Enumeration and Confirmation of *Aeromonas hydrophila*, *Aeromonas caviae*, and *Aeromonas sobria* Isolated from Raw Milk and Other Milk Products in Northern Greece

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ABSTRACT

A total of 138 raw cow’s and 57 raw ewe’s milk samples; 80 pasteurized cow’s milk samples; 39 Anthotyros cheese, 36 Manouri cheese, and 23 Feta cheese samples; and 15 rice pudding samples were examined for the presence and any countable population of *Aeromonas* species. Twenty-two (15.9%) of the 138 cow’s milk samples analyzed were contaminated with *A. hydrophila*. In 13 of these samples, populations of $3 \times 10^2$ to $5.0 \times 10^3$ CFU/ml were counted in starch ampicillin agar (SAA). Eighteen cow’s milk samples (13.0%) were contaminated with *A. caviae*, and in eight of these samples, populations of $2.0 \times 10^2$ to $3.0 \times 10^3$ CFU/ml were counted in SAA. Five cow’s milk samples (3.6%) were contaminated with *A. sobria*, and in two of these samples, populations of $2.5 \times 10^2$ and $5.0 \times 10^3$ CFU/ml were counted in SAA. Twelve cow’s milk samples (7.9%) were contaminated with other *Aeromonas* spp. not classified. Eight (14.0%) of the 57 ewe’s milk samples analyzed were contaminated with *A. hydrophila*. In these samples, populations of $5.0 \times 10^2$ to $5.0 \times 10^3$ CFU/ml were counted in SAA. Six ewe’s milk samples (10.5%) were contaminated with *A. caviae*, and populations of $1.5 \times 10^2$ to $1.0 \times 10^3$ CFU/ml were counted in SAA. Two ewe’s milk samples (3.5%) were contaminated with *A. sobria*, and populations counted in SAA were $5.0 \times 10^2$ and $1.0 \times 10^3$ CFU/ml. Four samples (7.0%) were contaminated with other *Aeromonas* spp. not classified. *A. hydrophila* was recovered in 4 (10.2%) and 3 (8.3%) of the Anthotyros and Manouri cheese samples analyzed, respectively, but no countable populations were noted in SAA. None of the pasteurized milk, Feta cheese, and rice pudding samples yielded *Aeromonas* spp. The results of this work indicate that motile *Aeromonas* are common in raw milk in Greece. Also, the presence of *A. hydrophila* in the whey cheeses Anthotyros and Manouri indicates that postprocessing contaminations of these products with motile *Aeromonas* may occur during production.

*Aeromonas* species are readily isolated from raw milk and may occur in up to 60% of samples (6). Aeromonads are generally heat sensitive and killed by pasteurization (3, 5, 12, 13, 20, 26, 28), but strains of motile aeromonads were found in pasteurized milk (7, 13). Brazilian white cheese, and Villalon cheese made from pasteurized milk (7, 24), apparently as a result of postpasteurization contamination. There are also reports that other milk products, such as whipped cream and mayonnaise in Denmark and ice cream in the United Kingdom, may be contaminated with aeromonads (8, 14). Since *Aeromonas* species can grow at 4°C (3, 12, 13, 18–21), their presence in milk products is more significant, because these foods are kept for some time in refrigerators and consumed without heating (7, 12, 13).

*A. hydrophila* and other motile aeromonads have been incriminated in cases of human gastroenteritis, particularly in children younger than 2 years, the elderly, and immunocompromised patients, in many countries, (1, 13, 18, 26, 28). In Greece, Kouppari et al. investigated 881 cases of children’s diarrhea in Athens during 1991 to 1993 and found that *A. hydrophila* was the third etiologic agent in these cases of gastroenteritis. *Salmonella* spp. and *Campylobacter jejuni* produced higher percentages of 37.8 and 21.7, respectively, whereas the percentage of *A. hydrophila* was 16.3 (15).

*Aeromonas* species were first recognized as pathogens of cold-blooded animals (fishes, frogs, turtles, reptiles, and snails) long before they were considered to be pathogenic to humans (18, 23, 25, 27, 28). *Aeromonas* strains identical to diarrhea-associated strains are readily isolated from a wide variety of foods, including raw and pasteurized milk and white cheeses (1, 4, 7–9, 11, 13, 24). So the past several years, foods were considered as possible vehicles for foodborne human gastroenteritis caused by species of the *A. hydrophila* group (*A. hydrophila*, *A. sobria*, and *A. caviae*). Since *A. hydrophila* can grow at 5°C (2, 12, 18, 20) and produce exotoxins at this temperature (12, 17, 28), refrigeration will not prevent growth and may not guarantee safety from this pathogenic microorganism. Thus, the objective of this study was to determine the extent of aeromonads’ contamination of raw and pasteurized cow’s milk, raw ewe’s milk, and milk products from the region of Northern Greece.

MATERIALS AND METHODS

Sampling of foods. A total of 138 raw cow’s and 57 raw ewe’s milk samples were collected from more than 90 dairy farms.
TABLE 1. Incidence of Aeromonas species in raw milk and milk products

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. tested</th>
<th>No. (%) positive$^a$</th>
<th>No. (%)$^b$ A. hydrophila</th>
<th>A. caviae</th>
<th>A. sobria</th>
<th>Not classified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw cow’s milk</td>
<td>138</td>
<td>56 (40.57)</td>
<td>22 (15.94)</td>
<td>18 (13.04)</td>
<td>5 (3.62)</td>
<td>11 (7.97)</td>
</tr>
<tr>
<td>Pasteurized cow’s milk</td>
<td>80</td>
<td>0 (0)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Raw ewe’s milk</td>
<td>57</td>
<td>20 (35.08)</td>
<td>8 (14.03)</td>
<td>6 (10.53)</td>
<td>2 (3.51)</td>
<td>4 (7.01)</td>
</tr>
<tr>
<td>Feta cheese</td>
<td>23</td>
<td>0 (0)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Anthotyros cheese</td>
<td>39</td>
<td>4 (10.25)</td>
<td>4 (10.25)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Manouri cheese</td>
<td>36</td>
<td>3 (8.33)</td>
<td>3 (8.33)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Rice pudding</td>
<td>15</td>
<td>0 (0)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$ Percentage of samples in which Aeromonas spp. was found.

