One-Year (2003) Nationwide Pork Carcass Microbiological Baseline Data Survey in Taiwan

KUANG-SHENG YEH,1,* SHIH-PING CHEN,2 AND JIUNN-HORNG LIN2

1Department of Microbiology and Immunology, School of Medicine, Taipei Medical University, Taipei, Taiwan; and 2Division of Animal Medicine, Animal Technology Institute Taiwan, Chunan, Miaoli, Taiwan

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ABSTRACT

From January through December 2003, swab samples from 1,650 pork carcasses were collected from 39 slaughter plants in Taiwan. These samples were analyzed for the prevalence of indicator microorganisms and specific pathogens. Viable aerobic bacteria, total coliforms, and Escherichia coli were recovered from 100, 95.3, and 87.5% of these carcasses, respectively. Of those carcasses that harbored bacteria, the mean aerobic plate, total coliform, and Escherichia coli counts were 4.0, 0.6, and 0.1 log CFU/cm², respectively. Staphylococcus aureus, Clostridium perfringens, Campylobacter jejuni, Campylobacter coli, Listeria monocytogenes, and Salmonella were recovered from 4.8, 0.3, 13.8, 0.7, and 1.7 of 1,038 carcasses, respectively. E. coli O157:H7 was not detected from any carcass. When positive for a specific pathogen, the mean carcass concentration was 0.57 log CFU/cm² for S. aureus, 0.66 most probable number (MPN)/cm² for C. jejuni and C. coli, and 0.18 MPN/cm² for Salmonella. The findings of this study will help provide a reference for establishing hygienic standards and a criterion for evaluating the effects of slaughtering operations in Taiwan.

Foodborne diseases cause approximately 76 million illnesses, 325,000 hospitalizations, and 5,000 deaths each year in the United States (23). Food safety is a growing concern in many countries, and bacterial contamination of animal carcasses is a significant food safety issue. During slaughtering operations, the carcass surface may become contaminated with microorganisms from the gut and hide or from the facilities and slaughter plant personnel (9). The Food Safety and Inspection Service (FSIS) of the U.S. Department of Agriculture (USDA) mandates that all meat and poultry processing plants employ the hazard analysis critical control point (HACCP) pathogen reduction system to ensure meat product safety (3). The Nationwide Pork Microbiological Baseline Data Collection Program (12) was designed by FSIS to collect data to provide a microbiological profile of pork carcasses and to use such information gained from this program as a reference for further investigation and evaluation of new preventive measures. For example, the performance standard for Salmonella was set based on the data collected by such program (13).

The Bureau of Animal and Plant Health Inspection and Quarantine (BAPHIQ) of Taiwan is the central agency responsible for the enforcement of the Meat Inspection Act, which empowers the agency to review slaughter plant facilities, inspect carcass products, and assure proper labeling of carcasses. The Meat Inspection Act primarily focuses on gross findings and pathologic changes to reject diseased animals going to slaughter plants. Because bacterial contamination of carcasses is not detectable by visual inspection, a standard and feasible microbiological survey system must be developed and put into force. BAPHIQ therefore adapted the framework of the program designed by FSIS and began to investigate the prevalence of both indicator microorganisms and specific pathogens on pork carcasses nationwide. The objective was to build up the Taiwanese pork carcass baseline database, which could be used as a criterion to evaluate the effects of slaughtering operations and provide a reference for future international commercial treaties. In this article, we report the microbiological profiles of pork carcasses surveyed in Taiwan in 2003.

MATERIALS AND METHODS

Sample collection. Thirty-nine slaughter plants were chosen for this surveillance program and were evaluated between January and December 2003. These plants were located in 16 counties of Taiwan. Sampling was performed by the meat inspection veterinarians in the slaughter plants. Each slaughter plant was sampled two or three times during the whole year, and 10 carcass sponge samples (as described subsequently) were collected each time. The randomly chosen carcasses were swabbed after evisceration and before refrigeration. The sampling sites for each carcass included the belly, ham, and jowl areas because these locations are most commonly contaminated during slaughtering operations (20). A reference template was placed over the selected location and each sample was obtained by swabbing five times horizontally and vertically in an enclosed area (10 by 10 cm) using a sterile sponge (Whirl-Pak Speci-Sponge, NASCO, Fort Atkinson, Wis.) moistened with 10 mL of 0.1% peptone. One sponge was used to swab three sites per carcass (total area of 300 cm²). The bagged samples were packed with ice and placed in a box, and the entire box was delivered by a contracted express company. The trucks used by the delivery company are able to maintain a temperature of 4°C. On average, it took about 24 h between the time the carcass was swabbed and the time the laboratory personnel began processing.
the samples. Samples from some slaughter plants may have been held up to 36 h before processing because of the long distance between the individual slaughter plant and the laboratory.

