, an enzyme (lecithinase C) which attacks lecithin and similar chemical substance to liberate phosphorylcholine. This reaction is seen as an opaque zone of precipitate around a colony on egg yolk agar. *Clostridium perfringens* type A may or may not be hemolytic (possess *theta* toxin which lyses red blood cells); it does, however, form collagenase (destroys collagen in tissues), hyaluronidase (facilitates the spread of the organism through tissues), and desoxyribonuclease (destroys DNA). A few years ago, *C. perfringens* type A was subdivided into gas gangrene strains and food poisoning strains *(34)*. Heat-sensitive organisms that produced large amounts of lecithinase and *theta* toxin were considered as gas gangrene strains. Food poisoning strains were considered those that were heat resistant, produced little lecithinase and no *theta* toxin. Today, however, it is believed that it is neither necessary for a particular strain to possess a specific biochemical characteristic nor for it to be of a certain serotype to produce foodborne illness. Thus, there is no such thing as a "food poisoning *C. perfringens*" per se *(23, 26)*.

Some strains of *C. perfringens* produce heat-resistant spores; others produce heat-sensitive spores. Heat-sensitive strains outnumber heat-resistant strains in most habitats. Although spores of most strains are killed in a few minutes at 212 F, the spores of some strains are extremely heat-resistant and survive boiling for 1 to 6 hr *(6, 22, 30, 34, 44, 51, 69)*.

Clostridium perfringens is rather demanding in its nutritional needs, requiring 13 to 14 amino acids and 5 to 6 growth factors *(9, 17)*. These nutritional needs may play a role in the types of foods associated with foodborne illness. Foods associated with outbreaks caused by this organism are usually high in protein, such as meat or meat dishes.

The optimum temperature for growth of *C. perfringens* is between 109.4 and 116.6 F *(9)*. Growth will occur at 122 but not at 131 F. At 122 F a "Phoenix phenomenon" occurs with initial declines in numbers to minimum counts at 4 hr followed by a sudden increase in growth to maximum counts at 6 hr *(13)*. In beef (pH 5.7-5.8) growth did not commence until 68 F *(6)*. After a long lag period, growth of *C. perfringens* in beef cubes in gravy was observed at 65 F *(21)*. No growth of *C. perfringens* was observed in cooked meat after storage for 7 days at 43.7 F *(70)*.

Spores of *C. perfringens* in frozen meat were resistant to freezing and holding at 23 F or –4 F *(6)*. Destruction was less rapid during storage at –4 F than at 23 F, and a much slower rate of destruction occurred at temperatures of 33.8 to 59 F. In laboratory media, Canada et al. *(12)* recovered an average of from 16 to 58% of spores after freezing at 0 F for 48 hr; in chicken gravy, 4.3 to 38% survived after 90 days, and 3.7 to 11% after 180 days when held at 0 F *(59)*. A considerable proportion of vegetative cells of *C. perfringens* was destroyed by freezing and frozen storage *(6, 12, 59)*. Storage for 48 hr at refrigeration temperatures (45-50 F) also resulted in substantial decreases of both vegetative cells and spores. Other investigators have observed decreases in numbers during refrigerated storage *(61, 71)*.

Clostridium perfringens is an anaerobe, but stringent anaerobic conditions are not required. The organism requires a low oxidation-reduction potential for initiation of growth (cell division). Oxidation-reduction potential is a measure of a system's reducing tendency (to give up or accept electrons). This potential is usually expressed as the Eh value in millivolts (mv). The major effect of an adverse oxidation-reduction potential is on the lag phase. At an Eh value of –45 mv there is a minimal lag in growth, but the lag increases with increasing redox potentials until a point is reached where growth ceases and cells commence to die (+31 to +231 mv). (For comparison purposes, the potential of venous blood is +180mv.) During the lag period, *C. perfringens* cells reduce the Eh of their immediate surroundings until it becomes sufficiently low to permit logarithmic growth. The optimum Eh of culture media is in the vicinity of –200 mv *(50)*. The pH has a marked effect on limiting Eh for growth. Specific Eh values supporting growth of *C. perfringens* may vary with strain, size of inoculum, metabolic state, pH, and the method of determination *(7, 25, 50)*. The ability of *C. perfringens* to grow appears to be governed, almost entirely, by the oxidation-reduction potential of the medium and not by the presence or absence of gaseous oxygen *(55)*.

No growth of *C. perfringens* was observed at or below pH 5 or at or above pH 9 *(17)*. Fairly rapid growth occurred between pH 5.5 and pH 8 *(54)*. Growth of *C. perfringens* in beef stored at 68 F was variable at pH 5.7-5.8, but growth was rapid at 7.2 *(6)*.

Spores were able to germinate and grow in media containing up to 5% NaCl (a_w 0.97) but not in 10% NaCl *(30)*. Slight growth was observed in 8% NaCl *(19)*. Vegetative cells survivied for 6 days on raw meat covered with brine (22% NaCl and 0.9% $NaNO_3$). The inhibitory effect of salt was greater when 1% $NaNO_3$ was present *(29)*. *Clostridium perfringens* can survive and grow in curing salt solutions that are higher than those used in normal curing operations. Growth also occurred in media containing up to 10,000 ppm $NaNO_3$ or 400 ppm $NaNO_2$. *Clostridium perfringens* survived all steps in curing and smoking hams, up to 113 F internal temperature *(19)*. Growth of *C. perfringens* is inhibited by certain other bacteria, particularly enterococci *(57)*.

