Bacteria of the genus *Aeromonas* in different locations throughout the process line of beef slaughtering

Bactérias do gênero *Aeromonas* em diferentes pontos do fluxograma de abate bovino

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**Summary:** To determine the occurrence and the population of bacteria of the genus *Aeromonas* at different points in the flow diagram for cattle slaughter at a plant with a high hygiene-sanitation level under permanent federal inspection, we investigated products and locations along the slaughter flow diagram from animal arrival to boned meat ready for commercialization. Of the 30 samples taken in each of the nine locations investigated, the bacteria of genus *Aeromonas* were isolated in 20 (66.6%) of the samples collected from the forequarter hide, in 19 (63.3%) of the samples collected from the hindquarter, another 5 (16.6%) and 3 (10.0%) from the samples collected from the muscle surface tissue of the forequarter and hindquarter, respectively, in 6 (20.0%) samples of boned meat, in 4 (13.3%) knife surfaces, in 1 (3.3%) sample of the environment of the slaughtering room, and from 12 (40.0%) samples of the intestinal content. *Aeromonas* spp wasn’t isolated from the still dry animal skin at the time of animal arrival at the slaughterhouse. The following species were isolated: *Aeromonas hydrophila* from 9 (3.3%) samples, *Aeromonas caviae* from 59 (21.8%), and atypical forms from 35 (13.0%). The mean populations detected were $1.1 \times 10 \text{ CFU/cm}^2$ and $3.1 \times 10 \text{ CFU/cm}^2$ on the fore and back carcass skin, respectively, $1.5 \times 102 \text{ CFU/cm}^2$ on the muscular surface of the fore carcass, and $1.4 \times 10 \text{ CFU/g}$ in the intestinal content. The results obtained demonstrated that, despite the high hygiene-sanitation level of the slaughterhouse-meat processing plant, the meat may be contaminated with *Aeromonas* bacteria as early as at the time when it is first processed.

**Keywords:** *Aeromonas* spp, *A. hydrophila*, *A. caviae*, beef slaughter.

**Introduction**

The different species of the genus *Aeromonas* are considered to be emergent pathogens and are held responsible for the occurrence of diseases classified as non-intestinal and gastroenteric (Ko and Chuang, 1995). At the non-intestinal level, meningitis (Ellison and Mostow, 1984), arthritis (Dean and Post, 1967), endocarditis (Davis et al., 1978), osteomyelitis (Lopez et al., 1968), peritonitis (Janda et al., 1983; Nygard et al., 1970), cutaneous infections (Joseph et al., 1979) and ocular infections (Lee et al., 1997), among others, have been attributed to the action of *Aeromonas* bacteria. At the gastroenteric level, these bacteria are considered to be responsible for diseases ranging from mild diarrhea to severe dysentery (Knochel, 1989).

These microorganisms are widely diffuse in the environment (Cunliffe and Adcock, 1989), with water and food being the possible routes of transmission to man. There are reports of isolation from polluted waters (Joseph et al., 1979; Neves et al., 1990), from non-chlorinated waters (Krovacek et al., 1989), from chlorinated waters (Kersters et al., 1995), and from waters used for the most diverse purposes in the food industry (Gill and Jones, 1995; Rossi Júnior et al., 1996b).
The occurrence of different Aeromonas species in foods of animal origin, especially commercially obtained meats, has been reported by several investigators in studies conducted in different parts of the world (Majeed et al., 1989; Fathi and Moustafa, 1991; Gill and Jones, 1995; Rossi Júnior et al., 1996a,b; Yadav and Verma, 1998).

The objective of the present study was to determine the occurrence and population of bacteria of the genus Aeromonas at different points along the flow diagram of cattle slaughter.

Material and methods

The study was conducted at a slaughterhouse-meat processing plant in the State of São Paulo, where the following samples were analyzed: still dry animal skin surface at the time of arrival to the plant, skin surface of the fore and back carcass, after the animal was washed by aspersion, muscular surface of the fore and hind carcass, boned meat ready for commercialization, knife surface, and intestinal content.

Surface samples were collected with sterilized swabs from 20 cm² areas and transported to the plant in test tubes containing 4 ml 0.1 % peptone water, according to the method recommended by Apha (2001).

