THESIS

Jeon Sungkook

2019

University of Veterinary Medicine, Budapest

Controlling of *Salmonella* species in Korean Chicken Slaughterhouse

By Jeon Sungkook

Supervisor

Dr. Szakmár Katalin

Department of Food Hygiene

Budapest, Hungary

2019

Table of Contents

1. Introduction

1.1. Background and purpose of this research

Salmonella spp. belongs to *Enterobacteriaceae* family, it is Gram-negative, non-spore forming, rod-shaped, facultative anaerobic microbe, motile bacterium, which can be found on animals and in the environment. *Salmonella* spp. grows well at the body temperature, optimum temperature for growth is between 35℃ and 37℃. The slow growth of *Salmonella* spp. has been observed at 5℃, with a maximum growth between 45℃ and 47℃. Growth may occur between pH 4.0(depending on the acid) and pH 9.0; optimum pH is between 6.5 and 7.5. D-value of 2 to 6 minutes at 60℃ has been reported for *Salmonella*. There are more than 2,600 different *Salmonella* serotypes(O-, H-, Vi- antigens) were found, and more than 99 percent of antigens are belonging to a single species and there are six subspecies in this species (Peter Laczay, 2014).

Salmonella spp. is one of the most zoonotic pathogens with *Campylobacter* spp. that is transmitted to human most often with contaminated food (Peter Laczay, 2014).
Salmonella spp. causes various of symptoms, such as enteritis, septicemia and abortion by infecting various of hosts, and is considered to be important in food hygienic background, because they cause many dangerous infections through the carrier of food, such as pork, poultry meat and egg containing food (Baek et al., 2003). *Salmonella* spp., and *Campylobacter* spp. which are major foodborne pathogens contributed significantly to an estimated 2.4 million food-related illnesses, 55,961 hospitalizations, and 1,351 deaths and costing USD 48 billion every year in United States (Scallan et al., 2011).
Salmonella spp. and *Campylobacter spp*. commonly occur in live poultry, contaminate the meat during the slaughter and get transferred to further processing areas. Although *Salmonella* spp. is commonly associated with poultry (Izat et al., 1990; Bailey et al., 1987), and other sources may include raw meats, eggs, milk and dairy products, fish, shrimp, coconut and peanut butter.
Poultry are asymptomatic carrier of most serotypes of *Salmonella* aside from *S.* Pullorum

and *S*. Gallinarum. Most cases of Salmonellosis attributed to poultry occur when poultry products are inadequately cooked or cross contamination occurs (Baiely et al.,1987).

1.2. Comparison of the Salmonella verification of foreign countries and Korea in poultry slaughterhouse

1.2.1. *Salmonella* verification of foreign countries in poultry slaughterhouse

In the United States, slaughter establishments must take samples at a frequency proportional to the establishment's volume of production – in case of chickens 1 sample per 22,000 carcasses, and in case of turkeys, ducks, geese and ratites 1 sample per 3,000 carcasses - concerning the raw poultry product performance standards for *Salmonella*, an establishment's raw poultry products, when sampled and tested by FSIS (Food Safety and Inspection Service, included in the USDA) for *Salmonella*, may not test positive for *Salmonella* at a rate of exceeding the applicable national pathogen reduction performance standard, as **Table 1** (Code of Federal Regulation title 9, § 381.94, revised in 2011).

Table 1. *Salmonella* performance standards in 9 CFR §381.94

Class of product	Performance Standard* (percent of positive for *Salmonella*)	Number of samples tested	Maximum number of positive to achieve standard
Broilers	20.0%	51	12
Ground chicken	44.6%	53	26
Ground turkey	49.9%	53	29

* Performance Standards are FSIS's calculation of the national prevalence of *Salmonella* on the indicated raw products based on data developed by FSIS in its nationwide microbiological baseline data collection programs and surveys. (Copies of Reports on FSIS's Nationwide Microbiological Data Collection Programs and Nationwide Microbiological Surveys used in determining the prevalence of *Salmonella* on raw products are available in the FSIS Docket Room.)

In case of Europe, Regulation of EC 2073/2005 on microbiological criteria for foodstuffs stipulates as a process hygiene requirement that with the aim of verifying the hygiene of poultry slaughter, poultry carcasses (broiler chicken and turkey) shall be tested for surface contamination for *Salmonella*. To this end, once a week, a minimum of 15 slaughtered and dressed carcasses shall be sampled by taking a piece of approximately 10 g from the neck skin after chilling. Before analysis, samples shall be pooled by groups of three in order to obtain 5 × 25 g test samples (5 samples a week). Always the test results of the last 10 weeks (the last 50 samples) shall be evaluated by allowing maximum 5 samples out of the 50 (n=50, c=5) to be positive. Fresh poultry meat may be put on the market for human consumption only if *S.* Enteritidis and *S.* Typhimurium cannot be detected in it by testing a 25g sample. This requirement applies to fresh meat originating from broiler, breeding and laying flocks of domestic fowl as well as from breeding and fattening flocks of turkeys (Peter Laczay, 2014; Regulation of EC 2073/2005, 2000 revised).

1.2.2. *Salmonella* verification of Korea in poultry slaughterhouse

In case of Korea, there are two departments related to the food safety, such as “Ministry of Agriculture, Food and Rural Affairs” and “Ministry of Food and Drug Safety”. In case of the inspection for the slaughter, “Ministry of Agriculture, Food and Rural Affairs” takes charge of the duties, and “Animal and Plant Quarantine Agency” which belongs to the “Ministry of Agriculture, Food and Rural Affairs” and each local government take charge of the practical works about the inspection for the slaughter, and it is performed according to the Korean law, “Livestock products sanitary control act”.

The principle of the inspection of the slaughter animal in Korea is inspection with complete enumeration, and the inspection for slaughter, such as ante-mortem and post mortem inspection, is performed by the inspector, who belongs to the competent authority, and the assistant staff for the inspect for the slaughter. (Arrangement of the personnel for the slaughter inspection and direction of improvement for the HACCP in Korean slaughterhouse; Lee, revised in 2010)

In Korea, every slaughterhouse which is approved HACCP-based system shall be required the *Salmonella* laboratory test to verify whether their HACCP system is working properly or not, and every slaughterhouse shall be approved HACCP-based system. The frequency of *Salmonella* laboratory test for the establishment of chicken and duck is 1 sample per 22,000 carcasses, and in case of establishments of which production per weeks is not exceeding 22,000 carcasses, frequency of *Salmonella* is 1 sample per weeks. The performance standards for *Salmonella* is shown in the **Table 2.** (Method of laboratory test for the slaughterhouse - Regulation of the Korean Food and Drug Administration, 2017)

Table 2. *Salmonella* performance standards in Korean regulation (Method of laboratory test for the slaughterhouse)

`	Number of samples tested	Acceptance criteria for *Salmonella* positive	
		Maximum number of positive to achieve standard	Percent of positive for *Salmonella* per year
Cattle	26	1	2.5 %
Pigs	26	2	7 %
Chickens, Ducks	26	5	18 %

***** If laboratory test result excesses the one of the acceptance criteria for *Salmonella* detection, in case the number of *Salmonella* positive excesses among 26 samples tested lately or percent of positive for *Salmonella* per year excesses 18%, the judgement would be non-compliance.

In case the judgement of laboratory test result for *Salmonella* in some slaughterhouse is non-compliance, the applicable slaughterhouse have to investigate the cause of non-compliance and take the corrective actions to this non-compliance, and if they get the non-compliance of the *Salmonella* species again in 1 year of *Salmonella* positive, they must verify their HACCP plan again, and then correct, supplement according to the results of verification.

2. Material and methods of research

2.1 Materials of this research

Samples were taken in one poultry slaughterhouse which was not met the maximum number of positive to achieve standard of Korean regulation, and has the slaughtering capability of 500 thousand carcasses per day. Samples were taken at each of procedures those were possible to occur the contamination of *Salmonella* to the chicken carcasses, and equivalently with the Korean regulation, were 26 samples per each procedure both before and after corrective action is taken. Totally, 4 procedures are targeted as the subjects of the sample taking – receipt of live birds, evisceration, carcass washing both inside and outside and final collection. In case of the receipts of live birds, samples were taken by swabbing the cloaca and in case of the others, samples were taken by 40ml solution rinsing the carcasses in BPW (Buffered peptone water). Every taken samples were transported to the laboratory while they are put in the portable fridger such as icebox with the refrigerant immediately.

