

Clostridium perfringens in Meat and Meat Products

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ABSTRACT

HALL, HERBERT E. (Robert A. Taft Sanitary Engineering Center, Cincinnati, Ohio), AND ROBERT ANGELOTTI. *Clostridium perfringens* in meat and meat products. Appl. Microbiol. **13**:352-357. 1965.—A total of 262 specimens of meat and meat dishes were examined for the presence of *Clostridium perfringens*. Of this total, 161 were raw, unprocessed beef, veal, lamb, pork, or chicken; 101 were processed meats and meat dishes. *C. perfringens* was isolated from 113 (43.1%) of these specimens. The highest percentage of contamination (82%) was found in veal cuts, and the lowest (4.7%) in sliced sandwich meats and spreads. Only 2 of the 113 isolates were shown to produce heat-resistant spores, which indicates a very low incidence (0.8%) of contamination. These findings indicate that outbreaks of *C. perfringens* food-borne disease in the Cincinnati area are caused principally by the contamination of the food with vegetative cells or spores of the organism after cooking. Studies of the effects of various holding temperatures on the growth of *C. perfringens* indicated that, in the range of 5 to 15 C, no multiplication would occur, but that viable cells would still be present at the end of a 5-day holding period. Extremely rapid growth occurred at temperatures around 45 C, and complete inhibition of growth was accomplished between 49 and 52 C.

In discussing the epidemiology of food-borne disease caused by *Clostridium perfringens*, researchers generally concede that the ubiquitousness of this organism makes it a probable contaminant of nearly all foods (McKillop, 1959), most environments (Smith, 1963), animal feces Kraneveld and Djaenoedin, 1934a, b; Hobbs et al., 1953; Yamamoto et al., 1961), and nearly all human intestinal contents (Collee, Knowlden, and Hobbs, 1961). Determination of the source of a particular strain involved in an outbreak is, therefore, relatively difficult; little value can be attached to findings obtained from the examination of food handlers, since they are as likely to be victims as culprits in the chain of events.

When a comparison is made between the outbreaks occurring in England and the United States, however, differences that do bear upon epidemiology are noted. By far the greater number of outbreaks in Great Britain are caused by nonhemolytic strains that produce heat-resistant spores (Hobbs et al., 1953; Collee et al., 1961). In the United States, however, many of the outbreaks are caused by strains for which the production of heat-resistant spores cannot be demonstrated. The work of Hobbs and Wilson (1959) and Weadon (1961) forcefully upholds the theory that most British outbreaks arise from the outgrowth of *C. perfringens* from spores that resist boiling for 1 to 3 hr. These workers found that from 10.5 to 35% of retail market meats con-

tained such spores. Our own studies (Hall et al. 1963) of these heat-resistant strains revealed that their heat resistance could also be demonstrated with spores produced in sporulation media. Yet with most of the strains of *C. perfringens* isolated from outbreaks in this country, such demonstration failed. Because of these differences, meats and meat dishes from the Cincinnati area were examined to determine the degree and nature of their contamination with *C. perfringens*.

MATERIALS AND METHODS

The food specimens were purchased from the various chain stores in the Cincinnati area. They were picked from the self-service counters without any selective criteria except intact wrapping. They consisted of: unprocessed raw beef (stewing ground, roasts, steaks, liver, kidney, and soup meat and bones); pork (chops, steaks, roasts, liver, kidney, and spare ribs); veal (chops, roasts, stew meat, liver, and kidney); lamb (stew meat, roasts, chops, and kidney); frying chicken (leg, thigh, breast, and liver); processed meats requiring full cooking (ham, bacon, sausages, corned beef, and Canadian bacon); processed meats and meat dishes requiring partial cooking or warming (frankfurters, chili, goetta, barbecue, and tamales); and processed meats and meat dishes not requiring cooking (sliced sandwich meats, sandwich fillings, cocktail sausage, and dried cured beef).

