Research Note

Occurrence of Zoonotic Bacteria in Retail Game Meat in Japan with Special Reference to *Erysipelothrix*

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ABSTRACT

From December 1993 to March 1994, a total of 93 wild boar and 30 deer meat samples were purchased from 13 retail shops and examined for the presence of species of *Erysipelothrix*, *Yersinia*, *Listeria*, *Salmonella* and *Campylobacter*. *Erysipelothrix* spp. were isolated from 41 wild boar and 15 deer samples. These isolates were identified as 13 serotypes. Serotypes 6 and 2 were the most predominant. Of 17 isolates examined, 14 isolates were highly virulent for mice. The bacteria of the same serotype were isolated repeatedly from the samples purchased in 4 of the shops. *Yersinia enterocolitica* was isolated from 36 wild boar and 10 deer samples; however, all isolates of *Y. enterocolitica* did not have pathogenic properties. *Listeria monocytogenes* was isolated from 5 wild boar samples. The isolates were identified as serotype 1/2c and 4b. *Salmonella* spp. were isolated from 2 wild boar samples; one isolate was identified as S. *typhimurium* and the other was untypable. *Campylobacter* spp. were not detected.

Deer, *Erysipelothrix*, game meat, wild boar

The consumption of wild boar and deer meat is increasing with the trend towards serving these kinds of the game meat as specialty foods. Most of these kinds of game meat are sold and consumed during winter, since the wild boars and deer are captured in the winter hunting season.

Game meat is not under the control of the Meat Inspection Service as is meat from domestic animals such as cattle, swine, horses, sheep, and goats in Japan. Since game meats are usually processed in factories without official permits, the microbiological quality of game meat in Japan is not known. The first objective of the present study was to determine the prevalence of pathogenic bacteria such as species of *Salmonella, Listeria, Yersinia, Erysipelothrix*, and *Campylobacter* in the meat of wild boars and deer. The second was to elucidate the public health significance of the *Erysipelothrix* isolates by examining the serotype distribution and pathogenicity against mice of the isolates from the retail game meat samples.

MATERIALS AND METHODS

Meat samples

From December 1993 to March 1994, 93 samples of wild boar meat were purchased from 13 retail shops and 30 deer meat samples from 7 retail shops in the Kyushu, Kansai, Chugoku, and Kanto areas of Japan. Each sample was packaged in a vinyl bag and transported in ice to our laboratory. Samples were examined for pathogens immediately upon arrival.

Sampling method

Approximately 100 g of each sample was chopped and 10 g of the chopped meat was inoculated into 90 ml of each enrichment broth specific for the pathogens. In addition, for direct detection 1 g of the chopped meat was homogenized in 9 ml of phosphate buffer solution (PBS), pH 7.2, and 0.1 ml of the homogenate was spread over the surface of the selective agar media mentioned below.

Tenfold dilutions of the homogenate were made with PBS and bacterial quality was determined by enumerating colony-forming units by plating in Trypticase soy agar (BBL, Cockselyville, MD) and incubating at 25°C for 48 h.

Isolation and identification of pathogens

For enrichment of *Erysipelothrix* spp., 10 g of the meat sample was inoculated into 100 ml of tryptose phosphate broth (Difco Laboratories, Detroit, MI) containing 0.1% Tween 80, 50 µg of gentamicin, and 400 µg of kanamicin and incubated at 37°C for 48 h. A loopful of the culture broth was streaked on a selective agar medium plate prepared by adding 1.75% agar to the enrichment broth. The agar plate was incubated at 37°C for 48 h. Suspect colonies with typical morphological characteristics of *Erysipelothrix* spp. were picked from the plate and streaked on a second...
selective agar plate. The isolates were identified as *Erysipelothrix* spp. on the basis of cell morphology, characteristic reactions on triple sugar iron agar (Difco) slants and test-tube brush-like growth in gelatin (16). Atypical isolates were also tested for plasma-clotting activity (12). This clotting reaction is useful in differentiating *Erysipelothrix* spp. from other gram-positive bacilli which do not show this reaction. Serotyping of the isolates was performed by an agar gel double-diffusion precipitation technique (11) with rabbit antisera representing serovars 1 through 23 of *E. rhusiopathiae* (10). Tests for pathogenic characteristics of 17 selected isolates belonging to 13 serotypes were conducted in 4-week-old female ddY mice (Nippon SLC, Hamamatsu, Japan). Two dilutions of beef infusion broth (Difco) culture of each strain containing approximately 10^3 and 10^6 CFU/0.1 ml were used. Five mice were injected subcutaneously with 0.1 ml of cell suspension for each isolate. To determine the 50% lethal dose (LD₅₀), mortality rates were recorded 14 days after injection. Virulence was designated as follows: LD₅₀ of <3.0 (log 10^5 CFU), highly virulent; 3.1 to 8.0, weakly virulent; and ≥8.1, nonvirulent.