2.2. Methods of this research

2.2.1. Method of analysis

The poultry slaughterhouse which is located in Iksan-city, Jeollabuk-province, the Southern Korea has the slaughtering capacity of 500 thousand carcasses per day, and in terms of the amount of poultry slaughter, this slaughterhouse is the number one poultry slaughterhouse in the Republic of Korea. The amount of export to the other countries of this slaughterhouse is shown in the **Table 3**.

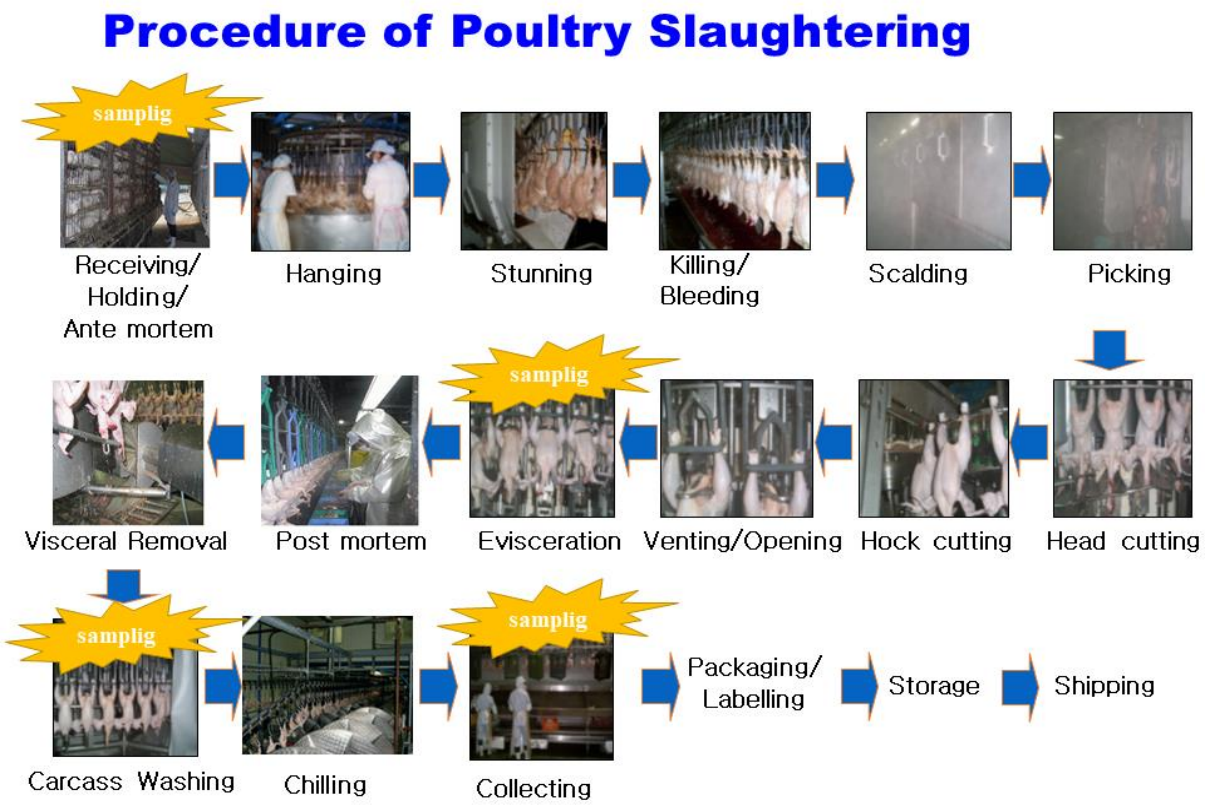
Table 3. The amount of export to the other countries of this slaughterhouse

		Hongkong	Japan	Thailand	Taiwan	Singapore	Vietnam	Total
2007	Cases	1	22	0	3	0	1	27
	amount(kg)	7,680	104,938	0	12,660	0	19.2	125,298
2008	cases	2	29	1	1	0	0	33
	amount(kg)	15,872	157,240	288	6,240	0	0	179,640
2009	cases	3	27	1	4	3	1	39
	amount(kg)	21,376	176,862	404	18,972	2,879	38.4	220,531
2010	cases	3	27	1	5	0	1	38
	amount(kg)	20,448	202,438	998	34,740	0	6,916	265,540
2011	cases	3	33	0	5	0	1	42
	amount(kg)	21,440	252,989	0	31,260	0	96	305,785

Reference: Animal and Plant Quarantine Agency

This slaughterhouse was not met the maximum number of positive to achieve standard of Korean regulation (5 times per 26 samples) on March 2012. Based on the Korean regulations (Method of laboratory test for the slaughterhouse - Regulation of the Korean Food and Drug Administration, 2017) in case the judgement of laboratory test result for *Salmonella* in some slaughterhouse is non-compliance, the applicable slaughterhouse have to investigate the cause of non-compliance and take the corrective actions to this non-compliance. Therefore, as an investigation of the cause of non-compliance, through the sample taking at 4 slaughtering procedures those are possible to contaminate the chicken carcasses with the *Salmonella* and comparative analysis for this result, this slaughterhouse can be make the recurrence prevention for the non-compliance, and can research the methods of controlling *Salmonella* species in the chicken slaughterhouse, and the procedure of poultry slaughtering and places of sampling is shown in the **Chart 1**.

Chart 1. The procedure of poultry slaughtering and places of sampling in that slaughterhouse located in Iksan-city, the Southern Korea



11

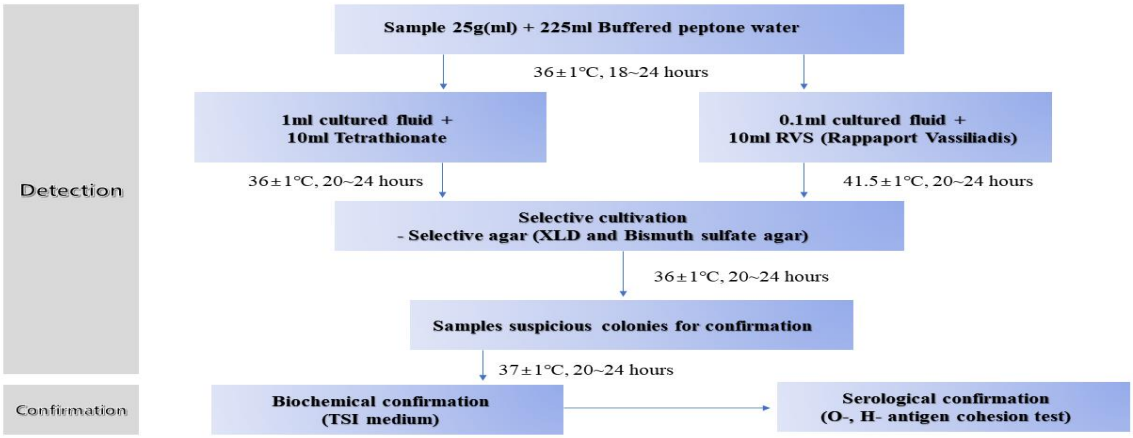
Furthermore, the sampling at the same place – receipt of live bird, evisceration, carcass washing and collecting - and laboratory test against *Salmonella* under the same conditions would be performed as a method of verification for this research.

2.2.2. Method of laboratory test

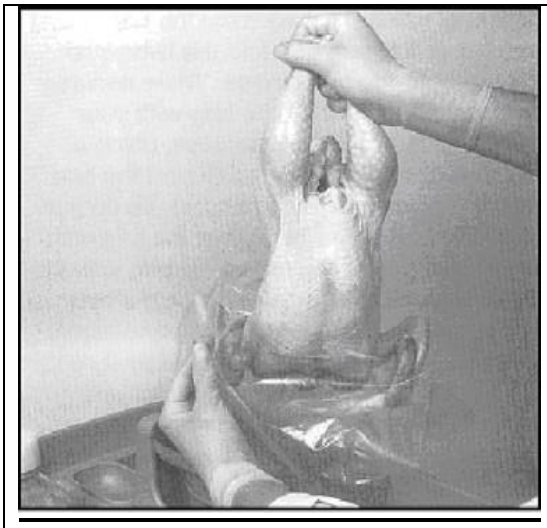
Method of laboratory test is based on the Korean regulation (Standards and Criteria of the Food – Regulation of Korean Food and Drug Administration, 2019). The first step is detection of *Salmonella* spp. Add 225ml of BPW (Buffered peptone water) to a sample of 25ml and incubate it at 36±1℃ for 18 to 24 hours, and then

