ESTABLISHMENT OF EVALUATION METHODS FOR MICROBIAL CONTROLS AT BEEF SLAUGHTERHOUSES IN JAPAN

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A. INTRODUCTION

To assure safety and good microbiological quality of meat, sanitary slaughtering, dressing and handling are essential. But there is little information on the status of Japanese beef in this respect. So at 14 slaughterhouses in Japan (including 3 USDA-registered slaughterhouses), we checked operational hygiene conditions during slaughtering and dressing processes, took samples from carcasses after a final rinse in the cooling room with swab methods (10X10 cm of a carcass), at 4 sites (flank, brisket, neck and inner surface of the thoracic cavity), and analysed for Aerobic Bacteria Counts at 32(C, Coliform. From the data, we evaluated how sanitary slaughtering and dressing operations were performed. Further, we investigated the relationships between the microbiological data and the operational hygiene conditions we checked.

B. MATERIAL AND METHODS

1. Slaughterhouse

Fourteen beef slaughterhouses (including 3 USDA-registered slaughterhouses) in Japan were investigated. In these slaughterhouses, 30 to 250 cattle per day were slaughtered, and the slaughtering and dressing methods in each of the slaughterhouses are as following,

1) Skin removal by machinery, in which
   A) evisceration before skin removal : eight slaughterhouses
   B) evisceration after skin removal : one slaughterhouse
2) Skin removal by hands, in which
   A) evisceration before skin removal : two slaughterhouses
   B) evisceration after skin removal : three slaughterhouses

The slaughtering and dressing methods and the numbers of carcasses produced per day are shown in Table 1.

2. Samples

Samples used to determine the enumeration of microorganisms were collected from the surface of carcasses after a final rinse in the cooling room. For collecting samples, 10X10 cm of the surface of carcasses were swabbed with cotton swab at 4 sections (flank, brisket, neck and inner surface of the thoracic cavity).
The sampling was performed twice (First in the winter (December to February); and second in the summer (July to September).
The swabbed cotton were homogenized by Stomaching for 30sec with 30ml of phosphate buffered saline, pH7.2.

3. Enumeration of microorganisms in samples

For the enumeration of Total Aerobic Bacteria Counts (ABC), 0.1ml portions of suitable dilution of each samples were spread on plate agar (Nissin Pharm. Co., LTD., Japan) incubated at 32(C for 48 hours.
For the enumeration of Total Coliform Counts (CC), 0.1ml portions of suitable dilution of each samples were spread on Desoxycholate agar (Nissui) incubated 32(C for 24 hours.

4. Organoleptic Inspection of sanitary procedures
Hygienic environment where slaughtering and dressing were taken place, sanitary procedures during slaughtering and dressing were checked by organoleptic inspection, then the information obtained were compared with microbiological test results.

5. Sanitary evaluation based on Carcass microbiological test
ABC and CC of each of carcass section were converted to logarithmic numbers.
An arithmetical mean of 4 sites were taken, then the mean in the winter and summer were also arithmetically averaged.

C. Results and Discussions
1. Sanitary evaluation based on ABC
In three US registered slaughterhouses, the mean Log-ABC/cm2 of Flank was 1.6 both in the winter and summer, as for Brisket, 1.7 in the winter and 1.6 in the summer (average 1.7), as for Neck, 1.4 in the winter and 1.5 in the summer (average 1.5), meanwhile as for Thoracic cavity, 0.7 in the winter and 0.4 in the summer (average 0.6). A significant difference ($p<0.05$) was found between thoracic cavity and the other three sites, but no significant difference was found among the areas of the neck, brisket and flank. No significant difference $p<0.05$) was found between the data in summer and winter. Among three slaughterhouses, Sanitary Evaluation Point of Est-G was lowest 0.7. K (1.7) and MY (1.9) followed, an arithmetical mean of three US registered slaughterhouses was 1.4.
On the other hand, in other eleven conventional slaughterhouses, the mean Log-ABC/cm2 of Flank was 3,2 in the winter and 3.3 in the summer (average 3.3), as for Brisket, 3.0 in the winter and 3.4 in the summer (average 3.2), as for Neck, 2.9 in the winter and 3.3 in the summer (average 3.1), meanwhile as for Thoracic cavity, 2.2 in the winter and 2.7 in the summer (average 2.5). A significant difference $p<0.05$) was also found between thoracic cavity and the other three sites, but no significant difference was found among the areas of the neck, brisket and flank. The mean Log-ABC/cm2 of 4 sites in the winter was 2.8, on the other hand, those in the summer was 3.2 (a significant difference($p<0.05$) was found between the data in summer and winter), so it was suggested that beef carcasses were more contaminated in the summer. Among eleven slaughterhouses, an arithmetical mean was 3.0, so a significant difference ($p<0.05$)was found between US registered slaughterhouses and other eleven slaughterhouses (Table 2).

