Detection of *Salmonella* in Cattle and their Carcasses at Karary Slaughterhouse, Khartoum State, Sudan

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Abstract

Salmonellosis is an important public health problem worldwide particularly in the developing countries. The current research work was conducted at Karary slaughterhouse, Khartoum State, Sudan in order to determine the prevalence rate and associated risk factors of *Salmonella* infection in cattle and their carcasses as well as to estimate the level of bacterial contamination of beef. For that aim bacteriological examinations including isolation of *Salmonella* and Total Viable Counts (TVCs) were used. A total of 120 swab samples were taken from beef immediately after slaughtering and last washing. On the other hand, the same number was collected for faecal samples from the rectum of cattle in sterile plastic containers. All samples were stored in ice box and transferred to laboratory for bacteriological investigation in the same day. Statistical Package for the Social Sciences (SPSS) version 20.0 was used for data analysis and both descriptive and analytical statistics were used. The results revealed that the overall percentage of *Salmonella* in different sites of beef *was* 13.3% (16/120). The highest percentage of *Salmonella* was detected in flank 7.5% (9/120) and rump 3.3% (4/120), while the lowest percentage was detected in shoulder 1.7% (2/120) and neck 0.8% (1/120). A statistical significant difference (χ^2 = 10.962, P-value = 0.012) was recorded. According to Sudanese Standards for meat and meat product, relatively high contamination level was observed in different sites of beef. For instance, mean log TVCs of 6.22 CFU/ cm^2, 6.23 CFU/cm^2, 6.06 CFU/cm^2 and 6.24 CFU/cm^2 with 95% confidence interval of (5.88 - 6.56 CFU/cm^2), (5.90 - 6.77 CFU/cm^2), (5.61 - 6.51 CFU/cm^2) and (5.59 - 6.90 CFU/cm^2) were recorded for neck, shoulder, rump and flank, respectively. There was no statistical significant was reported (F-value = 0.209, P-value = 0.890). While, strong positive association was observed between the mean log TVCs and presence of *Salmonella* in beef (t-Test 46.973 = P-value = 0.000 with 95% confidence interval of 5.80 - 6.32). On the other hand, the overall prevalence of *Salmonella* infection in cattle using faecal samples was 28.3% (34/120). A high prevalence rate was obtained for cattle from Darfur states 15.0% (18/120), Kordofan states 9.2% (11/120) and less in White Nile state 4.1% (5/120), statistically the difference was significant (χ^2 = 8.220, P-value = 0.042). A positive association (χ^2 = 8.956, P-value = 0.030) was observed for breed with respect to *Salmonella* infection in cattle using faecal samples. The highest prevalence rate was recorded for Baggara 12.5% (15/120) and Nylawi 11.7% (14/120). In contrast, low prevalence rate was reported for Kenana 4.20% (5/120). The study concluded that the presence of *Salmonella* infection in cattle in anti-mortem and post-mortem at Karary slaughterhouse was relatively high which constitutes an important public health problem. Hence, it was recommended that basic hygiene measures must be reinforced during meat processing and handling as well as application of control measures in both pastoral and intensive production system of Sudan.

Keywords: Salmonella; Cattle; Carcasses; Khartoum State; Sudan

Introduction

Salmonella species are a leading cause of acute gastroenteritis in several countries. Food animals such as cattle may carry *Salmonella* spp*.* at slaughter and can serve as sources of contamination and provide an opportunity for entry of pathogen into the food products [1]. Infection of both animals and human results in serious diseases with different species of *Salmonellae* [2]. *Salmonella* is gram negative short rods most strains are motile with exception of *S. pullorum* and *S. gallinarum*. They are intestinal parasites giving rise to enteritis and typhoid like disease [3]. The organism was named *Salmonella* in memory and honor of Dr. D. E. Salmon for his early work on this organism.

In Sudan Khan [4] examined 161 healthy cattle and isolated *Salmonella* from domestic and wild birds, animal, rodent and insects. Sary Eldin [5] isolated *Salmonella* from meat and one liver of sheep at slaughterhouse in Khartoum state. While, Rahman [2] isolated *Salmonella* from mesenteric lymph nodes, hepatic lymph nodes and bile ducts. On the other hand, Mc Evoy., *et al.* [6] stated that *Salmonella* spp. can be present in the rumen and faeces of Irish cattle at slaughterhouse, transfer of contamination from the faeces and rumen to the carcass can occur. However, an alternative source, such as the hide or adjacent carcasses on the line may also contribute to carcass contamination. Therefore, the objectives of the study are:

- To determine the prevalence rate and associated risk factors of *Salmonella* infection in the cattle and their carcasses at Kerrey slaughterhouse*,* Khartoum State, Sudan.
- To estimate the level of bacterial contamination of beef using Total Viable Counts (TVCs) at Kerrey slaughterhouse, Khartoum state, Sudan.