inoculate this culture fluid to 2 kinds of enrichment mediums, i.e. 1ml of this culture fluid to 10ml of Tetrathionate medium and, at the same time, inoculate 0.1ml of this culture fluid to 10ml of RVS medium, and then cultivate for enrichment at 36±1℃ for 20 to 24 hours(Tetrathionate medium) and at 41.5±1℃ for 20 to 24 hours (RVS medium), respectively. And after inoculating each culture fluid to selective agar, XLD agar and Bismuth sulfate agar, cultivate at 36±1℃ for 20 to 24 hours (selective cultivation). Samples the suspicious colonies for the confirmation. The second step is confirmation of *Salmonella*. The suspicious or probable colonies are confirmed with biochemical and serological tests. In case of biochemical confirmation, the suspicious colonies those were found by the detection of *Salmonella* were inoculated onto the TSI medium and incubated at 37±1℃ for 20 to 24 hours. And then confirm the *Salmonella* spp. through the biochemical reactions, for example Indol(-), Methyl red(+), Voges-Proskauer test(-), Citrate(+), Urease(-), Lysine decarboxylation(+), KCN(-) and malonate test(-). *Salmonella* spp. confirmed biochemically is subjected to a serological test(O-, H- antigen cohesion reaction). The strains of *Salmonella* spp. should be confirmed based on the results of the agglutination reaction using antiserum for *Salmonella* diagnosis. This method of laboratory test is shown in **Chart 2**.

Chart 2. Method of laboratory test based on the Korean regulation (Standards and Criteria of the Food – Regulation of Korean Food and Drug Administration, 2019)



Picture 1. Method of sampling of carcass for *Salmonella* laboratory test



3. Results/Discussion

3.1. Results of the Salmonella laboratory test at each slaughter process

The slaughtering procedure of this slaughterhouse is carried out in order of receipt of birds, shackling, stunning, bleeding, scalding, defeathering, removal of head and feet, vent cutting, evisceration, washing the carcasses, immersion chilling, air chilling, collection and packaging. Among these slaughtering procedures, *Salmonella* laboratory test was performed to confirm the problem and improvement measures of *Salmonella* occurrence. The samples were taken 4 procedures those were possible to occur contamination of *Salmonella* to the chicken carcasses, and 26 samples per each procedure, in other words 104 samples were taken in total. The *Salmonella* positive rate of samples taken during the receipt of live birds is 7.7% (2 positive samples per 26 samples taken), during the procedure of evisceration is 57.7% (15 positive samples per 26 samples taken), during the washing both inside and outside is 53.8% (14 positive samples per 26 samples taken) and during the final collection is 42.3% (11 positive samples per 26 samples taken). The percent of positive for *Salmonella* is 40.4% (42

positive samples per 104 samples taken) in total. The results of *Salmonella* test during these 4 slaughtering procedures are shown in the **Table 4** and **Chart 3**.

Table 4. The results of *Salmonella* test for the 4 slaughter procedures

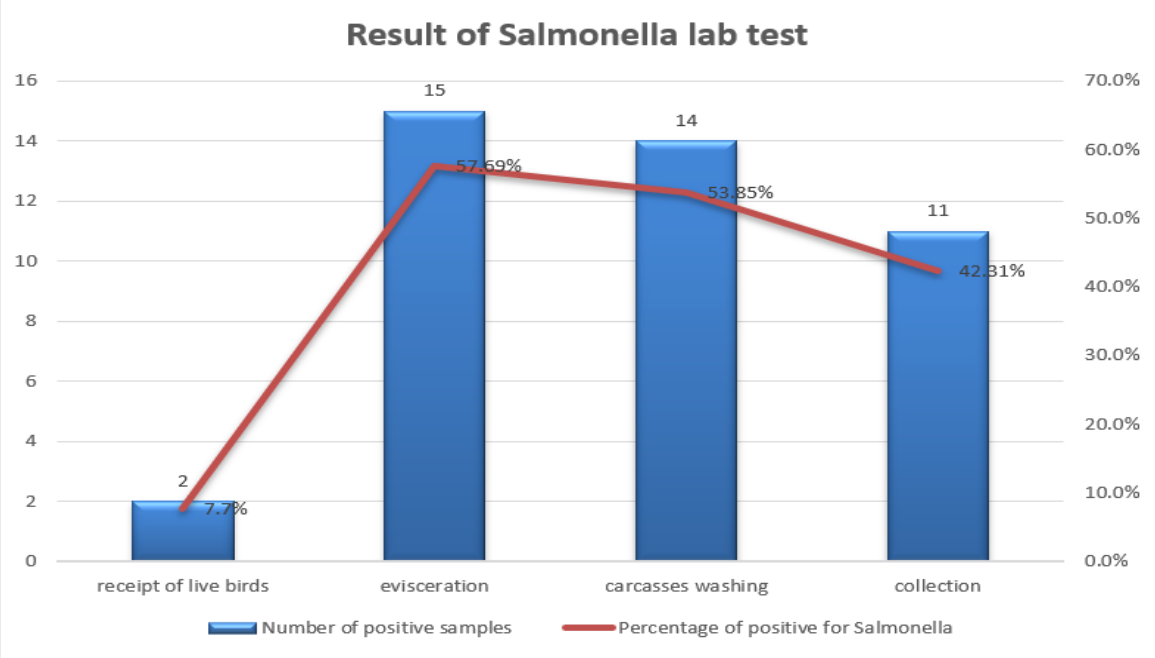
	Number of samples	Number of positive samples	Percentage of positive for *Salmonella*
Live bird receipt	26	2	7.7 %
Evisceration	26	15	57.7 %
Washing carcasses	26	14	53.8 %
Final collection	26	11	42.3 %
Total	104	42	40.4 %

These results mainly show that the positive rate of *Salmonella*, which has increased rapidly during the procedure of evisceration, is not controlled by the process-wide CCPs and SSOPs of this slaughterhouse, and the positive case of *Salmonella* exists all of the procedures. Furthermore, this result shows that all the figure of *Salmonella* detection in this slaughterhouse is over the *Salmonella* performance standards in Korean regulation* (Method of Laboratory Test for the Slaughterhouse-Regulation of Korea;), except for the step of live bird receipt.

***** If laboratory test result excesses the one of the acceptance criteria for *Salmonella* detection, in case the number of *Salmonella* positive excesses among 26 samples tested lately or percent of positive for *Salmonella* per year excesses 18%, the judgement would be non-compliance.

For the easy understanding of this results of *Salmonella* test for the 4 slaughter procedures, there is the charts for this result as below, in the **Chart 3**.

Chart 3. The chart for the results of *Salmonella* test for the 4 slaughter procedure



Therefore, it needs to research about the method of controlling *Salmonella* species during these procedures. After finding out the appropriate method to control *Salmonella* in the slaughterhouse, *Salmonella* laboratory test will be carried out with the same method and same way. Hence through this *Salmonella* laboratory test, it can be judged that this research was right or false.

3.1.1. Live bird receipt

3.1.1.1. Pre-harvest

As far as you see the result of this *Salmonella* test, the first step to control *Salmonella* in the poultry slaughterhouse is step of live bird receipt. We have to think about the contamination in the step of pre-harvest. Although the prominent modes of transmission are not well understood, it is known that *Salmonella* may be spread through horizontal transmission from environmental sources (Stern, 2012) and through

vertical transmission from breeder flocks (Hiett el a., 2002; Cox et al., 2005). The only method to prevent the horizontal transmission could be vaccination of *Salmonella* against the breeding stocks. Further evidence of potential vertical transmission is demonstrated by studies showing *Salmonella* from poultry hatchery samples. Specially, Cox et al. (1991) recovered *Salmonella* from shell eggs fragments, chick pads and chick fluff in poultry hatcheries. Environmental samples indicating horizontal spread of *Salmonella* at the farm level includes factor such as litter, feed, water, insects, humans, animals and rodents (Jones et al., 1991; Hoover et al., 1997; Amick-Morris). Basically, it needs to provide for poultry the feeds without *Salmonella* origin and pellet feeds rather than meals. Poultry feed and water treatments generally included acid supplement. Research conducted by Byrd et al. (2002) found that acid application in drinking water could reduce *Salmonella* in poultry crops by identified 80%. And concerning the withdrawal feed, the Korean regulation says that feed shall be withheld 3 hours before the shipping poultry, but in the European regulation feed shall be withheld 8~12 hours before the scheduled time of slaughter and in the U.S. regulation feed shall be withheld at least 14 hours before slaughter. Therefore, the feed withdrawal time in the Korean regulation need to be revised in equivalent with the regulation of U.S or EU. In addition, the implementation of biosecurity measures and maintenance of good sanitation measures to the poultry farm are essential sources to control the *Salmonella* at the stages of the pre-harvest.