2. Sanitary evaluation based on CC
In three US registered slaughterhouses, the mean Log-CC/cm2 of Flank was 0.1 in the winter and summer, as for Brisket and Neck the mean Log-CC/cm2 were respectively 0.1 on the average, meanwhile as for Thoracic cavity, no CC was detected both in the winter and the summer. No significant difference ($p<0.05$) was found between the data in summer and winter. Among three slaughterhouses, an arithmetical mean was 0.1.
On the other hand, in other eleven conventional slaughterhouses, the mean Log-CC/cm2 of Flank was 0.3 in the winter and 0.5 in the summer (average 0.4), as for Brisket, Neck and Thoracic cavity, the mean Log-CC/cm2 were respectively 0.4 on the average.
The mean Log-CC/cm2 of 4 sites in the winter was 0.2, on the other hand, those in the summer was 0.5 (a significant difference ($p<0.05$) was found between the data in summer and winter), so it was suggested that beef carcasses were more contaminated in the summer. Among eleven slaughterhouses, an arithmetical mean was 0.4, so a significant difference ($p<0.05$) was found between US registered slaughterhouses and other eleven slaughterhouses (Table 3).

3. Correlation between Log-ABC/cm2 and organoleptic inspection results
To identify the sources of microbial contamination, we checked the sanitary procedures during the slaughtering and dressing processes in each of the slaughterhouses with organoleptic inspection method, and analyzed the correlation between Log-ABC/cm2 and the sanitary procedures. As the Sanitary Evaluation Point for the investigation with the results of sanitary procedures, an arithmetical mean of 4 sites and 2 season of Log-ABC/cm2 was used (Table 4). Significant differences were found in the following items:

1) Effective washing of livestock before stunning
The Sanitary Evaluation Points of the slaughterhouses where an effective washing of livestock was conducted before stunning were 0.7-3.6 (average 2.0), meanwhile those of the slaughterhouses where no effective washing of livestock was conducted were 1.9-3.7 (average 2.9). Therefore, it was suggested that not bringing

soil, dirt, etc. into the slaughtering area and keeping there clean were essential to prevent carcass from contamination.

2) Difference of an order of dressing procedures (bleeding and hanging)
In 3 US registered slaughterhouses and Est.E, bleeding was conducted after carcasses were hung up on the rail, on the other hand, in 7 slaughterhouses carcasses were hung up after the bleeding process was finished, in the rest of 3 slaughterhouses, the bleeding process was conducted while carcasses were being hung up with a crane. The mean Sanitary Evaluation Points of the first group (the bleeding was conducted after carcasses were hung up) was 0.7-1.9 (average 1.6), those of the second group (carcasses were hung up after the bleeding process was finished) was 2.7-3.2 (average 3.0), the third group (the bleeding was conducted while carcasses were being hung up) was 3.0-3.4 (average 3.2). Therefore, it was considered that in an order of dressing procedures, hanging carcasses up completely before bleeding started, was the most effective method to prevent cross contamination of carcasses.

3) "Rodding" the esophagus and tying the anus before evisceration
In only 3 US registered slaughterhouses, "Rodding" the esophagus and tying the anus were taken place. With the "Rodding", the esophagus should be effectively closed to prevent the escape of rumen contents. And with cutting the rectum and the ureter free from surrounding tissue and securely tying off, urine and fecal leakage should be prevented. Therefore, from the microbiological points of view, these procedures were considered to be critical for microbial control of meat.

4) Contamination with hide and skin during skinning operation;
Sanitary Evaluation Points of the slaughterhouses where skinned parts of carcasses were hardly contaminated with the outside of the hide and skin was 0.7-3.2 (average 2.2), meanwhile those of the slaughterhouses where skinned carcasses were often contaminated with them was 1.9-3.7 (average 3.0), so during skinning operation, care must be taken to prevent contaminations.

5) Inadequate usage of water between sticking and an end of post-mortem inspection
In slaughtering and dressing process, between sticking and an end of post-mortem inspection, an inadequate usage of water for washing carcasses was found in 9 slaughterhouses, on the other hand, in 5 slaughterhouses (including 3 US registered slaughterhouses), water was not used for carcass washing.
Sanitary Evaluation Points of the first group (inadequate water washing) were 1.9-3.7(average 3.1), those of the second group (No water washing) were 0.7-2.7 (average 1.8). The volume of running water was too little to remove contaminants such as feces , urine, hair, ingesta, pathological tissues and exudates and other filth, so the washing was considered to accelerate the contaminants spread on the carcass. Therefore, it was considered that an inadequate usage of water for washing carcasses should be avoided to prevent cross contamination with water.

