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Research Note

Bacterial Quality and Prevalence of Foodborne Pathogens in Edible Offal from Slaughterhouses in Korea

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ABSTRACT

Edible offal meats have recently received significant attention worldwide. However, studies evaluating the microbial quality of diverse edible offal and specifically investigating contamination by pathogens that cause foodborne illnesses are rare. Our study was conducted to investigate the microbiological quality of six kinds of edible offal produced from 11 pigs and 8 cattle slaughterhouses in the Republic of Korea and the prevalence of pathogenic microorganisms such as *Salmonella, Clostridium perfringens, Staphylococcus aureus,* and *Escherichia coli* O157:H7 in these products. The values for aerobic plate counts, coliform counts, and *E. coli* counts in red offal were 1.00 to 6.70, 0 (below 10 CFU) to 4.78, and 0 to 4.00 log CFU/g, respectively. For green offal, the values were 3.00 to 7.00, 1.48 to 6.30, and 0 to 6.00 log CFU/g, respectively. The most frequently detected foodborne pathogen was *Salmonella* (23.8% prevalence in pig offal and 7.1% prevalence in cattle offal), followed by *C. perfringens* (11.1 and 7.1%, respectively) and *S. aureus* (12.7 and 2.4%, respectively). None of the offal samples tested positive for *E. coli* O157:H7. Considering the microbial quality of offal from Korean slaughterhouses and the prevalence of foodborne pathogens in this material, more refined hygienic standards such as a hazard analysis critical control point system for processing, packing, and transporting edible offal are necessary for preventing further contamination.

Edible offal meats have recently received significant attention, particularly those for human consumption, because of their nutritional qualities and the worldwide emphasis on reducing economical losses from wasting food *(28)*. Each country has different criteria for regulating edible and inedible by-products from the slaughtering of livestock animals. According to the regulations of the Korean Animal and Plant Quarantine Agency (QIA) *(17),* edible meat by-products include a greater diversity of internal organs than do those of other nations, such as liver, lung, heart, stomach, pancreas, spleen, kidney, small intestine, and colon, because of the high preference for foods made from offal meats in the Republic of Korea. However, because of their functional characteristics many edible offal meats easily become spoiled *(19)* and could become contaminated with bacteria such as *Salmonella, Clostridium perfringens, Staphylococcus aureus,* and *Escherichia coli* O157:H7, which are major causes of food poisoning in humans *(4, 9, 21, 25)*. The microbial quality of these products must be monitored at the slaughterhouse to prevent food poisoning. Several studies in various countries have been conducted to evaluate the microbiological quality of edible offal meats *(1, 5, 11, 12)*. However, a thorough investigation of the microbial quality of diverse edible offal products and the prevalence in slaughterhouses of pathogens that can cause foodborne illness is still lacking in Korea. The present study was conducted to investigate the microbiological quality of diverse edible offal products from pig and cattle slaughterhouses in Korea by performing aerobic plate counts (APCs), coliform counts, and *E. coli* counts. The prevalence of *Salmonella, C. perfringens, S. aureus,* and *E. coli* O157:H7 in these products also was determined.

MATERIALS AND METHODS

Sample collection. A total of 11 pig and 8 cattle slaughterhouses were investigated between 2012 and 2013. Our study focused on three kinds of edible red offal (heart, liver, and lung) and three kinds of edible green offal (stomach [rumen and omasum], small intestine, and large intestine) that are widely consumed in Korea. Among the six kinds of offal meats that were processed (described in Fig. 1), samples from four to six kinds were collected depending on the conditions of each slaughterhouse. About 500 g of each kind of offal was collected at the end of processing and placed in Whirl-Pak stomacher bags (Nasco, Fort Atkinson, WI) using sterile equipment. All samples were transported to the laboratory and stored at 4°C within 12 h of arrival.

