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Comparison of excision, swabbing and meat fluid sampling techniques on the prevalence and detection of *Salmonella* from beef in Namibia

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ABSTRACT

Salmonella bacteria cause major challenges in food production and public health because of their ability to cause foodborne disease known as salmonellosis. The microbiological safety of food can be ensured through routine surveillance programs with a view to detect the presence of pathogenic bacteria such as *Salmonella*. However, the rate of *Salmonella* detection in food may be affected by the sampling technique used leaving the product unsafe for human consumption. In order to assess the effectiveness of sampling techniques for *Salmonella* detection, a total of 9508 of beef samples were collected from the local slaughter houses over a period of two years starting from January 2008 to December 2009. Samples used were routine samples collected using three different sampling techniques; excision, swabbing and meat fluid. Samples were pre-enriched in Buffered Peptone Water followed by enrichment in the Rappaport Vissiliadis and Selenite Cystine broths. The isolation of *Salmonella* was done on Xylose Lysine Desoxycholate and Brilliant Green agars followed by biochemical confirmation and serotyping according to Kauffman-White scheme. The statistical analysis by Chi square showed that there was a significant difference ($p < 0.05$) on the prevalence of *Salmonella* between the swabbing sampling techniques (2.67 %) with excision (0.50 %) and meat fluid (0.43 %) sampling technique. However, there was no significant difference ($p > 0.05$) between the excision and meat fluid sampling techniques. The findings suggest that the three sampling techniques could produce different results when used for sampling beef for *Salmonella* detection. The swabbing sampling technique showed a higher detection rate of *Salmonella* and could be the best choice as compared to other techniques. The significant detection rate of the swabbing sampling technique as compared to other techniques could probably be due to the large surface area employed in this technique as a result of uneven distribution of microorganisms.

1. Introduction

The prevalence of *Salmonella* presents major challenges in the food production and public health sectors in their efforts to supply safe foods. While the consumers' food safety awareness is on the increase, *Salmonella* pose a risk to consumer with a foodborne disease known as salmonellosis. The *Salmonella* bacteria are generally transmitted to humans through consumption of mainly contaminated food of animal origin. The contamination is usually caused by the intestinal materials which often contain *Salmonella* bacteria that pollute the surface of the carcasses during the slaughtering process (Oosterom, 1991).

A periodic surveillance of the level of *Salmonella* contamination is regarded necessary to control the spread of the pathogens and infection to humans (Molla *et al*., 2003). However, the method of sampling may negatively the recovery, hence, the detection of the target organisms in the product.

Different sampling techniques i.e. excision, swabbing and collection of meat fluid from packaged products have been used for recovery of *Salmonella* in meat and meat products. A previous study on the comparison between excision and the swabbing technique has found a poor relationship between the two sampling techniques (Reid *et al.,* 2002) where excision technique has been found to significantly recover more bacteria than the swabbing technique (Pearce and Bolton, 2005; Salmela *et al*., 2013). However, the industry personnel are said to prefer a less laborious swabbing method (Pearce and Bolton, 2005) although it only recovers 20 % or less bacteria as compared to destructive methods (Miraglia *et al*., 2005).

The present study was undertaken to evaluate the effect of the three sampling techniques (excision, swabbing and meat fluid) on the recovery of *Salmonella* from the beef carcasses.

2. Materials and Methods

2.1. Sampling plan

Convenience sampling method was used whereby all samples received for analysis were regarded as samples for this study. The minimum sample size for this study was calculated with the confidence level of 95 % and a confidence interval of 5 %. The minimum sample size of 138 was obtained with an assumption of the prevalence rate of *Salmonella* was below 10 %.

A total of 9508 of beef samples were collected from the local abattoirs over a period of two years starting from January 2008 to December 2009. Samples were collected using three different sampling techniques; excision, swabbing and meat fluid. As a routine microbial analysis at the Central Veterinary Laboratory in Windhoek, the samples were analyzed for the presence of *Salmonella*. Of the samples analysed, 3424 samples were collected using excision method, 1688 samples were collected using a swabbing technique where 4396 samples were collected using meat fluid technique. Samples were collected by the State veterinary officials at three different slaughter houses using sterile dilution bags and media bottles as part of their routine sampling program to meet safety and export requirements.

2.2. Sample collection techniques

For excision sampling method, approximately 20 g (equivalent to 20 cm^2) meat sample was taken from four different sites of the carcass. Approximately 5 g (equivalent to 5 cm^2) of sample was taken from each sampling site and then pulled together into a sterile stomacher bag. Two carcass swabs (wet and dry) were used to sample the surface area of 100 cm^2 of the carcass per site of which four sites were sampled. The swabs were pooled together into 500 ml media bottle containing 200 ml buffered peptone water (BPW) to a maximum of 8 swabs. The four sites sampled for excision and the swabbing sampling techniques were rump, flank, brisket and neck. For meat fluid, approximately 100 ml of meat fluid from 10 vacuum packed meat was sampled. The sampling was done by draining the meat fluid from the vacuum packaged meat using a sterile needle with a syringe and then pooled into sterile 250 ml media bottle. Samples were transported to the laboratory for analysis on the same day. During transportation samples were kept at refrigeration temperatures (2 to 8 °C) using a cooler box with ice bricks. When received at the laboratory, samples were stored in the refrigerator (1 to 5 °C) before the analysis. The isolation of *Salmonella* was done within 24 hours from the time when samples were received.

