Research Note

Prevalence of *Yersinia enterocolitica* in Hake (Merluccius hubbsi) Fillets

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**ABSTRACT**

*Yersinia enterocolitica* is a human pathogenic bacterium. The prevalence of *Y. enterocolitica* in refrigerated hake fillets sold for human consumption in retail stores was investigated in order to determine the degree of pathogenicity. Three hundred samples were enriched in 0.067 M phosphate-buffered saline, pH 7.6, with 1% sorbitol and 0.15% biliary salts, postenriched in 0.5% KOH, and isolated in salmonella-shigella agar and MacConkey agar. Twelve strains of *Yersinia* were isolated from the whole group of samples, 11 (91.6%) of which corresponded to *Y. enterocolitica* and 1 (8.3%) to *Y. intermedia*. The *Y. enterocolitica* strains recovered were *Y. enterocolitica* B1 O:5 Lis X₀ (1 strain), *Y. enterocolitica* B3 O:5 (1 strain), *Y. enterocolitica* B1 O:6,30-6,31 (1 strain), *Y. enterocolitica* B1 O:7,8-8,19 (1 strain), *Y. enterocolitica* B1 O:7,8-8,19 Lis X₂ (7 strains) and *Y. intermedia* B1 O:40 Lis X₀ (1 strain). Of the 12 strains isolated, 8 (66.7%) were recovered by alkaline postenrichment. The first two strains were positive for virulence tests (autoagglutination and calcium dependence for growth at 37°C). The antibiotic susceptibility of the isolated strains was studied by the agar-diffusion method according to Bauer-Kirby, modified by Barry. Some of the isolated strains were potentially pathogenic, representing a risk for human health.

Key words: *Yersinia enterocolitica*, hake, prevalence, virulence, antibiotic susceptibility

*Yersinia enterocolitica* is a gram-negative bacterium which causes serious gastrointestinal infection in humans. It is considered an emergent microorganism (21) transmitted by water and contaminated foods. *Y. enterocolitica* is responsible for enterocolitis in warm or cold regions, which appears to be linked to changes in eating habits (20), such as an increase in the consumption of raw vegetables and food preserved at 4°C, a temperature which permits the growth of *Yersinia* spp. Two chromosomal factors, inv and ail, and a 70-kb plasmid, called pVY, are involved in the pathogenicity of *Yersinia* spp. (15, 19).

Numerous works have demonstrated that *Y. enterocolitica* has been isolated from a wide variety of foods such as raw milk, beef, pork, chicken, turkey, oysters, shrimp, crab, and vegetables (5, 14, 23, 24, 27).

Fresh fish is subjected to good safety controls in most regions of the world. In spite of this, this product bears a potential risk when eaten raw, due to toxins and to the presence of some microorganisms. Fresh fish muscle structure provides an excellent substrate for microbial development, thanks to its favorable water activity, neutral pH, and high levels of soluble nutrients (12). A microbiological control therefore becomes important.

The possibility of seafoods acting as vehicles of pathogenic microorganisms is related to environmental conditions. Temperature is the environmental factor which is most influential in the composition of fish microflora. The typical microbial flora of fish, as well as that of warm-water shellfish, is predominantly psychrophilic, since seawater temperature is 10°C or lower (12).

The purpose of the present work was to investigate the prevalence of *Y. enterocolitica* in refrigerated hake fillets for human consumption and to determine its degree of pathogenicity.

**MATERIALS AND METHODS**

**Samples**

Three hundred refrigerated hake fillet samples provided by four retail food stores in San Luis, Argentina were studied. The samples were placed in refrigerated containers and transported to the laboratory where they were immediately analyzed. The samples were collected over a 2-year period.

**Isolation and identification**

Ten grams of the sample were ground in sterilized mortars and enriched in 90 ml of phosphate-buffered saline (PBS) (0.067 M KH₂PO₄ — Na₂HPO₄ and 0.145 M NaCl), pH 7.6, with the addition of 1% sorbitol and 0.15% biliary salts. The samples were incubated at 4°C for 21 days. After incubation, each sample was processed as follows: (i) isolation in MacConkey agar (MC) and salmonella-shigella agar (SS), incubating for 48 h at 25°C; and (ii) postenrich-
ment of 0.5 ml of enrichment buffer in 4.5 ml of 0.5% KOH in 0.145 M NaCl solution for 30 s, followed by immediate isolation in MC and SS as above described. The colonies with characteristic morphology were identified by conventional biochemical tests (3). Typical Yersinia colonies were transferred to triple sugar iron agar slants (A/A−) and assayed for the following biochemical tests: oxidase, lysine and ornithine decarboxylase, urease, phenylalnine deaminase, acid production from glucose, sucrose, sorbitol, manni-
tol, arabinose and salicin. Gram staining was also performed.

The classification into biovar, serovar and phagovar was performed by Dr. Elisabeth Carniel of the Yersinia Reference National Center, Institute Pasteur, Paris, France.

Virulence tests and antibiotic susceptibility

The virulence tests were performed by the autoagglutination technique (15) and calcium dependence for growth at 37°C (11). The susceptibility to antibiotics was assessed by the agar diffusion method (Bauer-Kirby, modified by Barry) (2).

Reference strains

The reference strains used were Y. enterocolitica W22703 (pGC565) pYV (+) and Y. enterocolitica W 1024 O:9 pYV (+) (wt), kindly provided by Dr. Guy Cornelis of Louvain Catholic University, Belgium. These were employed as patterns for the virulence tests.

RESULTS AND DISCUSSION

The present study is the first one carried out on hake fillets in this region. Fish and crustaceae have been involved in 10% of outbreaks of foodborne diseases in the United States between 1978 and 1987 (18).