Microbiological analytical methods. Ten milliliters of 0.1% peptone was added to the sample bag containing the sponge. The sponge was massaged thoroughly by hand for 1 min, and the suspension was squeezed from the sponge. The suspension (10 ml) was then transferred to a sterile tube and used as the starting material for the following tests. The microbiological analytical methods used in this study followed the FSIS-USDA Microbiological Laboratory Guidebook (10), the AOAC International official methods (2), or other standard methods. Aerobic bacteria, Escherichia coli, and coliforms were enumerated by using Petrifilm aerobic count plates and Petrifilm E. coli-coliform count plates (3M Microbiology, St. Paul, Minn.) based on AOAC official methods 989.10 and 991.14. A portion (1.0 ml) of a diluted sample (diluted with 0.1% peptone) was added to the center of a film base, and a top film was carefully placed down on the inoculum. The sample was distributed over a prescribed growth area using a downward pressure in the center of a plastic spreader. The plates were left undisturbed for 1 min to permit the gel to solidify prior to incubation at 32°C for 48 h for aerobic bacteria and 35°C for 24 h for total coliforms and E. coli. For aerobic bacteria, colonies in the countable range (25 to 250 colonies) were enumerated. Red colonies with gas bubbles were counted as coliforms, and blue colonies with gas were considered E. coli.

For Staphylococcus aureus, a 0.1-ml sample was spotted onto the surface of Baird Parker RPF agar (bioMérieux, Marcy l’Etoile, France) and spread evenly over the agar surface, and each plate was incubated at 37°C for 16 to 18 h (19). Dark colonies 2 to 3 mm in diameter with a surrounding opaque halo were considered S. aureus (4).

For Clostridium perfringens, a 0.1-ml sample was spotted onto the surface of a tryptose sulfite cycloserine agar plate (Oxoid, Hampshire, UK), spread evenly, and air dried. A layer of egg yolk was then added on top of the agar (16, 18). Plates were incubated anaerobically at 37°C for 16 to 18 h. Black colonies with 2- to 4-mm opaque halos were selected and inoculated into thioglycollate broth (bioMérieux), and the identity of the colonies as C. perfringens was biochemically confirmed by the criteria specified in the FSIS-USDA Microbiological Laboratory Guidebook. C. perfringens are nonmotile and can reduce nitrate, ferment lactose, liquefy gelatin, and produce acid from raffinose.

Media used to isolate Campylobacter jejuni and Campylobacter coli included Hunt enrichment broth (HEB) and modified Campylobacter charcoal differential (MCCD) agar (Oxoid). A 0.5-ml sample was inoculated into 4.5 ml of HEB and incubated at 37°C with constant shaking at 100 rpm for 4 h in a 2.5-liter AnaeroJar (Oxoid) under a microaerophilic environment of 5% O2, 10% CO2, and 85% N2, generated by a CampyGen pack (Oxoid). Cefoperazone sodium solution (Sigma, St. Louis, Mo.) was added to each culture to achieve a final concentration of 30 mg/liter (10). A microaerophilic atmosphere was reestablished, and the cultures were incubated at 42°C with constant shaking at 100 rpm for 20 h. A 0.1-ml aliquot of the enrichment culture was spotted onto the surface of MCCD agar and spread evenly (6), and the MCCD agar plates were incubated at 42°C microaerobically for 24 h. Campylobacter colonies on MCCD agar are colorless to light cream, 1 to 2 mm in diameter, and spreading with an irregular edge. Further identification was performed by testing for sensitivity to nalidixic acid (30 μg) but resistance to cephalothin (30 μg). A PCR assay to detect the presence of the C. jejuni-C. coli-specific gene cadF (22) was conducted using forward primer 5’-TTGAAGGTAATTAGATATG-3’ and reverse primer 5’-CTAATACCTAAAGTTGAAC-3’. Conditions for PCR were denaturation at 94°C for 2 min, 30 cycles of 94°C for 1 min, 45°C for 1 min, and 72°C for 3 min, and a final extension at 72°C for 15 min. The PCR product was about 400 bp in length (22).

For Listeria monocytogenes, a 0.5-ml sample was inoculated into 4.5 ml of University of Vermont (UVM) broth (Oxoid) and incubated at 30°C for 20 to 24 h (11, 17). An aliquot of 50 μl from the resulting UVM culture was added to 5.0 ml of Fraser broth (Oxoid) and grown at 35°C for 27 h (15). The Fraser enrichment was streaked onto modified Oxford agar (Oxoid) and incubated at 35°C for 24 h. Round colonies surrounded by zones of esculin hydrolysis (black) were chosen for confirmation. API Listeria (bioMérieux) and the BAX detection system for screening L. monocytogenes (DuPont Qualicon, Wilmington, Del.) were both used to expedite the final identification.

