Prevalence of Clostridium perfringens in Pork during Processing

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ABSTRACT

The prevalence of Clostridium perfringens on pork carcasses, fluid from the body cavity, livers, hearts, spleens, visceral pans, scalding vat water and pork sausage was determined. Clostridium perfringens was not detected on pork carcasses, hearts, spleens nor visceral pans. Fluid from the body cavity had 11.8% positive incidence while livers had 21.4% for interior tissues and 11.8% for exterior tissues. The organism could always be isolated from scalding vat water. Commercial pork sausage had 38.9% prevalence of C. perfringens. Numbers of spores and their heat resistance are also reported.

Smith and Holdeman (19) claimed that Clostridium perfringens is probably more widespread over the earth than any other bacterium. It is a normal inhabitant of the intestinal tract of man (4,7,17,20) and has been isolated from animal feces, pigs (2), poultry carcasses (13) and from soil, water and air (15). Presence of C. perfringens in meat products has been confirmed by Hobbs et al. (10), McKillop (14), Weadon (23), Strong et al. (19), Hall and Angelotti (6) and Ladiges et al. (12). The prevalence of C. perfringens in pork carcasses at various stages during slaughter was investigated by Baltzer and Wilson (11). The tissues of 129 cattle, 100 pigs and 20 sheep were examined subsequent to slaughter for presence of the organism by Narayan (16), McKillop (14) and Canada et al. (3) reported isolating cultures of C. perfringens from 50% and 26%, respectively, of beef livers examined.

This study was conducted to determine the prevalence of C. perfringens in pork carcasses at the time of slaughter and to assess the levels of contamination on organ meats and some processing equipment.

MATERIALS AND METHODS

Prevalence of C. perfringens in pork

Samples for detection of C. perfringens were taken at the time of slaughter from two abattoirs: (a) Meat Laboratory, University of Georgia, where samples were taken from hog carcasses that were dressed by students and (b) a local meat slaughtering and processing plant under USDA inspection. Samples of scalding rat water, livers, spleens, hearts, and body cavity fluid and carcass samples were taken from each of the two abattoirs. In addition, pork sausage samples were purchased from supermarkets in the local area for examination for C. perfringens.

Sampling technique

Three sampling techniques were employed. Samples of outer surfaces of livers and carcasses were taken by placing a sterile 5-cm2 template on the surface to be sampled. A cotton swab dipped in a sterile solution of 0.1% peptone (Difco) was used to swab the designated area covered by the template, and then the swab was placed in the tube containing the appropriate medium. For isolation of C. perfringens, the cotton swab was placed in 9 ml of fluid thioglycollate medium without dextrose (Difco). For aerobic counts, the swabs were placed in 9 ml of 0.1% peptone. A 0.1% peptone (pH 7.0 ± 0.1) solution was used as a dilution medium, as recommended by Harmon (8), who reported that this solution produced viable counts three to four times higher than the buffer solution recommended by the American Public Health Association (9). All tubes were stored at 5 C if plating was delayed for more than 1 h. Evisceration pans were also sampled by the method of swabbing just described.

Scalding vat water and fluid from the body cavity were taken either with a 10-ml sterile pipette or a 10-ml sterile syringe and each sample was placed in appropriate medium.

Interior tissues of livers, hearts, and spleens, along with the sausage samples, were prepared in the following method. The outside of the tissue to be sampled was flamed over a bunsen burner until the surface reached a brown color. Fifty g of tissue from the interior of the meat to be examined were excised and placed in a sterile pint blender jar containing 200 ml of 0.1% peptone. The contents were blended until thoroughly macerated (approximately 60 sec at 3200 rpm) after which samples were taken and appropriate dilutions made using a sterile 0.1% peptone solution as diluent. Sausage samples were prepared in the same manner except that the outside was not flamed.

Aerobic plate counts

The dilutions were plated in triplicate on plate count agar (PCA-Difco), using the pour plate method. Two sets of plates were incubated for 2 days at 37 C for aerobic mesophilic counts and one set of plates was incubated for 10 days at 5 C for aerobic psychrotrophic counts. Plates containing between 30-300 colonies were counted, the numbers from duplicate plates averaged and the results recorded.