EPIDEMIOLOGY

Early reports linking *C. perfringens* with food poisoning were made before the turn of the century *(1, 39)*, but the illness was not reported in the United States until 1945 when McClung described four outbreaks of *C. perfringens* foodborne illness *(41)*. This ailment did not receive much attention, however, until the appearance of the classical paper of Hobbs et al. in 1953 *(34)*. Since then, this illness has become recognized as one of the most common foodborne diseases.

In the United States, during 1966-68, *C. perfringens* foodborne illness accounted for 67 reported, bacteriologically confirmed, outbreaks (13.5% of the outbreaks of known etiology). There were also 26 outbreaks that were clinically and epidemiologically similar to *C. perfringens* foodborne illness. During 1966 and 1968 and some previous years, *C. perfringens* was responsible for more reported cases of foodborne diseases than any other agent *(46)*. In England and Wales during 1966, *C. perfringens* was responsible for 33% of the reported general outbreaks but only 2% of all outbreaks when family outbreaks and sporadic cases were included in the totals. This organism, however, accounted for 30% of the total numbers of reported cases of food poisoning *(68)*.

Foods involved in outbreaks of *C. perfringens* foodborne illness are usually meat or poultry that has been boiled, stewed, or lightly roasted; or meat and poultry stews, sauces, gravies, pies, salads, casseroles, and dressings. The incriminated food invariably is held at room temperature or refrigerated in large masses for several hours, often overnight or longer. Outbreaks frequently follow banquets or meals prepared at hospitals and schools where large amounts of meat or poultry are involved. Thus, *C. perfringens* foodborne illness is a disease intimately associated with the food-service industry.

When sufficient numbers of *C. perfringens* are ingested, diarrhea and abdominal pain accompanied by large volumes of gas in the intestine occur after an incubation period of 4 to 22 hr (12 hr average). Nausea and vomiting are rare. Fever, shivering, headache, and other signs of infection seldom occur *(15, 27, 34, 52)*.

TABLE 1. REPORTS OF *C. perfringens* ISOLATION FROM HUMAN FECES[1]

Group	Number of samples	Number positive	Percent positive	Type[2]	Reference
General population	108	19	18	HR (F)	*(20)*
General population	45	1	2	HR	*(34)*
Hospitalized old people	53	8	15	HR	*(34)*
Healthy hospitalized personnel and families	50	10	20	HR	*(15)*
General population	50	3-4	6-8	HR	*(13)*
Male	59	13	22	HR	*(40)*
Female	131	19	15	HR	*(40)*
Hospital patients	308	96	30	HR	*(40)*
Chinese hospital patients (Hong Kong)	364	229	63	HR	*(67)*
General population (Leeds, England)	57	5	9	HR	*(67)*
General rural population					
Children	461	7	2	HR	*(62)*
Adults	50	3	6	HR	*(62)*
Persons fed in hospitals	48	12	25	HR	*(62)*
Persons fed in boarding schools	53	8	15	HR	*(62)*
Aborigines (Australian)	420	80	19	HR	*(62)*
Normal adults	11	9	82[3]		*(53)*
Normal adults	25	25	100[3]		*(66)*
Normal adults	50	50	100[3]		*(13)*
Food handlers (Louisiana)	219	171	78[3]		*(24)*
		15	7	HR	

[1]Modified from Hobbs *(31)*.
[2]HR = heat resistant; (F) = type F; blank = not differentiated (includes both heat-sensitive and heat-resistant strains).
[3]Percentages are most significant because both heat-sensitive and heat-resistant strains cause foodborne illness.
illness.

In human volunteer studies with heat-resistant strains of *C. perfringens*, doses of 1.9 x 10^8 or larger were required to produce illness. Filtrates from cultures containing a mean of 8.1 x 10^8 viable organisms failed to produce illness in six volunteers *(15)*. A strain of *C. perfringens* that produced heat-sensitive spores caused diarrhea and abdominal cramps in five of six volunteers when cultures containing 4 to 6 x 10^9 vegetative cells were fed to them *(27)*. In children and young adults *C. perfringens* foodborne illness is relatively mild, and symptoms usually subside within 24 hr; but in the elderly, ill, or debilitated, serious consequences, including death, have occurred *(38, 48, 63)*.

The absence of fever, immunity, and secondary spread suggests that the illness is an intoxication; however, culture filtrates or suspensions of dead organisms have failed to produce illness in human volunteers. Nygren *(47)* proposed an interesting hypothesis that lecithin in food was hydrolyzed by the phospholipase C enzyme that is produced by *C. perfringens*, and phosphorylcholine was formed and produced diarrhea. Animals given synthetic phosphorylcholine developed diarrhea. Human volunteer studies, however, have failed to confirm this hypothesis. One volunteer was given 100 mg and 500 mg phosphorylcholine but did not develop diarrhea *(14)*. The specific cause of *C. perfringens* foodborne illness remains an enigma.

Smith and Holdeman *(57)* claim that *C. perfringens* is probably more widespread over the earth than any other pathogenic bacterium. This, of course, is conjecture, but nevertheless its ubiquity is apparent. This organism is widely distributed in the intestinal contents of man and animals, in sewage, and in soil.

