A Research Note

Biovars, Serovars, and Phagovars of *Yersinia enterocolitica* Isolated From 450 Samples of Cold Food in San Luis, Argentina

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**ABSTRACT**

A search of *Yersinia enterocolitica* in foods of animal origin has been carried out. Isolates were obtained from 450 samples of cold foods: 100 samples of cooked ham, 150 samples of salami, 100 samples of porcine cheese (artisan cold foods), and 100 samples of mortadella. Enrichments were performed in 0.067 M phosphate buffered saline solutions, pH 7.6, containing 1% sorbitol and 0.15% biliary salts. The samples were postenriched in 0.5% KOH. Subcultures were done in *Salmonella-Shigella* agar and MacConkey agar. Isolates were identified through biochemical, serological, and phagotyping methods. The following biovars (B), serovars (O), and phagovars (Lis) were isolated from cooked ham B2 O:9 Lis X3 (1%), from salami B2 O:5 Lis X2 and B2 O:9 Lis X3 (1.33%), from porcine cheese B2 O:9 Lis X3 (2%), and from mortadella (0%). Virulence tests (calcium dependent growth at 37°C and autoagglutination activity at 37°C) were always negative. Serovar B2 O:9 Lis X3 associated with human disease was isolated. It is concluded from the results of this study that *Y. enterocolitica* isolates from cold foods lack of pathogenic importance.

*Yersinia enterocolitica* is an enterobacterium frequently involved in human acute enteritis. Worldwide studies indicate that foodborne and waterborne *Y. enterocolitica* infections in humans are essentially transmitted by the consumption of raw, undercooked, or recontaminated foods (72). Sometimes the biovars-serovars isolated from cold foods have been associated with human infection and sometimes not. Two chromosomal factors inv and ail and a plasmid of 70 kilo-bases (Kb), called pYV, are involved in the pathogenesis of yersiniosis (14,17).

Several strains of *Y. enterocolitica* were isolated for the first time in Argentina from fresh sausages (10). The purpose of this study was the isolation of *Y. enterocolitica* in foods of animal origin.

**MATERIALS AND METHODS**

**Specimens**

A total of 450 samples of cold foods were isolated. They were 100 samples of cooked ham, 150 samples of salami, 100 samples of porcine cheese (product elaborated with porcine heads and bovine jaws and tongue and cooked at appropriate temperature and time), and 100 samples of mortadella. Sampling procedure was performed in public markets during the period of March-December 1990.

**Reference strains of *Y. enterocolitica***

Two plasmid-bearing strains, *Y. enterocolitica* WA B2 O:8 Lis X2, human source (USA) and *Y. enterocolitica* 099 O:9, human source (Canada); and isogenic plasmid-cured derivatives of *Y. enterocolitica* WA B2 O:8 Lis X2 and *Y. enterocolitica* 1821 B2 O:8 Lis X2, human source (USA), were assayed. All strains were provided by Dr. Georg Kapperud (Norway).

**Isolates and identification**

Enrichments were performed in phosphate-buffered saline solution (KH2PO4-Na2HPO4 0.067 M and NaCl 0.85%) pH 7.6, with the addition of sorbitol 1% and biliary salts 0.15%, for 3 weeks at 4°C. The samples were postenriched in saline 0.5% KOH solution for 30 s (8). The isolates were done onto *Salmonella-Shigella* and MacConkey agar (Merck). Cefsulodin-irgasan-novobiocin agar could not be used because it was not available in the laboratory. Isolates were identified through standard biochemical tests (3). Biovar, serovar, and phagovar classifications were carried out by Dr. Elisabeth Carniel at the Institut Pasteur of Paris, France.

**Virulence tests**

Calcium-dependent test was performed by the method of Higuchi and Smith (11), and the autoagglutination test was done as described by Laird and Cavanaugh (13).

**Antibiotic susceptibility**

Antimicrobial susceptibility tests were performed according to Bauer-Kirby procedure (2).

**Detection of beta lactamase**

Beta lactamase activity was performed by the rapid iodometric method (4).

