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Prevalence and Characterization of *Salmonella* Isolated from Beef in Namibia

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Authors' contributions

This work was carried out in collaboration between all authors. Author RPS designed the study, performed the statistical analysis and wrote the first draft of the manuscript. Authors GPK and PMC managed the review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The purpose of this research was to determine the prevalence of *Salmonella* in raw beef produced from selected commercial abbatoirs in Namibia.
Methodology: A total of 9508 of beef samples from three different types of samples; meat cuts, carcass swabs and meat fluid were collected from the three local abattoirs over a period of two years starting from January 2008 to December 2009. Pre-enrichment for isolation of *Salmonella* was done in Buffered peptone water followed by enrichment in the Rappaport-Vassiliadis and selenite cystine broth. The isolation of *Salmonella* was done on Xylose Lysine Desoxycholate and Brilliant Green agar followed by biochemical confirmation and serotyping according to Kauffman-White scheme.
Results: The overall prevalence of *Salmonella* was 0.85% for beef samples derived from meat cuts, meat fluid and carcass swabs. The prevalence of *Salmonella* in carcass swabs (2.67%) was significantly different (P = 0.05) from that of meat cuts (0.50%) and meat fluid (0.43%). No significant difference (P = 0.05) on the prevalence of *Salmonella* existed between the meat cuts and meat fluid. A total of 34 different types of *Salmonella* serovars were identified with *S.* Chester being the most frequently isolated serovars (n = 12), followed by *S.* Reading and *S.* Bredeney (n = 6) and *S.* Typhimurium (n = 5).
Conclusions: The prevalence of *Salmonella* in raw beef found in this survey was lower than those observed in Sub Sahara Africa with *S.* Chester being the most prevalent serovar.

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Keywords: Namibia; Salmonella; beef meat cuts; beef carcass swab; beef meat fluid; serovar.

1. INTRODUCTION

Salmonella bacteria cause foodborne illness in humans commonly known as salmonellosis. The outbreaks of salmonellosis are commonly associated with consumption of food of animal origin which is contaminated with *Salmonella* [1,2]. The link between *Salmonella* infections and food consumption is supported by the findings of other researchers elsewhere [3,4]. According to a study in Australia, a wide range of *Salmonella* serovars, all of which have been isolated from humans, were found in both cattle and sheep [5]. In the US and Canada, *S.* Heidelberg is among the most frequently isolated serovar both in clinical cases of salmonellosis and from retail meats and food animals [6]. Another study on food animals, meat products and slaughterhouses personnel in Ethiopia suggested the likely link of human *Salmonella* infections and food of animal origin [3]. According to the Center for Diseases Control and Prevention (CDC) beef contributed up to 11% of the foodborne outbreaks for the year 2009 – 2010 in the United States [2].

In order to reduce the threat of *Salmonella* to food safety, the sources of contamination need to be identified in the food chain. This is because scientific investigation on the sources and distribution of *Salmonella* contamination in food are a basic step to mitigate the spread of *Salmonella* in food that contributes to food safety [7].

Hazard Analysis Critical Control Point (HACCP) system, a food safety management tool is currently being used in Namibia to manage the risk of pathogens in the abattoirs that produce beef for export. These abattoirs test for *Salmonella* on beef carcasses as part of the quality control criteria in order to meet the stringent export requirements for the European Union, Norway and South Africa. Norway, one of the major markets of Namibia's beef has a zero tolerance policy on *Salmonella* based on the Norwegian National Food Law [8]. Although Namibia has managed to export beef to Norway, there is limited information on the prevalence of *Salmonella* in Namibia. In most of the developing countries including Namibia the lack of epidemiological studies on foodborne diseases are probably due to poor coordination and lack of resources [7].

