ENUMERATION OF AEROMONAS FOR VERIFICATION OF THE HYGIENIC ADEQUACY OF SWINE CARCASS DRESSING PROCESSES

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ABSTRACT

Populations of Aeromonas spp. and aerobic bacteria from dehairing equipment and from carcasses passing through different processing steps in a swine slaughtering plant were evaluated to identify the hygienic risks of each operation. Sponge samples were taken from the scraper flails in dehairing machines and the surface of the shackling table at pre- and post-operation times, with 20 samples from each location being collected at each time. Increased post-operation levels of Aeromonas spp. indicated a buildup and possible spread of these bacteria to carcasses. The belly skins of 40 dehaired carcasses were each sampled at five points along the process line which were after the shackling, after the final singeing, after the final polishing, after the final wash and after chilling. The levels of microbial contaminants on carcasses varied at each processing step. The heaviest contamination of carcasses with Aeromonas (1.88 log CFU/cm²) and aerobic bacteria (2.66 log CFU/cm²) occurred after shackling. Counts were reduced at other steps as a result of singeing, washing and chilling operations. However, singed carcasses were recontaminated with Aeromonas and aerobic bacteria during the polishing operation. Aeromonas hydrophila were the most prominent motile aeromonads (74.1%) recovered at the plant. The findings for Aeromonas spp. were similar to those for aerobic bacteria ($r^2 = 0.9995$) which

¹Mention of a brand or firm name does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

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suggested that Aeromonas spp. are appropriate indicators for assessing carcass dressing processes.

INTRODUCTION

The United States Department of Agriculture requires all meat and poultry establishments to develop and implement hazard analysis critical control point (HACCP) systems for their processes to assure the safety of their products (USDA 1996). The first step towards developing an HACCP program for a process is to properly identify the hygienic risks associated with each operation in the process. Because bacterial hazards are a major concern in the production of food of animal origin, the identification of hazards involves establishing a list of pathogenic bacteria associated with a particular product and evaluating production processes, the possible contamination routes and the growth, survival and death of specific bacteria during processing (Notermans et al. 1994; Borch et al. 1996).

Bacteria may be transferred to the meat surfaces of swine carcasses from the skin or gastrointestinal tract of animals, from the hands of workers, or from equipment during slaughtering and dressing (Nottingham 1982; Carr et al. 1998). The bacterial biota in detritus or water that persists on equipment in swine slaughtering plants is dominated by psychrotrophic bacteria that include Aeromonas, Listeria, and Yersinia (Gill and Jones 1995). Aeromonas hydrophila is an enteric pathogen which causes mild, self-limited diarrhea in young children, elderly adults or immunocompromised patients (Kirov 1993). Many environmental strains of A. hydrophila infect the human gastrointestinal tract (Havelaar et al. 1992). Most raw pork at the retail level is contaminated with aeromonads that are predominantly A. hydrophila (Palumbo et al. 1985; Hudson and De Lacy 1991; Ibrahim and Mac Rae 1991; Singh 1997). Because Aeromonas spp. are prevalent in the processing environment and can be controlled by proper cleaning and disinfection, these organisms are useful as indicators for monitoring processing hygiene of equipment such as dehairing machines, breaking table, mesh gloves and trimming belts (Gill and Jones 1995; Borch et al. 1996; Gill et al. 1999). In addition, the numbers of coliforms and injured coliforms were low on carcasses in a recent study of carcass-dehairing processes at a swine slaughtering plant (Yu et al. 1999). Therefore, the objective of this study was to investigate the applicability of using aeromonads as indicators for hygiene evaluation to determine if steps in swine carcass dressing such as dehairing, shackling, polishing, singeing, washing and chilling procedures could be important critical control points to control psychrotrophic pathogens that contaminate pork products.
MATERIALS AND METHODS

Processing Steps in Wet and Clean Areas of the Plant

Samples were obtained from a slaughtering plant in Hatfield, PA which processes between 800 and 827 swine carcasses/h. Dressing processes within the wet and clean areas are shown in Fig. 1.

