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Abstract- The current study was conducted on a deboned veal meat imported to the Kingdom of Saudi Arabia (KSA), and analyzed at Jeddah Food Control Laboratory (JFCL). The samples were collected routinely during 2013. The aim of this trial is to compare between effects of two sampling techniques; sponge swabbing and excision, on the capability of Salmonella recovery. A total of 900 samples of unknown infection status (150 individual samples × 3 analysts × 2 sampling methods) were examined for salmonella. Simultaneously, an artificial inoculation experiments of veal meat (n=120) were conducted. The international standard procedure for the detection of Salmonella (ISO 6579:2002) was the reference. Results show that the swab sampling technique was more representative, it resulted in higher isolation mean percentage of salmonella (97.8%) and (100%) of spiked samples, compared to excision percentage (86.7%) and (95%) of spiked samples. Percentages of swab and excision techniques of natural contaminated samples were positive for salmonella from the different analysts and ranged from 93.3% to 100% and from 70% to 100%, respectively. The average time for sampling by excision was significantly higher (5:10 minutes) than the corresponding time by swabbing technique (1:10 minute). Taking on consideration the daily workload pressure and the time required for sampling, the results illustrate that swabbing is superior to excision. This study suggests that swab sampling could be an alternative method for the detection of salmonella in meat on the basis of better recovery rate, accuracy, sensitivity and repeatability.

Keywords: *veal meat; salmonella; microbiological sampling; excision; swabbing.*

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Comparison of two Sampling Methods for Salmonella Isolation from Imported Veal Meat Samples of Unknown Infection Status

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Abstract- The current study was conducted on a deboned veal meat imported to the Kingdom of Saudi Arabia (KSA), and analyzed at Jeddah Food Control Laboratory (JFCL). The samples were collected routinely during 2013. The aim of this trial is to compare between effects of two sampling techniques; sponge swabbing and excision, on the capability of Salmonella recovery. A total of 900 samples of unknown infection status (150 individual samples × 3 analysts × 2 sampling methods) were examined for salmonella. Simultaneously, an artificial inoculation experiments of veal meat (n=120) were conducted. The international standard procedure for the detection of Salmonella (ISO 6579:2002) was the reference. Results show that the swab sampling technique was more representative, it resulted in higher isolation mean percentage of salmonella (97.8%) and (100%) of spiked samples, compared to excision percentage (86.7%) and (95%) of spiked samples. Percentages of swab and excision techniques of natural contaminated samples were positive for salmonella from the different analysts and ranged from 93.3% to 100% and from 70% to 100%, respectively. The average time for sampling by excision was significantly higher (5:10 minutes) than the corresponding time by swabbing technique (1:10 minute). Taking on consideration the daily workload pressure and the time required for sampling, the results illustrate that swabbing is superior to excision. This study suggests that swab sampling could be an alternative method for the detection of salmonella in meat on the basis of better recovery rate, accuracy, sensitivity and repeatability.

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I. Introduction

The contamination of food products by food borne pathogenic organisms such as Salmonella spp. is an on-going problem worldwide which required governments to improve their food safety systems (Anonymous, 2002; Codex Alimentarius Commission, 2003; Orriss and Whitehead, 2000; Schlundt, 2002). The significance of Salmonella comes from the association with several food categories. Greig and Ravel (2009) reported that salmonellosis outbreaks over the four regions (Australia and New Zealand, Canada, EU, and USA) were the most numerous. The majority of human salmonellosis incidences are due to the consumption of contaminated foods of animal origin. Despite the enormous efforts to eliminate/reduce such risk, Salmonella will still be a risk to human health in the future (Anonymous, 2006). Consequently, the detection of pathogenic microorganisms in foodstuffs is one of the steps to control food safety. In this context, the KSA government has been working to develop the food control laboratories. In view of the fact that around 80% of the food are imported from over than150 countries (USDA, 2013), the strengthening of food control laboratories will eventually lead to the improvement of food safety system.