For Salmonella, a 0.5-ml sample was enriched in 4.5 ml of buffered peptone water (Difco, Becton Dickinson, Sparks, Md.) at 35°C for 20 to 24 h. A 50-μl aliquot from the culture was inoculated into 5.0 ml of Rappaport-Vassiliadis (RV) broth (Oxoid), grown at 42°C for 24 h (2), and streaked onto Salmonella detection and identification medium (bioMérieux). Suspected colonies were confirmed by serology (polyA-I and Vi, Difco, Becton Dickinson), API 20E (bioMérieux), or the BAX system for screening Salmonella (DuPont Qualicon).

For Escherichia coli O157:H7, a 0.1-ml sample was placed in 4.5 ml of modified EC broth with novobiocin and incubated at 35°C for 24 h (26). The enriched culture was streaked onto Fluorocult E. coli O157:H7 agar (Merck, Darmstadt, Germany) and grown at 35°C for 24 h. Green colonies without fluorescence under UV illumination were chosen for identification. A slide latex agglutination kit Escherichia coli O157-F (Denka Seiken Co., Tokyo, Japan) and BAX system for screening E. coli O157:H7 (DuPont Qualicon) were used to confirm suspected colonies.

MPN analyses. For quantitative analysis, aerobic bacteria, total coliforms, E. coli, S. aureus, and C. perfringens were reported as CFU per square centimeter of surface analyzed. C. jejuni and C. coli, L. monocytogenes, Salmonella, and E. coli O157:H7, which need enrichment, were reported as the most probable number (MPN) per square centimeter of surface analyzed. When a carcass sample was positive for a specific pathogen, a nine-tube three-dilution (10-1, 10-2, and 10-3) MPN analysis was prepared. Tenfold dilutions (10-1, 10-2, and 10-3) from the original suspension squeezed from the respective sponges were prepared in triplicate tubes with 0.1% peptone. The procedure was the same as described to screen for the presence of specific pathogens. The number of confirmed positive MPN tubes was used to estimate the number of microorganisms per square centimeter of surface analyzed from the MPN table in the FSIS-USDA Microbiological Laboratory Guidebook.

RESULTS AND DISCUSSION

The slaughter plants investigated in this study account for all the government-operated and few private slaughter plants in Taiwan. The baseline data collected from these slaughter plants should represent the general microbiological profile for raw pork carcasses in Taiwan in 2003. The analytical methods described in this study have been routinely performed in our laboratory. In the presence of 6 log CFU/ml natural background flora, about 100 CFU/ml S. aureus and C. perfringens and 10 CFU/ml C. jejuni, L.
TABLE 1. Prevalence of selected microorganisms on pork carcasses

<table>
<thead>
<tr>
<th>Microorganism(s)</th>
<th>No. of carcasses positive (%)</th>
<th>SEa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobic bacteria</td>
<td>1,650 (100)</td>
<td>NAb</td>
</tr>
<tr>
<td>Total coliforms</td>
<td>1,573 (95.3)</td>
<td>0.5</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1,444 (87.5)</td>
<td>0.8</td>
</tr>
</tbody>
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Pathogens (n = 1,038)

- Staphylococcus aureus 50 (4.8) 0.7
- Clostridium perfringens 3 (0.3) 0.2
- Campylobacter jejuni 143 (13.8) 1.1
- Campylobacter coli 7 (0.7) 0.3
- Listeria monocytogenes 18 (1.7) 0.4
- Salmonella 0 (0) NA

- E. coli O157:H7 1,444/1,444 0.01 MPN/cm²
- C. jejuni 143/16 0.03 log CFU/cm²
- C. coli 7/0 NA
- L. monocytogenes 70/70 0.03 log CFU/cm²
- Salmonella 18/16 0.07 log CFU/cm²
- E. coli O157:H7 0/0 NA

a Standard error using the binomial distribution.
b NA, not applicable.