Wet area:

Stunning & Sticking
  ↓
  Bleeding
  ↓
  Scalding
  ↓
  Dehairing*
  ↓
  Shackling*
  ↓**
  First Polishing
  ↓
  First Singeing
  ↓
  Middle Polishing
  ↓
  Final Singeing
  ↓**
  Final Polishing
  ↓**

Clean area:

  Shaving
  ↓
  Washing
  ↓
  Evisceration
  ↓
  Splitting
  ↓
  Final Washing
  ↓**
  Chilling
  ↓**
  Cutting

* Samples taken from the equipment.
** Samples taken from the carcasses leaving each process step.

FIG. 1. PROCESS STEPS IN SWINE SLAUGHTERING AND CARCASS DRESSING
Carcasses are scalded and dehaired at a water temperature of 58.3-60.6°C. Dehairing is performed mechanically by four dehairing machines which use rotating scraper flails to remove the hair while hot water is sprayed over the carcasses. After hair and detritus are screened from the water, it is circulated back to the carcasses. The water is drained from the machines after each working day. After dehairing, the carcasses are manually shackled to the railing by their hind legs on a moving stainless steel table. Before each break, the shackling table is generally sprayed with hot water. The dehaired carcasses are then passed through three polishers and two singeers alternately before entering the clean room where the hides are shaved, the trotters are removed, and the carcasses are washed. Each carcass is subsequently eviscerated and split after the head is removed. The carcasses pass through a final wash before entering the chiller for overnight chilling. The chiller is operated at 2°C by cold air blast.

Levels of *Aeromonas* spp. During Carcass Dehairing and Dressing Processes

Areas of about 100 cm², which were not delimited by a template, on the scraper flails inside dehairing equipment and on the surface of the shackling table were sampled with premoistened Whirl-Pak sponges (Nasco, Fort Atkinson, WI) before work commenced and after work on each of four days. Five samples from each piece of equipment were collected on each day.

The bellies of swine carcasses were labeled with a crayon and sampled, without the delimitation of a surface area, by rubbing a sterile Whirl-Pak swab moistened with 10 mL of 0.1% w/v peptone water over approximately 100 cm² of each belly surface (Yu et al. 1999). On each of four days, the bellies of 10 carcasses were sampled after shackling, after final singeing, after final polishing, and after the final washer. The next day, the same 10 carcasses were similarly sampled after overnight chilling at 2°C. Different areas on the belly of each selected carcass were sequentially sampled at the five points in the process.

Each swab was homogenized for 2 min in a stomacher (Seward Stomacher 400, Tekmar, Cincinnati, OH), and 0.5 mL portions of the fluid from the stomached samples or dilutions were surface plated onto duplicate plates of plate count agar (PCA; Difco Laboratories, Detroit, MI) and starch-ampicillin agar (SAA; Palumbo et al. 1985). The PCA and SAA plates were incubated at 37°C and 28°C, respectively, for 24 h before enumeration. After incubation, SAA plates were flooded with 5 mL of Lugol's iodine solution and colonies surrounded by clear zones of starch hydrolysis were counted as presumptive *Aeromonas* spp. These colonies are typically 3-5 mm in diameter and yellow to honey colored (Palumbo et al. 1985).

Incidence of *Aeromonas* Species

Five surface samples were taken from each of the following areas and carcasses...
using Rodac plates with SAA medium: dehairer, shackling table, wall inside the final polisher, and carcasses leaving the shackling table, the final singeer, the final polisher and the final washer for the incidence of *Aeromonas* spp.

**Confirmation of Isolates**

Presumptive *Aeromonas* colonies on SAA plates were confirmed as Gram-negative rods, oxidase-positive, catalase-positive, and resistant to vibriostatic agent 0/129 (150 μg), ampicillin (10 μg) and cephalothin (30 μg). The biochemical profiles on *Aeromonas* spp. obtained from API 20E strips (bioMérieux Vitek, Inc., Hazelwood, MO) after 24 h incubation at 37°C were compared against those described by Popoff (1984) and Joseph and Carnahan (1994) to speciate isolates.

