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Assessment of microbial qualities of exported sheep and goats carcasses and the hygiene conditions of an export slaughterhouse in Khartoum state

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Abstract:
The study was conducted to determine the level of contaminations in exported sheep and goats carcasses during September 2018 to January 2019 in an exported slaughterhouse and at Khartoum airport in Khartoum state. A total of 250 samples were collected, from slaughterhouse 130 samples and from Khartoum airport 120 samples.
The total viable count (TVC) was used to evaluate the levels of contamination in the four sites of the carcasses (neck, forelimb, flank and hind limb) at different operational control points during the slaughter process (skinning, evisceration, washing, and chilling) and at Khartoum air port. Also, samples were taken from hands of the workers and contact surfaces (40 samples) from both slaughterhouse and at airport. TVCs of sheep and goats carcasses in slaughterhouse and airport were ranged between 8.39±0.10 log10 cfu/cm^2 and 8.58±0.06 log10 cfu/cm^2, the TVCs of the butcher's hands and loaders in the slaughterhouse were 8.43±0.10 log10 cfu/cm^2 and 8.44±0.06 log10 cfu/cm^2 respectively, while the hands of the workers in the airport were 8.21 ±0.12 log10 cfu/cm^2.Certain pathogenic organisms were isolated in the slaughterhouse *E. coli (*39.88%), *Salmonella spp* (19.02%) and *Staphylococcus areus* (41.10%).While the percentages at the air port were *E. coli* (38.0%), *Salmonella spp* (9.2%) and *Staphylococcus areus* (71.7%).The study showed that the levels of contamination on the exported sheep and goats carcasses were higher than the acceptable values set by the Sudanese and international standards. However, for providing hygienic meat, it is important to maintain high standards of hygiene in the slaughterhouse by continuous monitoring and imposing the hazard analysis critical control points system (HACCP).
Keywords: Contamination, sheep and goal carcasses, slaughterhouse, HACCP.

Introduction:

In Sudan, the livestock sector is a renewable resource and one of the important pillars of the national economy, despite the relative progress of this sector, it is still far from achieving the desired targets for export of meat compared to the size of the resources it possesses; especially studies indicate that the Arab countries suffer from a gap in red meat consumption by 72.44%, which necessitated the promotion of meat exports. There are challenges facing the export of animal meat, including the external competition in addition to the growing specifications from importers Ministry of Animal Resource (2018).Establishing a hygienic program for exported meat is required in order to enable the Sudan to face the international trade parameters.
Food safety is defined as an assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use (FAO, 2004). Food safety plays a significant role in the national economy and health development by safeguarding the health of the nation, enhancing tourism, national and international trade for production, preventing

avoidable losses and conserving natural resources. Thus countries with well-established food safety assurance systems can export and trade their products without any barriers and become competitive in global trade (FAO/WHO, 2005).

Food safety in developing countries and especially in Africa is weak, unable to protect human health. Because of stringent food safety laws of developed nations, many African countries are unable to export their potential raw or processed food. These nations not only lose foreign exchange earnings, they also overstretch the national health services as a result of preventable foodborne illnesses and death (FAO/WHO, 2005).

Supply of safe and quality meat is essential for the protection of public health and access to regional and international market opportunities.

This study was conducted to evaluate the microbial qualities of exported sheep and goats carcasses and to assess the sanitation and hygienic practices in a selected export slaughterhouse in Khartoum state. The purpose is to provide information to promote meat hygiene and to establish and maintain regionally acceptable meat quality standards required by meat export trade.

Materials and Methods:

Study Area:-

Across sectional study was conducted during September 2018 to January 2019 in an export slaughterhouse in Khartoum State and at Khartoum airport.

Samples Collection:-

A total of 250 swab samples were collected 130 from slaughterhouse and 120 from Khartoum airport.

Sheep and goats carcasses (n=80) were sampled at four sites (neck, forelimb, flank and hind limb) at different operational control points during the slaughter process (skinning, evisceration, washing and chilling) and at Khartoum air port (n=80) . Also, samples were taken from contact surfaces (50) included 10 from slaughterhouse water and samples from hands of the workers (n=40) in both slaughterhouse and at airport.

The swab was initially rubbed vertically for at least 5 seconds, then horizontally and finally diagonally in an area of 10 cm^2 for no less than 20 seconds, sufficient pressure has been applied. All samples from the rubbed sites and worker hands were placed separately in a cold box that had ice below 4° C but did not freeze.

