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Tracing surrogate bacteria inoculated on hide through the beef slaughtering process

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Abstract

Many countries have imposed regulations relating to concerns that hide contamination will affect the cleanliness of abattoirs. However, South Korea has not indicated any clear criteria. The purpose of this study is to use surrogate bacteria to measure the contamination in abattoirs caused by contaminated cattle hides. The swab contact method and plate count method are used. Surrogate bacteria are found in most internal environments after the final process. These surrogates remained on the carcass even after the final washing process. This paper is the first study in South Korea that use surrogate bacteria to analyze contamination levels in abattoirs.

Keywords: Republic of Korea; abattoirs; surrogate bacteria; skin; *Escherichia coli* O157:H7

In 2013, there were 4,958 reported cases of food poisoning. This number nearly doubled in 2016 to reach 7,162 cases, according to the Statistical Yearbook of Food and Drugs. This indicates a drastic increase in illnesses derived from food poisoning. The prevalence of food poisoning was evaluated for its causative bacteria and it was found that pathogenic bacillus and *Salmonella* accounted for approximately 20% of all cases of food poisoning [1].

Escherichia coli and *Salmonella* are pathogenic bacteria that have strong links to the feces that causes meat contamination. Human infections are caused by the consumption of contaminated food products (contaminated fresh produce such as unwashed salad leaves or undercooked meat), drinking contaminated water, or spread directly from person-to-person due to poor hygiene [2]. During the evisceration process, feces are exposed to meat, but considerable amounts of feces are already present on livestock animals at every abattoir. Specifically, *E. coli* O157:H7 and *Salmonella* were found on the floors and entrances of holding spaces [3]. In particular, the contaminated hides of animals often transmit *E. coli* O157:H7 and *Salmonella* to the meat during the slaughtering process [4]. Direct contamination happens when the hides of livestock come into contact with meat, while indirect contamination happens within the internal environment, the space where livestock is kept [5]. Therefore, the level of contamination on livestock hides may be a public health risk factor in abattoirs and meat they produce.

Hide contamination on livestock significantly affects the cleanliness of meat which is why many countries have imposed regulations relating to hides. The US Food Safety and Inspection Service has established the stages at which contaminants are present on livestock hides through their mud/fecal material scoring sys-

tem. The system requires all animals to be washed either before they enter an abattoir or prior to hide removal [6]. Another department that enforces food safety is the European Food Standard Agency which categorizes the cleanliness of the outer surface of livestock animals according to the Clean Livestock Policy. Animals that fail to meet the minimum cleanliness standards are rejected from abattoirs [7]. However, South Korea's Livestock Products Sanitary Control Act does not have any clear criteria on the regulation of contamination in livestock hides.

Surrogate bacteria are non-pathogenic microorganisms that grow and survive to have resistant properties comparable to specific pathogens; therefore, they may be used in food processing establishments to simulate the effect of antimicrobial treatments of the actual pathogens, without introducing a hazard into the environment [8]. In this study, they were chosen to follow the dissemination over the abattoir from cattle hide without interfered by other bacteria.

The purpose of this study is therefore to prepare a basis for regulation criteria and standards using surrogate bacteria to measure the levels of contamination of carcass as well as the internal environments of abattoirs caused by contaminated cattle hides.

E. coli biotype 1 (American Type Culture Collection, USA), under accession numbers BAA-1427, BAA-1428 and BAA-1430, were used in this experiment. *E. coli* biotype 1 was obtained in a frozen state. For the stock working culture preparation, frozen vials of each bacterium were transferred to Tryptic Soy Broth (TSB; BD Diagnostics, USA) at 37°C for 24 hours.

Selectivity was needed to distinguish surrogates from *E. coli* O157:H7 or *Salmonella* in the abattoir. Referencing the method used by Kaspar and Tamplin [9], each strain was made resistant to 100 μg/mL rifampicin (Sigma-Aldrich, USA). Rifampicin-resistive strains were then named BAA-1427#, BAA-1428#, and BAA-1430#. To strengthen the colony up to 5 times, each strain was transferred to TSA with 100 μg/ml rifampicin. The day before the experiment, BAA-1427#, BAA-1428#, and BAA-1430# strains were transferred to a 50-mL TSB and incubated at 35°C for 18 ± 2 hours. 150 mL of cocktails of BAA-1427#, BAA-1428#, and BAA-1430# were prepared before the experiment.

Gelatin (Zeltec Co., Ltd., Korea) that is harmless to public health was used to mimic dirt and fecal material. This produces results of a higher accuracy because the desired bacteria can be applied without using actual dirt or fecal materials directly. The gelatin was prepared the day before the experiment: 20 g of gelatin was dissolved in 1 liter of heated 0.1% (W/V) peptone water (PW, Peptone; Difco, USA). Then, hot gelatin matrix was poured into an empty hand spray container (Marolex. Sp. z o.o., high pressure: 0.4 MPa) and cooled it at room temperature (25°C) over 18 ± 2 hours.