Media included Fluid Thioglycollate Medium (BBL or Difco); blood-agar, prepared by adding

5% by volume of sterile defibrinated horse, ox, or sheep blood to Brain Heart Infusion Agar (BBL or Difco); egg medium (McClung and Toabe, 1947); motility-nitrate medium; Noyes' veal broth; and sporulation broth (SEC broth; Angelotti et al., 1962). All tubed media were steamed 10 min and rapidly cooled just before use, to expel dissolved oxygen.

The specimens were cultured in the following manner. The packages were opened in a hood that had been previously surface-sterilized with ultraviolet irradiation, and two 25-g samples were aseptically cut and placed in screw-capped tubes (25 by 200 mm) containing 30 ml of Fluid Thioglycollate Medium. One tube was placed in a boiling-water bath for 1 hr, cooled in running tap water, and incubated at 35 C for 7 days. The other tube was placed immediately in a water bath at 46 C. Tubes showing growth, as evidenced by gas evolution or turbidity, were subcultured to plates of McClung-Toabe egg medium; these plates were incubated anaerobically at 35 C. Isolated colonies on these plates were transferred to the motility-nitrate medium and Noyes' veal broth. If the isolate was nonmotile and reduced nitrate, a provisional identification as *C. perfringens* was made, and the veal-broth tube was stored at room temperature until further study.

The characteristics of the isolates were determined as previously described (Hall et al., 1963). Anaerobiosis was produced as previously described (Angelotti et al., 1962).

The effect of various holding temperatures on the growth of *C. perfringens* in laboratory-prepared beef cubes in natural gravy was studied. Lean rump roast was cut into 3/4-in. (1.9 cm) cubes, and the cubes were thoroughly browned; flour, salt, and pepper were added and mixed with the meat. A measured volume of cold water, depending upon the amount of meat (2 qt/lb; ca. 1 liter/kg), was added, and the whole was boiled for 30 min. Additional flour (to give a gravy equivalent to a no. 2 cream sauce in thickness) and Kitchen Bouquet (a commercial product used to add flavor and color to gravies) were added, and the whole was boiled for 15 min. The gravy was poured off, and 25 g of beef cubes were added to each wide-mouth pint jar. Gravy was added to give a final weight of 300 g. The jars were closed with screw-cap lids and autoclaved at 15 psi for 15 min. When the jars were cool, the lids were tightened; the jars were stored at 4 to 5 C until needed.

Standardized inocula of *C. perfringens* strains NCTC 8239, 6867, and A91 (Hall et al., 1963) were prepared by washing the growth from plates of McClung-Toabe egg medium and adjusting the density of the suspension with buffered dilution water (American Public Health Association, 1960) containing sodium thioglycolate (0.1%) to give approximately 50,000 organisms per gram of beef cubes in gravy. The jars were tempered in a water bath at the temperature to be studied for at least 8 hr before the addition of the inoculum. Inoculum counts, initial counts of the inoculated food, and

counts of the food at various times up to 5 days were made in SPS agar (Angelotti et al., 1962). For low-temperature studies, daily counts sufficed; for higher temperatures, counts at 2- or 4-hr intervals were required.

RESULTS

Of 262 specimens examined, 161 were from raw unprocessed meat, and the remaining 101 were from various processed meats and meat dishes. *C. perfringens* was isolated from 113 of the 262 specimens. All isolates were obtained from the samples incubated at 46 C without previous heating. No isolates were obtained from the samples heated at 100 C for 1 hr. The percentages of specimens of the various raw unprocessed meats that were positive for *C. perfringens* are shown in Table 1. The veal cuts gave the highest percentage (82%) and the pork cuts, the lowest (37%); beef cuts were high (70%); and chicken and lamb were quite similar, with 58% and 52%, respectively. No attempt was made, with this relatively small number of specimens, to examine the same number of each kind of cut, although, in general, about equal numbers of the major cuts (chops, roasts, and steaks) were examined. A total of 38 visceral (liver and kidney) and miscellaneous (spare ribs, ox tail, soup bone, and short ribs) specimens were examined; 25 (65%) of them were positive, which is only 7% higher than the average for the whole group. It was noted, however, that all of the chicken-liver specimens (five of five) and all of the ground-beef specimens (seven of seven) were positive for *C. perfringens*. The somewhat less severely handled stew meat (beef, veal, and lamb) provided 6 positive specimens out of 10 examined, or 60%.