monocytogenes, Salmonella Typhimurium, and E. coli O157:H7 in the original carcass suspension could be detected by the selective agar media (data not shown). Table 1 presents the prevalence of the selected microorganisms on the surfaces of the pork carcasses. Because this study followed the framework of the Pork Microbiological Baseline Data Collection Program regarding microorganisms selected, sampling, transportation, and analytical methods as closely as possible, we compared our results with those reported by researchers from the United States (12). Viable aerobic microorganisms were recovered from all the carcasses analyzed. Total coliforms were recovered from 95.3% and E. coli was recovered from 87.5% of 1,650 carcasses. These numbers were both more than twofold higher than the prevalence reported in the United States (12). Fecal contamination on the carcasses is obviously a major concern during slaughter operation. The FSIS requires testing in slaughter plants for generic E. coli to verify that the slaughter operation can control and prevent fecal contamination (13). The Taiwanese government is considering implementing a similar program, where meat inspection veterinarians would test for total coliforms and E. coli in slaughter plants using an easy testing method. The present data support the importance and urgency of this policy. The mean concentration of aerobic bacteria was 4.0 log CFU/cm², that for total coliforms was 0.6 log CFU/cm², and that for E. coli was 0.1 log CFU/cm² (Table 2).

S. aureus, C. perfringens, C. jejuni, C. coli, L. monocytogenes, and Salmonella were recovered from 4.8, 0.3, 13.8, 0.7, and 1.7%, respectively, of 1,038 carcasses. E. coli O157:H7 was not detected on any carcass analyzed (Table 1). When positive for a specific pathogen, the mean concentrations on carcasses were 0.57 log CFU/cm² for S. aureus, 0.66 MPN/cm² for C. jejuni and C. coli, and 0.18 MPN/cm² for Salmonella (Table 2).

Campylobacter species are normally found in the intestinal tracts of pigs and in higher numbers than other pathogens such as Salmonella (25). Our study indicated that C. jejuni and C. coli did have the highest prevalence among the pathogenic bacteria; the same result was also observed in the United States (12). Enteritis caused by Campylobacter is common in the United States and most industrialized countries (1). Because Campylobacter species are sensitive to an aerobic atmosphere, drying, and freezing and they do not grow below 30°C, Campylobacter enteritis is not likely to be associated with the consumption of pork (25). Chilling of carcasses could considerably reduce Campylobacter load, and changes in chilling procedures at slaughter plants could alter this situation (8).

The FSIS has established pathogen reduction performance standards for Salmonella that slaughter plants must meet (3). The standards are based on the prevalence of Salmonella as determined from the FSIS Microbiological Baseline Data Collection Program. The prevalence of Salmonella in U.S. samples has declined from 8.7% in 1996 to 4.7% from 1998 to 2002 (14). We estimated the current prevalence of Salmonella in the meat carcasses surveyed in Taiwan at 1.7%, which is lower than that previously reported (27). Our observations of high fecal contamination (total coliforms, 95.3%; E. coli, 87.5%) and low Salmonella prevalence do not seem logical. However, the same isolation method has been used for Salmonella in past years, and there is a trend toward a gradual decrease in the annual incidence of Salmonella (27). The mean Salmonella concentration of 0.18 MPN/cm² of carcass surface was close to the 2.0 MPN/cm² reported by the FSIS (12).

E. coli O157:H7 was not detected from any of the samples analyzed. However, others have detected a low prevalence of E. coli O157:H7 shedding in pigs (21). Pork may not be regarded as a risk product for E. coli O157:H7 at the present time. Nevertheless, the role of pigs in the epidemiology of E. coli O157:H7 infection remains to be elucidated (7).

Sources of contamination during pig slaughter are pig related (bacteria from the pharynx and feces) and environmental (8). C. jejuni, C. coli, C. perfringens, Salmonella,
and *E. coli* O157:H7 are associated with the pigs, and *S. aureus* and *L. monocytogenes* can be endemic in the processing environment. The prevalences of *L. monocytogenes* and *S. aureus* were relatively low in our survey. In Norway and Sweden, *L. monocytogenes* was not detected from pork carcasses (24), but a 7.4% *L. monocytogenes* prevalence on pork carcasses was reported in the United States (12). The importance of the slaughter plant environment in the dissemination of *L. monocytogenes* has been confirmed by multilocus enzyme electrophoresis typing, and *L. monocytogenes* may be used as an indicator of the general hygienic status of slaughter plants (5). Although the presence of *S. aureus* on pork carcasses is not sufficient to be considered an indicator for rejection of the meat, *S. aureus* may be used as an indicator to evaluate the general hygienic status of the equipment (8).

Although the HACCP Pathogen Reduction Program is currently not mandatory in Taiwan, establishment of a nationwide microbiological screening program and increased awareness of the potential and consequences of microbial contamination may push slaughter plants to take preventive or corrective measures during slaughter operation, thus yielding more wholesome products. Performance standards for specific pathogens will be determined in the near future to evaluate the hygienic level in slaughter plants in Taiwan. All these criteria will be based on the information obtained from a nationwide microbiological screening program.

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**REFERENCES**