Clostridium perfringens is a normal inhabitant of the intestinal tract of man and animals. Percentages for human carriers of *C. perfringens* type A in various population groups are listed in Table 1. Variation in results is dependent in part on the method used for culturing. The investigators chose various media, and only a few used selective or differential media. Most of the studies dealt with the isolation of only heat-resistant strains, and criteria for heat resistance also differed with several investigators. Since it is known that heat-sensitive strains also cause food

TABLE 2. INCIDENCE OF *C. perfringens* TYPE A IN RAW FOODS[1]

Food	Number of samples	Number positive[2]	Percent positive[3]	Reference
RAW RED MEATS				
Beef	50	35	70	[21][4]
	54	13	(24)	[34][4]
frozen boneless	237	32	(14)	[36][4]
carcasses	158	2	(1)	[36][4]
imported-Gr. Brit.	134	28	(21)	[65]
retail	47	17	(36)	[65]
abattoirs	40	2	(5)	[65]
steak and mince	10	6	60	[42][5]
tripe	6	1 H	17	[42][5]
		1 N	17	
Veal				
carcasses	17	14	82	[21][4]
	10	0	(0)	[36][4]
	7	1	(14)	[34][4]
	20	3	(15)	[65]
frozen boneless	163	2	(1)	[36][4]
Pork				
carcasses	41	15	37	[21][4]
	4	0	(0)	[36][4]
	55	11	(20)	[34][4]
retail	55	27	(49)	[65]
abattoirs	14	3	(21)	[65]
sausage	21	10	48	[21][4]
	38	36 H	95	[42][5]
		9 N	24	
		1	(3)	
Lamb and Mutton				
carcasses	27	14	52	[21][4]
	23	1	(4)	[36][4]
	17	0	(0)	[34][4]
imported-Gr. Brit.	76	18	(24)	[65]
abattoir	19	12	(63)	[65]
frozen boneless	163	2	(1)	[36][4]
Liver				
market	100	26	26	[11][5]
abattoir	100	12	12	[11][5]
hospital	2	1	50	[42][5]
Black Pudding	4	2	50	[42][5]
POULTRY	26	15	58	[21][4]
	7	6 H	86	[42][5]
		3 N	44	
		1	(14)	
FISH	18	11 H	61	[42][5]
		3 N	17	
herring	14	8	58	[37][4]
herring	100	1	1	[37][5]
MEAT, POULTRY, FISH no breakdown	122	20	16	[60][5]
MILK	4	1	25	[18][5]
FRUITS AND VEGETABLES	52	2	4	[60][5]
SPICES	60	3	5	[60][5]

[1]Modified from Smith *(54)* and Hobbs *(31)*.
[2]H = hemolytic; N = non-hemolytic.
[3]( ) = heat-resistant strains; percentages are least significant because both heat-sensitive and heat-resistant strains cause foodborne illness.
[4]Enrichment method.
[5]Without enrichment.

poisoning, the true incidence of *C. perfringens* in human feces would be high as indicated in the studies that sought the presence of *C. perfringens* without regard to its heat resistance *(13, 24, 53, 66)*.

Studies by Sutton *(62)* suggested that heat-resistant strains of *C. perfringens* in humans is closely linked with communal feeding and poor hygiene. Of 26 Australian aboriginal families, 24 (92%) had one or more members who were carriers. Families classified as living under conditions of good hygiene had a carrier rate of only 20%. The carrier state of heat-resistant strains appeared to be transient *(62)*.

In an examination of 219 fecal specimens from food handlers in Louisiana, Hall and Hauser *(24)* found that 78% yielded *C. perfringens*. Multiple serotypes were found in 29% of the cultures. Only about 35% of the isolates produced heat-resistant spores (surviving boiling for 30 min or more). There is a definite risk that food workers may contaminate foods with *C. perfringens*.

Since *C. perfringens* is abundant in feces, it is present in sewage, and it has been used as an index of water pollution. In a survey of sewage from about 70 houses, swab samples were positive for heat-resistant strains of *C. perfringens* in 56% of 125 examinations *(34)*.

Animal feces also serve as sources of *C. perfringens (66)*. Heat-resistant strains of *C. perfringens* were recovered from 14 of 76 (18.4%) samples of pig feces obtained from feeding passages, slaughterhouse pens, and pigs; from 2 of 113 (1.7%) of cattle feces obtained from farms and slaughterhouses; and from 6 of 41 (14.6%) samples of rodent pellets *(34)*. *Clostridium perfringens* was found in the feces of cattle, sheep, pigs, and chickens at about the same proportion as in human feces, 10^{2-4}/gm; higher levels, 10^{8-9}, were found in dog and cat feces *(53)*. In poultry feces, Yamamoto et al. *(72)* found *C. perfringens* in 41 of 160 (25.6%) samples. All batches of greenbottle and bluebottle flies, sampled by Hobbs et al. *(34)*, contained *C. perfringens*.

In soil, where it is part of the normal bacterial flora, *C. perfringens* exists in both vegetative and spore forms. Taylor and Gordon *(66)* examined 196 samples of soil; 190 contained *C. perfringens*, mostly type A. Smith and Gardner *(56)* found between 100 and 56,700 *C. perfringens* per gram in various types of soil. Dust obtained from a kitchen environment revealed *C. perfringens* in 81-89.6% of samples *(42)*.