Bacteria: APCs, coliform counts, and *E. coli* counts. APCs were performed according to AOAC International *(2)* protocol 990.12, and coliform and *E. coli* counts were performed according to AOAC International protocol 991.14. A 25-g sample was homogenized with sterile 0.85% saline in a stomacher bag, 10-fold serial dilutions were made, and 1 ml of culture was inoculated onto

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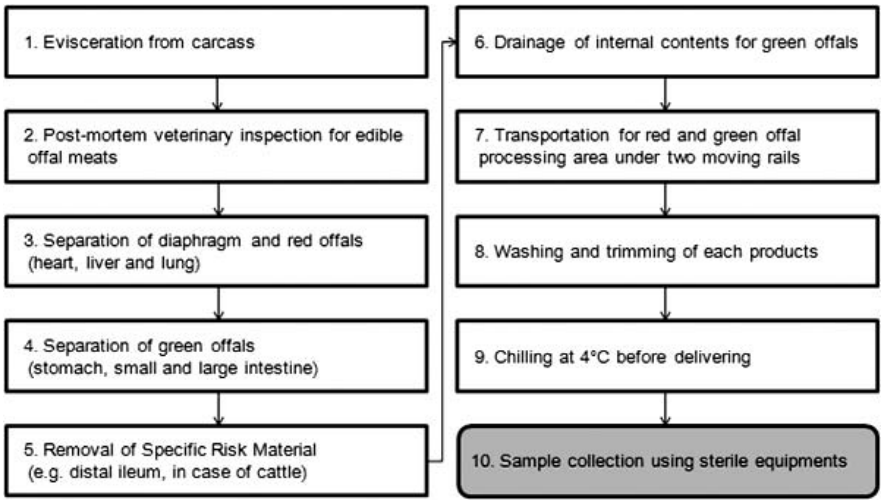

FIGURE 1. *Overall processing scheme for edible offals in Korean slaughterhouses.*

Petrifilm plates (3M, St. Paul, MN) and incubated at 37°C for 24 h, at which time colonies were counted.

Detection of pathogens *S. aureus, Salmonella, C. perfringens,* and *E. coli* O157:H7. Four foodborne pathogens were isolated as described in the QIA regulations *(17)*. For isolation of *S. aureus,* 25 g of sample was homogenized in 225 ml of tryptic soy broth (Difco, BD, Detroit, MI) supplemented with 10% NaCl and incubated at 37°C for 24 h. After incubation, 10 μl of homogenate was directly streaked with an inoculation loop onto Baird-Parker agar supplemented with egg yolk tellurite solution (Oxoid, Basingstoke, UK) and incubated at 37°C for 48 h. Typical or suspect colonies were removed, streaked onto blood agar, and incubated at 37°C for 24 h. Suspected colonies on the agar plates were confirmed by identification of the *nuc* gene with a PCR assay *(16)*.

For isolation of *Salmonella,* 25 g of sample was homogenized in 225 ml of buffered peptone water (BPW; Difco, BD) and incubated at 37°C for 24 h. After incubation, 100 μl of this BPW culture was enriched in 10 ml of Rappaport-Vassiliadis R10 (RV; Difco, BD) broth and incubated at 37°C for 24 h. Subsequently, each RV broth culture was streaked with an inoculating loop onto Rambach agar (Merck, Darmstadt, Germany) and incubated at 37°C for 24 h. Suspected colonies on the agar plates were confirmed by identification of the *invA* gene with a PCR assay *(22)*.

For isolation of *C. perfringens,* 25 g of sample was homogenized in 225 ml of sterile 0.85% saline, and 1 ml of homogenate was transferred to 10 ml of cooked meat medium (Difco, BD) and incubated at 37°C for 24 h in an anaerobic chamber (Forma Scientific, Marietta, GA) with 5% CO_2, 10% H_2, and 85% N_2. After incubation, each culture was transferred to a *perfringens* agar base supplemented with egg yolk emulsion (Oxoid) and incubated at 37°C for 24 h under the same anaerobic conditions. Suspected colonies on the agar plates were confirmed by identification of the *cpa* gene with a PCR assay *(10)*.

For isolation of *E. coli* O157:H7, 25 g of sample was homogenized in 225 ml of mEC broth with novobiocin (Merck) and incubated at 37°C for 24 h. After incubation, 0.1 ml of homogenate was directly streaked with an inoculating loop onto sorbitol MacConkey agar (Merck) and incubated at 37°C for 24 h. Suspected colonies on the agar plates were confirmed by identification of the *uidA* gene with a PCR assay *(6)*.