3.1.1.2. Live bird receipt and hanging

The most important thing to prevent contamination of *Salmonella* to the poultry during the waiting time for slaughter is cross-contamination prevention. As a way for the prevention of cross-contamination, first of all, the crates or cages shall be made with corrosion-free material and must be easy to clean and disinfect, and have gap-free floors in order to prevent fecal contamination. In addition, the crates or cages shall be cleaned and disinfected immediately after emptying or use, if necessary, before their re-use, and the poultry shall always be loaded into clean and disinfected vehicles only (Peter Laczay, 2014), because in this slaughterhouse in Korea, the poultry is waiting

for their slaughtering in the crates or cages. Secondly, this slaughterhouse has the integrated system with many poultry farms those are shipping to this farm, if any farm where has occurred the *Salmonella* infection, poultry stocks from that farm shall be slaughtered at the last time of those slaughter day to minimize the cross-contamination of *Salmonella* to the poultry carcasses and this slaughterhouse. Finally, the maintenance of good sanitation measurement. For example, the adequate ventilation shall be provided in the holding place of slaughterhouse for the health of poultry stocks, the UV-light is adequate for the sanitary environment, and development of Sanitary Standard Operating Procedures (SSOPs) is essential to maintain good sanitation measurement, and the Sanitation SOP's shall describe all procedures an official establishment will conduct daily, before and during operations, sufficient to prevent direct contamination or adulteration of product(s) (Code of Federal Regulation title 9, § 416.12; development of sanitary SOPs, revised in 2011).

3.1.2. Evisceration

Among the all of slaughter procedures, this evisceration is the easiest procedure to produce contamination by feces the poultry carcasses. Because the size of poultry body is smaller than any other animals, and in this procedure, the rupture of intestine inside the poultry body is occurred at higher frequency, and there are many pathogens of *Salmonella* in the feces. As you can see the result of the *Salmonella* test, the number of positive samples is increased rapidly at the evisceration. To prevent and control this contamination of *Salmonella* at this procedure, there are some methods as below.

3.1.2.1. Appropriate adjustment of the evisceration machine

Lots of poultry slaughterhouses are carrying out the evisceration using the human power manually, not using the evisceration machine automatically. Absolutely, it is fact that automatic evisceration is recommended rather than the evisceration using the automatic machine, because the more *Salmonella* contamination could be occurred through the hands of staffs, therefore this slaughterhouse is using the automatic

evisceration machine. Although the evisceration using the automatic machine causes the less *Salmonella* contamination, it is not always the case.

Evisceration machine shall be adjusted to the average size and weight of the carcasses being slaughtered. If the evisceration machine is not adjusted or the size and weight of whole carcasses is not uniform, the rupture of intestine or cloaca will be increased more and more, because this machine is not able to adjust to the size and weight of the carcasses. The more rupture of intestine or cloaca is occurred, the more carcasses are contaminated to the *Salmonella* in their feces.

3.1.2.2. Appropriate adjustment and maintenance of the slaughter speed

When it comes to slaughtering speed, it was a difficult situation to handle because it is a part of the slaughterhouse's revenue and is not specified in the Korean law. But the determination and will to improve the detection of *Salmonella* in this slaughterhouse was very firm and strong, the adjustment of slaughter speed is also available to upgrade the detection of *Salmonella*. And as I mentioned at the first time, the average amount of slaughtered poultry in this slaughterhouse is about 500 thousand carcasses per day. But the slaughter speed of this slaughterhouse is 100 birds per minute, this speed is too fast for inspectors to inspect the slaughtering carcasses and for HACCP monitoring staff to sample the carcasses to monitor the CCP 1, zero tolerance to the visible fecal contamination. In the U.S. regulation, there are five slaughter systems of post-mortem inspections such as SIS (Streamlines Inspection System), NELS (New Line Speed), NTS (New Turkey System), Traditional and Ratite Inspection. Among them, considering the actual situation of Korea, in which the broilers account for the most of the slaughtered amount, this SIS-1 (Streamlines Inspector System-1) that may be performed by one inspector was applied to this slaughterhouse. This SIS-1 requires that the establishment provide one inspection station for each line and adequate reinspection facilities so carcasses can be removed from each line for evaluation. The maximum speed of the SIS-1 is 35 birds per minute (Code of Federal Regulation title 9, §381.76; post-mortem inspection; revised in 2011).

3.1.2.3. Appropriate maintenance of Hazard Analysis Critical Control Point (HACCP) – zero tolerance to the visible fecal contamination

For this slaughterhouse controls this procedure as the critical control point in the way of examining the rupturing the intestine and cloaca. But there is fatal error in this method. The critical limit of this critical control point, examining the rupturing the intestine and cloaca is over 4 birds per 10 birds those are examined, and the corrective action against the deviation from the critical limit. The HACCP plans shall describe the corrective action to be taken if there are some deviations from the critical limit, and assign responsibility for taking corrective action, to ensure 1) The cause of the deviation is identified and eliminated; 2) The critical control point will be under control after the corrective action is taken; 3) Measures to prevent recurrence are established; and 4) No product that is injurious to health or otherwise adulterated as a result of the deviation enters commerce (Code of Federal Regulation title 9, §417.3; corrective actions, revised in 2011). But the measures to prevent recurrence (3) and the measures in order that no product that is injurious to health or otherwise adulterated as a result of the deviation enters commerce (4) are not established in the CCP plan of this slaughterhouse and the product (poultry carcasses) that is adulterated as a result of the deviation from this slaughterhouse is entering commercial market at that moment. Therefore, a complete modification or revision for this critical control point plan should be inevitable. In addition, in consequence of investigation for the slaughterhouse staffs performing the monitoring of critical control point, their method of monitoring was unsuitable. The sampling method for monitoring was that every monitoring for the rupturing intestines and cloaca should be sampled ten carcasses in total, one carcass per ten carcasses, but the staffs in this slaughter house were sampling the carcasses for their sweet will. The basic and periodic training or instruction to the monitoring staffs shall be needed, thoroughly. The individual performing the functions shall have successfully completed a course of instruction in the application of the seven HACCP principles to meat or poultry product processing, including a segment on the development of a HACCP plan for a specific product and on record review (Code of Federal Regulation title 9, § 417.7; training, revised in 2011). Based on this regulation

of the U.S. the poultry carcasses contaminated with visible fecal material shall be prevented from entering the chilling tank (Code of Federal Regulation title 9, §318.65.e; revised in 2011) and the mandatory CCP in the poultry slaughter HACCP plans shall be Zero fecal tolerance (FSIS directive 6420.2; verifications of procedures for controlling fecal materials, ingesta and milk in livestock slaughter operations; revised in 2017), this slaughterhouse revised their HACCP plans through a reassessment process. This revised critical control point is as below. First of all, the name of the CCP, "rupture of intestine and cloaca" was changed to the "zero tolerance to the visible fecal contamination". Secondly, the examining method is established like this, after consecutive sampling of 10 poultries at the exam station for the fecal contamination, and then observe external dorsal parts (knees, behind the legs, sebaceous gland, behind the carcass, upper wings), external abdominal parts (lower wings, front legs), and internal parts (internal surface of carcass, lipid, skin part of neck). The critical limit is zero tolerance to the visible fecal contamination, literally. Thirdly, the frequency of this CCP becomes once per 1 hour. Finally, the measures to prevent recurrence were established like this, if any deviation is occurred, the corrective action is as follows according to the U.S. regulation, Code of Federal Regulation title 9, §417.3; corrective actions. 1) The cause of the deviation is identified and eliminated: check and confirmation of the deviation at the evisceration machine, washing machine with the high pressure of water; 2) The critical control point will be under control after the corrective action is taken: conduct a re-examination against the fecal contamination after 10 minutes of deviation, and then CCP will be under control until the fecal contamination will be 0%; 3) Measures to prevent recurrence are established: report to HACCP team manager, the relative teams or department, then establish and perform the measures to prevent recurrence; 4) No product that is injurious to health or otherwise adulterated as a result of the deviation enters commerce: clean and rinse all the products those are produced up to 1 hour prior to occurrence of deviation and then store them separately in the refrigerator. Each entry on a record maintained under the HACCP plan shall be made at the time the specific event occurs and include the date and time recorded, and shall be signed or initialed by the establishment employee making the entry (Code of Federal Regulation title 9, § 417.5; records, revised in 2011).