Foods are frequently contaminated with *C. perfringens*. Table 2 lists the incidence of *C. perfringens* type A in raw foods, and Table 3 lists the incidence in processed foods. Care must be exercised when interpreting these data since the investigations differed in technique and media. The results represent

TABLE 3. INCIDENCE OF *C. perfringens* TYPE A IN PROCESSED FOODS[1]

Food	Number of samples	Number positive[2]	Percent positive[3]	Reference
PROCESSED MEATS	101	20	20	[21][4]
Requires full cooking	38	14	37	[21][4]
Requires light cooking	21	4	14	[21][4]
Requires no cooking	42	2	5	[21][4]
Steak and Mince	15	1 H	7	[42][5]
		1 N	7	
Sausage	25	4 H	16	[42][5]
		1 N	4	
Tripe	6	1	17	[42][5]
Black Pudding	2	2	100	[42][5]
Roast (cold)	15	4 H	27	[42][5]
		2 N	13	
Cold Meats	63	4 H	6	[42][5]
		4 N	6	[42][5]
Cooked Chickens	46	13 H	28	[42][5]
		7 N	15	[42][5]
		1	(2)	[42][5]
PROCESSED FISH				
Hospital cooked	6	1	16	[42][5]
Kipper	98	7	7	[37][4]
Kipper	59	13	22	[37][5]
Smoked haddock	16	7	44	[37][4]
	14	0	0	[37][5]
Smoked salmon	9	7	78	[37][4]
	9	0	0	[37][5]
Prawns	2	0	0	[37][4]
Scampi	1	1	100	[37][4]
	1	1	100	[37][5]
PASTEURIZED MILK	10	1	10	[18]
DEHYDRATED SOUPS, GRAVIES, SAUCES AND SPAGHETTI	55	10	18	[45][4]
COMMERCIALLY PREPARED FROZEN FOODS	111	3	3	[60][5]
HOME PREPARED FOODS	165	3	2	[60][5]

[1]Modified from Smith *(54)*.
[2]H = hemolytic; N = non-hemolytic.
[3]( ) = heat-resistant strains; percentages are least significant because both heat-sensitive and heat-resistant strains cause foodborne illness.
[4]Enrichment method.
[5]Without enrichment.

samples that were cultured with and without enrichment. Higher percentages were usually observed when enrichment techniques were used. Data that include heat-sensitive strains are the most meaningful.

Foods of animal origin become contaminated from direct or indirect contact with intestinal contents during processing. During the processing of pork, for instance, *C. perfringens* was isolated from pig carcasses after scalding, scraping, and inspection. Scald-tank water also yielded these organisms *(5)*. Peppers and other spices contained 2 to 12 *C. perfringens* per gram *(54)*. Any food or object directly or indirectly exposed to fecal material or soil may be contaminated with this organism.

DETECTING AND IDENTIFYING THE ORGANISM

In the investigation of cases of foodborne disease with a clinical history similar to that of *C. perfringens*, it is necessary to examine both suspected foods and feces of patients. Stool specimens from food workers and environmental swabs from kitchens or processing plants are also valuable in outbreak investigations or bacterial surveys if serotyping is to be done. Samples of food should be sent to the laboratory refrigerated, but not frozen. Although refrigeration temperatures have an adverse effect on *C. perfringens*, they prevent excessive growth of this organism or overgrowth by other organisms during the period between sampling and laboratory analysis. Fecal specimens can be placed in transport or enrichment broth media.

Quantitative colony counts are generally performed on food samples. In the United States, sulfite-polymixin-sulfadiazine (SPS) agar is usually used *(2, 3)*. Because *C. perfringens* reduces sulfite to sulfide, these organisms appear as black colonies in SPS agar.

The interpretation of *C. perfringens* counts in foods is difficult because the sample selected may not be representative of portions eaten, the degree of contamination may vary in different parts of the sample, and the numbers of organisms may change appreciably during the interim between serving and laboratory analysis. This last change can occur if foods are heated or frozen, thus killing vegetative cells and perhaps some spores, or if foods are stored at temperatures that would allow multiplication of organisms.

Portions of fecal samples are transferred to duplicate tubes of cooked meat or thioglycollate broth. One tube is heated in a 176 F (80 C) water bath for 15 min, the other is left unheated. After anaerobic incubation, each broth culture is streaked onto blood and egg-yolk agar plates *(16)*. Swabs taken from food, carcasses, or environmental surfaces are placed in cooked meat broth and treated in the same manner as described for fecal samples. Feces from patients involved in recent outbreaks usually have large numbers of *C. perfringens*, 10^5 to 10^8/g *(35)*. This is higher than the normal level in feces, 10^{2-4}/g *(53)*.

All isolates should be confirmed morphologically and biochemically. Tests for motility, indol production, and nitrate reduction are useful for screening

cultures *(2)*. *Clostridium perfringens* gives a positive reaction only in the latter test; it reduces nitrates to nitrites. All tubes of liquid media (with caps loosened) should be heated in a boiling water bath for 10 min and cooled before inoculation. This action drives off oxygen.

A number of systems are suitable for cultivation of *C. perfringens* including: Brewer anaerobic jars, Case jars, Torbal jars, Gaspak jars or disposable anaerobic systems, desiccators, plastic pouches *(8)*, roll tubes, deep agar tubes, or anaerobic incubators. A gas mixture containing 10% hydrogen, 10% carbon dioxide, and 80% nitrogen is excellent.

Since *C. perfringens* is an ubiquitous organism, more definitive identification of strains must be used in outbreak investigations than just the isolation of *C. perfringens* from foods and from the feces of patients and workers. This can be accomplished by serotyping. At present there are 91 specific antisera (including the 13 types of Hobbs[1]) available for typing isolates of *C. perfringens*. This test is performed by mixing a drop of formalinized suspension (obtained from sediments of centrifuged pure cultures of isolates) with a drop of pooled or specific antisera on a slide. Clumping occurs when the cells and sera are homologous.

It is rare to find the same serotype of *C. perfringens* in a significant percentage of people selected at random, but after a common-source outbreak, the same serotype can be recovered from the stools of an appreciable number of patients. Isolates from the suspect food and from patients should be correlated serologically. A contaminated food may contain only one serotype, while feces from patients are likely to contain resident and transient strains of *C. perfringens* as well as the serotype from the contaminated food. Therefore, several isolates from each culture must be serotyped *(23)*.