The first step of food microbiological analysis is obtaining representative sample. False negative or false positive results can occur when sampling executed incorrectly. To some extent, for liquid food products, it is quite easy to get a representative sample. On the other hand, the sampling process would be more difficult in cases of solid food. In view of obtaining representative samples, the sampling methods are very essential. The relative efficacy of excision and swab sampling methods for red meat carcasses have been compared in several studies (Van der Merwe et al., 2013; Pearce and Bolton, 2005; Gill and Jones, 2000; Gill and Jones, 2000; Gill et al., 2001; Dorsa et al., 1997). Nevertheless, the microbiological criteria in the reference standard for salmonella detection are applied to samples taken by excision of 25g (ISO 6579:2002). Yet, the excision method is very time consuming and usually covering limited area. On the other hand, swabbing technique seems to overtake the disadvantages of excision (Bolton, 2003). Additionally, Bolton (2003) reported the reliability of swabbing technique for monitoring salmonella.

The aim of this study is to evaluate the effectiveness of sponge swab sampling method in comparison with excision method for the recovery of Salmonella from deboned veal meat samples.

II. Materials and Methods

a) Sampling plan

A total of 150 duplicate frozen packed Veal meat compensated in 2 Kg bag were sampled (over a year) at JFCL from imported commodities. Each of six identical individual 2 Kg bags from same lot were taken as one sample, and divided to three groups (A,J,Z) each group has two bags (duplicate sample). The study was

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conducted in a controlled sterile environment of a laboratory in KSA, Jeddah, which has the approval of the International Accreditation Service (IAS).

b) Artificial inoculation

Artificial spiking experiments were conducted using 1–10 colony-forming units (n=60) and 20–50 CFU (n=60) concentrations of S. Typhimurium ATCC®14028, KWIK STIK, in 250 g initial weight of veal meat samples. Prior to an inoculation, all samples were initially confirmed as Salmonella-negative by real-time PCR.

c) Excision sampling

Excision samples were taken by every one of the analysts from each of the 150 samples (one set of the duplicate) by cutting 25g thin tissue from the surface of the sample using a sterile single use scalpels. Once excised, sample was placed into a separate sterile stomacher bag, and 225g of Buffer Peptone Water (BPW) poured into the bag. Without delay, the analysis was performed based on the horizontal method for the detection of Salmonella (ISO6579:2002).

d) Sponge swab sampling

The remaining set of the duplicate samples were also sampled using sponge (SPECI-SPONG" BAGS, Nasco Whirl-Pak, the USA) swabbing of the whole sample surface. Sponges were prepared in sterile stomacher bags pre-moistened with 10 ml of maximum recovery diluents (BPW; OXOID). Immediately prior to use, each sponge was grasped through the sterile plastic bag, which was inverted to present the sample sponge. After swabbing, the sponge was withdrawn into the stomacher bag. For pooled samples one sponge was used to sample all four sites, one side of the sponge was used to swab two sites, while the other side was used to swab the remaining two sites. After swabbing, 1:10 of the BPW was added to the bag and the reference analysis protocol was followed.

e) Microbiological analyses

All samples were stomached with 1:10 of BPW for 2 minutes in a Stomacher (Model 400 circulator Seward, England, UK). Then, the bag incubated at 37±1 °C for 18 ±2 h (pre-enrichment). After that comes enrichment in selective liquid media, 0.1 ml of the BPW was transferred into 10 ml of Muller-Kauffmann tetrathionate/novobiocin broth (MKTTn) and incubated at 37±1 °C for 24 ±3 h, and 1ml of the BPW was transferred into 10 ml of Rappaport-Vassiliadis medium with soya broth (RVS) and incubated at 41,5±1 °C for 24 ±3 h. The next step was plating on two selective solid media; xylose lysine deoxycholate agar (XLD) and Brilliant green agar (BGA), and then incubating at 37±1 °C for 24 ±3 h. Afterward, the typical salmonella colony streaked onto the surface of nutrient agar plate, and was incubated at 37±1 °C for 24 ±3 h. All medium used in this study were obtained from OXOID, UK. The last step was the confirmation of the isolation using a biochemical test (biochemical rapid test "api®20E", bioMérieux, France). A detailed description of the detection methods is given in the ISO 6579:2002, Figer.1 briefing the procedure.

