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Incidence of Mesophilic *Clostridium* Spores in Raw Pork, Beef, and Chicken in Processing Plants in the United States and Canada

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ABSTRACT

GREENBERG, R. A. (Swift & Co., Chicago, Ill.), R. B. TOMPKIN, B. O. BLADEL, R. S. KITTAKA, AND A. ANELLIS. Incidence of mesophilic *Clostridium* spores in raw pork, beef, and chicken in processing plants in the United States and Canada. Appl. Microbiol. **14**:789–793. 1966.—The anaerobic film pouch technique was used to quantitate and isolate clostridial spores in 2,358 samples of raw meat (1,078 of chicken, 624 of beef, 656 of pork). Of 19,727 putrefactive anaerobic (PA) sporeformers isolated, 1 was confirmed by mouse protection testing to be *Clostridium botulinum* type C. This isolate was obtained from a Western Canada chicken sample which contained 5.33 clostridia per gram. These data indicate a very low incidence of botulinal contamination in raw meats at the packing-plant level (0.042% of 2,358 samples) and an almost 20,000:1 ratio of nonbotulinal PA sporeformers to mesophilic *C. botulinum* spores. The mean level of PA contamination was 2.8 PA sporeformers per gram of meat; 77% of the samples contained three or less PA sporeformers per gram. Small but statistically significant differences in the incidence of clostridial spores were noted for season, geographical region, and type of meat.

Recent innovations in food processing and packaging technology have created an increasing interest in the magnitude of botulinal spore contamination of various foods. The few surveys of these organisms in raw meat have been too limited in scope to permit meaningful interpretation (2, 7).

Meyer and Dubovsky (5, 6) had little difficulty in isolating *C. botulinum* spores from soils on the North American continent. Thus, while botulinal spore contamination of raw meats might occur, the question remains as to its frequency and magnitude.

Development of a convenient, sensitive method (1) for the isolation and quantification of botulinal spores from raw meats (4) made practical an extensive survey of these organisms at the packing-plant level.

MATERIALS AND METHODS

Sample collection. Beef, pork, and chicken samples were collected during autumn (August, September and October), winter, spring, and summer from plants in seven geographical regions of North America (i.e., Middle Eastern seaboard, Southeastern, North Central, Southwest Central, and Far Western United States and Eastern and Western Canada). Wherever possible, two plants were sampled from each geographical region. Twelve 1-lb (453.6-g) samples of the following beef and pork items were collected per season from each region: meat immediately surrounding the bloody neck area, and trimmings designated for dry sausage. Thirty-six chicken samples consisted of 12 each of fore-quarters, hind-quarters, and giblets (liver, heart, gizzard) per season and region. The experimental design is summarized in Fig. 1. All samples were collected by in-plant personnel, packaged in clean jars or plastic bags, shipped in Dry Ice to the laboratory, and held frozen (−17.5 C) until analyzed.

Sample preparation. Samples were chopped aseptically, and portions (11 g) were shaken mechanically with 0.015 M phosphate buffer and glass chips. The meat suspensions were pasteurized at 60 C for 15 min, and portions equal to 0.5 g of meat were dispensed into each of six plastic pouches per sample. The pouches (125-mm internal diameter) were filled with modified *Angelotti* agar (4). After the agar solidified, the pouches were incubated for 72 hr at 37 C.

Enumeration and isolation of putrefactive anaerobic (PA) sporeformers. All black (sulfide-producing) colonies were transferred into tubes of modified peptone colloid broth (4) and were incubated for 72 hr at 37 C. All cultures which blackened or were putrid, or both, were tested for botulinal toxin. Presumptive

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A. Source of samples

Geographical region	*Beef*	*Pork*	*Chicken*
Middle Eastern Seaboard	North Carolina Pennsylvania	North Carolina Pennsylvania	Maryland Delaware
Southeastern	Florida	Georgia	Georgia
North Central	Missouri Illinois	Missouri Illinois	Missouri
Southwest Central	Nebraska Kansas	Nebraska Kansas	Oklahoma
Far Western	California	California	Idaho
Eastern Canada	New Brunswick	Ontario	Ontario
Western Canada	Alberta British Columbia	Alberta Saskatchewan	Alberta British Columbia

B. Seasons

Autumn: August, September, October
Winter: November, December, January
Spring: February, March, April
Summer: May, June, July

C. Types of samples

Beef: Bloody neck area, trimmings for dry sausage
Pork: Bloody neck area, trimmings for dry sausage
Chicken: Fore-quarter, hind-quarter, giblets

D. Number of samples

7 regions × 4 seasons × 24 samples = 672 samples each of beef and pork
7 regions × 4 seasons × 36 samples = 1,008 samples of chicken

FIG. 1. *Experimental design.*

TABLE 1. *Incidence of mesophilic putrefactive anaerobic (PA) spores, including Clostridium botulinum, in raw beef, pork, and chicken*