Statistical analysis. Data were analyzed using the Statistical Package for Social Sciences, version 18.0 (IBM, Armonk, NY). Results are expressed as the mean ± standard deviation (SD). A one-way analysis of variance was performed to compare bacterial counts, followed by Scheffé's multiple range test for posthoc analysis.

RESULTS

Bacteria: APCs, coliform counts, and *E. coli* counts. APCs and coliform and *E. coli* counts in red offals are shown in Figure 2. APCs in the red offal of pigs and cattle were 2.48 to 6.00 and 1.00 to 6.70 log CFU/g, respectively. Coliform counts were 0 (<10 CFU) to 4.78 and 0 to 3.90 log CFU/g, respectively, and *E. coli* counts were 0 to 4.00 and 0 to 3.30 log CFU/g, respectively.

APCs and coliform and *E coli* counts in green offals (stomach and small and large intestines) are shown in Figure 3. APCs in the green offal of pigs and cattle were 3.48 to 7.00 and 3.00 to 6.60 log CFU/g, respectively. Coliform counts were 2.48 to 6.30 and 1.47 to 5.60 log CFU/g, respectively, and *E. coli* counts were 1.30 to 6.00 and 0 to 4.00 log CFU/g, respectively.

Statistical analysis. The mean ± SD for APCs and coliform and *E coli* counts are shown in Table 1. There were significant differences in mean coliform and *E. coli* counts between red offal and green offal from pigs. Significant differences in mean APCs were also observed between partial red offal and green offal of pigs ($P < 0.05$). However, no significant differences were observed in cattle offal with respect to APCs or coliform and *E. coli* counts. No significant differences in parts of APCs, coliforms, and *E. coli* were found between pig and cattle offals.

Detection of pathogens: *S. aureus, Salmonella, C. perfringens,* and *E. coli* O157:H7. The prevalence of pathogens is shown in Table 2. Of the 63 samples of pig offal tested, the most frequently detected pathogen was *Salmonella* (23.8%), which was isolated from the small and large intestines, heart, lungs, liver, and stomach. The second most frequently isolated pathogen was *S. aureus* (12.7%), isolated from the lungs, large intestine, and liver. The third most frequently isolated pathogen was *C. perfringens*