**RESULTS**

**Reference strains**

Two *Y. enterocolitica* reference strains representative of the most pathogenic and two nonpathogenic strains were selected. They showed the expected behavior according to their properties.
Isolates and identification

Table 1 shows the biovars (B), serovars (O), and phagovars (Lis) isolated from cooked ham, B, 0:9 Lis X, (1%); from salami B, 0:5 Lis X, and B, 0:9 Lis X, (1.33%); from porcine cheese B, 0:9 Lis X, (2%) and mortadella (0%).

<table>
<thead>
<tr>
<th>Foods</th>
<th>Positive samples</th>
<th>No. of samples</th>
<th>Biovars</th>
<th>Serovars</th>
<th>Phagovars</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked ham</td>
<td>1</td>
<td>100</td>
<td>2</td>
<td>9</td>
<td>X,</td>
</tr>
<tr>
<td>Salami</td>
<td>2</td>
<td>150</td>
<td>1</td>
<td>5</td>
<td>X,</td>
</tr>
<tr>
<td>Porcine cheese</td>
<td>2</td>
<td>100</td>
<td>2</td>
<td>9</td>
<td>X,</td>
</tr>
<tr>
<td>Mortadella</td>
<td>0</td>
<td>100</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Virulence tests

Calcium-dependent growth and autoagglutination activity at 37°C were negative.

Beta lactamase activity

All strains produced beta lactamase.

Antimicrobial susceptibility

All five strains were susceptible to kanamycin (30 µg), cefuroxime (30 µg), chloramphenicol (30 µg), phosphomycin (50 µg), mezlocillin (75 µg), piperacillin (100 µg), and pipemidic acid (20 µg). They were resistant to ampicillin (10 µg). Two of them, B, 0:9 Lis X, and B, 0:5 Lis X, were resistant to sulfisoxazole (300 µg).

DISCUSSION

As demonstrated in the present study, Y. enterocolitica isolates from cold foods were characterized by only two serovars. The serovar B, 0:9 Lis X, was isolated from cooked ham, salami, and porcine cheese. This serovar was isolated in other works (10) from “chorizos” (thick fresh sausages) and bovine tongue. The serovar B, 0:5 Lis X, was isolated only from salami, but it was previously isolated in other works from bovine tongue and cecum (10) and chicken. Two serovars were isolated from salami. The isolation of enterobacteria from cold foods has been previously reported. Curi de Montbrun et al. (6) isolated Salmonella from mortadella, salami, raw, and cooked ham in Mendoza City, Argentina.

The heating applied during ham precooking or softening decreases the bacteria pollution but it does not sterilize. For this reason, these foods must be preserved in cold.

Sörqvist (16) observed that Yersinia strains showed somewhat lower rates of inactivation during the early stages of heating; the cause of these lags could be attributed to the fact that some bacterial cells occurred in clumps of two or more cells (18). The curing preservation effect is mostly attributed to the sodium chloride maximum concentration (10%) (5); some bacteriostatic effect is due to nitrite and in a minor proportion to nitrate. The salts, the high carbohydrates concentration (50%) (5), and meat proteins are combined to decrease the water activity values of cured meat (9). Yersinia pestis and Yersinia pseudotuberculosis tolerate up to 3.5% NaCl, and the other species can tolerate up to 5% NaCl (3).

Regarding pH, Adams et al. (1) investigated the growth of two pathogenic and one environmental serotype of Y. enterocolitica under acidic conditions and at 4 and 25°C. At the lower temperature, the maximum growth inhibitory pH was 0.3-0.5 pH units higher than 25°C.

From the finding of five Y. enterocolitica strains in 450 samples of cold foods, it is inferred that probably it was caused by a possible contamination because the samples were taken from displayed pieces at sale. Otherwise, these bacteria should not develop due to the salt content and pH, and if they appear it could be attributed to the presence of a big inoculum. The low number of Y. enterocolitica isolates in this study could be attributed to postenrichment with KOH. However, this treatment was successfully applied by Delmas et al. (7) for the Y. enterocolitica recovery from various pork subproducts. The presence of the virulence plasmid in all Yersinia strains was tested by calcium-dependent growth and by autoagglutination activity. These tests were negative in all instances (15). It is concluded from the results of this study that Y. enterocolitica isolates from cold food are without pathogenic importance even considering the isolation of serovar 0:9 which is associated to human disease. The lack of pathogenic activity showed for these strains may be attributed to the loss of the virulence plasmid.

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REFERENCES

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