CONTROL

Of the three principles of foodborne disease control—limitation of contamination, inhibition of growth, and destruction of the organism *(10)*—only inhibition of growth is practical for controlling outbreaks of *C. perfringens* foodborne illness.

Foods become contaminated with *C. perfringens* in various ways. Meat and poultry may be contaminated by excrementborne organisms of animal origin during slaughtering and processing operations. Boned or rolled meats are apt to be contaminated. Skewers or thermal pins (heating rods) push surface contamination into internal portions of meat. Raw meat and poultry serve as vehicles for conveying *C. perfringens* into kitchens and can contaminate workers' hands or preparation equipment. From these sources other foods may become contaminated. Heat-resistant strains of *C. perfringens* have been isolated from chopping boards and from other articles of kitchen equipment *(33)*. Hobbs *(29)* stated that in view of the common occurrence of *C. welchii (perfringens)* in raw meat, the human carrier in the kitchen is probably a minor source of contamination. On the other hand, Hall and Angelotti *(21)* reported that the greatest hazard was from contamination after cooking. Any food may be contaminated by dust, contact with contaminated equipment, vectors, and excrement-borne organisms of human origins.

Obviously, sanitation, proper processing techniques, and personal hygiene of food workers reduce, but do not always prevent, the risk of contamination. It is not feasible to prevent carriers from handling food since most people harbor *C. perfringens* in their intestinal tract. Thus, control of human contamination rests in adequate hand washing, care in handling foods (particularly cooked foods), and in knowledge of proper food preparation and storage techniques. Care should be taken to clean and sanitize kitchen equipment such as cutting boards and slicers, and to avoid using the same equipment for both raw and cooked food (unless the implement has been effectively sanitized between usages). Although contamination may be limited by good sanitation and personal hygiene, there does not seem to be any way to assure that *C. perfringens* can be kept out of foods.

Outbreaks of *C. perfringens* foodborne illness would not occur if cooked foods were eaten while still hot, just after initial cooking; or reheated to internal temperatures of 165-212 F immediately before serving. Heat penetration during cooking is more effective for small fowl, small pieces of meat, or small masses of food. Hobbs *(32)* suggested that roasts and joints should be 6 lb or less when cooked in food-service establishments.

Vegetative cells of *C. perfringens* are destroyed by thorough cooking, but heat-resistant spores can survive. Even spores that are not considered heat resistant may survive many cooking processes. For instance, spores of heat-sensitive strains survived in bread and onion stuffing when cooked to doneness (163.4 to 180 F) in ovens set at 201.2 F, 225 F, and 450 F *(71)*. Survival has also been observed in cooked foods: baked hams (138.2 F), turkey rolls (165 F and 185 F), and ground-beef casseroles (160 F and 180 F) *(61)*. Internal temperatures that were obtained are indicated following each food item.

When raw chicken was cooked to obtain a temperature of 185 to 194 F in the breast muscle, a *C.*

[1]Only Hobbs' 1-13 are available commercially. NCDC Anaerobic Bacteriology Laboratory has 78 additional antisera, and Hobbs has 4 additional antisera.

perfringens spore inoculum was reduced from 10,000 spores per gram to 1.5 per gram. Upon incubation of the cooked chickens at 113 F, a lag period was observed for about 4 hr and the growth then became logarithmic. After 14.7 hr, 10 million cells were present. When cooked chicken was inoculated with 1,000 vegetative cells, they multiplied to a total of 10 million cells in 6.3 hr *(49)*. *Clostridium perfringens* survived better during oven roasting at low temperatures (200-210 F) overnight than during roasting at 375 to 425 F for a few hours. During conventional methods of roasting, the internal temperature of the meat reached 185 to 195 F in about 3 hr; during overnight roasting at low temperature, the internal temperature of the meat only reached slightly higher than 150 F. It took 7 to 10 hr before this temperature was reached. Overnight roasting, therefore, is not recommended *(64)*.

Preparation of foods several hours or a day before serving is hazardous and should be avoided. Leftover, cooked meat should never be merely warmed up, but heated to an internal temperature of at least 165 F to destroy vegetative cells of *C. perfringens*, or cut up into small pieces and boiled for a sufficient period so that the interior temperatures become lethal to vegetative cells. Once reheated, the food should be eaten while hot and not allowed to remain at incubating temperatures.

Many competing organisms will succumb to heat treatments that allow *C. perfringens* spores to survive. Heat is a very effective spore germination activator. Spores are heat shocked (activated) by high temperatures, and when conditions become favorable they germinate and multiply rapidly. High percentages (30 to 100%) of spores germinated after meat was heated to 158 to 176 F, but only low percentages (<5%) germinated when raw meat was inoculated *(6)*. Heat also drives off oxygen which results in anaerobiosis in meat and poultry. Thus, cooking may contribute to outbreaks of *C. perfringens* foodborne illness if proper precautions are not taken subsequently to inhibit the multiplication of these organisms in food.

The mere presence of *C. perfringens* in food is not enough to cause illness since millions of viable organisms are required. Contamination alone cannot account for such numbers—multiplication must occur after contamination.