For enrichment of *Yersinia* spp., PBS, pH 7.6, was used. The enriched samples were inoculated at 4°C for 2 weeks. After alkali (KOH) treatment each culture broth was streaked onto a plate of *Yersinia*-selective agar base medium (Oxoid, Basingstoke, U.K.) containing irgasan and novobiocin; the plates were incubated at 25°C for 48 h. The suspect colonies were identified by the recommended biochemical tests (5). The auto-agglutination test (6) and calcium dependency test (3) were done to determine pathogenicity of *Y. enterocolitica* isolates.

For enrichment of *Listeria* spp. samples were inoculated in *Listeria* enrichment broth (Merck, Darmstadt, Germany) and incubated at 30°C for 48 h. A loopful of culture broth was streaked onto a plate of Palcam *Listeria*-selective agar medium (Merck) and incubated at 37°C for 48 h. Suspect colonies on the agar plate were subjected to biochemical identification (15). The *L. monocytogenes* isolates were tested by a colony-blot double-stain method with rabbit antisera prepared in our laboratory to determine the serotype.

*Enterobacteriaceae* species enrichment mannitol broth (Nissui Pharmaceutical Co., Ltd, Japan) was used for enrichment of *Salmonella* spp. The inoculated broth was incubated at 37°C for 24 h. One milliliter of the culture broth was inoculated into 9 ml of Hajna tetrathionate broth (Eiken, Chemical Co., Ltd, Japan) and incubated at 37°C for 48 h. The loopful of culture broth was streaked on a plate of mannitol-lysine-crystal violet-brilliant green agar medium (Nissui) which was then incubated at 37°C for 24 h. Suspect colonies were examined for triple sugar iron agar (Eiken) and lysine-indole motility agar (Nissui) reaction patterns. Serotyping of suspected isolates was performed with antisera of O and H antigens (Denka Seiken, Japan).

For enrichment of *Campylobacter* spp. a 10-g sample was inoculated in Preston broth (Oxoid) and incubated at 42°C for 24 h. A loopful of culture broth was streaked on a plate of blood agar base No. 2 medium (Oxoid) containing 5% horse blood and Skirrow's selective supplement (Oxoid). The plates were incubated at 42°C for 48 h using a Gas Pak Jar (BBL). Suspect colonies were examined for typical morphology, growth in a normal atmosphere, and the oxidase reaction.

**RESULTS**

Table 1 shows the prevalence of the pathogenic bacteria found in the assays by retail shops. *Erysipelothrix* spp. were isolated from 44.1% of the wild boar meat samples and 50.0% of the deer meat samples. *Yersinia* spp. were also frequently detected. *Y. enterocolitica* was isolated from 38.7% of the wild meat samples and 33.3% of the deer samples; however, all of these isolates were negative for both the autoagglutination and calcium dependency tests. *L. monocytogenes* was detected in 5 wild boar samples. Of five *L. monocytogenes* isolates, three were identified as serotype 1/2c and two as serotype 4b. *Salmonella* spp. were detected in 2 wild boar samples; one isolate was serotyped as *S. typhimurium* and the other was untypable. *Campylobacter* spp. were not isolated from any meat samples.