Materials and Methods

Study area

The study was conducted at Karary slaughterhouse, Khartoum State, Sudan.

Collection of swab samples

Total of 120 swabs from 30 were collected from different sites of bovine carcasses (shoulder, neck, rump and flank) after last washing. The sterile swabs were moistened in 15 ml of sterile buffered peptone water and pressed on the carcass sites for sampling. All samples were stored in ice box and transported to laboratory for *Salmonella* isolation and Total Viable Counts (TVCs) in the same day.

Collection of faecal samples

A total of 120 faecal samples was collected, 5gram of faeces collected from the rectum of cattle in sterile plastic containers. The samples were stored in ice box and transported to laboratory for *Salmonella* isolation and bacteriological examinations. The description of the study population at Karary slaughterhouse is summarized in table 1.

Bacteriological examinations
Total viable counts (TVCs)

A Total Viable Counts (TVCs) was done as described by Miles and Misera [7]. Each sample was immersed in 10 ml of sterile peptone water and incubated at 37°C for one hour. Sterile peptone water was used as diluents to make tenfold dilution from each sample. A drop of 0.02 ml was mounted onto sterile nutrient agar plates for aerobic bacterial count and spread over the plate. Then 10^{-4}, 10^{-5} and 10^{-6} were cultured in duplicates. The plates were incubated at 37°C for 24 - 48 hours. Then the average number of colonies was multiplied by the dilution factor to give the number of colonies forming units (CFU) per ml and divided by ten to give the number of colonies forming unit per cm^2.

Swabs culture

The swabs were put in sterile peptone water for 30 minutes to stimulate growth. Then a loopful from specimens was inoculated into Selenite F Broth with overnight incubation at 37°C and then one drop were taken by loop and cultured in Xylose Lysine Deoxycholate

Parameter	Location Darfur	Kordofan	White Nile	Sinnar	Total
Sex					
1- Male	2	7	1	3	13
	1.7%	5.8%	0.8%	2.5%	10.8%
2- Female	42	38	19	8	107
	35.0%	31.7%	15.8%	6.7%	89.2%
Sub total	44	45	20	11	120
	36.7%	37.5%	16.7%	9.2%	100.0%
Age group*					
0 - 1	0	0	0	0	0
	0.0%	0.0%	0.0%	0.0%	0.0%
1 - 2	0	0	0	0	0
	0.0%	0.0%	0.0%	0.0%	0.0%
2 - 3	44	40	20	0	104
	42.3%	38.5%	19.2%	0.0%	100.0%
Sub total	44	40	20	0	104
	42.3%	38.5%	19.2%	0.0%	100.0%
Breed					
Baggara	14	44	0	0	58
	11.7%	36.7%	0.0%	0.0%	48.3%
Nylawi	30	0	1	0	31
	25.0%	0.0%	0.8%	0.0%	25.8%
Kenana	0	1	19	0	20
	0.0%	0.8%	15.8%	0.0%	16.7%
Majok	0	0	0	11	11
	0.0%	0.0%	0.0%	9.2%	9.2%
Sub total	44	45	20	11	120
	36.7%	37.5%	16.7%	9.2%	100.0%

***Table 1:** Description of the study population at Karary slaughterhouse, Khartoum State, Sudan.*
Age: Missing value = 16.*

(XLD) media and incubated at 37°C for 24 hours and then it were kept in refrigerator in 4°C. Colonies containing *Salmonella* appear black colonies on XLD media as well as biochemical tests were also used [8].

Faecal culture

One gram of each faecal sample was diluted in 3 ml of sterile saline. Samples were cultured and identified according to [9]. For isolation of *Salmonella* strains, a loopful from the diluted specimens was inoculated into Selenite F Broth with overnight incubation at 37°C. Then, a loopful was streaked out onto Xylose Lysine Deoxycholate media (XLD) and incubated at 37°C for 18 - 24hours. Colonies containing *Salmonella* appear black colonies on XLD media and biochemical tests were also used.