The study was performed at the Livestock Process Complex regulated by Hazzard Analysis Critical Control Points in Yeoncheon. The abattoir processed up to 10 cattle and 1,700 pigs (on average 3 cattle and 250 pigs per 1 hour).

Mixtures of gelatin and cocktails of *E. coli* biotype 1 were prepared prior to carcass inoculation. The spray was shaken by hand to mix cocktails of *E. coli* biotype 1 and gelatin. The mixture was then sprayed on the animal's hide at the lairage. In this study, the contamination level of the cattle hide is set at 2 major levels. Classification of the levels were determined based on McEvoy et al. [10] and domestic situation: contamination affecting the entire front legs, belly, flank, rump, and entire rear legs is set to "severe" while contamination affecting only the entire front legs, rump, and entire rear legs is set to "moderate." The average application time was 20 to 24 seconds for the "severe" level, and 17 to 19 seconds for the "moderate" level. After every application, 100 cm^2 of the cattle hide surface was swabbed to make the monitoring easier (Table 1). Whirl-Pak with sterile sponge (Nasco, USA) was used as the swab. Only 5 cattle hides were collected per day.

The study used Whirl-Pak with sterile sponge in all swabs performed on cattle carcasses. Just prior to sampling, 20 mL of 0.1% (W/V) PW was injected into the sealed Whirl-Pak. Areas of 200 cm^2 on the front legs, brisket, and rear legs were swabbed by sterile sponge (3.8 cm [width] × 7.6 cm [length] × 1.5 cm [thickness]). Sampling processes included de-hiding and final washing. To prevent any overlap of sampling and accuracy, samples were always performed at the left side of the carcasses.

The Whirl-Pak with sterile sponge was used. Environmental swabs were performed a day after the experiment was completed, and immediately cleaning the abattoir. Samples were swabbed

Table 1. The average concentration of surrogate microorganism on cattle hides after spraying different contamination levels (n = 15)

	Severe	Moderate
Day 1	7.12 ± 0.1	7.05 ± 0.07
Day 2	6.84 ± 0.08	6.78 ± 0.06
Day 3	7.28 ± 0.04	6.92 ± 0.1
Average	7.08 ± 0.08	6.92 ± 0.13

Values are presented as mean ± standard deviation.
Severe, contamination affecting the entire front legs, belly, flank, rump, and entire rear legs (average application time: 20 to 24 seconds); moderate, contamination affecting only the entire front legs, rump, and entire rear legs (average application time: 17 to 19 seconds).

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from the floor of the hook removal phase (F1), the de-hiding phase (F2), and between the washing and the inspection phase (F3) (900 cm^2). Gloves were also swabbed of the workers who removed the hooks (G1) and who performed the de-hiding and evisceration processes (G2). The shackles used to suspend cattle were also swabbed.

Samples were kept refrigerated until microbiological examination. These were performed within 20 hours after sampling. Appropriate decimal dilutions were prepared with 0.1% PW and were plated onto TSA with 100 μg/ml rifampicin. TSAs with 100 μg/mL rifampicin were incubated at 37°C for 24 hours.

The data was expressed as mean and standard deviation of the $\log_{10}$ value. The study used GraphPad Prism 5.01 (GraphPad Software Inc., USA) for the statistical analysis and graph formulation. Two-way ANOVA and Bonferroni post-tests were used in the analysis of data. Significant differences were defined as $p < 0.05$.

In our analysis, the floor of the rear-hook exchange process showed the most contamination compared to the other floor surfaces (Table 2). The high contamination is because the carcass sticks during the hook exchange process longer than it does the other processes. There is also a higher chance of gelatin removal, which is the substitute for feces in this study, due to increased hide movement during hook exchange. Moreover, even though gelatin was only placed on the surface of the hide, surrogates were found on the floors, even after the hide was removed. This proves that in addition to contamination through livestock hides, cross-contamination also happens on the floor surfaces.

The study then compared the level of contamination between the gloves worn by workers who exchanged rear hooks and those worn by workers assigned to evisceration and hide removal ($p < 0.05$). It was found that the former had higher contamination levels, and the level of glove contamination did not differ according to the level of hide contamination ($p \geq 0.05$). This is not surprising since, in the rear-hook exchange process, direct contact between the rear legs and gloves is inevitable. The gloves are therefore highly contaminated during this process, and while cross-contamination occur when the gloves make contact with carcass during the pre-hide removal process. A previous study showed a consistent result indicating that workers' gloves move microorganisms from the hide and feces of livestock animals to carcass, and when the level of microorganisms on the workers' gloves before and after de-hiding process was compared, a significant increase in glove contamination after the process was evident [10,11]. The level of shackle contamination varied according to hide contamination and the difference was not significant. Surrogates were found in the shackle used to hang the carcass, which clearly indicates that cross-contamination may occur with the shackles as medium.