The meat samples were collected in the form of fragments at one point in the boning room and intestinal content samples in the entrails and tripe processing section at the time of the opening of the digestive tract for washing and preparation. For the collection of environmental samples in the slaughter room, Petri dishes containing selective media for the genus under study (dextrin-ampicillin agar and phenol red-starch-ampicillin agar) were left open for 15 minutes (Apha, 2001).

The samples were transported in styrofoam boxes containing ice cubes and immediately processed in the laboratory for bacterial isolation and counts. For isolation, 25 g of the meat and intestinal content samples were obtained and homogenized in a Stomacher, apparatus with 225 ml trypticase-soy broth (TSB) supplemented with ampicillin (Sigma – A9393) at the concentration of 30 mg/liter. The swabs and the peptone water used for transport were poured into test tubes containing 20 ml of the selective enrichment broth. After incubation at 28 °C for 24 hours, the selective enrichment cultures were streaked on plates containing phenol red-starch-ampicillin agar (Palumbo et al., 1985) and dextrin-ampicillin agar (Havelaar and Vonk, 1988).

The dishes were incubated at 28 °C for 24 hours and examined for the presence of large colonies (3-5 mm) surrounded by a yellow halo caused by the hydrolysis of starch or dextrin, characteristic of the genus Aeromonas. Up to five colonies were streaked in tubes containing trypticase-soy agar (TCA) and tested for motility, oxidase, catalase and resistance to the vibrio-static agent O/129 (2,4-diamine-6,7-diisopropylpteridine) according to the characterization scheme adopted by Popoff (1984), Neves et al. (1990) and Hudson e Lacy (1991). The species were characterized according to the scheme of Popoff (1984) and using some other tests recommended by Buchanan and Palumbo (1985).

Counts were performed in phenol red-starch-ampicillin by spreading on the surface of 0.2 ml volumes of each sample collected from the surface, or by 1/10 dilution in the case of solid samples. Characteristic colonies were counted and up to five colonies per dish were tested for genus confirmation.

Results and discussion

Data concerning the frequency of isolation from the various sample categories analyzed and the species detected are presented in Table 1.

Bacteria of the genus Aeromonas were not isolated from the dry skin surface of the animals at the time of their arrival at the plant. These results suggest that, when animals arrive at the plant, their outer body surface does not represent a source of significant contamination of bovine meat with Aeromonas microorganisms. On the other hand, the presence of these microorganisms in a high percentage of samples from the skin surface of the forequarter (66.6%) and hindquarter (63.3%) after contact with water suggests the possible role of water as a source of contamination, as discussed by Jindal et al. (1993) and Hänninen and Siitonen (1995).

The lack of literature data concerning the study of the presence of Aeromonas spp on the skin surface of beef cattle impairs a comparative analysis with the findings of the present study. However, on the basis of the results obtained, we may conclude that the animal skin, after washing, may become an important source of meat contamination acting during the different phases of slaughtering. With respect to the other possible sources of contamination, Table 1 shows that Aeromonas was isolated from a significant number of intestinal content samples (12/40.0%) and from forequarter (10.0%) and hindquarter (13.3%) knife surface samples. The number of isolations from the intestinal content (12/40%) was higher than that reported by Gray (1984) and Gray and Stickler (1989) in England, by Abbey and Etang (1988) in Nigeria and by Jindal et al. (1993) in India, who detected Aeromonas in 21.1%, 4.6%, 2.86% and 10.0% of cattle feces samples, respectively. These investigators refer that the occurrence of Aeromonas in the digestive tract of animals depends on the exposure of the latter to water sources containing the microorganism and that carcass contamination with
intestinal content and transfer of the contaminants between carcasses by cross-contamination during processing cannot be overlooked.

The knives used in the different procedures of cattle slaughter are known to be easily contaminated from the skin surface, gastrointestinal contents, water and other sources, thus representing an additional vehicle of contamination. The presence of the genus Aeromonas in equipment, developing on organic matter residues on the surface of poorly cleaned surfaces has been previously demonstrated by Gill and Jones (1995).