III. Results

Percentages of salmonella obtained by each analyst recovered by excision and sponge swabbing are presented in Table 1. In spite of the consistent results by analyst (J), there were significant differences in the number of salmonella recovered according to the sampling technique.

A total of 300 individual naturally contaminated samples for each analyst (150 was sampled by excision, and 150 was sampled by sponge swabbing) were examined for Salmonella. Using the excision method, 30 of 150 samples were the highest positive recovery, whereas 21 of 150 was the lowest positive isolation. On the other hand, the number of the lowest positive recovery samples using sponge swabbing was 28 of 150 samples. The use of the excision method for sampling required more time to be performed by the analyst (Table 2). The average sampling time for one sample by sponge swabbing and excision were 1:10 minute, and 5:10 minutes, respectively. Additionally, there was a great difference in the time (about 10 hours) that was needed to accomplish the 150 samples task; the average needed swabbing time was (2.75 h), and average time of excision was (12.75 h) (Table3). In general, Salmonella were more recovered when sampled by sponge swabbing than excision.

In this study the artificial inoculation of veal meat samples revealed identical results for inoculation level of 20-50 CFU. It was observed that excision and swab sampling methods seemed to be suitable for the recovery of salmonella from veal meat samples with high levels of contamination (Table1). Nevertheless, the swabbing technique yielded a higher salmonella recovery rate (100%), compared to the excision (90%), with spiked level of (1-10 CFU).

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Table1: Percentage of salmonella recoveries (%) from natural and artificial spiked veal meat samples

Analyst	Sampling method	Source of samples		
		Natural	Spiked (1-10 CFU)	Spiked (20-50 CFU)
A	Swab	100	100	100
	Excision	90	90	100
J	Swab	100	100	100
	Excision	100	100	100
Z	Swab	93.3	100	100
	Excision	70	80	100

Table 2 : Means of sampling time by swab and excision methods (minute).

Method	A	J	Z
Swab	1.10	1.20	1
Excision	5	7	3.30

Table 3 : Average time required for sampling 150 samples (hour).

Method	A	J	Z
Swab	2.75	3	2.5
Excision	12.5	17.5	8.25

IV. Discussion

This study compared two approved sampling techniques; the swabbing method, which is reported for sampling carcass surface (ISO, 2003a), and the excision method, the most common technique for bacterial recovery (ISO, 2003b). In the present study salmonella were recovered from a greater number of samples using sponge swab than excision. There are many factors that may account for the relatively higher salmonella recoveries by swabbing than excision. The main factor is that a larger area was sampled by swabbing compared to that sampled by excision, and hence, resulting in a lower variation of bacterial numbers (Taking in consideration that the distribution of microorganisms in food was assumed to be unevenly). In addition, sponge moist could cause loose bacterial attachment on meat tissue that leaded to a higher salmonella recovery by swabbing. Also, sponge is an abrasive material with a capability of recovering bacteria numbers higher to those obtained by excision. Nevertheless, analysts' behavior on excision technique has a great contribution on the sensitivity of salmonella isolation. This suggests that the recovery of salmonella may vary substantially as the analysts' performance differs.

Calculating average sampling time resulted in somewhat different estimates of the efficacy of the sampling methods compared to mean salmonella numbers. Since excision sampling resulted in higher variation compared to swab sampling, the increase of sampling time would raise the recovery rate. As a result, the relative differences between the two sampling methods in estimating the numbers of salmonella decreased for the different analysts, analyst J as an example, where a stabile average number was estimated by both excision and swabbing.

In this study, an artificial inoculation of veal meat samples (n=120) revealed essentially identical results, especially for spiked level 20-50 CFU. This observation suggests that the recovery of salmonella from meat sample will be more difficult when occur in low numbers. Nevertheless, in these spiking experiments, sponge swab seemed to be the most suitable sampling method for the detection of salmonella in meat samples with high and low levels of contamination.

The findings of this study suggests that swabbing using the polyurethane sponge should be considered as a suitable alternative method for the salmonella sampling of veal meat, which requires less time and yields higher recovery. Yet, there is a need to expand the current study on the basis of ISO 16140:2003, the protocol for the validation of alternative methods (ISO, 2003c).

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