Type of sample	No. of samples	Total no. of PA spores isolated	Mean PA spores/g	No. of botulinal isolates
Beef, bloody neck area	298	2,929	3.277	0
Beef trimmings	326	2,742	2.803	0
Total beef	624	5,671	3.029	0
Pork, bloody neck area	319	3,655	3.820	0
Pork trimmings	337	2,308	2.317	0
Total pork	656	5,963	3.030	0
Chicken, anterior	373	2,673	2.390	0
Chicken, posterior	379	3,071	2.700	1
Chicken, giblets	326	2,349	2.403	0
Total chicken	1,078	8,093	2.500	1
Total, all types	2,358	19,727	2.789	1

evidence of toxicity was established by intraperitoneal injection of 0.5 ml of the peptone colloid cultures into 15 to 20-g Swiss strain white mice. Cultures causing death within 4 days were confirmed as containing botulinal toxin by protection testing against trivalent ABC antitoxin. Suspect cultures were further tested against monovalent A, B, and C antitoxins. When both protected and nonprotected mice died within 4 days, the cultures were diluted and further evaluated. Toxins resistant to boiling for 15 min or indistinguishable in activity in protected and nonprotected mice were considered nonspecific and nonbotulinal. Antitoxins were obtained from Fort Dodge Laboratories, Fort Dodge, Iowa.

RESULTS

A total of 19,727 mesophilic PA spore formers were isolated from 624 beef, 656 pork, and 1,078 chicken samples (Table 1). The overall mean con-

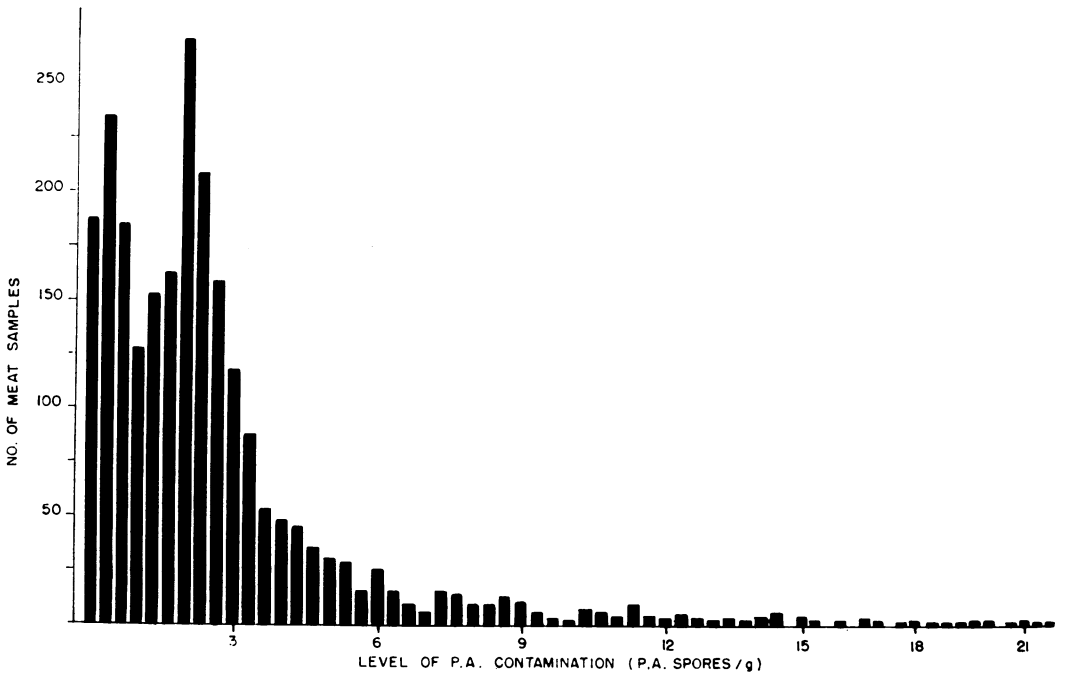

FIG. 2. *Level of putrefactive anaerobic contamination of meat samples.*

TABLE 2. *Seasonal distribution of putrefactive anaerobic (PA) spores in meat samples*

Season	No. of samples	Total no. of PA spores isolated	Mean PA spores/g	No. of botulineal isolates
Spring...	585	2,499	1.422	0
Summer..	624	4,382	2.338	0
Autumn..	563	7,015	4.149	0
Winter...	586	5,831	3.314	1

centration of PA spores was 2.8 per gram of meat. Variation in the levels of PA contamination for all but the 12 most heavily contaminated samples is presented in Fig. 2. Of the 2,358 samples, 77% had 3 or less PA spores per gram. The most heavily contaminated sample had 115 PA spores per gram.

The level of PA spore contamination was significantly lower in chicken (2.50 per gram) than in beef (3.03 per gram) or pork (3.03 per gram) ($P = 0.05\%$). Samples from the bloody neck area had significantly higher PA spore levels than did the trimmings from the beef and pork samples ($P = 0.05\%$). Bloody neck area beef contained 3.28 per gram, whereas this type of pork sample carried 3.82 per gram. Beef and pork trimmings had 2.80 and 2.31 per gram, respectively. The posterior chicken samples had a significantly higher PA spore level (2.70 per gram) than the anterior chicken samples (2.39 per gram) or the giblets (2.40 per gram; $P = 0.05\%$).