The generation time of *C. perfringens* can be as short as 8.5 min (12 min median for 22 strains) in broth cultures incubated at 114.8 F; it is about 20 min at 98.6 F *(4)*. In various poultry and meat stock soups, the generation time ranged from 24-32 min *(54)*. As temperatures deviate from the optimum, in either direction, the generation time lengthens until such a point is reached at which multiplication ceases. Strains of *C. perfringens* failed to grow at temperatures at or above 131 F or at or below 59 F *(54)*. Based on Arbuckle's data *(4)* for growth of 22 strains of *C. perfringens* in thioglycollate broth, this organism would increase over one thousandfold in 3 hr at the optimum temperature of 114.8 F. To increase the same amount 5.5 hr would be required at 98.6 F; 10 hr at 86 F; and about 30 hr at 68 F. Thus, prevention of *C. perfringens* multiplication can be achieved by the effective use of refrigeration (<45 F) or hot holding (>140 F).

Foods such as barbecued chicken, stews, and gravies that are cooked and held warm, should be held under conditions which provide internal product temperatures of 140 F or above. This temperature prevents the germination of spores of *C. perfringens* as well as the growth of vegetative cells. However, *C. perfringens* survived for over 6 hr when roast turkey slices in broth were held at conditions of steam table storage of 154.4 F *(61)*. When boiled lamb, inoculated with 2×10^5 *C. perfringens* vegetative cells, was stored in gravy on a hot plate at 104-122 F for 3 hr, the count increased to 46 million per slice. Meat stored without gravy, but at the same time and temperature, yielded about half as many organisms. When the meat was stored at room temperature for 3 hr, no increase of organisms was observed *(28)*.

All foods that are not eaten while hot, or that are not held in devices that maintain temperatures of 140 F or above, must be chilled rapidly and refrigerated at 45 F or below. The key to the prevention of *C. perfringens* foodborne outbreaks is to prevent multiplication of these organisms in cooked and cooling meat, poultry, meat broths, and foods containing these items as ingredients.

Foods should never be held at room temperature to cool; they should be refrigerated immediately after removal from warming devices or serving tables. More efficient cooling will usually occur if large walk-in type coolers are used instead of small refrigerators. The walk-in coolers have a greater capacity to dissipate heat and frequently have forced-air circulation. It must be kept in mind that meat, poultry, gravies, and meat casseroles cool slowly by conduction. Every possible effort, practical in an operation, should be made to cool foods rapidly. Techniques for rapid cooling of foods include putting containers of food into freezer compartments, packing containers in ice, immersing containers in running water, and putting stews, dressings, gravies, and meat stocks into shallow containers to induce more rapid heat transfer from the products *(10, 70)*. In each instance, af-

ter the temperature has been sufficiently lowered, food is put into refrigerators.

SUMMARY

Clostridium perfringens is a common, anaerobic, sporeforming organism that is likely to be a contaminant of foods. The foodborne illness that results from the consumption of large numbers of these organisms is associated with food-service operations where large portions of meat are prepared. Control lies in the prevention of spore germination and multiplication of vegetative cells.

REFERENCES

1. Andrews, F. W. 1899. On an outbreak of diarrhoea in the wards of St. Bartholomew's Hospital. Lancet i:8-9.

2. Angelotti, R. 1964. Methods for isolation and enumeration of *Clostridium perfringens*. In: "Examination of Foods for Enteropathogenic and Indicator Bacteria." (Lewis, K. H., and Angelotti, R., eds.), pp. 50-54. U.S. Dept. Health, Education, and Welfare, Public Health Service Publ. 1142.

3. Angelotti, R., H. E. Hall, M. J. Foter, and K. H. Lewis. 1962. Quantitation of *Clostridium perfringens* in foods. Appl. Microbiol. 10:193-199.

4. Arbuckle, R. E. 1960. The influence of temperature on the growth of *Clostridium perfringens*. M. S. Thesis. Library, Indiana Univ., Bloomington, Ind.

5. Baltzer, J., and D. C. Wilson. 1965. The occurrence of clostridia on bacon slaughter lines. J. Appl. Bacteriol. 28:119-124.

6. Barnes, E. M., J. E. Despaul, and M. Ingram. 1963. The behaviour of a food poisoning strain of *Clostridium welchii* in beef. J. Appl. Bacteriol. 26:415-427.

7. Barnes, E. M., and M. Ingram. 1956. The effect of redox potential on the growth of *Clostridium welchii* strains isolated from horse muscle. J. Appl. Bacteriol. 19:117-128.

8. Bladel, B. O., and R. A. Greenberg. 1965. Pouch method for the isolation and enumeration of clostridia. Appl. Microbiol. 13:281-285.

9. Boyd, M. J., M. A. Logan, and A. F. Tytell. 1948. The growth requirements of *Clostridium perfringens* (*welchii*) BP6K. J. Biol. Chem. 174:1013-1025.

10. Bryan, F. L. 1968. What the sanitarian should know about staphylococci and salmonellae in non-dairy products. I. Staphylococci. II. Salmonellae. J. Milk Food Technol. 31:110-116, 131-140.

11. Canada, J. C., and D. H. Strong. 1964. *Clostridium perfringens* in bovine livers. J. Food Sci. 29:862-864.

12. Canada, J. C., D. H. Strong, and L. G. Scott. 1964. Response of *Clostridium perfringens* spores and vegetative cells to temperature variation. Appl. Microbiol. 12:273-276.

13. Collee, J. G., J. A. Knowlden, and B. C. Hobbs. 1961. Studies on the growth, sporulation and carriage of *Clostridium welchii* with special reference to food poisoning strains. J. Appl. Bacteriol. 24:326-339.

