

***Aeromonas* species research in samples of water and beef obtained at industrial level**

Pesquisa de espécies de *Aeromonas* em amostras de água e carne bovina obtidas em nível industrial

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Abstract

The presence of bacteria of the genus *Aeromonas* in food of animal origin has been demonstrated by several authors. Among the most often cited foods are those which, during their preparation, come into contact with water which is considered to be the habitat of different species and the major source of food contamination. Thus, in order to determine the occurrence of bacteria of the genus *Aeromonas* in an abattoir where cattle is slaughtered and beef is processed, 20 samples of the water supply, 20 samples of residual water from carcass washing, and 20 samples of meat, were analyzed. The results showed the occurrence of aeromonads in 40% of the samples of residual water from carcass washing, represented only by the species *A. caviae*, and in 25% of the meat samples (*A. hydrophilla* in 15% and *A. caviae* in 10%). The fact that no aeromonads were isolated from the water supply samples suggests the existence of other sources of beef contamination.

Key words: *Aeromonas*; water supply; residual water; beef

Introduction

The genus *Aeromonas* belongs to the family Vibrionaceae and is subdivided into two groups based on characteristics of motility and temperature requirements. The non-mobile and psychrophilic group consists of a single species, *A. salmonicida*, with the subspecies *salmonicida*, *achromogenes*, and *masoucida*; the mobile and mesophilic group comprises three species: *A. hydrophilla*, *A. caviae* and *A. sobria*, being the first of them the type species (Popoff, 1984).

According to Majjed et al. (1990), in the United States food-poisoning diseases are responsible for large economic losses, especially because of lost working hours. Buchanan (1984) pointed out that 35 to 40% of the cases recorded each year are of unknown origin, many of them

being provoked by bacterial species not routinely determined during the investigation of outbreaks. Among these bacteria are the mobile aeromonads (Gracey et al., 1982; Janda et al., 1983; Abeyta Junior et al., 1986).

Mobile aeromonads are microorganisms widely diffused in the environment (Cunliffe & Adcock, 1989) and are important members of the normal microbiota of water (Pathak et al., 1988), having been isolated from polluted waters (Nygard et al., 1970), from unchlorinated waters (Burke et al., 1984) and also from chlorinated water (LeChevallier et al., 1982).

Concerning to pathogenicity, some mobile aeromonads are pathogenic to man and animals. The species *A. hydrophilla* is known to be pathogenic to amphibians (DeFigueiredo & Plumb, 1977), reptiles (Shotts Junior et al., 1972) and fish (Haley et al., 1967) to which it causes mainly hemorrhagic septicemia, and to cattle, to which it causes abortion (Wohlegemuth et al., 1972). In humans, they cause gastroenteric diseases (Knochel, 1989) as well as nonintestinal diseases such as meningitis (Ellison & Mostow, 1984), arthritis (Dean & Post, 1967), endocarditis (Davis et al., 1978), osteomyelitis (Lopez et al., 1968) and peritonitis (Janda et al., 1983).

Most of the studies performed on the ecology of the gastroenterites caused by aeromonads have been concerned with establishing water as the major vehicle of transmission (Moyer, 1987; Neves et al., 1990). However, Buchanan and Palumbo (1985) consider aeromonads to be potential food poisoning species. In this respect, Callister and Agger (1987) consider water to be one of the most important sources of food contamination.

The objective of the present study was to determine the occurrence of aeromonad species at an abattoir where cattle is slaughtered and beef is processed, by the analysis of the water supply, residual water from carcass washing, and meat in order to verify the role of water as a possible source of contamination.

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Material and Methods

Samples of the water supply were collected with proper care to maintain asepsis (Apha, 1985), in amounts of 400 ml at one site in the slaughter room. To neutralize the action of chlorine were added 10% sodium thiosulfate to each sample at the rate of 0.1 ml/100 ml water. The amount of residual chlorine was determined at the time of collection using the comparing disk for chlorine and 0.1% orthotolidine as reagent (Cetesb, 1973).

Samples of residual water from carcass washing were collected during the stage in which the water runs along the outer and inner surfaces of half carcasses. These samples, each consisting of approximately 400 ml of water corresponding to an average of 14 half carcasses, were collected into sterilized Erlenmeyer flasks.

The meat samples consisted of fragments removed from the neck (bleeding region) at the time when half carcasses were dressed, placed on Petri dishes and carried to the laboratory in styrofoam boxes containing ice cubes together with the water samples. Twenty samples from each collection site were analyzed.

Sample preparation and selective enrichment - The water samples were filtered through cellulose ester membranes. These membranes, which retained the filtrate, were cut into small fragments and the fragments were placed in 250 ml of trypticase-soy broth (TSB) supplemented with ampicillin (Abeyta Junior et al., 1990). Selective enrichment of the meat samples was performed by homogenizing 25 g of the sample with 225 ml TSB and incubating in a BOD oven at 28°C for 24 hr.

Selective plating and presumptive identification of the genus - The enrichment cultures were inoculated into phenol red-starch-ampicillin agar (Palumbo et al., 1985) and into dextrin-ampicillin agar (Havelaar & Vonk, 1988). After incubation at 28°C for 24 hr, six colonies suggestive of the genus, i.e., of a yellowish color and surrounded by an hydrolysis halo, were submitted to the oxidase, O/F glucose and catalase tests and to the test of resistance to the vibriostatic agent O/129 according to the procedure proposed by Popoff (1984) and Knochel (1990).