A breakdown of the results according to seasons and geographical regions is presented in Tables 2 and 3. These data are summarized in Fig. 3 as a comparison of the mean PA contamination level of the meat samples according to geographical region and season. The mean level of PA spore contamination was significantly higher in meats from plants located west of the Mississippi River (Far Western U.S., Western Canada, and South Central U.S.) than in meats from plants located east of the Mississippi River ($P = 0.01\%$). The North Central plants were located on both sides of the river (i.e., Missouri and Illinois). These samples carried 1.96 PA spores per gram. Far Western U.S. meats contained 3.34, Southwest Central U.S. 3.12, and Western Canada 3.10 clostridial spores per gram. Southeastern U.S. meats had 2.45; Eastern Canada, 2.32; and Mideastern U.S., 2.29 per gram.

The levels of PA spores varied with the season. The order of PA spore contamination from highest to lowest was autumn, 4.15 per gram; winter, 3.31 per gram; summer, 2.34 per gram; and spring, 1.42 per gram. These differences were significant at the 99% confidence level.

One *C. botulinum* type C spore was isolated from a posterior sample of chicken from Western Canada containing 5.33 clostridia per gram. The

TABLE 3. *Distribution of samples and concentrations of putrefactive anaerobes according to geographical regions*

Region	No. of samples	Total no. of PA spores isolated	Mean PA spores/g	No. of botulinal isolates
Far Western U.S.	355	3,555	3.34	0
Southwest Central U.S.	370	3,467	3.12	0
Western Canada	324	3,014	3.10	1
Southeastern U.S.	446	3,277	2.45	0
Eastern Canada	382	2,524	2.32	0
Mideastern Seaboard U.S.	406	2,791	2.29	0
North Central U.S.	373	2,188	1.96	0

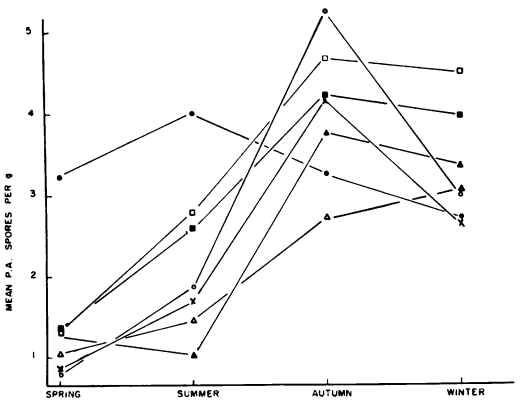

FIG. 3. *Level of putrefactive anaerobic contamination of meats by season and geographical region. Symbols:* □ = *Southwest Central U.S.*, ■ = *Western Canada* ▲ = *Eastern Canada*, △ = *North Central U.S.*, ○ = *Southeastern U.S.*, ● = *Far Western U.S.*, × = *Middle Seaboard U.S.*

data suggest, therefore, that the incidence of botulinal contamination in raw meats is very low (0.042% of the 2,358 samples). The results further indicate an almost 20,000:1 ratio of PA to mesophilic *C. botulinum* spores in raw meats.

DISCUSSION

The most obvious conclusion resulting from the survey is that the level of PA spore contamination in raw meat at the plant level is very low. Furthermore, botulinal contamination occurs rarely. These results assume greater significance when it is considered that the bloody neck area and trimmings were selected as those portions of beef and pork most likely to have the greatest contamination. The bloody neck area may be expected to be heavily contaminated as a result of the sticking knife carrying microflora from the surface of the skin into the deep tissues of the neck. Trimmings for dry sausage also are susceptible to heavy contamination through handling and by the fact that they move relatively slowly through the plant in relation to the remainder of the carcass.

These results are in agreement with the low levels of PA contamination previously reported for raw meat (2, 8; L. A. Harriman et al., Soc. Illinois Bacteriologists, Peoria, 1948). The fact that only one botulinal spore was detected from among the 19,727 PA sporeformers isolated emphasizes the exceedingly low probability of detecting botulinal spores in anything but an extensive survey such as that described herein. To our knowledge, this is the first reported isolation of *C. botulinum* from raw meat at the packing-house level. It is of interest that the isolate was a type C organism and was isolated from a chicken sample. Type C botulism has been recognized as a problem in animals (e.g., limberneck in wild fowl), but has been implicated only rarely as a cause of illness in man (3).

The large number of samples permitted the demonstration of statistically significant differences in levels of PA spore contamination. However, while it may be of academic interest that beef and pork, for example, had higher levels of PA contamination than chicken, the differences were so small as to lack commercial importance.

It must be emphasized that the survey was directed specifically toward the enumeration and isolation of mesophilic PA spores, particularly those of *C. botulinum*. Type E spores and vegetative cells would have been destroyed during the pasteurization of the samples. However, it is also true that these forms would be readily destroyed during a commercial thermal or radiation process applied to meat products.

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