Of the 300 samples examined, 12 Yersinia strains were recovered, of which 11 (91.6%) corresponded to Y. enterocolitica and 1 (8.3%) to Y. intermedia.

The characteristics of the isolated strains according to biovar, serovar, and phagovar patterns are shown in Table 1. Among the Y. enterocolitica isolations, serovar O:7,8-8-8,19 prevailed (8 strains), followed by O:5 (2 strains) and O:6,30-6,31 (1 strain). Serovar O:5, as well as O:6,30-6,31 and O:7,8, also identified in these isolations, have been recovered by Bisset (4) from clinical human samples. They have also been identified in strains isolated from patients with gastrointestinal symptoms (22).

The flesh and internal organs of recently caught healthy fish are normally sterile, but psychrotrophic bacteria have been found in the skin and gills of Cape hake with

prevalence of Moraxella and Pseudomonas (60% and 80%), and Corynebacterium and Micrococcus (20% and 40%) (25, 26).

Several studies have shown the association of human infections with consumption of Y. enterocolitica-contami-
nated food (6, 13, 16). There is no information about yersiniosis caused by consumption of raw hake fillets.

In the present study all the Yersinia strains were recovered from MC agar, a culture medium of low selectiv-
ity which permits most of the Y. enterocolitica strains to recover (8). Of the 12 strains isolated, 8 (66.6%) were recovered by alkaline postenrichment. Aulisio et al. (1) isolated 54 Yersinia strains from 6 different kinds of food (celery, chicken, lettuce, oyster, pork and spinach), of which 43 (80%) were recovered by the alkaline postenrichment method.

The Yersinia isolation frequency in the present study was 4%. This percentage does not present differences with the Y. enterocolitica isolations performed by Peixotto et al. (23) from shrimp (4%), but it is significantly lower than that obtained for oysters (13%) and crab (21%) by the same author.

The presence of the 70-kb plasmid was assayed in all the Yersinia strains by means of tests of autoagglutination and calcium dependence at 37°C. A significant difference was observed between the number of virulent and nonviru-
 lent strains. Positive results were obtained for 2 of the 12 isolated strains, classified as Y. enterocolitica B3 O:5 and Y. enterocolitica B1 O:5 Lis X0, respectively. The remaining strains gave negative results. The reference strains were included in these tests as controls with positive results.

The antibiotic susceptibility data are shown in Table 2.

The detection of two Yersinia strains carrying the virulence plasmid and the fact that hake fillets are likely to be consumed raw imply a possible risk for human health. In Argentina it is not customary to eat dishes with raw hake fillets, though it is usual in bordering countries. The contamination of raw fish is probably due to the transference of bacteria during evisceration and filleting processes by direct handling, from the intestine and skin to the fillet surface, or from the environment through contaminated working surfaces, knives, machines, etc. The degree of

### TABLE 1. Biovars, serovars and phagovars of Yersinia strains isolated from hake fillets

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of strains</th>
<th>Biovar</th>
<th>Serovar</th>
<th>Phagovar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y. enterocolitica</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>X0</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>ND⁷</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>1</td>
<td>1</td>
<td>6,30-6,31</td>
<td>ND⁷</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>1</td>
<td>1</td>
<td>7,8-8-8,19</td>
<td>X0</td>
</tr>
<tr>
<td>Y. enterocolitica</td>
<td>7</td>
<td>1</td>
<td>7,8-8-8,19</td>
<td>X0</td>
</tr>
<tr>
<td>Y. intermedia</td>
<td>1</td>
<td>1</td>
<td>40</td>
<td>X0</td>
</tr>
</tbody>
</table>

⁷ ND: not determined.

### TABLE 2. Antibiotic susceptibility of 12 Yersinia strains isolated from hake fillets

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration (µg)</th>
<th>Susceptible no. (%)</th>
<th>Resistant no. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanamycin</td>
<td>30</td>
<td>12 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>10</td>
<td>12 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>30</td>
<td>12 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>12 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mezlocillin</td>
<td>75</td>
<td>12 (100.0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Polymixin B</td>
<td>35.7</td>
<td>12 (100.0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
| Trimethoprim-
| sulframethoxazole   | 1.25/23.75         | 12 (100.0)          | 0 (0)            |
| Chloramphenicol     | 30                 | 12 (100.0)          | 0 (0)            |
| Ampicillin          | 10                 | 3 (25.0)            | 9 (75.0)         |
| Phosphomycin        | 50                 | 10 (83.3)           | 2 (16.6)         |
contamination depends on care and hygiene at processing plants, but it is also a function of the bacteriological quality of the raw material employed (7, 17, 26).

The most frequently used conservation method is refrigeration, which delays bacterial growth and subsequent alteration of the fish. Storage at 4°C for prolonged periods could favour *Yersinia* growth in the absence of low levels of background psychrotrophic flora, in spite of the fact that fish cannot be stored for long periods. If the flsh is present in high levels, it competes with advantage on *Yersinia*, masking its presence. Refrigerated foods are potential vehicles for the development of these microorganisms (6, 9, 13). This implies a risk for human health if the food is eaten raw or if it contaminates other foods in the refrigerator.

There are few works reporting on *Yersinia* sensitivity to heat. Hanna et al. (10) observed that strains treated at 60°C did not survive after 3 min of heat even when initial bacterial counts were 6 to 7 log units per ml. However, fish is eaten raw in several regions. In Taiwan, a pie prepared with frozen raw or semi-cooked shrimp is very popular. These raw products which are kept at refrigeration temperatures for long periods have shown overgrowth of psychrotrophic *Vibrio* spp. (28).

Due to the importance of *Y. enterocolitica* as a potential human pathogen it is considered convenient to perform microbiological controls of this product to reduce consumer risks.

ACKNOWLEDGMENTS

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REFERENCES