Data analysis

Statistical Package for the Social Sciences (SPSS) for Windows version 20.0 was used for data analysis. Descriptive statistic such as counts and percentage were used for some variables. While, mean, standard deviation, standard error and 95% confidence interval were used for Total Viable Counts (TVCs) which converted to log 10 for analysis. Analytical statistic such as One Way ANOVA and t-Test were used for comparison of (TVCs) between different sites of the carcasses as well as between TVCs and presence of *Salmonella*. Chi-square was also used for risk factors analysis. According to Sudanese Standards (SDS) 341. (2015) cut off level from meat and meat products, Minimum limit10^5 CFU/cm^2 (log 10^5= 5 CFU/cm^2) and Maximum limit 10^6 (log 10^6 = 6 CFU/cm^2) were obtained (Shuaib., *et al.* 2015). All results were presented as tables and graphs.

Results

The presence of *Salmonella* in beef

The overall percentage of *Salmonella* in different sites of beef at Karary slaughterhouse, Khartoum state, Sudan was 13.3% (16/120). Highest percentage 7.5% (9/120) was recorded for flank followed by rump 3.3% (4/20), shoulder 1.7% (2/120) and neck 0.8% (1/120), respectively. Statistically, the difference was significant (χ^2 =10.962, P-value = 0.012) (Table 2).

Site	No. of Samples tested	No. of positive samples	Percentage (%)	χ^2	df	P-value
Neck	30	1	0.8%			
Shoulder	30	2	%1.7			
Rump	30	4	%3.3	10.962	3	.012*
Flank	30	9	%7.5			
Total	120	16	13.3%			

Table 2: *The presence of Salmonella in different sites of beef at Karary slaughterhouse, Khartoum State, Sudan.*
χ2 = 10.962, P-value = 0.012 < 0.05 (significant).

Total viable counts (TVCs)

According to Sudanese Standards for meat and meat product, relatively a high contamination level was observed in different sites of beef. For instance, mean log TVCs of 6.22 CFU/cm^2, 6.23 CFU/cm^2, 6.06 CFU/cm^2 and 6.24 CFU/cm^2 with 95% confidence interval of (5.88 - 6.56 CFU/cm^2), (5.90 - 6.77 CFU/cm^2), (5.61 - 6.51 CFU/cm^2) and (5.59 - 6.90 CFU/cm^2) were recorded for neck, shoulder, rump and flank, respectively. There was no statistical significant was reported (F-value = 0.209, P-value = 0.890). In contrast, strong positive association was observed regarding the mean log TVCs and presence of *Salmonella* in beef ((t-Test 46.973 = P-value = 0.000 with 95% confidence interval 5.80 - 6.32). The results are presented in table 3 and figure 1 and 2.

Site	No. of Samples tested	Mean	Standard Deviation	Standard Error	95% Confidence Interval for Mean
Neck	25	6.22	0.83	0.17	(5.88 - 6.56)
Shoulder	24	6.33	1.04	0.21	(5.90 - 6.77)
Rump	26	6.06	1.11	0.22	(5.61 - 6.51)
Flank	30	6.24	1.76	0.32	(5.59 - 6.90)
Total	105	6.21	1.25	0.12	(5.97 - 6.46)

Table 3: *Descriptive statistic of log TVCs CFU/cm^2 for different sites of beef at Karary slaughterhouse, Khartoum state, Sudan.*
χ^2 = 10.962, P-value = 0.012 < 0.05 (significant)
Cut off level: Minimum limit 10^5 CFU /cm^2 (log 10^5 = 5 CFU /cm^2); Maximum limit 10^6 CFU /cm^2 (log10^6 = 6 CFU /cm^2).