C. perfringens counts

Sample dilutions were plated on tryptose-sulfite-cycloserine (TSC) agar without egg yolk, as recommended by Harmon (8). The plates were placed in a desiccator under anaerobic conditions (dry ice jar), as described by Washam (22). This method consists of placing 50-100 g of dry ice along with the plates to be incubated in a desiccator fitted with tubing passing into a water trap containing crystal violet indicator. Anaerobic conditions are obtained by the release of CO2 from dry ice, replacing the oxygen within the environment of the desiccator. The
colonies. Five typical black colonies were selected from each countable plate, was placed in a 37 C incubator for 2 days. After incubation, plates were observed macroscopically for growth and black colonies. Five typical black colonies were selected from each countable plate for confirmation.

Confirmation of C. perfringens

The selected and isolated black colonies were stabbed into tubes of buffered motility-nitrate medium and lactose-gelatin medium, as recommended by Harmon (6), and the tubes were incubated at 37 F for 24 h. Motility was observed in the motility-nitrate medium by growth along the stab line. Nonmotile organisms grow only in and along the line of stab while motile organisms produce diffuse growth out into the medium away from the stab. Presence of nitrite was tested by adding 0.5 ml of reagent A (8 g of sulfanilic acid in 1 liter of 5 N HOAc) and 0.2 ml of reagent B (5 g of naphthol in 1 liter of 5 N HOAc) to the motility-nitrate medium. The presence of nitrates is indicated by precipitation of iron sulfide (black) colonies in TSC agar without egg yolk, were non-motile, reduced nitrate to nitrite, produced acid and gas from lactose, and liquefied gelatin were identified as C. perfringens.

C. perfringens' spore counts

After samples were plated for C. perfringens, tubes of thioglycollate medium without dextrose were placed in a steam bath for 20 min at 80 C. These samples were then plated on TSC agar and incubated as described above. Black colonies that appeared and that were confirmed as C. perfringens were counted as spores.

Various cultures of C. perfringens that were isolated from different samples were cultured in cooked meat medium (Difco) for 24 h at 37 C and then placed in a 5-C cooler. These stock cultures were tested within 2 weeks for heat resistance.

Heat resistance of spores

Sporulation broth, as recommended by Harmon (6), was inoculated with 0.5 ml of an 8-h-old culture of C. perfringens that had been grown in cooked meat medium and the newly inoculated broth was incubated for 48 h at 37 C. The sporulation broth was placed in an 80-C water bath for 48 h at 37 C. The sporulation broth was placed in an 80-C water bath for 20 min to kill vegetative cells and then was checked for spores by plating on TSC agar. The sporulation broth was then placed in a 95-C water bath for 5, 15, 30 and 60 minutes. At each time interval, a portion of the broth was removed, placed in fluid thioglycollate medium (Difco) which was incubated for 5 days at 37 C and then observed for growth.

RESULTS AND DISCUSSION

C. perfringens in pork

No enrichment method was employed in this study, although fluid thioglycollate without dextrose (Difco) was used when samples were not plated immediately. Omitting the enrichment step could have affected the number of samples that were positive for C. perfringens. Hall and Angelotti (6) found that a number of the specimens that were negative by the plate count procedure yielded positive results by the enrichment method, indicating a level of contamination of less than 10 per gram.

There was some indication that as the number of mesophilic organisms increased, the prevalence of C. perfringens also increased, which is in agreement with results of Dennis et al. (5). Samples of both the interior of livers and sausage contaminated with C. perfringens had high aerobic mesophilic counts.

Examination of 258 samples, which included livers, spleens, hearts, sausage, carcasses and body cavity fluid revealed that 37 (14%) were positive for C. perfringens. Tables 1 and 2 show the distribution among samples.

Of the 48 samples taken from pork carcasses, none were positive for C. perfringens. All of the samples were obtained from the Meat Laboratory at the University of Georgia where the level of sanitation is excellent. Swab samples from the ham area showed aerobic mesophilic numbers of 65 x 10^3/cm^2 for the inside surface and 63 x 10^3/cm^2 for the outside surface. The jowl area revealed a contamination rate of 60 x 10^3/cm^2 and 30 x 10^3/cm^2 for the inside and outside surfaces, respectively. Investigation of literature on the prevalence of C. perfringens on pork carcasses reveals various results. Baltzer and Wilson (1) isolated C. perfringens from pork carcasses during and after slaughter, although the percentage of positive cultures in these samples was not reported. Hobbs and Wilson (11) were unable to isolate C. perfringens from four carcass samples, while Hobbs et al. (10) examined 55 samples and found a contamination rate of 20%.

Table 1 shows that 11.8% of 76 body fluid cavity samples were positive for C. perfringens with levels of contamination ranging from 10 to 1400/ml. All of these samples came from a commercial abattoir. Total aerobic numbers plated on PCA ranged from 1700 to 330,000/ml of fluid.