14. Dack, G. M. 1966. Importance of food-borne disease outbreaks of unrecognized causes. Food Technol. 20:1279-1284.

15. Dische, F. E., and S. D. Elek. 1957. Experimental food-poisoning by *C. welchii*. Lancet 2:71-74.

16. Dowell, V. R., and T. M. Hawkins. 1968. Laboratory methods in anaerobic bacteriology. U. S. Dept. Health, Education, and Welfare, Public Health Serv. Publ. 1803.

17. Fuchs, A. R., and G. J. Bonde. 1957. The nutritional requirements of *Clostridium perfringens*. J. Gen. Microbiol. 16:317-329.

18. Goudkov, A. V., and M. E. Sharpe. 1965. Clostridia in dairying. J. Appl. Bacteriol. 28:63-73.

19. Gough, B. J., and J. A. Alford. 1965. Effect of curing agents on the growth and survival of food-poisoning strains of *Clostridium perfringens*. J. Food Sci. 30:1025-1028.

20. Hain, E. 1949. On the occurrence of *Clostridium welchii* type F in normal stools. Brit. Med. J. i:271.

21. Hall, H. E., and R. Angelotti. 1965. *Clostridium perfringens* in meat and meat products. Appl. Microbiol. 13: 352-357.

22. Hall, H. E., R. Angelotti, K. H. Lewis, and M. J. Foter. 1963. Characteristics of *Clostridium perfringens* strains associated with food and food-borne disease. J. Bacteriol. 85:1094-1103.

23. Hall, H. E., and V. R. Dowell. 1967. Foodborne disease outbreaks caused by *Clostridium perfringens*. In: "Cooperative Study of *Clostridium perfringens* Isolates" packet. National Communicable Disease Center, Atlanta, Ga.

24. Hall, H. E., and G. H. Hauser. 1966. Examination of feces from food handlers for salmonellae, shigellae, enteropathogenic *Escherichia coli*, and *Clostridium perfringens*. Appl. Microbiol. 14:928-933.

25. Hanke, M. E., and J. H. Bailey. 1945. Oxidation-reduction potential requirements of *Cl. welchii* and other clostridia. Proc. Soc. Exptl. Biol. Med. 59:163-166.

26. Hauschild, A. H. W. 1968. Food-poisoning *Clostridium perfringens*. Assoc. Food Drug Officials U.S. Quart. Bull. 32:135-139.

27. Hauschild, A. H. W., and F. S. Thatcher. 1967. Experimental food poisoning with heat susceptible *Clostridium perfringens* type A. J. Food Sci. 32:467-469.

28. Hobbs, B. C. 1957. The growth of heat-resistant *Clostridium welchii* in cooked meat. Bacteriol. Proc. 57:14.

29. Hobbs, B. C. 1962. Staphylococcal and *Clostridium welchii* food poisoning. *In*: "Food Poisoning." pp. 49-59. Royal Soc. Health, London.

30. Hobbs, B. C. 1965. *Clostridium welchii* as a food poisoning organism. J. Appl. Bacteriol. 28:74-82.

31. Hobbs, B. C. 1965. Contamination of meat supplies. II. *Clostridium welchii* and toxoplasma. Mon. Bull. Minist. Health Public Health Lab. Serv. (G. Brit.) 24:145-151.

32. Hobbs, B. C. 1969. *Clostridium perfringens* and *Bacillus cereus* infection. *In*: Food-borne infections and intoxications (H. Rieman, ed.) Academic Press, N. Y.

33. Hobbs, B. C., J. M. Ritchie, and E. D. Ritchie. 1962. Food hygiene on board ship. J. Hyg. 60:259-278.

34. Hobbs, B. C., M. E. Smith, C. L. Oakley, and G. H. Warrack. 1953. *Clostridium welchii* food poisoning. J. Hyg. 51:75-101.

35. Hobbs, B. C., and R. G. A. Sutton. 1968. Characteristics of spores of *Clostridium welchii* in relation to food hygiene. *In*: "The Anaerobic Bacteria." Proc. Intern. Workshop, Oct. 16-20, 1967. (V. Fredette, ed.), pp. 51-59. Inst. Microbiol. Hyg., Montreal Univ., Montreal, Quebec.

36. Hobbs, B. C., and J. G. Wilson. 1957. Contamination of wholesale meat supplies with salmonellae and heat resistant *Clostridium welchii*. Mon. Bull. Minist. Health Public Health Lab. Serv. (G. Brit.) 18:198-206.

37. Hobbs, G., D. C. Cann, B. B. Wilson, and J. M. Shewan. 1965. The incidence of organisms of the genus *Clostridium* in vacuum packed fish in the United Kingdom. J. Appl. Bacteriol. 28:265-270.

38. Kellert, E., and W. Meeler. 1953. Fatal gastroenteritis associated with *Clostridium welchii*. Am. J. Clin. Pathol. 23:1234-1237.

39. Klein, E. 1895. Ueber einen pathogenen anaëroben Darmbacillus, *Bacillus enteritidis sporogenes*. Zentr. Bakteriol. Parasitenk. (Abt. I, Orig.) 18:737-743.

40. Leeming, R. L., J. D. Pryce, and M. J. Meynell. 1961. *Clostridium welchii* and food poisoning. Brit. Med. J. i:50.

41. McClung, L. S. 1945. Human food poisoning due to growth of *Clostridium perfringens* (*C. welchii*) in freshly cooked chickens: Preliminary note. J. Bacteriol. 50:229-231.

42. McKillop, E. J. 1959. Bacterial contamination of hospital food with special reference to *Clostridium welchii* food poisoning. J. Hyg. 57:31-46.

43. Murrell, T. G. C., J. R. Egerton, A. Rampling, J. Samels, and P. D. Walker. 1966. The ecology and epidemiology of the pigbel syndrome in man in New Guinea. J. Hyg. 64:375-396.