As for wild boar samples, the isolation rate of *Erysipelothrix* in shop I was significantly higher than that in shops G, H, K, and L (*P < 0.05*). The isolation rate of *Erysipelothrix* spp. in shop D was also significantly higher than that in shops G, H, and L (*P < 0.05*). Furthermore, in shop D the prevalence of *Yersinia* spp. was significantly higher than in shops G, K, L, and M and that of *Listeria*, higher than in the other shops except shop C (*P < 0.05*). As for shop D, the isolation rate of *Erysipelothrix* spp. from deer samples was also significantly higher than that in shops F, I, and L (*P < 0.05*). Serotypes of the *Erysipelothrix* isolates were as follows. Seventy-nine isolates were detected from 41 positive wild boar samples and 15 positive deer samples. Sixty-two strains were serotypes 1a, 1b, 2, 5, 6, 8, 9, 11, 16, and 19 of *E. rhusiopathiae* and five were serotypes 7 and 10 of *E. tonsillarum*. The remaining 12 strains were nontypable by antisera representing serotypes 1 through 23. Fifty-nine isolates from the wild boar meat samples were classified into 13 serotypes, and 20 isolates from the deer meat, into 6 serotypes. Serotypes 6 and 2 were the most predominant isolates from 13 wild boar and 7 deer, and from 13 wild boar and 5 deer samples.

In shops D, E, I and J, the *Erysipelothrix* isolation rate was comparatively high, and the same serotypes were repeatedly isolated from samples purchased on different dates, such as serotype 2 in shop E, serotype 6 in shops D and J, and serotype 11 in shop I.

The following data are not shown in Table 1. *Listeria* spp. were detected in 2 wild boar samples with 10^5 CFU/g, and *Erysipelothrix* spp. in 1 boar sample with 10^5 CFU/g by direct culture. Since pathogenic bacteria in the other samples were detected only by the enrichment culture method, the number of pathogens might be under 10^5 CFU/g of the game meat with the exception of the above-mentioned cases.

The pathogenicity tests for 17 selected isolates of *Erysipelothrix* showed that two isolates of serotypes 6 and
TABLE 1. Incidence of several pathogenic bacteria in boar and deer meat purchased in retail shops

<table>
<thead>
<tr>
<th>Kinds of meat</th>
<th>Retail shops</th>
<th>No. of samples</th>
<th>log CFU/g</th>
<th>Erysipelothrix</th>
<th>Y. enterocolitica</th>
<th>Others</th>
<th>L. monocytogenes</th>
<th>Others</th>
<th>Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild boar meat</td>
<td>A</td>
<td>2</td>
<td>5.3 ± 0.3</td>
<td>1 (50.0)</td>
<td>1 (50.0)</td>
<td>2 (100.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>2</td>
<td>6.5 ± 0.5</td>
<td>2 (100.0)</td>
<td>1 (50.0)</td>
<td>1 (50.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2</td>
<td>4.9 ± 0.0</td>
<td>2 (100.0)</td>
<td>0</td>
<td>2 (100.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>12</td>
<td>5.9 ± 1.0</td>
<td>9 (75.0)</td>
<td>8 (66.7)</td>
<td>10 (83.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>12</td>
<td>5.5 ± 0.6</td>
<td>5 (41.7)</td>
<td>7 (58.3)</td>
<td>7 (58.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>11</td>
<td>4.9 ± 0.7</td>
<td>4 (36.4)</td>
<td>6 (54.5)</td>
<td>5 (45.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>G</td>
<td>8</td>
<td>5.5 ± 0.8</td>
<td>0 (0)</td>
<td>1 (12.5)</td>
<td>2 (25.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>H</td>
<td>7</td>
<td>5.3 ± 0.8</td>
<td>0 (0)</td>
<td>4 (57.1)</td>
<td>1 (14.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>I</td>
<td>12</td>
<td>5.0 ± 0.4</td>
<td>10 (83.3)</td>
<td>4 (33.3)</td>
<td>4 (33.3)</td>
<td>0</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td></td>
<td>J</td>
<td>6</td>
<td>6.1 ± 0.6</td>
<td>4 (66.7)</td>
<td>1 (16.7)</td>
<td>2 (33.3)</td>
<td>0</td>
<td>1</td>
<td>16.7</td>
</tr>
<tr>
<td>Deer meat</td>
<td>K</td>
<td>6</td>
<td>5.2 ± 0.8</td>
<td>3 (33.3)</td>
<td>2 (22.2)</td>
<td>2 (22.2)</td>
<td>1 (11.1)</td>
<td>2</td>
<td>22.2</td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>7</td>
<td>3.8 ± 0.4</td>
<td>0</td>
<td>1 (14.3)</td>
<td>1 (14.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3</td>
<td>5.2 ± 0.5</td>
<td>1 (33.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>93</td>
<td>5.3 ± 0.9</td>
<td>41 (44.1)</td>
<td>36 (38.7)</td>
<td>40 (43.0)</td>
<td>5 (5.4)</td>
<td>22</td>
<td>23.7</td>
</tr>
</tbody>
</table>

a Mean ± standard deviation of CFU in Trypticase soy agar medium at 25°C in 48 h.
b Others contained Y. aldovae, Y. intermedia, Y. frederiksenii, Y. kristensenii, and Y. rhodei.
c Three isolates in shop D were serotype 1/2c, and two isolates in shops J and K were serotype 4b.
d Others contained L. innocua, L. welshimeri and L. grayi.