Samples obtained with swabs were transported to the laboratory of the microbiology in the University of Sudan, College of Veterinary Medicine for microbial analysis within 24-48 hours of sampling.

Sample preparation:-

This was done according to Adzitey *et al*. (2014).The swabs were placed in 10 ml of sterile peptone water and shaken completely to obtain the pure product (10^{-1}). One (1) ml of pure liquid was transferred to 9 ml of sterile peptone water until a dilution of 10^{-6} was obtained. Serial dilutions (10^{-5} to 10^{-6}) were spread plated onto nutrient agar plates.

Determination of Total Viable Count (TVC): -

One ml of each dilution was added to a sterile Petri dish and the Agar plate count (maintained at 45°C in a water bath) was added and mixed carefully. The preparation was then allowed to gel and finally incubated at 37°C for 24 hours and several colonies were counted and recorded. However, the exact number of colonies between 30 and 300 colonies were counted. The average counts obtained were multiplied by the dilution factor and expressed as the Colony Forming Unit per gram or cm^2 (C.F.U / cm^2) (Fawole and Oso, 2001).

Isolation and Identification of the Bacteria:-

The isolation and identification of *E.coli*, *Salmonella* and *Staphylococcus aurous* were chieved by using selective media for each bacteria followed by Gram staining of presumptive colonies

and standard biochemical tests (Cruikshank *et al.,* 1975).The isolation and entification of the bacteria were done as described by Barrow and Feltham (2003). The swab samples were ltured using prepared Nutrient Agar, Nutrient Broth, Deoxycholate Citrate Agar (DCA), Eosin methylene blue agar (EMB Agar) and Mannitol Salt Agar (MSA). The broth tubes and agar plates were incubated at 37°C for 24 hours. Afterwards, the morphology of colonies on agar media were examined microscopically, smears were then made from clean slides fixed with heat and subjected to Gram stain and examined under oil immersion lens and the biochemical tests for species identification were conducted.

Data analyses

The data were analyzed using the software Statistical Package for the Social Sciences version 23.0 (SSPS Inc. and Chicago, IL, USA). All bacterial counts were converted to log10 cfu/cm^2 for analysis. Analysis of Variance (ANOVA) was performed to evaluate the differences in the levels of TVCs between the different operational points/critical control points. Moreover, the statistical significance was set at a p-value of ≤0.05.

Result:

Bacterial Viable Count:-

Table 1 showed that, the highest mean of TVCs log values in the anatomical sites were on samples from flank regions which recorded 8.54±0.06 log10 cfu/cm^2 at skinning, 8.54±0.04log10 cfu/cm^2 at evisceration and 8.52±0.06 at washing.

Table (1).Mean±Sd of Total viable counts (Iog 10 cfu cm^2) on sites of the sheep and goat carcasses (n= 80) in an export slaughterhouse in Khartoum state:-

Site	Operation Points			
	Skinning	Evisceration	Washing	Chilling
Neck	8.43±0.53	8.45±0.09	8.42±0.05	8.47±0.08
Fore Limb	8.46±0.08	8.39±0.49	8.47±0.05	8.49±0.05*
Flank	8.54±0.06	8.54±0.04	8.52±0.06	8.49±0.06
Hind Limb	8.39±0.12	8.42±0.09	8.46±0.08	8.39±0.10

*= (Sig.) significant at level (P<0.05)

Table 2 showed that, the mean TVCs log values of the loader worker hands (8.44±0.06 Iog10cfu cm^2) were higher than the slaughter house butcher hands (8.43±0.11 Iog10cfu cm^2).The highest mean TVCs log values on some contact surfaces sites of the slaughterhouse and some utensils were on samples from knives (8.51±0.02 Iog10cfu cm^2) followed by the slaughterhouse floor (8.46±0.05 Iog10 cfu cm^2).

Table (2) Mean±Sd of Total viable counts (Iog10 cfu cm^2) on some sites of the slaughterhouse and some utensils in an export slaughterhouse in Khartoum state:-

Site	Number	Mean±St.Dev.	Significance
Slaughter House Butcher Hands	10	8.43±0.11	NS
Loader Worker Hands	10	8.44±0.06	*
Slaughterhouse Walls	5	8.45±0.05	*
Meat Scales	5	8.42±0.10	NS
Slaughterhouse Floor	5	8.46±0.05	*
Slaughtering Knives	5	8.51±0.02	*
Slaughterhouse Water	10	7.49±0.09	NS

*= (Sig.) significant at level (P<0.05), NS= Not significant.