Citation: Hoiam Aldokhrai Ead and Atif Elamin Abdelgadir. "Detection of *Salmonella* in Cattle and their Carcasses at Karary Slaughterhouse, Khartoum State, Sudan ". *EC Nutrition* 14.10 (2019): 952-960.
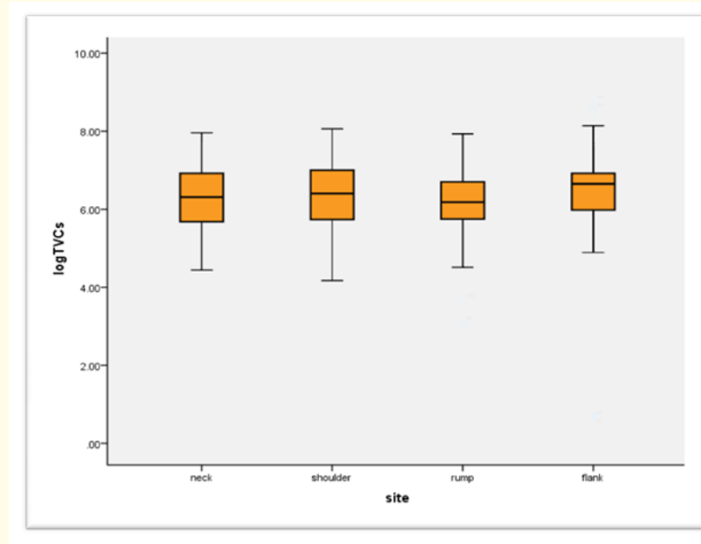
Figure 1: Comparison of mean log TVCs CFU/cm2 of different sites of beef at Karary slaughterhouse, Khartoum State, Sudan. F-value = 0.209, P-value = 0.890 > 0.05 (not significant).
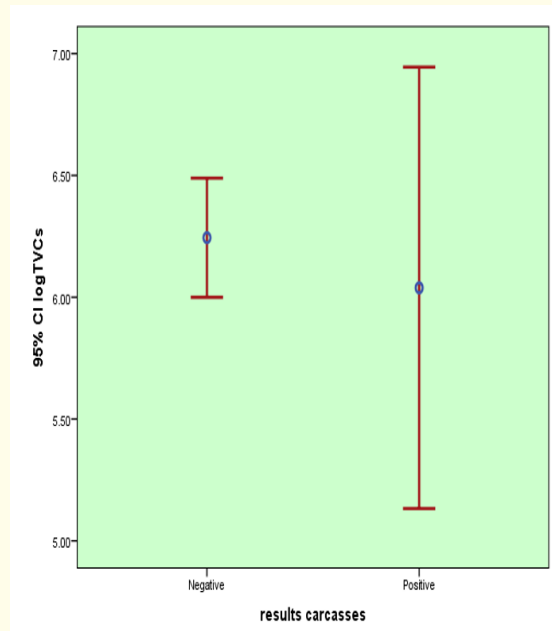

Figure 2: Association between the mean log TVCs and presence of Salmonella in beef at Karary slaughterhouse, Khartoum State, Sudan. t-Test = 46.973, P-value = 0.000 < 0.01 (Highly significant).

Faecal samples

The overall prevalence rate of *Salmonella* infection in cattle using faecal samples at Karary slaughterhouse, Khartoum state, Sudan was 28.3% (34/120). A high prevalence rate was obtained for cattle from Darfur states 15.0% (18/120), followed by Kordofan states 9.2% (11/120) and White Nile state 4.10% (5/120) respectively. Statistically, the difference was significant (χ^2 = 8.220, P-value = 0.042). The results are summarized in table 4.

Location	No. examined	No. of Positive	Prevalence rate%	χ^2	df	P-value
Darfur	44	18	15.0%			
Kordofan	45	11	9.2%			
White Nile	20	5	4.1%	8.220	3	.042*
Sinnar	11	0	0.0%			
Total	120	34	28.3%			

Table 4: *Association between presence of Salmonella infection in cattle using faecal samples and location at Karary slaughterhouse, Khartoum State, Sudan.*
χ^2 = 8.220, P-value = 0.042 < 0.05 (Significant).

Risk factors analysis

A negative association (χ^2 = 1.204, P-value = 0.226) was observed between presence of *Salmonella* spp. infection in cattle using faecal samples and sex at Karary slaughterhouse, Khartoum state, Sudan. A prevalence rate of 26.7% (32/120) and 1.7% (2/120) were reported for male and female, respectively (Table 5). In contrast, a positive association (χ^2 = 8.956, P-value = 0.030) was obtained for breed with respect to *Salmonella* infection in cattle using faecal samples. The highest prevalence rate was recorded for Baggara 12.5% (n = 15/120), Nylawi 11.6% (n = 14/120) and Kenana 4.2% (n = 5/120) respectively (Figure 3). On the other hand, all faecal samples 100% were taken from cattle with age group 2 - 3 years.
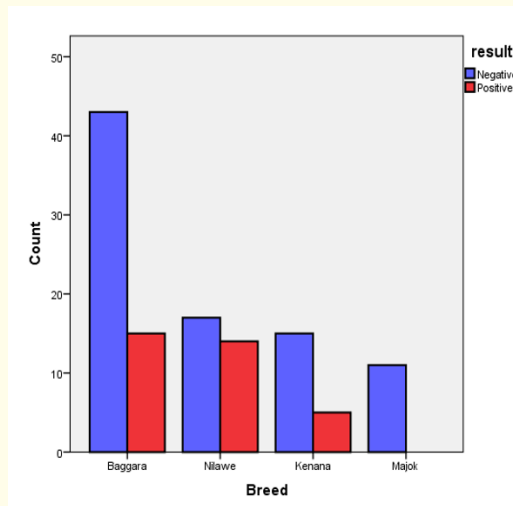

Figure 3: *Association between presence of Salmonella infection in cattle using faecal samples and breed at Karary slaughterhouse, Khartoum state, Sudan.*
$\chi2$ = 8.956, P-value = 0.030 < 0.05 (Significant).