Moreover, it was found that carcass contamination from the "severe" level was significantly higher than that from the "moderate" level ($p < 0.05$), and that surrogates remained on the carcass even after the final washing process (Table 3). This same finding is consistent with a previous study, which indicated that the difference in the degree of contamination on the surface of

Table 2. Prevalence of surrogate bacteria in the internal environment of the abattoir

Contamination levels	Sites					
	F1	F2	F3	G1	G2	S
Severe	7.9×10^3	6.0×10^2	5.0×10^2	2.0×10^4	7.9×10^2	2.9×10^2
Moderate	8.5×10^3	7.0×10^2	6.0×10^2	1.9×10^4	3.6×10^2	3.2×10^2

Values are presented as mean. F1, F2 and F3 were expressed as CFU/900 cm^2. G1, G2 and S were expressed as CFU/unit.
F1, the floor of the hook removal phase; F2, the floor of the de-hiding phase; F3, the floor between the washing and the inspection phase; G1, gloves from the worker performing the removal of hooks; G2, gloves from worker performing the de-hiding and evisceration; S, shackle.

Table 3. Prevalence of surrogate bacteria at the carcasses

Part	Class			
	Severe		Moderate	
	AHO	AFW	AHO	AFW
F	$9.2 \times 10 \pm 6.5 \times 10$	$9.6 \times 10 \pm 3.8 \times 10$	$6.4 \times 10 \pm 4.7 \times 10$	$6.6 \times 10 \pm 3.4 \times 10$
B	$7.3 \times 10^3 \pm 4.8 \times 10^3$	$9.0 \times 10^2 \pm 4.9 \times 10^2$	$4.4 \times 10^3 \pm 1.9 \times 10^3$	$3.5 \times 10^2 \pm 1.2 \times 10^2$
R	$9.3 \times 10^3 \pm 7.4 \times 10^3$	$1.2 \times 10^3 \pm 5.7 \times 10^2$	$9.0 \times 10^3 \pm 4.0 \times 10^3$	$9.7 \times 10^2 \pm 2.6 \times 10^2$

All counts are expressed as CFU/200 cm^2. Values are presented as mean ± standard deviation.
AHO, after hide opening; AFW, after final washing; F, front legs; B, brisket; R, rear legs.

cattle in lairage impacted carcass contamination at the last slaughtering process [10]. Moreover, *E. coli* O157:H7 on the cattle hide contaminated the carcass as it moved through the slaughtering process. 87% of the samples are positive before evisceration, 57% of the samples were positive after evisceration, and 17% of the samples were positive during post-slaughtering process [12]. According to the results from carcass swabs, significantly higher contamination was found in the rear legs, regardless of the level of hide contamination and process order ($p < 0.05$). This agrees with the results of a previous study, which showed that in all 3 abattoirs, contamination of the rear legs after the initial slaughtering process and final washing process was significant when compared with other parts of carcass [13]. After the de-hiding process, brisket contamination on a "severe" level was found to be significantly higher than that on a "moderate" level ($p < 0.05$). This higher contamination level might be related to the direct contact between the hide and the carcass during the de-hiding process [14]. When 0.5 to 2 kg of hide and meat touch each other's surface for 2 seconds, the rate of contamination was only at 0.5-0.00002%, indicating that there are other factors involved in addition to direct contact between hide and meat [15]. In short, the spread of contamination from the hide to the carcass is primarily due to the following contributing factors: the worker's gloves, the worker's expertise in performing the job, and the degree of hide contamination. Since this study reproduced feces and dirt on hide using gelatin, further study is needed to explore the distribution of hide contamination when the carcass and the internal environment are in dry sate. A significant difference was found between "severe" and "moderate" levels at the front legs and chest after final washing ($p < 0.05$). This finding deviates from that of a previous study which measured the level of microorganisms for each process and found that contamination was highest in the rear legs when measured after the hide pre-treatment process and similarly in the chest when measured after evisceration and hide removal [14]. The cause might be this study used surrogates that could only be tracked from the hide. In contrast, previous studies measured contamination based on various contaminants, such as intestinal ruptures that occurred during the slaughtering process. High contamination of brisket at severe level is indicative of poor hygiene during hide opening, which eventually leads to carcass contamination. Since it only takes the slightest contact to contaminate an entire part of the carcass, care must be taken to prevent the contamination from the hide to the meat, and a countermeasure plan must be established in South Korea in particular. As far as is known, this is the first study using gelatin and surrogate bacteria to inoculate cattle hide in South Korea. This study provides initial data to help understand cross-contamination with pathogenic bacteria inside abattoirs caused by the contamination level of livestock hides.

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