Table 1 also provides information about the isolation of bacteria of the genus Aeromonas from the muscle surface of both the forequarter (05/16.6%) and hindquarter (03/10.0%) and from the meat (06/20.0%) obtained in the boning room. Most of the studies concerning the presence of Aeromonas in foods of animal origin have been conducted on products obtained at the commercial level. Much higher percentages of isolation were detected by Ibrahim and Mac Rae (1991) in Australia, by Krovec et al. (1992) in Sweden, by Walker and Brooks (1993) in England, and by Pin et al. (1994) in Spain, who respectively detected 100%, 60%, 50%, 57% and 40% positive samples. In contrast, Knochel and Jeppesen (1990), Hudson and Lacy (1991), Ibrahim and Mac Rae (1991) and Pin et al. (1994) detected such strains in boned meat samples compared to Aeromonas hydrophila and to strains considered atypical. Several studies have demonstrated the predominance of A. hydrophila over A. caviae and A. sobria, (Majeed et al., 1989; Knochel and Jeppesen, 1990; Gobat and Jemmi, 1993; Rossi Júnior et al., 1996a). In contrast, Fathi and Moustafa (1991) detected a predominance of Aeromonas caviae in different types of beef products.

The presence of Aeromonas hydrophila, although in a small number of samples (09/3.3%), should be a source of concern for public health services, especially because of its isolation from samples of boned meat ready for commercialization, given the risk this may represent to different segments of the population. Aeromonas hydrophila has been characterized as the species presenting the greatest pathogenic potential (Cahill, 1990) and the one most frequently incriminated in human infections (Ellison and Mostow, 1984; Wadström and Ljung, 1991; Ko and Chuang, 1995).

Although Aeromonas hydrophila is the species causing the greatest concern in terms of pathogenicity, we should not overlook the presence of Aeromonas caviae in 59 (21.8%) of the samples studied since this microorganisms may also produce virulence factors, though to a lesser extent, as reported by Cahill (1990), Wadström and Ljung (1991) and Havelaar et al. (1992).

Aeromonas isolates considered to be atypical were present in 13.0% of the samples studied (Table 1). Hudson and Lacy (1991), Ibrahim and Mac Rae (1991) and Pin et al. (1994) detected such strains in meat from different types of butcher shops, as well as in cow’s milk and in cheese.

Table 2 presents the data referring to the populations of Aeromonas spp detected in the different types of samples. Among boned meat samples, only one could be counted, presenting a population of 2.0 x 10 CFU/g. This number was much lower than that obtained by

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### Table 1 - Total samples analyzed and number and percentage of positives for each one of the species of the bacteria of the genus Aeromonas identified in each type of samples collected at different locations throughout the beef slaughtering process line

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number of samples</th>
<th>Aeromonas spp</th>
<th>Atypical Aeromonas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Analyzed</td>
<td>Positive(%)</td>
<td>A. hydrophila</td>
</tr>
<tr>
<td>Dry hide surfaces – animals upon arrival</td>
<td>30</td>
<td>00</td>
<td>00(0,0)</td>
</tr>
<tr>
<td>Surface of forequarter hide – after aspersion bath</td>
<td>30</td>
<td>20(66,6)</td>
<td>03(10,0)</td>
</tr>
<tr>
<td>Surface of hindquarter hide – after aspersion bath</td>
<td>30</td>
<td>19(63,3)</td>
<td>00(0,0)</td>
</tr>
<tr>
<td>Surface of muscle tissue forequarter</td>
<td>30</td>
<td>05(16,6)</td>
<td>01(3,3)</td>
</tr>
<tr>
<td>Surface of muscle tissue hindquarter</td>
<td>30</td>
<td>03(10,0)</td>
<td>01(3,3)</td>
</tr>
<tr>
<td>Deboned meat – commercial cuts</td>
<td>30</td>
<td>06(20,0)</td>
<td>02(6,6)</td>
</tr>
<tr>
<td>Surface of knives</td>
<td>30</td>
<td>04(13,3)</td>
<td>01(3,3)</td>
</tr>
<tr>
<td>Slaughter room area</td>
<td>30</td>
<td>01(3,3)</td>
<td>00(0,0)</td>
</tr>
<tr>
<td>Intestinal content</td>
<td>30</td>
<td>12(40,0)</td>
<td>01(3,3)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>270</td>
<td>70(25,9)</td>
<td>09(3,3)</td>
</tr>
</tbody>
</table>