The purpose of this study was to determine the prevalence and to characterize *Salmonella* in raw beef (meat cuts, carcass swabs and meat fluid) produced from the beef export abbatoirs in Namibia.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 9508 of beef samples were collected from the three beef export abattoirs over a period of two years starting from January 2008 to December 2009. Three different types of samples were collected; meat cuts, carcass swabs and meat fluid. Of these, 3424 of meat cuts, 1688 of carcass swabs and 4396 of meat fluid samples were collected. Carcass swabs were collected before chilling and meat cuts and meat fluid were collected from the packaged finished product after deboning. Samples were collected by the State veterinary officials stationed at the three abattoirs for routine microbiological analysis using sterile dilution bags and media bottles.

2.2 Sample Collection Techniques

Meat cuts were collected from frozen packaged beef prepared for export to South Africa and the European markets. Approximately 5 g of meat was sampled from 10 randomly selected packages of frozen meat using the excision method. These samples were then pooled together into a sterile stomacher bag to make at total of approximately 50 g samples per bag. For carcass swabs sampling, two gauze swabs (wet and dry) were used to sample the surface area of 100 cm^2 per site of a carcass of which four sites were sampled. The wet swab was used first then the dry swab to sample the same sampling site. The 8 swabs per carcass were pooled together into a 500 ml media bottle containing 200 ml buffered peptone water (BPW). The four sites sampled were rump, flank, brisket and neck. Meat fluid samples were taken from vacuum packaged deboned beef stored for 24 h at 0 to 2ºC. Approximately 10 ml of meat fluid from 10 randomly selected vacuum packed meats was sampled and pooled (approximately 100 ml in total). 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 bottles.

Samples were transported to the laboratory for analysis on the same day. During transportation samples were kept at refrigeration temperatures (2 to 8ºC) using a cooler box with ice bricks. When received at the laboratory, samples were stored in the refrigerator (1 to 5ºC) before the analysis. The isolation of *Salmonella* was done within 24 h from the time when samples were received.

2.3 Isolation and Identification

As a routine microbial analysis, samples corrected were analyzed for the presence of *Salmonella* at the Central Veterinary Laboratory of the Ministry of Agriculture, Water and Forestry in Windhoek, Namibia. The pre-enrichment stage for all type of samples was done using BPW. For meat cuts, 25 g sample was pre-enriched into 225 ml of buffered peptone water (Merck, Darmstadt) and incubated at 37ºC for 24 h. For the carcass swabs, 300 ml of BPW (Merck, Darmstadt, Germany) at ambient temperature was added into a sample containing 200 ml of BPW in a 500 ml media bottle. The samples were then incubated at 37ºC 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 was maintained at 37ºC for 18 to 24 hours.

Subsequently, 0.1 ml of the pre-enrichment culture was added to 10 ml of Rappaport-Vassiliadis (RV) broth (Merck, Darmstadt) and 10 ml to 100 ml of selenite cystine (SC) broth (Merck, Darmstadt) and incubated for 24 h at 41.5ºC and 37ºC, respectively. 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 37ºC for 24 h. The presumptive *Salmonella* colonies were then confirmed serologically and biochemically. On XLD agar, presumptive *Salmonella* colonies appear black or pink with or without black center while on BGA *Salmonella* change the medium from pink to red. For serological confirmation of *Salmonella*, 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.

The confirmed strains of *Salmonella* were then further identified biochemically using miniaturized commercial systems (Vitek-BioMérieux, Marcy l'Etoile). Serological identification with commercially available antisera (State Serum Institute, Copenhagen) for detection of somatic and flagellar antigens was performed and the isolates were identified and named in accordance with the Kauffman-White scheme [9]. Further biochemical and serological identification were done at the Istituto 'G. Caporale', Teramo, Italy.

2.4 Data Analysis

The prevalence of *Salmonella* strains was evaluated in terms of percentage of occurances on the total number of *Salmonella* isolates within a sample type. The differences between observations were analyzed using a Fisher Exact test with the confidence interval of 95%. The differences were considered significant at $P = 0.05$.