10 were nonvirulent, one untypable isolate was weakly virulent, and the remaining 14 isolates of 11 different serotypes were highly virulent for mice.

DISCUSSION

The results obtained in the present investigation demonstrated a high incidence of Erysipelothrix spp. in the meat of wild boars and deer. Erysipelothrix spp. have been isolated from various kinds of meat. In Japan, Erysipelothrix spp. could be isolated from 34% of retail raw pork by Shiono et al. (7). Ternström and Molin (13) reported that Erysipelothrix spp. are isolated from 36% of pork and 13% of chicken in Sweden. However, there has been no information about isolation of Erysipelothrix spp. from retail game meat and characterization of the isolates.

Various serotypes of Erysipelothrix have been isolated from meat. Shiono et al. (7) reported 14 serotypes detected in retail pork, with serotypes 6, 8, and 11 being predominant. Stenström et al. (8) reported that 4 serotypes were isolated from pork, with serotype 2 being predominant. The present study also demonstrated the presence of 13 serotypes of Erysipelothrix in the game meat, with serotypes 6 and 2 were predominant.

In the present study, bacteria of the same serotypes were repeatedly isolated from samples purchased from the same shop. This fact would suggest that the bacteria contaminated the meat during the process of slaughtering and dressing in the plants and retail shops. Furthermore, the fact that plural serotypes were detected from the same sample would suggest that these samples were contaminated by plural sources. These facts indicate that the high incidence of Erysipelothrix spp. in these kinds of the game meat might be due to secondary contamination. The degree of contamination of Erysipelothrix isolates differed among the shops examined. This might be due to the degree to which cleaning and disinfection of equipment and facilities is practiced by retail shops. Future investigations are warranted to examine contamination of processing equipment to find the sources of the bacteria, especially in the retail shops that showed a high prevalence of the bacteria.

Although the number of bacteria was lower than 10^2 CFU/g in most samples, most of the isolates of 11 serotypes proved highly virulent for mice in the present study. There may be arguments against the results of virulence tests in mice being applied to humans; but there may be a risk of consumers being infected with Erysipelothrix spp. during touching of game meat.

As for Yersinia spp., pigs are known to be an important reservoir, and pork and related products have been regarded as the main vehicles. Tsai et al. (14) have isolated Y. enterocolitica from 44% of pork samples in Taiwan. In Japan,
Fukushima et al. (2) reported that 56.7% of minced beef samples, 55.0% of minced pork, and 58.3% of minced chicken are contaminated with Yersinia spp., and pathogenic Y. enterocolitica was detected from 7.6% of pork. In the present study, Yersinia was isolated frequently, and Y. enterocolitica was detected from more than 30% of the game meat samples, but a pathogenic strain was not detected.

Listeria spp. have been isolated frequently from meat (4, 15). Wang et al. (15) have isolated Listeria spp. from 52% of beef, 60% of pork, and 70% of chicken, and L. monocytogenes from 28% of the pork and 5% of the chicken in retail shops in Beijing. According to Johnson et al. (4), most of the isolates from the meat are typed as serogroup 1/2, and a few of them as serogroups 3 and 4. Serogroup 4 has been isolated most frequently in food-borne outbreaks of human listeriosis (4). In this study the isolation rate of L. monocytogenes was not so high as that in other reports; however, the serotype 4b involved in human listeriosis was isolated from game meat.

Salmonella spp. have been isolated from various kinds of meat. Bensink et al. (1) have isolated salmonellas from 11% of feral pig carcasses and 34% of kangaroo carcasses destined for human consumption in Australia. In the present study, Salmonella spp. were isolated from 2 boar samples, and the isolation rate was not so high.

C. jejuni has been isolated from 41 to 77% of chicken meat; however, it has scarcely ever been isolated from beef and pork (9). In this study, Campylobacter spp. was not isolated from game meat.

REFERENCES