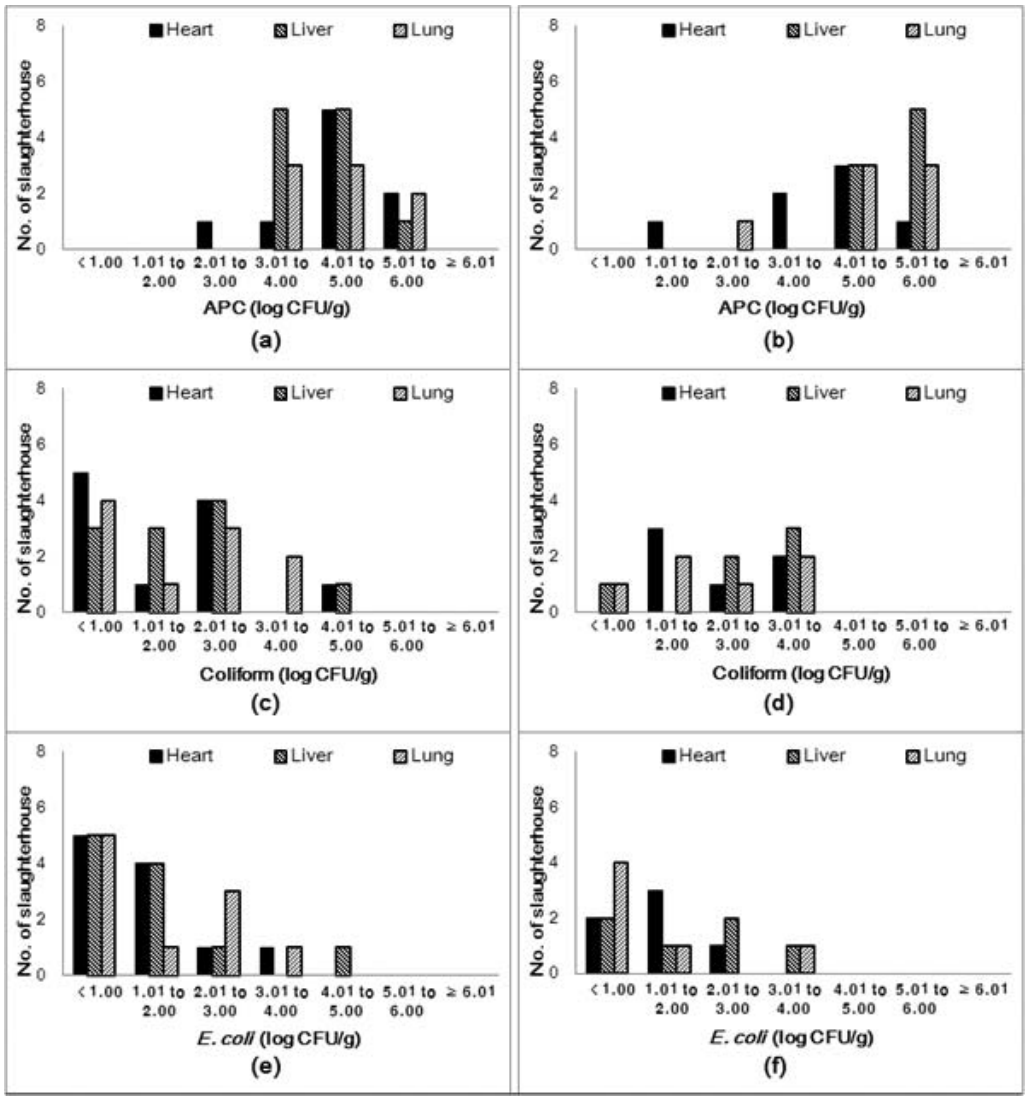

FIGURE 2. *Hygienic quality of edible red offal originated from 11 pig and 8 cattle slaughterhouses: aerobic plate counts (APCs) from pigs (a) and cattle (b), coliform counts from pigs (c) and cattle (d), and* E. coli *counts from pigs (e) and cattle (f).*

(11.1%), isolated from the small and large intestines. Of the 36 samples of cattle offal tested, the most frequently detected pathogens were *Salmonella* and *C. perfringens,* each found in three samples (7.1%). *C. perfringens* was recovered from the stomach and small intestine, but *Salmonella* was recovered from only the stomach. *S. aureus* was isolated from only one stomach sample (2.4%). No *E. coli* O157:H7 was found in either pig or cattle offals.

DISCUSSION

The initial level of bacteria in foods is important because this level directly impacts spoilage and shelf life *(19)*. The APC has been regarded as a valuable indicator of the hygienic quality for slaughterhouses and livestock products *(15)*. The International Commission on Microbiological Specifications for Foods (ICMSF) criteria *(24)* have been widely used for evaluating the hygienic quality of edible offals via the APC. According to the ICMSF criteria, five samples should be collected and tested for microbiological analysis of edible offal products during meat processing, and the hygienic quality is considered satisfactory when more than three samples have an APC of <6 log CFU/g. The APCs of all samples should not exceed 7 log CFU/g. In this study, we tested six kinds of edible offal. The APCs did not exceed 7 log CFU/g, and only 5.3% of samples have APCs of 6 to 7 log CFU/g. Although all tested samples did not exceed the ICMSF reference values, it is hard to assure the hygienic quality of offals in Korean slaughterhouses; the mean APC was higher than that reported for carcass meat products in Korean slaughterhouses *(23)*. Overall mean APCs in this study were higher than those found in previous studies in other countries. Hanna et al. *(12)* reported that APCs for livers, kidneys, and hearts obtained from cattle, pigs, and sheep soon after slaughter were nearly always <4 log CFU/g. Abdullah *(1)* found similar results, with mean APCs of 4.13 to 4.61 log CFU/g for ovine liver, kidney, spleen, and heart. For green offals, Bensink et al. *(5)* found mean APCs of 3.26 to 4.06 log CFU/g for beef tripe under different processing conditions. Overall microbial loads in livestock products tend to