Table3 showed that the highest mean TVCs log values on some contact surfaces sites at Khartoum air port were samples from worker hands (8.21 ±0.12 Iog10cfu cm^2) followed by the carcasses (8.15±0.22 Iog10cfu cm^2).

Table (3) Mean±Sd of Total viable counts (Iog 10 cfu cm^2) of worker hands, vans of meat and carcasses in the airport in Khartoum state

Site	Number	Mean±St.Dev.	Significance
Airport Worker Hands	20	8.21 ±0.12	NS

Airport Van of Meat	20	8.13 ±0.11	NS
Airport Carcasses	80	8.15±0.22	NS

NS= Not significant

Pathogenic bacteria:-

The study revealed three types of bacteria namely *E. coli, Salmonella spp* and *Staphylococcus aureus* with their frequency and percentages of contamination of the carcasses as shown in Table 4.The highest relative frequency of isolates was *Staphylococcus Aureus,* 67(41.10%), followed by *E. coli* 65(39.88%) and *Salmonella spp* 31 (19.02%).

Table 4 Number and frequency of bacteria isolated from different sites associated with meat for export in the slaughterhouse:-

Site	*E. coli*	*Salmonella spp*	*Staph Aureus*	Total
Skining	11(6.75%)	5(3.07%)	11(6.75%)	27(16.56%)
Eviceration	11(6.75%)	2(1.23%)	15(9.20%)	28(17.17%)
Washing	14(8.59%)	6(3.68%)	10(6.13%)	30(18.40%)
Chilling	7 (4.29%)	5(3.07%)	13(7.98%)	25(15.34%)
B.hands	6(3.68%)	2(1.23%)	8(4.91%)	16(9.81%)
Up.hands	7(4.29%)	2(1.23%)	1(0.61%)	10(6.13%)
Walls	3(1.84%)	1(0.61%)	3 (1.84%)	7(4.29%)
Scales	2(1.23%)	2(1.23%)	3 (1.84%)	7(4.29%)
Floor	0(0%)	5(3.07%)	1 (0.61%)	6 (3.68%)
Knives	4(2.45%)	2(1.23%)	1 (0.61%)	7(4.29%)
Water	0(0%)	0(0%)	0(0%)	0(0%)
Totals	65(39.88%)	31(19.02%)	67(41.10%)	163(100%)

Table 5 showed that, the highest relative frequency of isolates at Khartoum airport was *Staphylococcus Aureus,* 86 (71.7), followed by *E. coli* 46 (38.0) and *Salmonella spp* 11 (9.2).

Table 5 Number and frequency of bacteria isolated from different sites associated with meat for export in Khartoum airport:-

Sampling sites	At Airport		
	E.coli	*Salmonella spp*	*Staph Aureus*
Carcasses (n=80)	32 (40.0)	7 (8.8)	58 (72.5)
Hands of worker (n=20)	7 (35.0)	0 (00.0)	15 (75.0)
Contact surfaces (n=20)	7 (35.0)	4 (20.0)	13 (65.0)
Total (n=120)	46 (38.0)	11(9.2)	86 (71.7)

Discussions:

To prevent the occurrence of food borne illnesses and possible meat spoilage, it is important to ensure that foods are safe and in good hygienic conditions. The microbiological testing for different indicators such as *Salmonella*, coliforms and *E. coli* can be performed at different sites of the carcass surface (Buncic *et al*., 2014). Recommended sites include the rump, brisket, thigh, flank, and shoulders. Sampling should be performed at different stages during the slaughter process that is; after pelt removal, skinning, evisceration and pluck removal, washing, chilling and on the final product ready for redistribution to retailers (Lasok and Tenhagen, 2013).