**Statistical Analysis of Data**

Bacterial counts from the dehairer, shackling table and carcasses leaving each processing step were converted to log CFU/cm². Values for the mean log and standard error (S.E.) of each set of *Aeromonas* and aerobic bacteria counts obtained were calculated. Linear regression between the mean counts of *Aeromonas* and aerobic bacteria from five sampling points along the process line was performed and the correlation coefficient ($r^2$) was calculated by Microsoft Excel (Version 5, Microsoft Corp., Redmond, WA).

**RESULTS**

Our data from the scraper flails in the dehairing equipment indicate there is an increase in the numbers of *Aeromonas* and aerobic bacteria averaging from 0.49 to 1.80 log CFU/cm² and from 2.46 to 6.40 log CFU/cm², respectively during times of machine operation (Fig. 2). The numbers of *Aeromonas* and aerobic bacteria recovered from the shackling table also increased from 0.34 to 1.58 log CFU/cm² and from 1.14 to 3.38 log CFU/cm², respectively during times of operation.

Carcasses leaving the shackling table carried *Aeromonas* and aerobic bacteria at 1.88 log and 2.66 log CFU/cm² on the belly (Table 1). Although singeing reduces the counts of *Aeromonas* and aerobic bacteria to mean numbers of -0.52 and 0.78 log CFU/cm², both counts increase to 0.45 and 1.55 log CFU/cm² for *Aeromonas* and aerobic bacteria during the polishing process. A gradual reduction in the numbers of *Aeromonas* was detected from the point of polishing to the final washing at the end of the dressing process (60.7%) and to overnight chilling (27.3%). The chilling procedure, however, produced a higher aerobic bacteria count (Table 1).

When a total of 27 isolates were subjected to biochemical characterization, 74.1% of isolates were *A. hydrophila*, 14.8% *A. veronii* bv *sobria*, 3.7% *A. veronii* bv *veronii* and 7.4% atypical aeromonads (Table 2).
FIG. 2. NUMBERS OF *AEROMONAS* SPP. AND TOTAL AEROBIC BACTERIA DETECTED IN DEHAIRING EQUIPMENT AND ON SHACKLING TABLE BEFORE WORK COMMENCED AND AFTER WORK
TABLE 1.
NUMBERS OF AEROMONAS SPP. AND TOTAL AEROBIC BACTERIA DETECTED ON 40 CARCASSES AT DIFFERENT DRESSING STEPS

<table>
<thead>
<tr>
<th>Steps Sampled During Carcass Dressing</th>
<th>Log Mean CFU/cm² ± S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aeromonas</td>
</tr>
<tr>
<td>After shackling table</td>
<td>1.88 ± 1.28</td>
</tr>
<tr>
<td>After final singeur</td>
<td>-0.52 ± 1.40</td>
</tr>
<tr>
<td>After final polisher</td>
<td>0.45 ± 0.40</td>
</tr>
<tr>
<td>After final washer</td>
<td>0.04 ± 0.70</td>
</tr>
<tr>
<td>After chiller</td>
<td>-0.10 ± 0.52</td>
</tr>
</tbody>
</table>

\( r^2 = 0.9995 \) between Aeromonas and aerobic bacteria counts.

TABLE 2.
DISTRIBUTION OF AEROMONAS SPP. ON DEHAIRING EQUIPMENT AND CARCASSES PASSING THROUGH DRESSING PROCESS

<table>
<thead>
<tr>
<th>Organism</th>
<th>No. of Identified Isolates (%)</th>
<th>Origin (No. of Strains)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hydrophila</td>
<td>20 (74.1%)</td>
<td>Dehairer (3), Shackling Table (1), Carcasses after shackling Table (3), Wall inside final polisher (8), Carcasses after final polisher (1), Carcasses after final washer (4)</td>
</tr>
<tr>
<td>A. veronii bv sobria</td>
<td>4 (14.8%)</td>
<td>Dehairer (1), Shackling Table (1), Wall inside final polisher (1), Carcasses after final polisher (1)</td>
</tr>
<tr>
<td>A. veronii bv veronii</td>
<td>1 (3.7%)</td>
<td>Carcasses after shackling Table (1)</td>
</tr>
<tr>
<td>Atypical aeromonads</td>
<td>2 (7.4%)</td>
<td>Dehairer (1), Shackling Table (1)</td>
</tr>
</tbody>
</table>