Species characterization - Species characterization was performed by the method of Popoff (1984), and using some other tests recommended by Abeyta Junior et al. (1990), such as motility, indole production, esculin and arginine hydrolysis, lysine and ornithine decarboxylation, acid production from inositol, salicin, sucrose, mannitol and arabinose fermentation, acetoin, sulfhydryl gas and gas production from glucose, and growth at 37°C.

Results and Discussion

Table shows that no viable aeromonads were detected in the water supply samples used at the abattoir. Although there are reports of the isolation of aeromonads from chlorinated water (LeChevallier et al., 1982; Burke et al., 1984),

the level of 1.3 ppm active chlorine present in the water analyzed, which corresponds to hyperchlorinated water, may be the limiting factor in the survival and development of the genus *Aeromonas*.

Table also shows that 8 of the 20 samples of residual water from carcass washing (40%) were contaminated with bacteria of the genus *Aeromonas*.

Table - Number and percentage of positive samples and species of *Aeromonas* identified from supply water, residual water carcass washing and beef, obtained at industrial level

Samples	Total number analyzed	Positive samples	Species
Water supply	20	0(0)*	
Water from carcasses washing	20	8(40)	<i>A. caviae</i> (40)*
Meat	20	5(25)	<i>A. hydrophila</i> (15) <i>A. caviae</i> (10)
Total	60	13(21.6)	

* percent positive samples

Of the 20 meat samples analyzed, 5 (25%) were contaminated (Table). An identical result was obtained by Knochel and Jeppesen (1990) when they analyzed chopped beef acquired commercially in Copenhagen, Denmark. However, Ibrahim and Mac Rae (1991) obtained higher rates of contamination (60%) for meat samples acquired in Brisbane, Australia.

The presence of bacteria of the genus *Aeromonas* in residual water from carcass washing and in meat still inside the abattoir and the elimination of industrial water as the source of the bacteria suggest the existence of other sources of contamination. On this basis, water may no longer be, in a direct form, the most important source of contamination of food of animal origin at the industrial level, as established by Callister and Agger (1987) for foodstuffs in general. Drazek et al. (1986), after detecting a low incidence of aeromonads in cattle, swine and fowl feces, concluded that the manipulation of food may be an important source of contamination.

The data presented in Table, also related the isolation of aeromonad species from the residual water from carcass washing and from meat demonstrate that only the species *A. caviae* occurred in the former, whereas *A. caviae* and *A. hydrophila* were isolated from the latter, with a predominance of the second species.

The isolation of *A. hydrophila* from significantly higher percentages of samples compared to *A. caviae* and *A. sobria* has also been noted by Majjed et al. (1989) when analyzing lamb meat, by Knochel and Jeppesen (1990) when analyzing different types of foods of both animal and plant origin, by Ibrahim and Mac Rae (1991) when analyzing beef and milk, and by Okrend et al. (1987) when analyzing beef, pork and fowl meat. Several authors have char-

acterized *A. hydrophila* as the species presenting the highest potential for pathogenicity (Pathak et al., 1988; Majjed et al., 1989; Cunliffe & Adcock, 1989) and most frequently involved in human infections (Ellison et al., 1984; Abeyta Junior et al., 1986). These data demonstrate the importance of bacteria of the genus *Aeromonas* as potential food poisoning species; so, their isolation from food of animal origin should not be ignored by public health services.

Moyer (1987), in a study of the clinical significance of aeromonad species isolated from patients with diarrhea, concluded that strains of *A. hydrophila*, *A. sobria* and some strains of *A. caviae* can cause diarrhea and that antibiotic treatment and the ingestion of untreated water are significant risk factors for susceptible hosts. The present findings and those reported in the literature about the presence of *Aeromonas* in food allow us to add also food of animal origin as a risk factor for the transmission of the microorganisms to man according to the factors mentioned by Moyer (1987).

Resumo

Pesquisa de espécies de *Aeromonas* em amostras de água e carne bovina obtidas em nível industrial

A presença de bactérias do gênero *Aeromonas* em alimentos de origem animal tem sido demonstrada por diversos autores. Dentre os alimentos mais citados têm destaque aqueles que durante sua obtenção ou preparação entram em contato com a água, que é tida como habitat das diferentes espécies e principal fonte de contaminação para os alimentos. Assim, com o objetivo de verificar a ocorrência de bactérias do gênero *Aeromonas* em um estabelecimento que abate e industrializa carne bovina, foram analisadas 20 amostras de água de abastecimento, 20 de água residuária da lavagem de carcaças e 20 de carne. Os resultados obtidos mostraram a ocorrência de *Aeromonas* em 40% das amostras de água residuária da lavagem de carcaças, representados unicamente pela espécie *A. caviae* e em 25% das amostras de carne, sendo que em 15% delas esteve presente a *A. hydrophila* e em 10% a *A. caviae*. O não isolamento a partir das amostras de água de abastecimento sugere a existência de outras fontes de contaminação para a carne bovina.

Palavras chave: *Aeromonas*; água de abastecimento; água residuária; carne bovina

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