44. Nakamura, M., and J. D. Converse. 1967. Heat resistance of spores of *Clostridium welchii*. J. Hyg. 65:359-365.

45. Nakamura, M., and K. D. Kelly. 1968. *Clostridium perfringens* in dehydrated soups and sauces. J. Food Sci. 33:424-426.

46. National Communicable Disease Center. 1968, 1969. Foodborne outbreaks; annual summary 1967, 1968. National Communicable Disease Center, Atlanta, Ga.

47. Nygren, B. 1961. Lecithinase-producing bacteria and food poisoning. Acta Pathol. Microbiol. Scand. Suppl. 144 51:297-298.

48. Parry, W. H. 1963. Outbreak of *Clostridium welchii* food-poisoning. Brit. Med. J. ii:1616-1619.

49. Pivnick, H., I. E., Erdman, S. Manzatiuk, and E. Pommier. 1968. Growth of food poisoning bacteria on barbecued chicken. J. Milk Food Technol. 31:198-201.

50. Reed, G. B., and J. H. Orr. 1943. Cultivation of anaerobes and oxidation-reduction potentials. J. Bacteriol. 45: 309-320.

51. Roberts, T. A. 1968. Heat and radiation resistance and activation of spores of *Clostridium welchii*. J. Appl. Bacteriol. 31:133-144.

52. Robertson, J. S. 1963. An outbreak of *Cl. welchii* food poisoning. Mon. Bull. Minist. Health Public Health Lab. Serv. (G. Brit.) 22:144-147.

53. Smith, H. W., and W. E. Crabb. 1961. The faecal bacterial flora of animals and man: its development in the young. J. Pathol. Bacteriol. 82:53-66.

54. Smith, L. DS. 1963. *Clostridium perfringens* food poisoning. *In*: "Microbiological Quality of Foods." (Slanetz, L. W., C. O. Chichester, A. R. Gaufin, and Z. J. Ordal, eds.) pp. 77-83. Academic Press, New York, N.Y.

55. Smith, L. DS. 1968. Anaerobes and oxygen. *In*: "The Anaerobic Bacteria." Proc. Intern. Workshop, Oct. 16-20, 1967. (V. Fredette, ed.), pp. 51-59. Inst. Microbiol. Hyg., Montreal Univ. Montreal, Quebec.

56. Smith, L. DS., and M. V. Gardner. 1949. The occurrence of vegetative cells of *Clostridium perfringens* in soil. J. Bacteriol. 58:407-408.

57. Smith, L. DS., and L. V. Holdeman. 1968. The pathogenic anaerobic bacteria. Univ. of Chicago Press, Chicago, Ill.

58. Sterne, M., and G. H. Warrack. 1964. The types of *Clostridium perfringens*. J. Pathol. Bacteriol. 88:279-283.

59. Strong, D. H., and J. C. Canada. 1964. Survival of *Clostridium perfringens* in frozen chicken gravy. J. Food Sci. 29:479-482.

60. Strong, D. H., J. C. Canada, and B. B. Griffiths. 1963. Incidence of *Clostridium perfringens* in American foods. Appl. Microbiol. 11:42-44.

61. Strong, D. H., and N. M. Ripp. 1967. Effect of cookery and holding on hams and turkey rolls contaminated with *Clostridium perfringens*. Appl. Microbiol. 15:1172-1177.

62. Sutton, R. G. A. 1966. Distribution of heat-resistant *Clostridium welchii* in a rural area of Australia. J. Hyg. 64:65-74.

63. Sutton, R. G. A., and B. C. Hobbs. 1966. Food poisoning caused by heat-sensitive *Clostridium welchii*: a report of five recent outbreaks. J. Hyg. 66:135-146.

64. Sylvester, P. K., and J. Green. 1961. The effect of different types of cooking on artificially infected meat. Med. Offr. 105:231-235.

65. Sylvester, P. K., and J. Green. 1961. The incidence of heat-resistant *Clostridium welchii* in some raw meat supplies. Med. Offr. 105:289-290.

66. Taylor, A. W., and W. S. Gordon. 1940. A survey of the type of *Cl. welchii* present in soil and in the intestinal contents of animals and man. J. Pathol. Bacteriol. 50:271-277.

67. Turner, G. C., and M. M. Wong. 1961. Intestinal excretion of heat-resistant *Clostridium welchii* in Hong Kong. J. Pathol. Bacteriol. 82:529-531.

68. Vernon, E. 1967. Food poisoning in England and Wales, 1966. Mon. Bull. Minist. Health Public Health Lab. Serv. (G. Brit.) 26:235-249.

69. Weiss, K. F., and D. H. Strong. 1967. Some properties of heat-resistant and heat-sensitive strains of *Clostridium perfringens*. J. Bacteriol. 93:21-26.

70. White, A., and B. C. Hobbs. 1963. Refrigeration as a preventive measure in food poisoning. Royal Soc. Health J. 83:111-114.

71. Woodburn, M., and C. H. Kim. 1966. Survival of *Clostridium perfringens* during baking and holding of turkey stuffing. Appl. Microbiol. 14:914-920.

72. Yamamoto, R., W. W. Sadler, H. E. Adler, and G. F. Stewart. 1961. Characterization of *Clostridium perfringens* (*welchii*) isolated from market poultry. Appl. Microbiol. 9:337-342.

73. Zeissler, J., and L. Rassfeld-Sternberg. 1949. Enteritis necroticans due to *Clostridium welchii* type A. Brit. Med. J. i:267-269.