The data for prevalence of vegetative cells of C. perfringens in organ samples are recorded in Table 2. Six samples each were obtained of livers, hearts and spleens at the time of slaughter at the Meat Laboratory at the University of Georgia. Only one sample of liver was positive for C. perfringens. Samples from the interior of 22 livers from the commercial abattoir were also examined, with five (22.7%) being positive for C. perfringens. From the same abattoir, 76 livers were swabbed on the exterior and, of these, 11.8% were positive for C. perfringens, while the numbers of aerobic organisms ranged from 38 x 10^3/cm^2 to 176 x 10^5/cm^2. Total aerobic numbers on the interior of the livers ranged from 75 to 34 x 10^4/g. Narayan (16) found that 4% of the tissue samples secured from the interior of livers of pork carcasses at the time of slaughter contained C. perfringens (1% type A; 3% non-toxic), while 6% of the tissues from interiors of spleens were positive.

The fact that C. perfringens was found in the interior tissues of the livers indicates the possibility of dissemination of this organism within pork carcasses. With the frequent isolation of C. perfringens in scalding vat water, this could be a possible source of contamination. Lillard (13) found that the circulatory system as well as edible parts of broiler chickens could become contaminated with C. perfringens. The author indicated that contaminants entered the respiratory
system of poultry during scalding and then spread to the circulatory system. Thornton (21) investigated the possibility of scalding water entering the lungs of slaughtered pigs and reported that, if the period between bleeding and immersion in the scalding tank is unduely short, the animal's nervous system is still capable of response to stimuli and immersion causes the animal to inspire reflexly and draw water into the lungs. Once in the respiratory system, the contaminated water could make its way to the circulatory system and thus be disseminated throughout the carcass.

Another possibility is that *C. perfringens* could be present in the liver of the apparently normal healthy animals, as suggested by Canada et al. (3) for bovine livers.

Of the 18 brands of fresh pork sausage tested for *C. perfringens* contamination (Table 2), seven (38.9%) of the sausage samples were positive. The range of *C. perfringens* for the positive samples was 5 to 95 organisms/g. Total numbers of aerobic organisms in the sausage sampled ranged from $26 \times 10^3$ to $70 \times 10^7$ per gram of sausage. The hydrogen ion concentrations of the sausages were measured and it appeared that there was no relationship between these values and prevalence of *C. perfringens*.

These findings for the *C. perfringens* in sausage are consistent with results of other workers. Hall and Angelotti (6) found a *C. perfringens* contamination rate of 48% while McKillop (14) also found a high contamination of sausage samples in his study.

The only equipment examined for possible *C. perfringens* contamination was the viscera pans. These pans were used to hold the internal organs that were removed during evisceration of the hogs. Although these pans were treated briefly with hot water (100 C) before being reused, numbers of total aerobic bacteria were as

### TABLE 1. Prevalence of total aerobic numbers of vegetative cells of *C. perfringens* on pork carcasses and fluid from the body cavity.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>No. positive for</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inside surface ham <em>(UGA)</em></td>
<td>12</td>
<td>$65 \times 10^3$</td>
<td>$125 \times 10^4$</td>
<td>$50$</td>
<td>N.G.*</td>
<td>$1 \times 10^4$</td>
<td>0</td>
</tr>
<tr>
<td>Outside surface jowl <em>(UGA)</em></td>
<td>12</td>
<td>$63 \times 10^3$</td>
<td>$34 \times 10^2$</td>
<td>$250$</td>
<td>$5 \times 10^2$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inside surface ham <em>(UGA)</em></td>
<td>12</td>
<td>$60 \times 10^2$</td>
<td>$42 \times 10^2$</td>
<td>$60$</td>
<td>N.G.*</td>
<td>$10^4$</td>
<td>0</td>
</tr>
<tr>
<td>Outside surface jowl <em>(UGA)</em></td>
<td>12</td>
<td>$30 \times 10^3$</td>
<td>$35 \times 10^2$</td>
<td>$100$</td>
<td>N.G.*</td>
<td>$10^4$</td>
<td>0</td>
</tr>
<tr>
<td>Fluid from body cavity <em>(Commercial)</em></td>
<td>76</td>
<td>$80 \times 10^3$</td>
<td>$170 \times 10^2$</td>
<td>--</td>
<td>--</td>
<td>9</td>
<td>11.8</td>
</tr>
</tbody>
</table>

* N.G. = no growth.
* Per cm².
* Per ml.