Because no examination of the meat cutting, wrapping, or handling practices of these various stores was made, no critical evaluation of these factors can be attempted. It is interesting, however, to note that one store chain yielded 38 positives from 57 raw meat specimens (66.7%); a second, 25 of 47 (53.2%); a third, 19 of 37

TABLE 1. Recovery of *Clostridium perfringens* from raw, unprocessed meat

Type of specimens	No. of specimens examined	No. of specimens positive for <i>C. perfringens</i>	Per cent positive for <i>C. perfringens</i>
Veal	17	14	82
Beef	50	35	70
Chicken	26	15	58
Lamb	27	14	52
Pork	41	15	37
Total	161	93	58

(51.3%); and a fourth, 11 of 20 (55.0%). Since different store chains varied in their offerings of some of the raw meats, more of some kinds of specimens had to be obtained from particular store chains. For instance, the first store chain supplied a higher percentage of the veal specimens than the others; this disparity may have accounted for the slightly higher percentage for that chain.

Of the 101 processed meats and meat dishes, 20 (19.8%) were positive for *C. perfringens* (Table 2). Those specimens that require full cooking before being served had the highest percentage of positives (14 of 38, or 36.8%). The various sausages (pork sausage, mettwurst) were most frequently contaminated (10 of 21, or 47.6%). The higher degree of contamination could have been due to a number of factors, such as more handling, addition of spices, and use of trimmings and poorer cuts of meat. Contamination of bacon and ham specimens was rather low (1 of 9, or 11.1%).

Only four isolations were made from meats and meat dishes that require warming or are usually lightly cooked. Two of these were from chili, one from frankfurters and one from garlic frankfurters.

Of the 42 specimens of ready-to-eat meats, only 2 (4.7%) yielded *C. perfringens*. One of these was from a sandwich meat (Delicious Loaf); the other was from smoked beef sausage.

Quantitative determinations of the *C. perfringens* present were carried out in only 36 of the 262 specimens of various meats. The levels of contamination ranged to 760 per gram of meat. Most of the positive specimens contained between 1 and 100 per gram. A number of the specimens that were negative by the plate count procedure (SPS agar; Angelotti et al., 1962), however, yielded positive results by the enrichment method, which indicates a level of contamination of less than 10 per gram. The highest level (760 per gram) was obtained from a specimen of ground beef.

Characteristics of the isolates. Complete studies

of the characteristics of 5 of the 113 isolates were not possible because attempts to recover the strains from the original or subsequent veal broth stock cultures failed. In all five instances, however, anaerobic growth, the production of lecithinase on McClung-Toabe egg medium, the reduction of nitrate, and the lack of motility indicated that the isolates were probably *C. perfringens*.

The remaining 108 strains had the biochemical characteristics of typical *C. perfringens* (*Bergey's Manual*), the only exceptions being the failure of some strains to produce the typical stormy fermentation in iron litmus milk and the fermentation of salicin by a few strains. The hemolytic activity of the 108 strains was tested on horse, ox, and sheep blood agar. The results were quite varied; only nine gave reactions typical of the English heat-resistant type (no reaction or partial hemolysis on horse blood, and partial on ox and sheep blood agar). One of these nine strains possessed the characteristics of an English type 11. The other eight did not produce heat-resistant spores or agglutinate in any of the 13 sera. Ninety-one of the isolates gave hemolytic reactions characteristic of the classical gas gangrene organisms (complete, or partial and complete, hemolysis on all three bloods). The remaining eight strains varied from both of the above groups.

All 108 strains produced demonstrable spores in SEC broth, but only 2 (1.9%) resisted heating at 100 C for 30 min or more. These two isolates were not obtained directly from the boiled-meat specimens, but from the specimens incubated at 46 C without previous heat treatment. One of the heat-resistant strains came from chicken thigh and the other from goetta. Possibly, if the boiled specimens had been held for a longer time (over 7 days), outgrowth would have occurred.