3. RESULTS AND DISCUSSION

The prevalence of *Salmonella* serovars in meat cuts, meat fluid and carcass swabs samples are reported in Tables 1, 2 and 3 respectively. The overall prevalence of *Salmonella* was 0.85% for beef samples derived from meat cuts, meat fluid and carcass swabs.

The prevalence of *Salmonella* in meat cuts ($n = 3424$) was 0.50%, whereas in meat fluid samples ($n = 4396$) the prevalence was 0.43%. The prevalence of *Salmonella* in carcass swabs ($n = 1688$) was 2.67%. The present study found that the prevalence of *Salmonella* in carcass swabs was significantly different ($P = 0.05$) from meat cut and meat fluid. On the other hand, there was no significant difference ($P = 0.05$) on the prevalence of *Salmonella* between meat cuts and meat fluid. This was based on the Fisher's exact test.

The low prevalence of *Salmonella* found in the present study was not comparable to the once reported elsewhere in the Region [3,10,11]. A study done in Ethiopia on the prevalence of *Salmonella* from beef carcasses in abattoirs found the prevalence of *Salmonella* to be 13.3% [10]. Another study in Ethiopia found the prevalence of *Salmonella* contamination in minced beef to be 14.4% [3]. In Botswana, a study done in raw beef sausages found the prevalence of *Salmonella* to be as high as

25.3% [11]. However, the two latter studies were done on naturally contaminated raw beef from retail outlets where the level of bacterial contamination is likely to be higher than the levels at the abattoir due to post handling or processing conditions.

The lower prevalence of *Salmonella* in beef found in the present study could be due to the effective implementation and maintenance of high hygiene standards together with the HACCP system in the abattoirs as part of the EU export requirements. Namibia together with Botswana and Swaziland are the only African countries that have access to the EU beef market where implementation of the HACCP system is mandatory. The EU requires the third countries exporting to the EU to implement the same requirements as the EU Member States. Nevertheless, the differences in the isolation procedures used in the present study could have resulted to the low isolation rate of *Salmonella* because of the use of SC and RV combination as selective media. According to Hammack et al. [12] findings, the SC broth with a recovery rate of 96% and RV medium with a recovery rate of 90% have less proportion of *Salmonella* recovery as compared to tetrathionate broth (TT) of 97%. This study recommended a combination of RV and TT for recovery of *Salmonell*a from food with low microbial load. The recommendation was based on the fact that the selectivity of RV medium and TT broth increase the recovery of *Salmonella* by inhibiting the growth of the competitors in favor of *Salmonella* [12]. Unlike the present study, the study on the natural contaminated carcasses in Ethiopia used a combination of RV and TT as selective media [10]. This could probably have resulted to the higher prevalence of *Salmonella* in the carcass as compared to the present study.

Table 1. Distribution of *Salmonella* serovars isolated in meat cuts and the proportion of positive isolates

***Salmonella* serotype**	**Number of positive samples**	**(%) (*N* = 17)**
S. Schwarzengrund	1	5.88
S. Bredeney	1	5.88
S. Saintpaul	1	5.88
S. Parkroyal	1	5.88
S. Chester	5	29.41
S. Winston	1	5.88
S. Bahrenfeld	1	5.88
Salmonella group K*	1	5.88
S. Dublin	1	5.88
S. Lamberhurst	1	5.88
S. Uganda	1	5.88
S. Anatum	1	5.88
S. Sao	1	5.88
Total	17	100.00

** non-typeable isolates of Salmonella*

Table 2. Distribution of *Salmonella* serovars isolated in meat fluid and the proportion of positive isolates