Sex	Results		Total	χ^2	df	P-value
	Negative	Positive				
Female	11	2	13			
	9.2%	1.7%	10.8%			
Male	75	32	107	1.204	1	.226*
	62.5%	26.7%	89.2%			
Total	86	34	120			
	71.7%	28.3%	100.0%			

Table 5: Association between presence of Salmonella infection in cattle using faecal samples and sex at Karary slaughterhouse, Khartoum State, Sudan.
$\chi^2 = 1.204$, P-value = 0.226 > 0.05 (Not significant).

Discussion

Salmonella spp. infections are worldwide and constitute an important public health problem in many parts of the world. The current research work revealed that the presence of *Salmonella* in beef was high (Overall percentage = 13.3%). Hussein [10] stated that there was no *Salmonella* spp. in beef and refrigerated meat in Sudan. In contrast, Sary Eldin [5] isolated *Salmonella* from meat and one liver of sheep at slaughterhouse in Khartoum state. Moreover, the prevalence of *Salmonella* in bovine carcasses was different in several studies performed worldwide. Ranged from 0.7% in the USA [11] and 7.6% in Ireland [6]. The distribution of *Salmonella* species among cattle varies greatly over time and differs among geographic regions, age groups, clinical manifestation and production system [12]. Other causes of this variation are associated with stress during transportation to the slaughterhouse, hygienic of condition holding pens, processing practices, slaughterhouse facilities and employees' hygiene and practices.

As seen from the results, according to Sudanese Standard for meat and meat product, relatively a high contamination level was observed in different sites of beef with regard to average mean Total Viable Counts (log TVCs = 6.21). Relatively, similar observations were obtained by Nazik [13] who explained that the mean log Total Viable Counts of bacteria were (7.79 ± 0.17 CFU/cm^2 for the shoulder, (7.92 ± 0.15 CFU/cm^2) for the flank and (7.69 ± 0.2 CFU/cm^2) for the rump. In addition, the same author isolated *Salmonella* from bovine carcasses (0.7%) at El sabloga slaughterhouse, Khartoum state.

In the current study, faecal samples were collected from cattle to identify the prevalence of animals shedding *Salmonella*. The overall prevalence rate of *Salmonella* infection in cattle was 28.3%. A high prevalence rate was obtained for cattle from Darfur states 15.0% and Kordofan states 9.2% and statistically the difference was significant (χ^2 = 8.220, P-value = 0.042 < 0.05). However, Khan in 1969 [14] isolated *Salmonella* from cattle with rate of (2.07%). Low percentage was also obtained by Rahman [3] who investigated *Salmonella* in cattle in Sudan and stated that the recovery rate was (0.27%). Furthermore, Hag Elsafi., *et al*. [15] reported that the isolation of four *Salmonella* isolates from faecal samples of cattle (3.4%) collected in Khartoum State. In contrast, similar findings to our results were obtained by Sahar [16] who recorded a high level of *Salmonella* spp. infection in cattle using faecal samples (40%) in Omdurman localities, Sudan. In addition, Adil., *et al*. [17] explained that (18.13%) of faecal samples were positive for *Salmonella* spp. in Khartoum state.

Data on the prevalence of *Salmonella* in different studies were difficult to compare because the observed prevalence may be biased by diversity in sampling methods, sampling seasons and techniques [18]. Concerning slaughterhouse, Rahimi [19] explained that the variations observed between different research works related to the prevalence rate of *Salmonella* around the world may be due to several factors, including the sanitation within the slaughterhouse, possible contamination during meat processing and differences among isolation methods applied to detect *Salmonella.*

Conclusion

The presence of *Salmonella* in beef

- The overall percentage of *Salmonella* infection in different sites of beef at Karary slaughterhouse, Khartoum state, Sudan was 13.3% (16/120).

- The level of contamination using Total Viable Counts (TVCs) was relatively high in different sites of beef at export Karary slaughterhouse, Khartoum State, Sudan.

- Some risk factors such as location and breed were determined using faecal samples examination with regard to presence of *Salmonella* infection at Karary slaughterhouse, Khartoum State, Sudan.

Recommendations

- Improving hygienic procedures should be implemented as to mitigate *Salmonella* spp. contamination at slaughterhouse.

- Applied Hazard Analysis Critical Control Points (HACCP) to improve carcass hygiene as well as to eliminate or reduce the presence of pathogenic bacteria on the surface of the carcass.

- Application of control measures for Salmonellosis is required in both pastoral and intensive production system of Sudan.

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