All 108 strains were tested by the tube agglutination technique (Hall et al., 1963) for relationships to the 13 heat-resistant types, and 12 (11.1%) reacted with one or another of the sera. The distribution of types did not reveal any particular reservoir in any one store or at any one period of time. Of the 12 strains that reacted, 4 reacted serologically with type 3 serum; 2 with type 4; 2 with type 8; and one each with types 2, 7, 10, and 11. A number of the strains were also tested with 16 other sera, but, since no further serological information was obtained, the rest were not examined.

The results indicated that only 0.8% of the specimens contained heat-resistant spores. This low incidence seemed insufficient to account for the outbreaks of *C. perfringens* food-borne disease in this area; therefore, the effects of various hold-

TABLE 2. Recovery of *Clostridium perfringens* from processed meats and meat dishes

Type of specimen	No. of specimens examined	No. of specimens positive for <i>C. perfringens</i>	Per cent positive for <i>C. perfringens</i>
Require full cooking . . .	38	14	36.8
Require warming or light cooking	21	4	19.0
Require no cooking	42	2	4.7
Total	101	20	19.8

ing temperatures on essentially vegetative populations of *C. perfringens* were examined. For this purpose, three strains were selected: a typical heat-resistant strain isolated in England (NCTC 8239), an American isolate (6867), and a pathology isolate (A91). The characteristics of these isolates have been previously described (Hall et al., 1963).

Holding temperatures ranging from 4.4 to 51.6 C in increments of 2.8 C were studied. The inoculum levels ranged from 40,000 to 80,000 per gram of beef cubes in gravy. The inoculated jars were held in thermostatically controlled water baths (American Instrument Co., Silver Spring, Md.) and removed only long enough to be mixed by rotation for the removal of a specimen of the gravy for testing. Dilutions of the gravy were made in buffered dilution water (American Public Health Association, 1960) with 0.1% sodium thioglycolate added. These dilutions were plated in duplicate in SPS agar, incubated anaerobically at 35 C for 24 to 48 hr, and counted with a Quebec colony counter, by use of a white background.

Growth curves were prepared from the results, but, for the purpose of this paper, a comparison of the results in terms of the time required to produce a 100-fold, or greater, increase over the inoculum is presented in Table 3.

Between 5 and 15 C, a stabilization at the inoculum level or slow death over the 5-day period occurred. In no instance, however, did the population reach a level that did not allow recovery, and in most instances the final counts after 5 days were approximately 10% of the inoculum counts. At 18.3 C, stabilization of the inoculum was main-

tained for 2 or 3 days, but, on the 4th or 5th day, a 100-fold increase was detected. The time of this increase was shortened by about 1 day for each 2.8 C increase in temperature up to 29.4 C. At 29.4 to 35 C, rapid growth was obtained; by the end of 24 hr, 100- to 1,000-fold increases had occurred. It was noted, however, that 4-hr counts showed a slight drop from the inoculum level in every case.

At 46 C, however, no drop in level was noted, and by the end of 4 hr or less a 100-fold increase had occurred. At higher temperatures (49 and 51.6 C), very rapid death of the vegetative cells occurred. No recovery at either temperature could be made with strain 8239 after 24 hr; but, after a 100-fold or more reduction, a gradual increase in numbers of organisms to levels significantly higher than those of the original inoculum was observed with strains 6867 and A91 on the 4th and 5th days, at 49 C. These two strains, however, failed to survive at 51.6 C.

DISCUSSION

The results of this study confirm the findings of other workers (Hobbs and Wilson, 1959; Weadon, 1961; Strong, Canada, and Griffiths, 1963) that a high percentage of market meats contain *C. perfringens*. Unlike the findings of the English workers, however, fewer than 1% of these meat samples yielded heat-resistant strains. The work of Hobbs and Wilson (1959) on carcass meat (veal, beef, lamb, and pork) is more like the findings in this study, since they found only 1.5% of such specimens to contain the heat-resistant types. The higher percentages of contaminated retail market meats are probably due to processing. The cutting, handling, and wrapping operations may each be responsible for the addition of *C. perfringens* spores and vegetative cells. In England, however, a high percentage of this added contamination is with heat-resistant varieties, whereas in the Cincinnati area the contamination is more likely to be with strains resembling those found in soil, feces, and pathological conditions that do not usually produce heat-resistant spores.

