Research Note

Occurrence of Anaerobic Bacterial, Clostridial, and Clostridium perfringens Spores in Raw Goose Livers from a Poultry Processing Plant in Hungary

JUDIT TURCSÁN, LÁSZLÓ VARGA,* ZSOLT TURCSÁN, JENŐ SZIGETI, AND LÁSZLÓ FARKAS

Institute of Food Science, Faculty of Agricultural Sciences, University of West Hungary, 15–17 Lucsoony Street, H-9200 Mosonmagyaróvár, Hungary

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ABSTRACT

Anaerobic bacterial, clostridial, and Clostridium perfringens spores were enumerated in raw goose liver samples taken after evisceration of the birds (EB) in the slaughterhouse and after removal of blood vessels from the liver (RBVL) in the cannery. The samples taken after RBVL had significantly higher ($P < 0.05$) spore counts than did those taken after EB, indicating contamination of livers during processing. The number of C. perfringens spores was one log cycle higher in the samples taken after RBVL than in those taken after EB ($P < 0.05$). The confirmation of C. perfringens according to the profiles of Rapid ID 32 A tests was carried out by means of the ATB Plus computer program. With an identification percentage of 99.9 and a $T$-value of 0.65, the suspect colonies proved to be C. perfringens. Therefore, the importance of an appropriate cleaning and sanitation program and of personnel hygiene should be emphasized in the industry.

Anaerobic spore-forming bacteria are unable to use molecular oxygen as a final electron acceptor. They are not capable of synthesizing the prosthetic heme component necessary for a cytochrome system and the enzyme catalase or superoxide dismutase, which is why they grow under anaerobic conditions (12).

Clostridia have been incriminated in many anaerobic infections. They produce various toxins that damage tissues or the nervous system. The most common clostridial infections are short-lived and relatively mild food poisonings. In addition, clostridia may cause inflammation that sometimes destroys the walls of the large and small intestine, a condition called necrotizing enteritis. This infection can occur as an isolated case, or there may be outbreaks caused by consumption of contaminated meat (14).

Clostridium perfringens food poisoning is a very common type of human foodborne disease (8). In most cases, the foods involved are cooked meat or poultry products containing elevated levels of viable cells (9). Because poultry feces contain C. perfringens, meat and viscera might be contaminated during processing (2, 4, 7, 10, 11, 13).

In this study, raw goose liver samples, supplied by a major Hungarian poultry processing plant, were tested for the presence of anaerobic bacterial, clostridial, and C. perfringens spores; thus, the microbial condition of goose liver during processing was monitored.

MATERIALS AND METHODS

Experimental design. Goose livers were obtained from a local poultry processing plant. Ten pieces of liver were collected into sterile stomacher bags (Seward Medical, London, UK) after evisceration of the birds (EB; five pieces) in the slaughterhouse and after removal of blood vessels from the liver (RBVL; five pieces) in the cannery. The samples were cooled to 4–8°C and taken to the laboratory. The microbiological examination of livers was started within 4 h. The experiment was repeated three times.

Isolation and enumeration. The pieces of liver were cut with a sterile scalpel, and 10 g of each sample was measured into sterile stomacher bags. Anaerobic endospores were isolated using the pour plate count (PC) agar (Merck), clostridial spores by reinforced clostridial (RC) agar (Merck), and C. perfringens spores by tryptose sulfite cycloserine (TSC) agar (Merck). TSC agar has been documented as the most suitable of the media for quantitative recovery of C. perfringens, with adequate suppression of the growth of almost all facultative anaerobes (5, 6, 9).

Inoculated PC agars were incubated at 30–37°C for 24 h and RC and TSC agars at 37°C for 24 h. Anaerobic endospores were isolated using the pour plate technique. The total number of anaerobic bacterial spores were determined by plate count (PC) agar (Merck, Darmstadt, Germany), clostridial spores by reinforced clostridial (RC) agar (Merck), and C. perfringens spores by tryptose sulfite cycloserine (TSC) agar (Merck). TSC agar has been documented as the most suitable of the media for quantitative recovery of C. perfringens, with adequate suppression of the growth of almost all facultative anaerobes (5, 6, 9).

Inoculated PC agars were incubated at 30°C for 24 h and RC and TSC agars at 37°C for 24 h. Anaerobic culture jars (2.5 liters) were used to generate anaerobic conditions, atmospheric oxygen being absorbed by means of AnaeroGen AN 25 sachets (Oxoid, Basingstoke, UK).

Confirmation of C. perfringens. From each TSC agar, representative black colonies were streaked onto Columbia agar (Merck) containing 8% sterile horse blood. The plates were incubated under both aerobic and anaerobic conditions for 24 h at 37°C. The colonies formed under anaerobic conditions were fur-
TABLE 1. Enumeration of anaerobic bacterial, clostridial, and C. perfringens spores in goose livers

<table>
<thead>
<tr>
<th>Goose liver sample</th>
<th>Anaerobic bacteria</th>
<th>Clostridia</th>
<th>C. perfringens</th>
</tr>
</thead>
<tbody>
<tr>
<td>EB</td>
<td>2.77 ± 0.32 b</td>
<td>1.91 ± 0.09 c</td>
<td>1.03 ± 0.70 d</td>
</tr>
<tr>
<td>RBVL</td>
<td>3.11 ± 0.28 a</td>
<td>2.94 ± 0.15 AB</td>
<td>2.04 ± 0.69 c</td>
</tr>
</tbody>
</table>

a EB, after evisceration of the bird; RBVL, after removal of blood vessels from the liver.
b Values are means ± SD based on 15 observations (five samples, three replicates). Values followed by the same letter are not significantly different at the P = 0.05 level.

The liver samples taken after RBVL had significantly higher (P < 0.05) spore counts than those taken after EB, with respect to all three spore groups tested, thereby indicating contamination or cross-contamination of livers during processing.

The results of this study support the findings of Prukner-Radovčić and Milaković-Novak (11) that C. perfringens is one of the most prevalent clostridial species in poultry.

Before evisceration, the goose carcasses are precooled for 12 to 24 h. After evisceration in the slaughterhouse, the livers are kept refrigerated for 24 h and then transported by cooling truck to the cannery, where their veins and capil-
laries full of blood are removed. Because the canning factory is situated 1.6 km from the slaughterhouse, there is a considerable distance between these two stages (i.e., EB and RBVL) of the manufacturing process. This transportation of livers by truck from one place to another during processing is a serious hazard to microbiological quality because livers are thus exposed to several additional handling procedures. Therefore, the importance of an appropriate cleaning and sanitation program and of personnel hygiene should be emphasized in the industry. The microbial contamination and cross-contamination of livers can be reduced by implementing good manufacturing practices and by training workers in hygiene and quality assurance on a regular basis (1).

REFERENCES