2.3. Microbiological analysis

Detection of *Salmonella* was performed according to the standard culture method ISO-6579:2002. The pre-enrichment stage for all sampling techniques was done using buffered peptone water. For excision sampling technique, 25 g sample was pre-enriched into 225 ml of buffered peptone water (Merck, Darmstadt) and incubated at 37 °C for 24 h. For the swabbing technique, 300 ml of BPW (Merck, Darmstadt, Germany) at ambient temperature was added into 200 ml of sample using a 500 ml media bottles. The samples were then incubated at 37 °C ± 1 °C for 18 to 24 hours. For meat fluid, approximately 50 ml of the meat fluid sample was transferred into a 500 ml media bottle. Then 450 ml of BPW (Merck, Darmstadt, Germany) was added to the sample and incubation at 37 °C ± 1 °C for 18 to 24 hours.

Subsequently, 0.1 ml of the pre-enrichment culture was added to 10 ml of Rappaport-Vassiliadis broth (Merck, Darmstadt) and 10 ml to 100 ml of selenite cystine broth (Merck, Darmstadt) and incubated for 24 h at 41.5 and 37 °C, respectively. Selenite cystine broth was used instead of Mueller-Kauffmann Tetrathionate Novobiocin (MKTTn) broth. The culture was then streaked onto two selective agar: Xylose Lysine Desoxycholate (XLD) (Merck,

Wadeville) and Brilliant Green Agar (BGA) (Scharlau Chemie SA, Barcelona) and incubated at 37 °C ±1 for 24 h. The presumptive *Salmonella* colonies were then confirmed serologically and biochemically. For serological confirmation Omnivalent anti-sera (Siemens, Marburg) were used. For biochemical confirmation, the following tests were performed: triple sugar iron reactions, urea production, the Voges-Proskauer reaction, the indole reaction, the lysine decarboxylase reaction and the detection of β-galactosidase. Serological identification was done with commercially available antisera (State Serum Institute, Copenhagen) for detection of somatic and flagellar antigens in accordance with the Kauffman-White scheme (Popoff, 2001).

2.4. Data analysis

The prevalence of *Salmonella* strains was evaluated in terms of percentage occurances, in which the denominator was the total number of *Salmonella* isolates within a group. The differences between observations were analyzed using a Chi-square method with the confidence interval of 95 % at the expected prevalence rate of < 10 %. Similar method wad used by Akoachere *et al.* (2009) to compare the prevalence in the different anatomical sites and biotypes. The differences were considered significant at $p < 0.05$. The Statistical Package for the Social Sciences [SPSS], version 17.0 was used for the data analysis.

3. Results

From a total of 9508 samples of beef samples examined for the presence of *Salmonella*, 0.85 % (*n* = 81) were found to be positive for *Salmonella*. The prevalence of *Salmonella* per individual sampling technique and total prevalence of *Salmonella* in beef is presented in **Table 1** and **Figure 1** respectively. In general, there was a significant difference ($p < 0.05$) on the prevalence of *Salmonella* between all three sampling techniques used. The significant difference ($p < 0.05$) on the prevalence of *Salmonella* was observed between the swabbing sampling techniques (2.67 %) with excision (0.50 %) and meat fluid (0.43 %) sampling technique. However, there was no significant difference ($p > 0.05$) between the excision and meat fluid sampling techniques. Similar trend was observed when these samples were analyzed together (**Figure 1**).

Table 1. The prevalence of *Salmonella* per sampling technique and prevalence of *Salmonella* in beef

Sampling technique	No. *Salmonella* isolates	% prevalence rate per technique
Excision	17	0.50^{a} (N = 3424)
Meat fluid	19	0.43^{a} (N = 4396)
Swabbing	45	2.67^{b} (N = 1688)
Total	**81**	-

Percentage prevalence rate value with different letters differ significantly ($P < 0.05$) from each other.

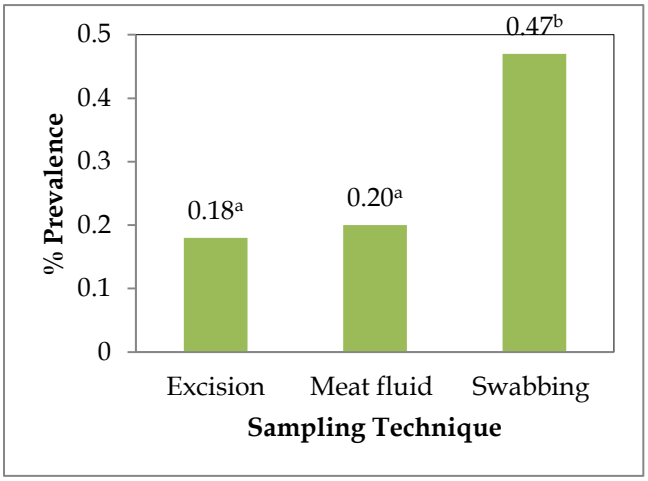

Figure 1. Percentage total Prevalence rate of *Salmonella* in beef (N = 9508)

4. Discussions

The present study was part of the previous study done by Shilangale *et al*. 2015. Unlike the previous study, the present study provides new information on the findings as it has focused on how the selected sampling procedures could have influenced on the rate of *Salmonella* detection from the prevalence rate perspective. This is because the rate of detection may directly relate to the recovery of microorganisms from the sample.