6) Usage of a high pressured water pistol in the final carcass rinse
In the final carcass rinse stage, a conventional hose connected with a faucet was used in 4 slaughterhouses, on the other hand, in 7 slaughterhouses (including 3 US registered slaughterhouses), a high pressured water pistol was used for the final carcass rinse.
Sanitary Evaluation Points of the first group (hose) were 0.7-3.6 (average 2.4), those of the second group (a high pressured water pistol) were 2.7-3.7 (average 3.3). Therefore, it was considered that a high pressured water pistol was effective to remove hair, dirt or other foreign materials.

7) Effective sterilization of utensils, equipment during operation
In the three US registered slaughterhouses, utensils, equipment used in dressing diseased or contaminated carcasses were thoroughly cleansed with hot water having a minimum temperature of 180(F or disinfectant, followed by rinsing clean water. On the other hand, in other 11 slaughterhouses, those were just rinsed with cold water, no effective disinfection were conducted. Therefore, whenever utensils, equipment are contaminated, before they are used in dressing the next carcass, they shall be thoroughly washed out and sterilized.

8) Wearing of hand gloves made of cotton;
In the three US registered slaughterhouses, the employees of the establishment who handle any products in slaughtering ,dressing process are prohibited to wear hand gloves made of cotton. On the other hand, except for

Est. N, the employees wore hands gloves made of cotton and they seldom washed or changed even after they touched contaminants. In general, those gloves were adhered by dirty meat, blood and fat.
Sanitary Evaluation Points of No cotton gloves were 0.7-1.9 (average 1.6), those of the cotton gloves were 2.2-3.7 (average 3.1), therefore, it was considered that hand gloves made of cotton would accelerate cross contamination of carcasses.

9) Carcasses contact with the floor, walls, doors and equipment
In the three US registered slaughterhouses and Est.KA, skinned carcasses were never contacted with the floor, walls and equipment,
but in other slaughterhouses, skinned carcasses were often contacted with them. Sanitary Evaluation Points of the No contact group were 0.7-2.7 (average 1.8), those of the contact group were 1.9-3.7 (average 3.0), therefore, before construction of a slaughterhouse, designing enough space in the slaughtering and dressing area to avoid carcasses contacting with walls etc. was considered to be essential for protection of carcass contamination. To make matters worse, in those establishment, a cleaning and sterilization of floor, walls and equipment are not conducted thoroughly, so once carcasses are contacted with them, carcasses are easily contaminated with foreign materials such as feces , urine, hair,ingesta, pathological tissues and exudates and other filth.

10) Effective Sterilization of utensils, equipment after operation
In the three US registered slaughterhouses, after daily operation, utensils, equipment used in slaughtering and dressing are thoroughly cleansed with hot water having a minimum temperature of 140(F and Sodium Hypoclorite solution or other disinfectant, followed by rinsing clean water. In conventional slaughterhouses, in only four out 11 establishments, similar washing and sterilization was performed, and Sanitary Evaluation Points of those slaughterhouses where an effective Sterilization was conducted were 2.8 on the average, and better than th point of the slaughterhouses where only water rinse was performed (3.1 on average). Therefore, after daily operation, effective sterilization of utensils, equipment was considered to be essential to prevent cross contamination.

5. Conclusion
Above mentioned points could be critical control points to protect products from microbial contamination. In each of slaughterhouses, to pinpoint virtual CCPS, more investigations will be required.

To provide industry and government with information to improve microbial quality of Japanese beef, detailed microbial research is being conducted.

To detect Salmonella, 10ml samples were inoculated into 100 ml Enterobacteriaceae-Enrichment Mannitol (EEM) medium (Nissui). After pre-enrichment at 37°C for 18 hours. 1 ml of each culture was transferred to 15 ml of Selenite-Brilliant Green (SBG) enrichment medium (Nissui) which was incubated at 43°C for 18 to 20 hours. The culture was plated on Mannitol-Lactose-Citrate-Methylblue (MLCB) agar (Nissui) which was incubated at 35°C for 18 to 20 hours. Suspected colonies were picked and incubated into Triple-Sugar-Iron (TSI) and Lysine-Indol-Motility (LIM) media (Nissui) for confirmation. (Tokumaru, et al., 1992)
To detect Listeria, 10ml samples were inoculated into 90 ml UVM Enrichment medium. After enrichment at 30°C for 2 to 7 days, the culture was plated PALCAM AGAR which was incubated at 30°C for 24 to 48 hours. Suspected colonies were picked and incubated into Tryptosoycase agar supplement 0.6% yeast (TSYEA) medium for confirmation at 30°C for 24 to 48 hours.