DISCUSSION

The dehairing process has long been recognized as a hygiene risk (Troeger 1993). Both vat scalding and spraying methods are used in the plant which was studied. The scalding and dehairing processes are carried out in separate machines one after the other. The scalding of swine carcasses destroys most mesophilic and psychrotrophic bacteria on the skins of the animals. However, large numbers of psychrotrophic, Gram-negative organisms such as Escherichia coli, Salmonella and Campylobacter are deposited on scalded carcasses during their passage through dehairing equipment (Gill and Bryant 1992, 1993). Consequently, total bacterial counts of 10³ to 10⁴/cm² were reported on the skin of pigs after scalding and dehairing (Gill and Bryant 1992; Troeger 1993).

Aeromonas organisms apparently grew well in the dehairing equipment detritus at two plants where the waters of dehairing machines were maintained at 57°C or
lower (Gill and Jones 1995). The warmer water in the equipment we studied (58-60°C) apparently does not restrict the growth of aeromonads in the accumulated detritus on the scraper flails inside the dehairing equipment. The organisms were then spread onto the shackling table and throughout the dressing lines. Proper cleaning and disinfection of the dehairing equipment would be necessary to prevent the establishment of *Aeromonas* flora in the slaughtering and dressing environment.

Singeing achieved the highest reduction of bacterial load on the pork skin but was not sufficient to eliminate the bacterial contamination on the carcasses (Huis in’t Veld *et al.* 1992; Borch *et al.* 1996). Singed carcasses are often recontaminated and supplemented with bacteria from the polishing apparatus (Gerats *et al.* 1981; Yu *et al.* 1999). Polishing also contributes to the spread of bacteria that survived singeing (Borch *et al.* 1996).

The physical removal of *Aeromonas* cells during washing of carcasses and the less than optimal temperature for the growth of *Aeromonas* and the detrimental drying effect during chilling may contribute to the continuous reduction in *Aeromonas* counts (Table 1). In an HACCP system for swine slaughtering plants, the time in the chiller and the temperature employed should be critical control points necessary for the establishment of critical limits, monitoring procedures and corrective action (Carr *et al.* 1998). Furthermore, the air quality of the blast used for chilling warrants some attention. Evidently, bacteria on the surface of animals, employees and equipment within swine slaughtering and processing establishments could become airborne and cause contamination (Saide-Albornoz *et al.* 1995).

The high incidence of *A. hydrophila* in the dehairing and polishing equipment indicates that pooled water and moist detritus provided niches for their growth (Table 2). More than one *Aeromonas* spp. were isolated from the dehairing equipment, shackling table, polisher and from carcasses leaving the shackling table and polisher. They may be introduced to carcasses from water, animal feces, equipment and symptomatic or asymptomatic carcass-handlers (Kirov 1993). Our frequent detection of motile aeromonads from the swine slaughtering environment represents a possible safety concern.

Obviously, aeromonads accumulated in the persisting detritus in the dehairers and on the shackling table are deposited on swine carcasses during operation. Increased levels of *Aeromonas* spp. indicate the need for a reevaluation of the cleaning and disinfection procedures for the equipment (Borch *et al.* 1996). Changes in numbers of aerobic bacteria are less obvious than those of *Aeromonas* spp. on carcasses leaving subsequent dressing processes (Table 1). Similarly, enumeration of *Aeromonas* on sheep carcasses passing through a process with subsequent sampling of equipment was needed for identifying sources of contaminants when increased numbers during processing were observed (Gill *et al.* 1999). Therefore, an assessment based on indicator organisms may be appropriate for assurance of hygienic adequacy of equipment used for dressing swine carcasses. Additional studies are needed to elucidate the use of aeromonads as possible...
hygienic indicators to identify sources of contaminants for each processing step and the decontaminating effects of critical control points, which may not be evident from changes in aerobic or fecal bacteria counts.

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REFERENCES