3.1.3. Washing carcasses inside and outside

Following the evisceration, it is carried out to rinse and wash the carcasses inside and outside to remove fecal contamination which adhere to each carcass. Thus, after the evisceration of the carcass, the carcass is washed and rinsed inside and outside. Carcass rinses or sprays can be effective interventions for removing incidental contamination from the carcass surface during evisceration. Studies have shown that *Salmonella* prevalence on carcasses can be reduced by 50-90% following rinses (Buncic and Sofos, 2012). The NCC recommends whole-carcass water rinses using 20 ppm free available chlorine (NCC, 1992). A 20 ppm free available chlorine rinse post-evisceration can decrease microbial contamination and improve food safety (Waldroup, et al., 1992). But in case of this slaughterhouse, the carcass is going to enter the chilling water contains the 20~50 ppm free available chlorine, thus it'd be better not use free available chlorine in the washing water to prevent for excessive remaining the residue of chlorine. And then, how can we control the *Salmonella* in this procedure? In principle, it's right that the detection of *Salmonella* is decreased after the washing and rinsing carcass, but as a result of this *Salmonella* laboratory test, the detection of *Salmonella* shows only a 3.9% of decrease (57.7% to 53.8%). This shows clearly that the washing system has some problems. As a result of the investigation for the carcass washing machine, the direction of water injection is not appropriate, not to the carcasses inside and outside exactly, and the water that is washing the carcass is being reused. Therefore, first of all, the direction of water injection shall be adjusted frequently by the staff who takes exclusively charge of adjusting and the water injector shall be cleaned and vacuumed by the staff. And secondly, the water that is used to wash and rinse the carcass inside and outside shall not be reused. Reuse of water, ice and solutions that which has come into contact with raw product may not be used on ready-to-eat product. (Code of Federal Regulation title 9 § 416.2 Establishment grounds and facilities; revised in 2011).

3.1.4. Final collection (Post-chill)

Prior to reaching the final collection, the carcasses shall go through the procedure of chilling (immersion and air chilling) in this slaughterhouse. Microbial growth of *Salmonella* in the eviscerated carcasses is inhibited by the procedure of chilling, the first reason why microbial growth of *Salmonella* is inhibited by the chilling procedure is because the temperature of carcasses is reduced to 5℃ (Livestock products sanitary control act; revised in 2019), and this temperature is out of range of temperatures for the growth of *Salmonella* (Peter Laczay; 2014). The second reason is concentration of free available chlorine in the chilling water. This concentration is designated as the second critical control point. But the cross contamination of *Salmonella* also can be increased through the procedure of chilling, because of the contamination in the chilling water of the immersion chiller. As you can see the result of *Salmonella* laboratory test, the number of positive samples is not decreased as required by law. To prevent and control this contamination of *Salmonella* at this procedure, there are some methods as below.

3.1.4.1. Prevention and removal of the condensation in chilling room

Ventilation must be adequate to control oders, vapors, and condensation to the extent necessary to prevent adulteration of product and the creation of insanitary conditions must be provided (Code of Federal Regulations Title 9. § 416.2 Establishment grounds and facilities; revised in 2011). But, on the ceiling of chilling room, there was full of condensation caused by temperature differences between evisceration room and chilling room. Thus, ventilation of this slaughterhouse shall be improved through the improvement of the air conditioning facilities, at the same time, the condensation on the ceiling of chilling room shall be removed by the personnel who takes exclusively charge of removal of the condensation.

3.1.4.2. Appropriate maintenance of Hazard Analysis Critical Control Point (HACCP) system – concentration of free available chlorine

The second critical control point was measurement of concentration of free available chlorine. The critical limit of this CCP was 20~50 ppm of chlorine concentration. But the method of monitoring was measurement using the litmus paper. It was difficult for the monitoring staff to measure accurately with this method using the litmus paper. Therefore, this method of monitoring was changed to method using the electronic chlorine meter undergone calibration at the certificate authority.

3.2. Re-examine the Salmonella laboratory test according to results of this research

After operating the workshop for one month using the improvement measures to control *Salmonella* in this slaughterhouse listed so far, among the same slaughtering procedures, the re-examination of the *Salmonella* laboratory test was performed to verify whether the improvement measures to control *Salmonella* is appropriate or not, the samples were taken 4 procedures those were possible to occur contamination of *Salmonella* to the chicken carcasses, and 26 samples per each procedure, in other words 104 samples were taken in total. The *Salmonella* positive rate of samples taken during the receipt of live birds is 7.7% (2 positive samples per 26 samples taken), during the procedure of evisceration is 26.9% (7 positive samples per 26 samples taken), during the washing both inside and outside is 15.7% (4 positive samples per 26 samples taken) and during the final collection is 7.7% (2 positive samples per 26 samples taken). The percent of positive for *Salmonella* is 14.4% (15 positive samples per 104 samples taken) in total. The results show that the detection frequency of *Salmonella* has definitely decreased compared to the initial test results, and that the value is not out of the Korean legal threshold, *Salmonella* performance standards*. The results of re-examination of *Salmonella* test during these 4 slaughtering procedures are shown in the **Table 5** and **Chart 4**. Through this result of re-examination of *Salmonella* laboratory test, we can confirm that these improvement measures to control *Salmonella* in this slaughterhouse was correct methods.

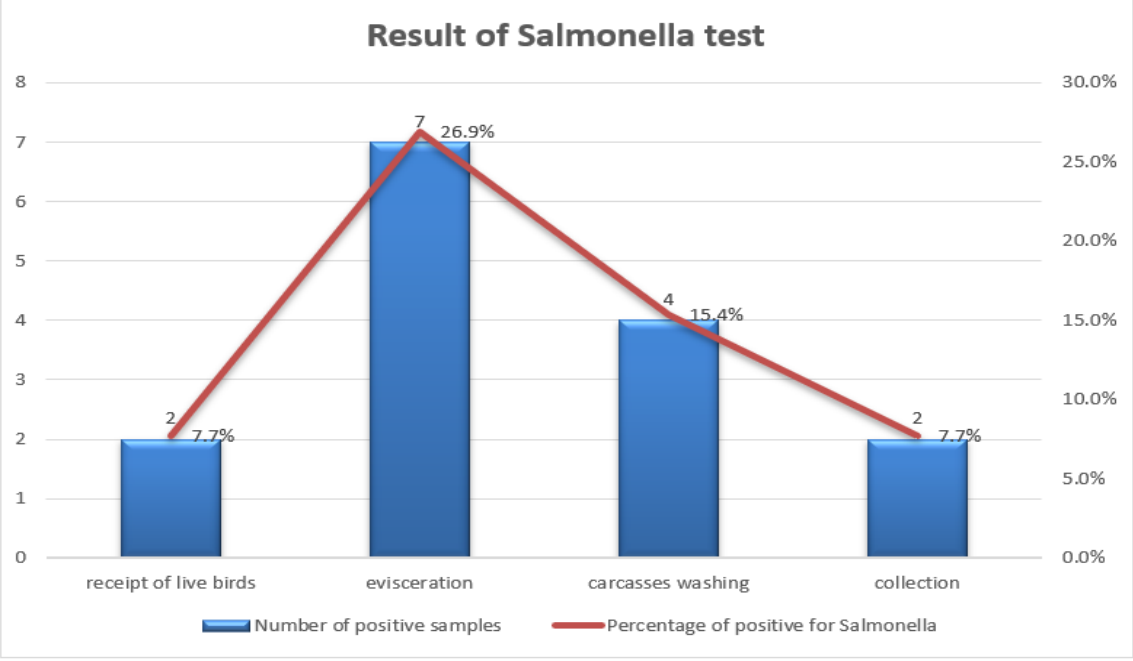
***** If laboratory test result excesses the one of the acceptance criteria for *Salmonella*

detection, in case the number of *Salmonella* positive excesses among 26 samples tested lately or percent of positive for *Salmonella* per year excesses 18%, the judgement would be non-compliance.