Different studies have investigated the effectiveness of different sampling techniques on the quantitative recovery bacteria from the carcass (Miller, 1999; Gill and Jones, 2000; Reid *et al*., 2002; Miraglia *et al*., 2005; Palumbo *et al*., 1999; Lindblad, 2007; Salmela *et al*., 2013). Most of these studies have done comparisons on the excision and the

swabbing sampling techniques from the hygiene point of view. However, the present study has focused on the safety of the product by trying to check the effectiveness of different sampling techniques on the detection of *Salmonella*. *Salmonella* were detected in order to determine their presence on the samples but not quantified because most of the pathogens do not appear in large quantities.

When comparing between the sampling techniques used in the present study it was established that there was a significant difference ($p < 0.05$) observed on the prevalence of *Salmonella* when the swabbing method was used to recover *Salmonella* for detection as compared to excision and meat fluid sampling techniques. This means that the rate of *Salmonella* detection was higher when the carcass swabbing method was used as compared to other two sampling methods. Based on the significance differences between the sampling techniques, the findings of the present study suggest that the carcass swabbing sampling method may be the best suitable method as compared to other two methods because of the higher prevalence rate of *Salmonella*. The findings of this study also suggest that the chances of detecting *Salmonella* in the beef carcasses may be higher when using the swabbing sampling technique than when the other sampling techniques are applied.

However, the findings of this study on the differences of the sampling methods between swabbing and excision methods were different from some studies anywhere. According to Gill and Jones (2000), the statistics indicates that the numbers of bacteria recovered on pig or beef carcasses by swabbing and excision methods are similar. A study by Lindblad (2007) also found similarities on the bacterial recovery between the excision and swabbing method. However, different study by Palumbo *et al.* (1999) found that swabbing method gave higher microbial count than excision when three sites were sampled as opposed to lower numbers obtained than excision when one site was sampled. These findings were also in agreement with the findings by Pearce and Bolton (2005) where the *bacteria* were recovered from a greater number of samples using the swabs than excision. However, unlike other studies, *Salmonella* were not quantified in the present study.

Nevertheless, these findings may probably explain why the rate of *Salmonella* isolation in the current study was observed to be significantly higher with a swabbing method as compared to other two methods. The reason for the higher prevalence rate with the swabbing method would probably be due to the lager sampling area used with the swabbing method as compared to excision. This idea is also supported with the findings of other scholars (Gill and Jones, 2000). The sampling surface area for the swabbing method is a minimum of 100 cm^2 per site (approximately 400 cm^2) as opposed to 20 g (approximately 5 cm^2 per site) when excision method was used. According to Gill and Jones (2000), the analysis of larger sampling areas of carcasses has shown to relatively increase the rate of recovery of bacteria. This is because microorganisms are unevenly distributed and covering a larger sampling area may be an advantage on the recovery of the microorganisms.

The other reason for the efficiency of the swabbing technique could be due to the application of two swabs; wet and dry per site when using this method. Pearce and Bolton (2005) findings suggest that apart from the role of the surface area the abrasiveness of the material used may influence the bacterial recovery. In the present study cotton gauze swabs which are abrasive materials were used in the swabbing method. The use of wet swabbing may also have helped on the recovery of microorganisms from the dry surface of the carcass which may not be easily recovered when using dry swabbing. However, there is a limitation in the comparison of the present study with others because unlike other studies the present study did not quantify the bacteria recovered.

The low rate of *Salmonella* isolation in meat fluid samples could be due to the result of the packaging method used. Meat fluid samples were obtained from the vacuum packaged meat. The vacuum packaging conditions may have probably inhibited or reduced the microbial load due to reduced oxygen levels in cold storage conditions. This is because vacuum packaging is a preservation technique and may significantly extends the shelf-life of the product stored at 4 °C for up to 8 days (Buick and Damoglou, 1987).

5. Conclusion

The findings of the present study suggests that swabbing, excision and meat fluid sampling techniques may not produce similar results when used for sampling beef for *Salmonella* detection. The swabbing sampling technique has shown to have a higher rate of *Salmonella* detection as compared to

other techniques probably due to the large surface area employed in this technique. The uneven distribution of microorganisms may also favor the sampling techniques which cover more surface area. The lower rate of *Salmonella* detection using meat fluid method may suggest that some bacteria could have died because of the packaging technique employed in the product.

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