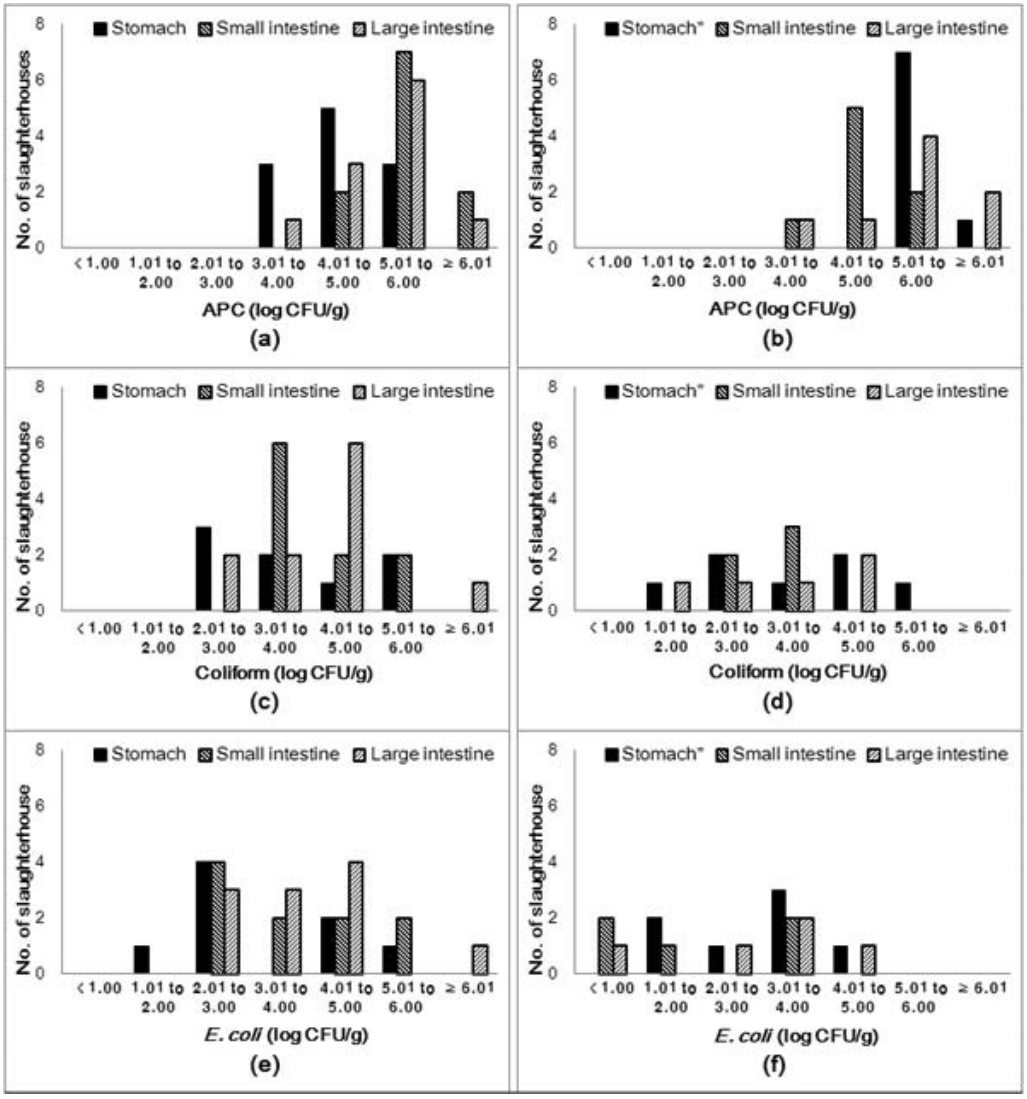

FIGURE 3. *Hygienic quality of edible green offal originated from 11 pig and 8 cattle slaughterhouses; aerobic plate counts (APCs) from pigs (a) and cattle (b), coliform counts from pigs (c) and cattle (d), and* E. coli *counts from pigs (e) and cattle (f). * Cattle stomach values shown are the mean of rumen plus omasum values.*

increase as the products progress after slaughtering through the line to packaging and transport *(23)*. These considerations make it hard to guarantee the hygienic quality of offal products for consumers in Korea.