Table 5. The results of re-examination of *Salmonella* test for the 4 slaughter procedures

	Number of samples	Number of positive samples	Percentage of positive for *Salmonella*
Live bird receipt	26	2	7.7 %
Evisceration	26	7	26.9 %
Washing carcasses	26	4	15.7 %
Final collection	26	2	7.7 %
Total	104	15	14.4 %

Chart 4. The chart for the results of re-examination of *Salmonella* test



3.3. Discussion

As you can see the result of re-examination of *Salmonella* laboratory test, after taking appropriate measures to reduce the detection of *Salmonella* in each process, the samples were taken 4 procedures those were possible to occur contamination of *Salmonella* to the chicken carcasses, and 26 samples per each procedure, in other words 104 samples were taken in total. The *Salmonella* positive rate of samples taken during the receipt of live birds is 7.7% (2 positive samples per 26 samples taken), during the procedure of evisceration is 26.9% (7 positive samples per 26 samples taken), during the washing both inside and outside is 15.7% (4 positive samples per 26 samples taken) and during the final collection is 7.7% (2 positive samples per 26 samples taken). The percent of positive for *Salmonella* is 14.4% (15 positive samples per 104 samples taken) in total. The results show that the detection frequency of *Salmonella* has definitely decreased compared to the initial test results, and that the value is not out of the Korean legal threshold, *Salmonella* performance standards. Then, what are the main factors to control *Salmonella* in the Korean chicken slaughterhouse? It seems that there are so many methods about this, but the main factors are appropriate management of SSOP and HACCP. So far, there are the contents about the SSOP and HACCP in the paper within Korean law and plans in slaughterhouse, but there are not contents how we should manage these, so as a result of finding out and applying the relevant foreign regulation, such as regulations of U.S., Code of Federal Regulations, there are essential elements in the corrective actions against the deviations of SSOP and HACCP. When the slaughterhouse would apply the methods of corrective actions appropriately, the controlling *Salmonella* would be not hard process.

4. Summary

Salmonella spp. belongs to *Enterobacteriaceae* family, it is Gram-negative, non-spore forming, rod-shaped, facultative anaerobic microbe, motile bacterium, which can be found on animals and in the environment. *Salmonella* spp. is major foodborne pathogens which commonly occur in live poultry, contaminate the poultry meat during slaughter and get transferred to further processing areas. This research is performed to make some controlling method of *Salmonella* spp. in the Korean poultry slaughterhouse. The laboratory test for *Salmonella* is carried out twice, before and after improvement measures for one poultry slaughterhouse which was not met the maximum number of positive to achieve standard of Korean regulation, and the sample was taken at 4 slaughtering procedures those were possible to occur contamination of *Salmonella* to chicken carcass and equivalently with Korean regulation, such as live bird receipt, evisceration, washing carcass both inside and outside, and final collection, and 26 samples per each procedure was taken, in other words 104 samples were taken in total. First laboratory test is carried out to find out the problem and improvement measures, and second laboratory test is carried out to verify whether these improvement measures are appropriate or not and as a result of the two-time *Salmonella* laboratory test, it is available to control *Salmonella* in the poultry slaughterhouse through the correct and accurate HACCP management for the slaughterhouse, and improvement of some problems those are discovered by this research without any special measurement for improvement.

5. Acknowledgements

Two and a half years have already passed since I started my second veterinary medicine studies. And graduation is just around the corner. Now it's time to submit my thesis after completing a required course. A lot of people around me have provided help to me. I'd like express my sincere thanks to all of them to the best of my poor ability. Particularly, I'd like to express my hearty gratitude to supervisor of mine, Dr. Szakmár Katalin for the significant support of my graduation thesis and related studies with the greatest care, and to my Professor Peter Laczay for warm encouragement and valuable advice despite his busiest schedules.

Appendices

Table 1.: *Salmonella* performance standards in 9 CFR §381.94

Class of product	Performance Standard* (percent of positive for *Salmonella*)	Number of samples tested	Maximum number of positive to achieve standard
Broilers	20.0%	51	12
Ground chicken	44.6%	53	26
Ground turkey	49.9%	53	29

Reference: Code of Federal Regulation, title 9 § 381.94; Contamination with Microorganisms; process control verification criteria and testing; pathogen reduction standards.

Table 2.: *Salmonella* performance standards in Korean regulation (Method of Laboratory Test for the Slaughterhouse)

Class of product	Number of samples tested	Acceptance criteria for *Salmonella* positive	
		Maximum number of positive to achieve standard	Percent of positive for *Salmonella* per year
Cattle	26	1	2.5 %
Pigs	26	2	7 %
Chickens, Ducks	26	5	18 %

***** If laboratory test result excesses the one of the acceptance criteria for *Salmonella* detection, in case the number of *Salmonella* positive excesses among 26 samples tested lately or percent of positive for *Salmonella* per year excesses 18%, the judgement would be non-compliance.

Table 3.: The amount of export to the other countries of this slaughterhouse

Year/Details		Hongkong	Japan	Thailand	Taiwan	Singapore	Vietnam	Total
2007	cases	1	22	0	3	0	1	27
	amount(kg)	7,680	104,938	0	12,660	0	19.2	125,298
2008	cases	2	29	1	1	0	0	33
	amount(kg)	15,872	157,240	288	6,240	0	0	179,640
2009	cases	3	27	1	4	3	1	39
	amount(kg)	21,376	176,862	404	18,972	2,879	38.4	220,531
2010	cases	3	27	1	5	0	1	38
	amount(kg)	20,448	202,438	998	34,740	0	6,916	265,540
2011	cases	3	33	0	5	0	1	42
	amount(kg)	21,440	252,989	0	31,260	0	96	305,785

Reference: Animal and plant quarantine agency

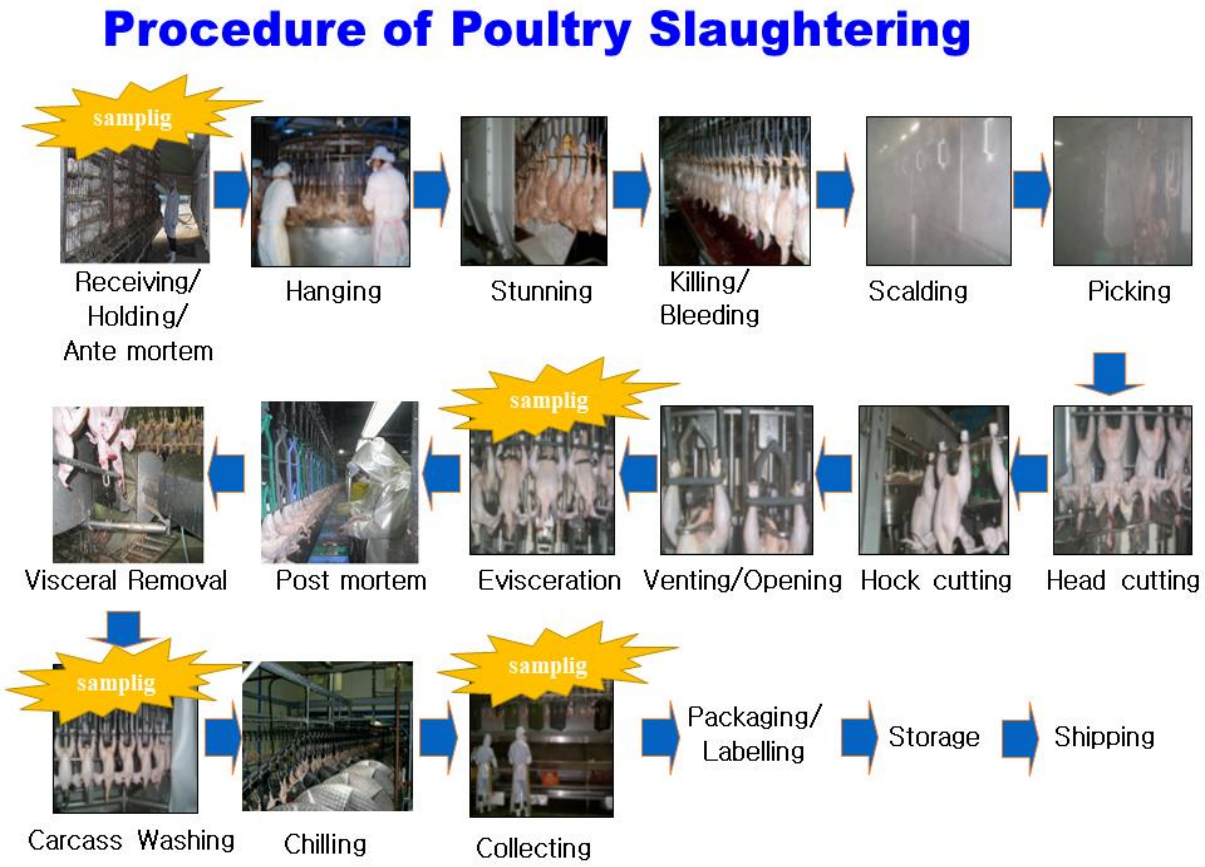
Table 4.: The results of *Salmonella* test for the 4 slaughter procedures