$^b$ Percentage of Aeromonas sp. found. ND, not detected.

The presence of Aeromonas species in raw milk and other milk products in Northern Greece is presented in Table 1. Fifty-six (40.5%) of the 138 raw cow’s milk samples analyzed were contaminated with Aeromonas spp. The confirmation of the isolates indicated that 22 samples (15.9%) were contaminated with A. hydrophila, 18 samples (13.0%) with A. caviae, 5 samples (3.6%) with A. sobria, and 11 samples (7.9%) with other Aeromonas spp. not classified.

Twenty (35.0%) of the 57 raw ewe’s milk samples analyzed were contaminated with Aeromonas spp. (Table 1). The confirmation of the isolates indicated that eight samples (14.0%) were contaminated with A. hydrophila, six samples (10.5%) with A. caviae, two samples (3.5%) with A. sobria, and four samples (7.0%) with other Aeromonas spp. not classified.

None of the 80 pasteurized cow’s milk, 23 Feta cheese, and 15 rice pudding samples yielded Aeromonas spp. Four (10.2%) of the 39 Anthotyros cheese samples and 3 (8.3%) of 36 Manouri cheese samples analyzed were contaminated with A. hydrophila, but no countable population was noted in SAA.

The populations of A. hydrophila, A. caviae, and A. sobria in raw cow’s and ewe’s milk samples counted in SAA are presented in Table 2. In 23 of the 56 raw cow’s milk samples and 16 of the 20 raw ewe’s milk samples that were contaminated with Aeromonas spp., populations of 1.5 × 10^2 to 5.0 × 10^3 CFU/ml were counted in SAA (Table 2). Specifically, in 13 of the raw cow’s milk samples, populations of A. hydrophila of 3.0 × 10^2 to 5.0 × 10^3 CFU/ml were counted. In eight of the raw cow’s milk samples, populations of A. caviae of 2.0 × 10^2 to 3.0 × 10^3 CFU/ml were counted, and in two of the raw cow’s milk samples, populations of A. sobria of 2.5 × 10^3 and 5.0 × 10^3 CFU/
ml were counted in SAA. Also, in eight raw ewe’s milk samples, populations of \textit{A. hydrophila} of $5.0 \times 10^2$ to $5.0 \times 10^3$ CFU/ml were counted. In six raw ewe’s milk samples, populations of \textit{A. caviae} of $1.5 \times 10^2$ to $1.0 \times 10^3$ CFU/ml were counted, and in two raw ewe’s milk samples, populations of \textit{A. sobria} of $5.0 \times 10^2$ and $1.0 \times 10^3$ CFU/ml were counted in SAA (Table 2).

### DISCUSSION

The results of our study indicate that \textit{Aeromonas} species are common in raw milk in Greece. In this study 40.5% (56/138) of the raw cow’s milk samples and 35.0% (20/57) of the raw ewe’s milk samples analyzed were contaminated with \textit{Aeromonas} spp. Strains of \textit{A. hydrophila}, \textit{A. caviae}, and \textit{A. sobria} with populations of up to $5.0 \times 10^3$ CFU/ml were predominant and mainly isolated. These findings agree with reports of other investigators who have identified that \textit{A. hydrophila}, \textit{A. sobria}, and \textit{A. caviae} are the predominant \textit{Aeromonas} species recovered from raw milk and milk products (13, 24). Also, \textit{A. hydrophila} was found in various food at levels of less than $1 \times 10^3$ to $5 \times 10^3$ CFU/g (4, 21), and the pathogen was capable of growing 10- to 1,000-fold at 5°C, indicating a competitive psychrotrophic growth in a variety of foods including raw milk (12, 13, 18, 19, 21). This suggests that \textit{A. hydrophila} can probably grow in Anthotyros and Manouri cheese. \textit{Listeria monocytogenes} can grow in Myzithra, Anthotyros, and Manouri whey cheeses even at refrigeration temperature because of their high pH value (approximately 6.0) and low-salt and high-moisture contents (22). The time–temperature conditions for whey cheese manufacture (82 to 92°C for 15 to 30 min) are lethal to \textit{Aeromonas} strains. Thus, the presence of \textit{A. hydrophila} in Anthotyros cheese and Manouri cheese samples (Table 1) was probably a result of postprocessing contamination. \textit{A. hydrophila} failed to survive during ripening of Feta cheese (unpublished data) because of the low pH of the product (approximately 4.5); thus, none of the Feta cheese samples analyzed yielded \textit{Aeromonas} spp. Also, none of the pasteurized milk and rice pudding samples yielded \textit{Aeromonas} spp. (Table 1), since postprocessing contamination of these products is more easy to prevent under close control during packing.

The results of this work indicate that motile aeromonads are present in raw milk in Greece. Also, the presence of \textit{A. hydrophila} in the whey cheeses Anthotyros (10.2%) and Manouri (8.3%) is more of concern because of their high pH value and low-salt and high-moisture content. The behavior of \textit{A. hydrophila} during storage of whey cheeses is currently under investigation in our laboratory.

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### REFERENCES