The potential hazard of heat-resistant spores is great in those areas where they frequently occur. In the Cincinnati area, however, the potential hazard appears to be from contamination after cooking. Such contamination with spores can occur at any time after a meat, gravy, or meat dish has cooled below 70 to 80 C; these temperatures would not immediately kill spores, but would merely heat-shock them and allow rapid germination. Furthermore, extremely rapid growth of these organisms would occur in any

TABLE 3. Effect of various holding temperatures on the growth of *Clostridium perfringens*^a

Temp	<i>C. perfringens</i> strain		
	8239	6867	A91
C			
4.44-15.6	— ^b	—	—
18.3	5 days ^c	5 days	4 days
21.1	4 days	3 days	3 days
23.9	3 days	2 days	2 days
26.7	2 days	1 day	1 day
29.4-35	<24 hr	<24 hr	<24 hr
46	<4 hr	<4 hr	<4 hr
49	0 ^d	4 days	5 days
51.6	0	0	0

^a Inoculum: 40,000 to 80,000 vegetative cells per gram of beef cubes in natural gravy.

^b Slow death or stabilization.

^c Time required to obtain a 100-fold or more increase over the inoculum.

^d Rapid death.

such food that was allowed to remain at temperatures between 43.3 to 46.6 C.

Although Smith (1963) indicated that 35 C gives an optimal growth rate for *C. perfringens*, Hobbs (1957) found that temperatures in the range of 39 to 49 C allowed more rapid growth in meat slices and gravy, and our findings indicate that the excellent growth obtained at 35 C is preceded by a lag of 2 to 4 hr, whereas no lag phase was noted at 46 C. Temperatures of 46 C and above have been used for isolation purposes. Chapman (1928) found 48 C to be excellent for fecal studies, and McClung (*personal communication*) prefers 46 C for enrichment cultures of food.

The findings of this study indicate that a high percentage of meat samples can be expected to be contaminated with *C. perfringens*. This fact leads to the assumption that the area in which meat and meat dishes are prepared may become similarly contaminated and thus increase the possibility of after-cooking contamination.

The principles of preventing *C. perfringens* food poisoning have been excellently stated in *California's Health*, 1 May 1964, p. 190, ". . . food should be cooked at a temperature above 140 F, cooled quickly to below 40 F, and kept cold. . ."

Furthermore, if meat or meat dishes are to be reheated for subsequent use, they should be reheated rapidly to above 60 C and, where possible, to boiling before they are placed in a steam table for serving.

The different percentages of contamination of the various kinds of raw meat can not be explained on the basis of any information obtained in this study. Hobbs and Wilson (1959), however, also found veal and beef to yield higher percentages than mutton. Unfortunately, Weadon (1961) did not characterize his findings on this basis. Strong et al. (1963) obtained no isolates from three veal cuts, and two from five of lamb; only 8% of the beef and 4% of the pork cuts were positive. The lower percentages obtained by these latter authors, using a plating technique, are substantiated by our findings that the level of contamination is frequently too low to be detected by means other than enrichment.

The high percentage of excessively handled and processed meat specimens, such as ground beef and pork sausage, containing *C. perfringens* would seem to substantiate the idea that contamination is added to meats between the time the carcass is first cut and the time when the various cuts are presented to the public for sale.

When obtained by the consumer, those processed meats least likely to receive cooking adequate to destroy *C. perfringens* rarely contain the organism. It must be pointed out, however, that although raw frankfurters are often eaten with

impunity, these same frankfurters heated to 46 C and held for a few hours may become highly contaminated with *C. perfringens*. Similarly, meat dishes such as chili with beans, which are removed from the containers, heated for serving, and then held at temperatures between 43.3 to 46 C, may develop an infective level in as short a time as 2 hr.

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