Coliform and *E. coli* counts are usually regarded as an indicators of fecal contamination in foods *(18)*. However, no specific criteria have been defined based on ICMSF standards for use of coliform and *E. coli* counts as indicators

TABLE 1. *Counts for aerobic bacteria, coliforms, and* E. coli *in each type of offal from pig and cattle slaughterhouses*

		Mean ± SD contamination (log CFU/g)[a]						
Species	Pathogen	Stomach	Small intestine	Large intestine	Heart	Liver	Lung	Total
Pigs	Aerobic bacteria	4.48 ± 0.63 ABC	5.47 ± 0.79 C	5.30 ± 0.68 BC	4.23 ± 0.73 A	4.30 ± 0.60 AB	4.80 ± 0.72 ABC	4.77 ± 0.82
	Coliforms	3.82 ± 1.21 B	4.26 ± 1.04 B	3.99 ± 0.81 B	1.31 ± 1.53 A	1.75 ± 1.43 A	1.42 ± 1.40 A	2.70 ± 1.78
	E. coli	3.30 ± 1.31 B	3.48 ± 1.05 B	3.69 ± 1.16 B	1.01 ± 1.22 A	1.06 ± 1.26 A	1.16 ± 1.27 A	2.23 ± 1.69
Cattle	Aerobic bacteria	5.55 ± 0.47 A	4.64 ± 0.70 A	5.12 ± 1.00 A	4.02 ± 1.20 A	4.57 ± 1.34 A	4.73 ± 1.21 A	4.77 ± 1.09
	Coliforms	3.81 ± 1.07 A	3.11 ± 0.80 A	3.55 ± 1.23 A	2.17 ± 1.08 A	2.46 ± 1.36 A	2.20 ± 1.59 A	2.85 ± 1.31
	E. coli	3.18 ± 1.38 A	1.82 ± 1.81 A	2.85 ± 1.66 A	0.98 ± 0.90 A	1.59 ± 1.28 A	0.77 ± 1.35 A	1.84 ± 1.58

[a] Detection limit was 1 log CFU/g (10 CFU/g). Within each column for each animal, means with different letters are significantly different ($P < 0.05$).

TABLE 2. *Prevalence of foodborne pathogens in offal from pig and cattle slaughterhouses*

		No. of positive samples/no. of samples tested (% positive)						
		Red offal			Green offal			
Species	Pathogen	Heart	Liver	Lung	Stomach	Small intestine	Large intestine	Total
Pigs	*S. aureus*	0/11	2/11 (18.2)	3/10 (30)	0/10	0/10	3/11 (27.3)	8/63 (12.7)
	Salmonella	3/11 (27.3)	2/11 (18.2)	2/10 (20)	1/10 (10)	4/10 (40)	3/11 (27.3)	15/63 (23.8)
	C. perfringens	0/11	0/11	0/10	0/10	4/10 (40)	3/11 (27.3)	7/63 (11.1)
	E. coli O157:H7	0/11	0/11	0/10	0/10	0/10	0/11	0/63
Cattle	*S. aureus*	0/6	0/6	0/6	1/13 (7.7)[a]	0/6	0/5	1/42 (2.4)
	Salmonella	0/6	0/6	0/6	3/13 (23.1)[a]	0/6	0/5	3/42 (7.1)
	C. perfringens	0/6	0/6	0/6	2/13 (15.4)[a]	1/6 (16.7)	0/5	3/42 (7.1)
	E. coli O157:H7	0/6	0/6	0/6	0/13[a]	0/6	0/5	0/42

[a] Sum of rumen and omasum samples.