	Number of samples	Number of positive samples	Percentage of positive for *Salmonella*
Live bird receipt	26	2	7.7 %
Evisceration	26	15	57.7 %
Washing carcasses	26	14	53.8 %
Final collection	26	11	42.3 %
Total	104	42	40.4 %

Table 5.: The results of re-examination of *Salmonella* test

	Number of samples	Number of positive samples	Percentage of positive for *Salmonella*
Live bird receipt	26	2	7.7 %
Evisceration	26	7	26.9 %
Washing carcasses	26	4	15.7 %
Final collection	26	2	7.7 %
Total	104	15	14.4 %

Chart 1.: The procedure of poultry slaughtering and places of sampling in that slaughterhouse located in Iksan-city, the Southern Korea



11

Chart 2.: Method of laboratory test based on the Korean regulation (Standards and Criteria of the Food – Regulation of Korean Food and Drug Administration, 2019)

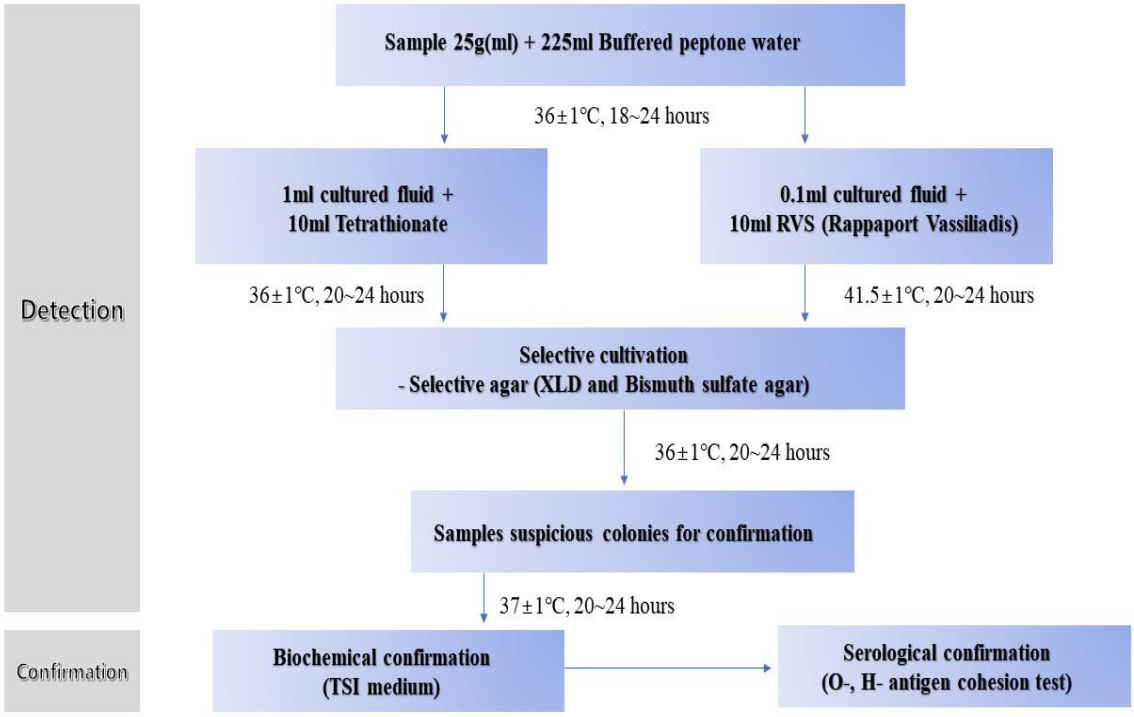


Chart 3.: The chart for the results of *Salmonella* test for the 4 slaughter procedures

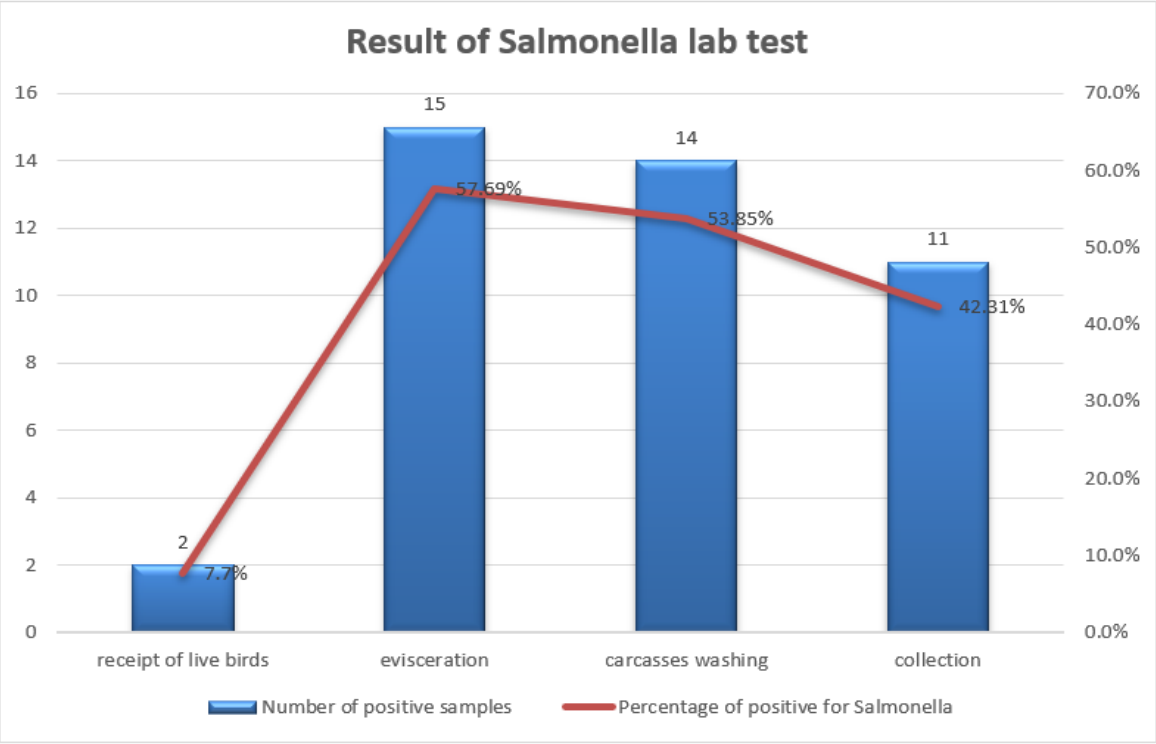
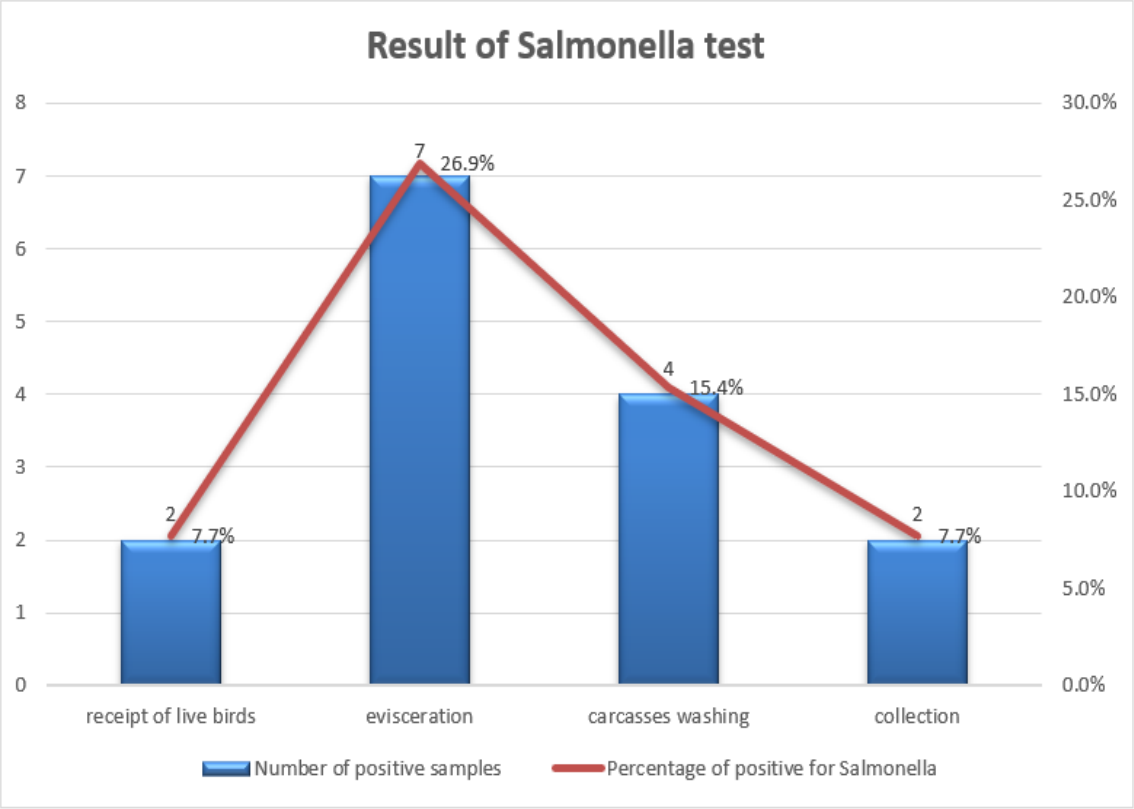