According to (Capita *et al*., 2004; Zwivel *et al.,* 2005), for practical and economic reasons, the swab technique is the most used method for sampling the carcass surface. Total plate count was used to measure the general bacteria load on meat and is a useful tool in monitoring food safety. The results may reflect the hygienic level of food handling and retail storage. According to Sudanese standards for red meat the bacterial total number should not exceed one million (10^6 CFU / g) per colony (SSMO, 2008), according to FAO (2007), Total viable plate count numbers exceeding 100 000/g (5.0 log10) on fresh meat are not acceptable and alarm signals, and meat hygiene along the slaughter and meat handling chain must be urgently improved. These standards from The Sudanese Standards and Metrology

Organization (SSMO) and FAO were lower compared to the results found in the present study and hence these counts put the consumers at risk. The bacterial counts of the carcasses in the present study ranged from 8.39±0.10 log10 cfu/cm^2 and 8.58±0.06 (log10 cfu/cm^2) were generally high above 10^7 where spoilage of meat occurs (Warriss, 2001), and above the International Commission on Microbiological Specification of Food (ICMSF, 1988) (<1.0x10^{6}cfu/g).

The higher counts could be due to the unhygienic practices followed during the meat handling and processing. In the present study, the highest mean log values in the anatomical site were on samples from flank region which recorded (8.54±0.06 log10 cfu/cm^2) at skinning, .54±0.04log10 cfu/cm^2 at evisceration and 8.52±0.06 at washing. Similar to this study Zweifel and Stephan (2003) noted that the neck and flank had the most increased contamination levels. This also agree with Bekker (1998) who indicated that washing of the carcasses with cold water does not significantly influence the microbiological load on beef carcasses. The high TVCs obtained from environmental contamination in abattoir is from slaughtering knives (8.51±0.02 log10 cfu/cm^2) followed by Slaughterhouse Floor (8.46±0.05 log10 cfu/cm^2) and this is an indication of ineffective and inadequate cleaning of floor before commencement of work and at the close of work, this is similar to Bhandare *et al*. (2009) who found higher level of environmental contamination on abattoir floor. Regarding the pathogenic bacteria, the microbiological profile in meat products is the key criteria for determining quality and safety of fresh produce. Ideally, meat should be considered as wholesome when pathogens of concern are absent or if present should be at low number depending on their toxin or metabolites produced (Biswas *et al*., 2011). Bacteria including *Staphylococcus aureus, E. coli* and *Salmonella* spp are the causes of 60% of food borne illness requiring hospitalization in the United States and about 2.1 million children in developing countries die of diarrheal- related illnesses annually (WHO, 2009).In this study, the microbiological examination of carcasses revealed the presence of *Salmonella* spp*, E.coli* and *Staphylococcus aureus* in all stages of processing (skinning, evisceration, washing, chilling and at the airport). At the slaughterhouse the highest relative frequency of isolates was *Staphylococcus Aureus,* 67(41.10%), followed by *E. coli* 65(39.88%) and *Salmonella* spp 31 (19.02%). The highest recorded levels with *E.coli* 6.75% were at washing and evisceration, the highest level with *Salmonella* 3.68% recorded at washing and that of *Staph Aureus* 9.20% at evisceration. The occurrence of *Salmonella* was higher than National Advisory Committee on Microbiological Criteria for Foods (NACMCF) (1993) who reported that incidence rates of *Salmonella* on raw beef are generally low (about 5%). Similar results in which little or no isolation of *Salmonella* in carcasses have been recorded in other studies. For instance, Sofos
et al. (1999) detected 3% *Salmonella* from 30 carcasses in the United States. The incidence of *E.coli* and *Salmonella* could be attributed to the poor cleaning and sanitary conditions in the abattoirs puncture of the viscera resulting in spread of infection and an increase in contamination of carcasses by fecal matter and to the poor handling by butchers, storage and environmental conditions. *Staphylococcus spp.* was isolated from the majority of the samples and this agreed with studies done by other researchers who also found a high prevalence of *Staphylococcus aureus* in raw meats (Ahmad *et al.,* 2013; Soyiri *et al*., 2008).The high prevalence of *Staphylococcus spp*. is an indication of contamination from meat handlers. For providing hygienic meat and meat products, maintaining high standard of hygiene in the abattoir is a matter of paramount importance. This maintained by continuous monitoring to establish a hygiene base and to ensure the quality of the products (Sofos, 1994), besides imposing the hazard analysis critical control points system (HACCP) is a matter of great importance.

In conclusion the Microbiological quality of meat is of public health significance. The meat gets contaminated from a variety of sources within and outside animal during the slaughter of animal and during its sale. Slaughter house, the workers, the vehicle used for the transport of the meat from the slaughter house to air port can act as the external sources for the contamination of the meat. Establishing a hygienic program for exported meat is required in order to enable the Sudan facing the international trade parameters maintaining regionally acceptable meat quality standards required by meat export trade

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