### TABLE 2. Total aerobic numbers and prevalence of vegetative cells of *C. perfringens* in organ samples, viscera, scalding vat water, and pork sausage.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
<th>Mean</th>
<th>Range</th>
<th>Mean</th>
<th>Range</th>
<th>No. positive for</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livers <em>(Interior)</em></td>
<td>28</td>
<td>$65 \times 10^3$</td>
<td>$75-34 \times 10^4$</td>
<td>N.G.*</td>
<td>$1 \times 10^4$</td>
<td>6</td>
<td>21.4</td>
</tr>
<tr>
<td>Livers <em>(Exterior)</em></td>
<td>76</td>
<td>$40 \times 10^3$</td>
<td>$38 \times 10^2$</td>
<td>$160 \times 10^2$</td>
<td>0</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Hearts <em>(Interior)</em></td>
<td>6</td>
<td>$120 \times 10^3$</td>
<td>$110 \times 10^2$</td>
<td>$40$</td>
<td>N.G.*</td>
<td>$10^1$</td>
<td>0</td>
</tr>
<tr>
<td>Spleens <em>(Interior)</em></td>
<td>6</td>
<td>$100 \times 10^3$</td>
<td>$60 \times 10^1$</td>
<td>$100$</td>
<td>N.G.*</td>
<td>$10^1$</td>
<td>0</td>
</tr>
<tr>
<td>Viscera pans</td>
<td>13</td>
<td>$30 \times 10^3$</td>
<td>$75 \times 10^1$</td>
<td>$210 \times 10^3$</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Scalding vat water</td>
<td>13</td>
<td>$60 \times 10^3$</td>
<td>$21 \times 10^1$</td>
<td>$170 \times 10^2$</td>
<td>13</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Pork sausage</td>
<td>18</td>
<td>$50 \times 10^3$</td>
<td>$265 \times 10^2$</td>
<td>$70 \times 10^3$</td>
<td>7</td>
<td>38.9</td>
<td></td>
</tr>
</tbody>
</table>

* N.G. = no growth.
* Per gram.
* Per cm².
* Per ml.
TABLE 3. Total aerobic numbers and prevalence of C. perfringens spores from livers (exterior), body cavity fluid and scalding vat water.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.</th>
<th>Total aerobic mesophiles</th>
<th>No. positive for C. perfringens spores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (Exterior)</td>
<td>28</td>
<td>40 x 10^4/cm^2</td>
<td>2</td>
</tr>
<tr>
<td>Fluid from body cavity</td>
<td>28</td>
<td>60 x 10^3/ml</td>
<td>4</td>
</tr>
<tr>
<td>Scalding vat water</td>
<td>3</td>
<td>52 x 10^3/ml</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 4 shows the heat-resistance of C. perfringens spores. Thirty-four samples isolated from this study were allowed to sporulate and heat resistance of the spores was measured. The results in Table 4 show that the spores were very susceptible to heat. Only 2 (6%) of 34 samples survived heating at 95°C for 30 min and none survived this temperature for 1 h. Hall and Angelotti (6) found that only 1.9% of the strains of C. perfringens that produce demonstrable spores could resist heating at 100°C for 30 min or more.

Scalding vat water was taken at different time intervals over a 3-h period. The temperature of the water was monitored throughout the sampling period and was 62 ± 2°C. The hogs were processed at the rate of 90 per hour with each carcass remaining in the scalding vat for 3-4 min. During the 3-h period of sampling, 270 hogs would have passed through the scalding vat. Results (Table 5) indicated that the scalding vat water became contaminated as the number of hogs going through the scalding vat increased. These findings are in agreement with those of Baltzer and Wilson (1), who reported that the numbers of clostridia (including C. perfringens) increased as more carcasses passed through the scalding tank.

Although the scalding water showed an increase in the number of C. perfringens as the number of hogs going through increased, there was no corresponding increase in the number of C. perfringens noted in liver or body cavity fluid samples. However, a limited number of samples were examined and further work should be done before definite conclusions can be drawn.

The ubiquitous nature of C. perfringens makes this a difficult organism to control. The mere presence of C. perfringens as spores surviving cooking or resulting from after-cooking contamination will not cause outbreaks of foodborne illness. C. perfringens must multiply after the meat is cooked, until there are sufficient numbers to cause illness. Control, therefore, depends upon a post-cooling time-temperature situation in which C. perfringens cannot multiply (14).

REFERENCES