Chart 4.: The chart for the results of re-examination of *Salmonella* test



Picture 1.: Method of sampling of carcass for *Salmonella* laboratory test

Bibliography

Peter Laczay. 2014. Food Hygiene – Food Chain Safety

B.K. Baek. 2003. Veterinary Public Health Science

Scallan, E., R. M.Hoekstra, F. J., Angulo, , R. V. Tauxe, M-A Widdowson, S. L. Roy, J. L.,Jones, P. M. and Griffin. 2011. Foodborne illness acquired in the United States-major pathogens. Emerging Infectious Diseases 17:7-15.

Izat, A. L., M. Coldberg, R. A. Thomas, M. H. Adams, and C. D. Driggers. 1990. Effects of lactic acid in processing waters on the incidence of *Salmonella* on broilers. J. Food Qual. 13: 295-306.

Bailey, J. S., J. E. Thomson, and N. A. Cox. 1987. The microbiology of poultry meat products. Academic Press Inc., New York.

Stern, N. J., M. C. Robach, D. F. Cox, and M. T. Musgrove. 2002. Effect of drinking water chlorination on *Campylobacte*r spp. colonization of broilers. *Avian Dis. 46*:401–404.

Hiett, K. L., N. A. Cox, and N. J. Stern. 2002. Direct polymerase chain reaction detection of *Campylobacte*r spp. in poultry hatchery samples. *Avian Dis. 46*:219–223.

Cox, N. A., J. S. Bailey, L. J. Richardson, R. J. Buhr, D. E. Cosby, J. L. Wilson, K. L. Hiett, G. R. Siragusa, and D. V. Bourassa. 2005. Presence of naturally occurring *Campylobacte*r and *Salmonell*a in the mature and immature ovarian follicles of late-life broiler breeder hens. *Avian Dis. 49*:285–287.

Jones, F. T., R. C. Axtell, D. V. Rives, S. E. Scheideler, J. Tarver, F.R., R. L. Walker, and M. J. Wineland. 1991. A survey of *Salmonella* contamination in modern broiler production.

J. Food Protect. 54:502

Hoover, N. J., P. B. Kenney, J. D. Amick, and W. A. Hypes. 1997. Preharvest sources of *Salmonella* colonization in turkey production. *Poult. Sci. 76*:1232–1238.

Amick-Morris, J. 1998. Insects' contribution to *Salmonella* transmission in turkey flocks. MS Thesis West Virginia University, Morgantown, WV.

Buncic S and Sofos J. 2012. Interventions to control *Salmonella* contamination during poulty, cattle and pig slaughter. Food Res. Int. 45: 641-655.

Byrd JA, Hargis BM, Corrier DE, Brewer RL, Caldwell DJ, Bailey RH, McReynolds JL, Herron KL, and Stanker LH. 2002. Fluorescent Marker for the Detection of Crop and Upper Gastrointestinal Leakage in Poultry Processing Plants. Poult Sci 81:70-74.

J. Lee. 2010. Arrangement of the personnel for the slaughter inspection and direction of improvement for the HACCP in Korean slaughterhouse

Food and Drug Administration in Korea. 2019. Livestock products sanitary control act

Food and Drug Administration in Korea. 2017. Method of laboratory test for the slaughterhouse

Food and Drug Administration in Korea. 2019. Standards and criteria of the food

USDA/FSIS. 2011. Code of Federal Regulations title 9, part 381, Poultry products inspection regulations

USDA/FSIS. 2011. Code of Federal Regulations title 9, part 416, Sanitations

USDA/FSIS. 2011. Code of Federal Regulations title 9, part 417, Hazard Analysis and Critical Control Points (HACCP) systems

USDA/FSIS. 2017. Directive 6420.2 Verification of procedures for controlling fecal material, ingesta, and milk in livestock slaughter operations

Commission Regulation 2073/2005. 2005. Microbiological criteria for foodstuffs

HuVetA - SZIA

ELECTRONIC LICENSE AGREEMENT AND COPYRIGHT DECLARATION*

Name: Jeon sungkook

Contact information (e-mail): humgibson@gmail.com

Title of document (to be uploaded): Controlling of Salmonella species in Korean chicken slaughterhouse

Publication data of document: -

Number of files submitted: 1

By accepting the present agreement the author or copyright owner grants non-exclusive license to HuVetA and SZIA over the above mentioned document (including its abstract) to be converted to copy protected PDF format without changing its content, in order to archive, reproduce, and make accessible under the conditions specified below.

The author agrees that HuVetA and SZIA may store more than one copy (accessible only to HuVetA and SZIA administrators) of the licensed document exclusively for purposes of secure storage and backup, if necessary.

You state that the submission is your original work, and that you have the right to grant the rights contained in this license. You also state that your submission does not, to the best of your knowledge, infringe upon anyone's copyright. If the document has parts which you are not the copyright owner of, you have to indicate that you have obtained unrestricted permission from the copyright owner to grant the rights required by this Agreement, and that any such third-party owned material is clearly identified and acknowledged within the text of the licensed document.

The copyright owner defines the scope of access to the document stored in HuVetA/SZIA as follows (**mark the appropriate box with an X)**:

- [v] I grant unlimited online access,
- [v] I grant access only through the intranet (IP range) of the Szent István University,
- [v] I grant access only on one dedicated computer at the Veterinary Science Library,
- [v] I grant unlimited online access only to the bibliographic data and abstract of the document.

* The present declaration is based on Ordinance no. 5/2011 of the Rector concerning the scholarly database of institution (i.e. Szent Istvan University) affiliated publications.

Please, define the **in-house accessibility of the document** by marking the below box with an **X**:

v

I grant in-house access (namely, reading the hard copy version of the document) at the Library.

If the preparation of the document to be uploaded was supported or sponsored by a firm or an organization, you also declare that you are entitled to sign the present Agreement concerning the document.

The operators of HuVetA/SZIA do not assume any legal liability or responsibility towards the author/copyright holder/organizations in case somebody uses the material legally uploaded to HuVetA/SZIA in a way that is unlawful.

Date: Budapest, ..…10….day …….11…..month……2019..…..year

.

Author/copyright owner
signature

HuVetA Magyar Állatorvos-tudományi Archívum – Hungarian Veterinary Archive *is an online veterinary repository operated by the Veterinary Science Library, Archives and Museum. It is an electronic knowledge base which aims to collect, organize, store documents regarding Hungarian veterinary science and history, and make them searchable and accessible in line with current legal requirements and regulations.*

HuVetA relies on the latest technology in order to provide easy searchability (by search engines, as well) and access to the full text document, whenever possible.
Based on the above, HuVetA aims to:

- *increase awareness of Hungarian veterinary science not only in Hungary, but also internationally;*
- *increase citation numbers of publications authored by Hungarian veterinarians, thus improve the impact factor of Hungarian veterinary journals;*
- *present the knowledge base of the Faculty of Veterinary Science, Szent István University and its partners in a focussed way in order to improve the prestige of the Hungarian veterinary profession, and the competitiveness of the organizations in question;*
- *facilitate professional relations and collaboration;*
- *support open access.*

SZIA Szent István Archive *is the online repository of scholarly publications affiliated with the Szent István University.*

Appendix 4. Supervisor counter-signature form

I hereby confirm that I am familiar with the content of the thesis entitled **Controlling of *Salmonella* species in Korean Chicken Slaughterhouse,** written by **Jeon Sungkook** which I deem suitable for submission and defence.

Budapest, 13th of November 2019.

.....

Dr. Szakmár Katalin

Department of Food Hygiene