1 Number of samples in which the specie was found to be isolated in.
2 Percentage in relation to the total number of samples analyzed in each type.
Knochel and Jeppesen (1990) who found populations higher than $10^5$ CFU/g in beef, pork and fowl. Fathi and Moustafa (1991) and Gobat and Jemmi (1993) respectively detected populations of the order of $2.1 \times 10^8$ and $3.0 \times 10^4$ CFU/g in meat products and concluded that these elevated populations were due to the lack of hygienic-sanitary care in the preparation and commercialization of the products. It is important to point out that the meat analyzed in the present study was collected at its source of production when it had not been submitted to the frequently adverse situations of conservation that occur both during commercialization and at the domestic level. The authors cited above are unanimous in stating that Aeromonas can very well develop during storage under refrigeration regardless of faults in this process and point out that many strains are psychrophilic.

The Aeromonas population detected in the intestinal content, although relatively reduced, with a maximum value of $1.4 \times 10^5$ CFU/g, was sufficient to permit its identification and count from direct seeding, whereas this was not possible in a study conducted by Gray and Stickler (1989) who, working with feces of different animal species, detected positive samples only after enrichment.

The lack of information about the population of bacteria of the genus Aeromonas in the remaining types of samples analyzed in the present study impairs a comparative analysis aiming at the establishment of the significance and real magnitude of this population. However, the data obtained should be considered worrisome since the populations detected were sufficient to permit isolation and count by direct seeding, a fact that was not possible in several other studies (Figura and Marri, 1985; Hudson and Lacy, 1991; Krovacek et al., 1992), and also because bacteria of the genus Aeromonas can reach the meat during the various phases of the slaughtering process through the outer surface of the animal’s body.

On the basis of the present results, we may infer that, regardless of the hygienic-sanitary level of a slaughter-house-meat processing plant, bovine meat can be contaminated with bacteria of the genus Aeromonas as early as at the time when it is obtained.

### Table 2 - Maximum and minimum populations and averages arithmetic’s of Aeromonas spp, in each type of samples collected at different locations throughout the beef slaughtering process line

<table>
<thead>
<tr>
<th>Type of samples</th>
<th>Positive samples in the direct inoculation for quantification</th>
<th>Population of Aeromonas spp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry hide surfaces – animals upon arrival</td>
<td>00(0,0)*</td>
<td>Minimum: 0,2x10** Maximum: 3,0x10 Average: 1,1X10</td>
</tr>
<tr>
<td>Surface of forequarter hide– after aspersion bath</td>
<td>07(23,3)</td>
<td>0,2x10** 1,5x10 Average: 2,2X10</td>
</tr>
<tr>
<td>Surface of hindquarter hide–after aspersion bath</td>
<td>09(30,0)</td>
<td>0,2x10** 3,0x10 Average: 1,5X10</td>
</tr>
<tr>
<td>Surface of muscle tissue forequarter</td>
<td>02(6,7)</td>
<td>0,2x10** 3,0x10 Average: 1,5X10</td>
</tr>
<tr>
<td>Surface of muscle tissue hindquarter</td>
<td>00(0,0)</td>
<td>0,2x10** 3,0x10 Average: 1,5X10</td>
</tr>
<tr>
<td>Deboned meat – commercial cuts</td>
<td>01(3,3)</td>
<td>2,0x10*** 3,0x10 Average: 1,4x10</td>
</tr>
<tr>
<td>Surface of knives</td>
<td>00(0,0)</td>
<td>0,2x10** 3,0x10 Average: 1,5X10</td>
</tr>
<tr>
<td>Slaughter room area</td>
<td>01(3,3)</td>
<td>0,2x10** 3,0x10 Average: 1,5X10</td>
</tr>
<tr>
<td>Intestinal content</td>
<td>07(23,3)</td>
<td>1,0x10*** 4,8x10 Average: 1,4x10</td>
</tr>
<tr>
<td>TOTAL</td>
<td>27(10,0)</td>
<td>0,2x10** 3,0x10 Average: 1,5X10</td>
</tr>
</tbody>
</table>

* Percentage in relation to the total number of samples analyzed in each type.
** Colony forming units/cm2.
*** Colony forming units/gram.

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