of contamination of edible offal products by enteric bacteria. The United Kingdom has set standards for *E. coli* counts *(27)*; levels of *E. coli* must not exceed 4 log CFU/g as a minimum qualification for all processed products. In the present study, in 41.4% of pig green offals, 11.8% of cattle green offals, and 3.1% of pig red offals *E. coli* exceeded the 4 log CFU/g limit. High coliform and *E. coli* counts in green offals, which are digestive organs containing intestinal microflora, could be associated with their functional characteristics. However, Gill *(11)* confirmed that effective washing of the rumen greatly reduces residual contamination. Bensink et al. *(5)* investigated the microbial quality of green offals in Australia and found lower coliform contamination levels than those in Korean offal from slaughterhouses. Their results suggest that the hygiene of green offals could be controlled by similar methods used for handling meats, i.e., lots of water (20 to 30 liters) during the washing process, or by including the dry dumping procedure during processing to reduce contamination levels. Although the washing step is included in the processing of offal in all Korean slaughterhouses, our results suggest poor hygiene management during offal processing in Korea compared with that in Australia and United States *(5, 12)*. Therefore, more care and strict regulations for processing green offal are required to prevent foodborne illnesses caused by substandard processing procedures.

In the present study, the prevalence of four major foodborne pathogens was investigated for their impact on the risk of food poisoning; three of these pathogens were isolated from pig and cattle offal (*E. coli* O157:H7 was not found). The most frequently isolated pathogen was *Salmonella* (23.8% of pig offal samples and 7.1% of cattle offal samples), followed by *C. perfringens* (11.1% of pig samples and 7.1% of cattle samples) and *S. aureus* (12.7% of pig samples and 2.4% of cattle samples). In comparison to the prevalence of foodborne pathogens in red meats in Korea, we found a higher prevalence for all pathogens in offal. Choi et al. *(8)* reported the prevalence of *Salmonella* and *S. aureus* in pork from slaughterhouses and retail markets as 1 to 3% and 2 to 8%, respectively. Chae et al. *(7)* found that 3.2 and 1.2% of pork and beef samples, respectively, were positive for *C. perfringens*. In particular, a severalfold increase in the prevalence of three pathogens compared with their prevalence in red meats suggests both more severe fecal contamination and improper handling. However, Bensink et al. *(5)* found the prevalence of *Salmonella* and *Campylobacter* spp. to be 0.25 to 1.8% and 0 to 3%, respectively, in green offal. Their results support the notion that proper handling procedures during offal processing could reduce the prevalence of foodborne pathogens. The processing of meats and edible offal is performed separately in the first stage of slaughtering, but edible offals are often handled improperly without suitable regulations, whereas meat products are handled using carefully defined procedures under strict regulations such as those implemented by the hazard analysis and critical control point (HACCP) system in Korea. Hong et al. *(13)* reported that after implementation of the HACCP system in Korean pork meat plants, samples with APCs exceeding the 3 log CFU/cm^2 limit dropped from 73.39 to 4.29% for the overall process. Therefore, stricter regulations are required to prevent contamination of edible offal by foodborne pathogens.

E. coli O157:H7 is a well-known foodborne pathogen frequently isolated from beef *(3, 26),* and this pathogen has been found in beef carcasses and feces from commercial cattle farms in Korea *(14)*. However, the general prevalence of *E. coli* O157:H7 in meats was relatively low compared with that of other pathogens in Korea *(8, 20)*. Although *E. coli* O157:H7 was not isolated from any of the samples tested in the present study, further investigation of *E. coli* O157:H7 should be required because of its high pathogenicity in humans.

In conclusion, based on the ICMSF criteria, most edible offal processed in Korean slaughterhouses complied with the regulations for APCs, but the APCs for green offals were higher than those found in other countries, suggesting potential risks for human consumption. Many of the green offal samples tested in this study exceeded the limit established in the United Kingdom for *E. coli* based on criteria for isolating foodborne pathogens. Therefore, more refined hygienic standards such as the HACCP system for processing, packing, and transporting edible offal are

necessary in Korea for preventing additional contamination of offal products.

ACKNOWLEDGMENT

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