***Salmonella* serotype**	**Number of positive samples**	**(%) (*N* = 19)**
S. Braenderup	2	10.53
S. Ball	1	5.26
S. Chester	1	5.26
S. Fischerkietz	1	5.26
S. Cannstatt	2	10.53
S. Petahtikve	1	5.26
S. Bredeney	3	15.79
S. Anatum	3	15.79
Salmonella enterica subsp. *salamae**	1	5.26
S. Winston	1	5.26
Salmonella group K*	1	5.26
S. Vaertan	1	5.26
S. Cerro	1	5.26
Total	19	100.00

** non-typeable isolates of Salmonella*

Table 3. Distribution of *Salmonella* serovars isolated in carcass swabs and the proportion of positive isolates

***Salmonella* serotype**	**Number of positive samples**	**(%) (*N* = 45)**
S. Typhimurium	5	11.11
S. Saint-paul	2	4.44
S. Chester	6	13.33
S. Minnesota	1	2.22
S. Braenderup	3	6.67
S. Parkroyal	1	2.22
S. Djermaia	1	2.22
S. Petahtikve	1	2.22
S. Fischerkietz	1	2.22
S. Kaapstad	1	2.22
Salmonella group I*	1	2.22
S. Cannstatt	1	2.22
S. Reading	6	13.33
S. Bredeney	2	4.44
S. Chichiri	1	2.22
S. Newport	1	2.22
S. Kintambo	1	2.22
S. Banana	1	2.22
Salmonella enterica subsp. salamae *	5	11.11
S. Anatum	1	2.22
Salmonella Group D2*	1	2.22
Salmonella Group C1*	1	2.22
S. Schwarzengrund	1	2.22
Total	45	100.00

**non-typeable isolates of Salmonella*

The lower prevalence of *Salmonella* in meat cuts and meat fluid as compared to carcass swabs in the present study was probably a result of different handling, sampling techniques used and the processing stage where samples were taken. This is because before packaging the meat had to be trimmed during deboning in order to remove the fat and dry part of the meat from the surface. Trimming the outer layer could have had reduced the population of bacterial contamination on the surface of the final product. Bacterial contamination and growth occurs on the surface of the meat while the inner part of the carcass is usually considered sterile. Furthermore, the lower prevalence of *Salmonella* in vacuum packaged beef could be the result of the preservation conditions which the beef was subjected to. Vacuum packaging has been reported to reduce the population of *Salmonella* stored at 4ºC by 1 log cycle in one day [13]. A different study has shown that vacuum packaging could significantly reduce *S.* Typhimurium counts in beef stored at 4ºC [14].

In general, a total of 34 different types of *Salmonella* serovars were identified with *S.* Chester being the most frequently isolated with 12 isolates, followed by *S.* Reading and *S.* Bredeney with 6 serovars each and *S.* Typhimurium with 5 isolates. Six strains were found to be positive for *S. enterica* subsp. Salamae, two for *Salmonella* group K, one *Salmonella* Group I, one each for *Salmonella* Group C1 and D2. These groups could not be identified further because they did not express the phase 2 'H' antigens. Nevertheless, there is little information in the Sub Sahara Africa on the prevalence of *Salmonella* in beef from the slaughter houses which can be used to make a comparison with this study. Most of the studies in Sub Sahara Africa are pertaining to clinical cases [5,15,16] and retail markets [3,11]. Unlike Namibia, a study done in Botswana found *Salmonella enterica* subsp. salamae II, *S.* Thompson and *S.* Anatum to the most frequently isolated *Salmonella* strains from different raw meat sausages made from beef with the prevalence of 13.8, 6.2 and 6.2% respectively [11].

Of the four most frequently isolated serovars found in this study, *S.* Typhimurium is the only serovar that is often being reported to be among the most frequently isolated elsewhere as compared to other serovars. A study in Sweden done between 1993 and 1997 on the isolation of *Salmonella* from animals and animal feed reported *S.* Typhimurium to be the second most frequently isolated serotype in cattle [17]. However, 78 of the 115 isolates originated from

infected herds where the remaining isolates were collected at autopsies, sanitary slaughter and surveillance at slaughterhouses. In a different study in Ireland on the prevalence of *Salmonella* in cattle carcass *S.* Typhimurium was the third most frequently isolated serotype after *S.* Dublin and *S.* Agona [18]. In the US, *S.* Typhimurium was the second most common isolate after *S.* Montevideo [19]. In a different study in meat and meat products, *S.* Typhimurium was among the top five most frequently isolated in Algeria [20].

Moreover, the differences on the type of strains found in the present study and those of other studies can probably be due to the differences in the geographical locations where these studies were carried out. This idea may be supported by the findings of other researchers who have suggested that the distribution of different types of *Salmonella* vary based on the geographical location [18,21]. According to Hendriksen et al. [21], the majority of salmonellosis cases in humans are caused by a limited number of *Salmonella* serovars which may vary over time from one country to another. According to McEvoy et al. [18], the higher prevalence in the incidence of *Salmonella* may be among other factors due to geographical variation. These suggestions imply that different types of *Salmonella* serovars may be expected to be more prevalent in this region and Namibia in particular as compared to other regions. Namibia's weather pattern is different from many countries and is among of the driest countries in the world which means it may have different growth conditions when compared to other countries.

Nevertheless, *S.* Typhimurium was the second most frequently isolated serotype in carcass swabs and was among the top four when the three beef products were analyzed together. This observation suggests that *S.* Typhimurium is an important bacterium of public concern in Namibia just as it is in other parts of the world. In the US, 43% of all *Salmonella* isolates from human sources are only from three types of strains; *S.* Enteritidis, *S.* Newport and *S.* Typhimurium, and of which the latter contributes to 11% of all *Salmonella* outbreaks [2]. However, there is no information that can be used to estimate the potential public health threat of *S.* Typhimurium in Namibia.

The most notable *Salmonella* strain in this study was *S.* Chester. Although this strain is infrequently reported in foods worldwide, it is the most common stain isolated in this study. In a different study by Shilangale et al. [22], *S.* Chester was the most prevalent serovar of *Salmonella* in Namibia in animal feed made from bovine and ovine byproducts. In this study the prevalence of *S.* Chester was found to be as much as 19.7% [22].

Unlike Namibia, in Canada *S.* Chester is considered to be a rare serotype of *Salmonella* with regard to human infections [23]. Despite being considered as a rare serovar in human infections, *S.* Chester can still be one of the *Salmonella* serovars of public concern worldwide due to some few outbreaks which have been reported [24,25]. This is because of its recent links to several salmonellosis outbreaks in North America and Australia. In 2010, *S.* Chester caused a multistate outbreak of human infections in 18 States in the US [24]. In the same year, another outbreak of *S.* Chester in Canada which involved 26 people was due to the consumption of luncheon meat, sausage and hard cheese [25]. Another notable case of *S.* Chester happened in Australia where 36 people of Aboriginal community were infected due to consumption of turtle meat in 1998 [26]. However, there are no clinical records to determine if *S.* Chester is a major cause of foodborne salmonellosis in Namibia apart of being the most frequently isolated strain in beef. Unlike in the developed countries, in Namibia sources of attribution of most of the *Salmonella* infections are unknown because most of these cases are treated based on the clinical diagnosis.

4. CONCLUSION

The prevalence of *Salmonella* in raw beef found in this survey was lower than those observed in Africa with *S.* Chester being the most prevalent serovar. Although the prevalence of *Salmonella* contamination is low in beef in Namibia the prevalence of *Salmonella* in beef suggest that there is still a risk of *Salmonella* infection through beef consumption as well as possible export of *Salmonella* strains to other countries through trade. Because of scant *Salmonella* studies in the Region, the information on the prevalence in the present study may be useful in future epidemiological studies of *Salmonella* in Namibia and in the Region. The information of the present study may further be useful in the beef production in their efforts to improve the hygiene and safety standards in the food chain as part of the requirements to meet the high demanding export requirements.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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