



# Evaluation of Microbiological Quality of Halal Beef Intended for Export from Khartoum State, Sudan

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## Authors' contributions

This work was carried out in collaboration between both authors. Author HAK collected the research data, conducted laboratory analysis and performed the statistical analysis. Author EAM conceptualized the initial idea, drafted the initial manuscript, and reviewed the final manuscript. Both authors read and approved the final manuscript.

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## ABSTRACT

**Aims:** To evaluate halal criteria of beef microbiological quality intended for export in two slaughterhouses.

**Study Design:** A cross-sectional study.

**Place and Duration of Study:** Khartoum State, Sudan between August and November 2022.

**Methodology:** Samples were collected by using the non-destructive dry swab method for microbiological quality sampling of beef and a standardized checklist to evaluate good manufacturing practices (GMPs) and good hygienic practices (GHPs). Restraining methods were categorized as "modern method" where the full inversion rotary casting box was used which resembled slaughterhouse "A" or "conventional method" where a large-sized hammer for blowing

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the skull was used and this resembled slaughterhouse "B". A total of 80 male beef cattle were investigated after arrival in the lairages of the designated slaughterhouses. Forty carcasses from each slaughtering method were randomly chosen and swabbed.

**Results:** The results revealed that halal criteria for the slaughtering methods in slaughterhouse (A) was 100%, while slaughterhouse (B) was 83.2%. Also, this study revealed poor personal hygiene as slaughterhouse A scored 53.2%, while slaughterhouse B scored 33.2% for personal hygiene evaluation. The mean total bacterial count (TBC) was found to be  $4.556 \times 10^5$  cfu/ml and  $5.53275 \times 10^5$  cfu/ml in slaughterhouses A and B, respectively with highly significant differences compared to the standard permissible limits ( $1 \times 10^5$ cfu/ml) with  $p \leq 0.05$ , while there were no statistically significant differences (0.847) within slaughterhouses with  $p \leq 0.05$ . Furthermore, the mean total coliform count (TCC) was found to be  $16.4795 \times 10^2$ cfu/ml and  $47.8670 \times 10^2$ cfu/ml in slaughterhouses A and B, respectively with a highly significant difference compared to the standard permissible limits ( $1 \times 10^2$ cfu/ml) with  $p \leq 0.05$  and also with highly significant differences (0.000) between slaughterhouses themselves with  $p \leq 0.05$ .

**Conclusion:** The study concluded that both slaughterhouses failed to meet the requirements of the various beef halal criteria.

*Keywords: Beef; slaughterhouse; halal criteria; animal welfare; TBC; TCC.*

## 1. INTRODUCTION

The term "halal" in the Qur'an means "permissible and lawful" [1]. Non-Muslim consumers started purchasing halal beef as a result of worries about food safety and increased awareness that halal-certified food complies with stringent requirements for sanitation and hygiene [2].

Furthermore, [3,4] pointed out that using halal techniques of animal slaughter, such as those that prioritize animal welfare both in lairages and during transportation, results in meat that is of superior quality.

Stunning, an animal welfare issue is a procedure used to render animals unconscious before slaughtering them [5,6]. The full inversion rotary casting box is utilized as a restraint technique and it is used according to certain ritual requirements [7,8].

In the halal slaughtering process, animals must be alive and the act must adhere to niyyah, which is the purpose of Allah's name [9]. The trachea, esophagus, carotid arteries, and jugular veins are the organs to be severed to ensure complete bleeding [10,8]. Additionally, poor bleeding will reduce the quality, which will damage the meat's appearance and acceptability [11].

The high demand and popularity of halal meat are due to the fact that the halal food industry uses standards for food safety and quality assurance as well as hygienic and

contamination-free practices throughout meat manufacture [12,13]. Therefore, the aim of this study is to evaluate the halal criteria of beef microbiological quality intended for export in two slaughterhouses in Khartoum State.

## 2. MATERIALS AND METHODS

### 2.1 Study Area and Layout

This cross-sectional study was conducted between August and November 2022 in Khartoum State, in two slaughterhouses; a modern one resembled "A", and a conventional one resembled "B".

### 2.2 Slaughtering Methods

#### 2.2.1 Restraining and stunning

Following the approach of [7,8] restraining and stunning methods were categorized as "modern" where the full inversion rotary casting box was used or "conventional" where a large-sized hammer for blowing the skull was used. The first method is carried out according to specific ritual (Shariy'a) requirements [7].

### 2.3 Data Collection Methods

The sterile dry swabs method was conducted to count the bacterial load from carcasses.

Direct observation with a standardized and structured checklist was conducted from the moment of animal arrival to the final product processed to evaluate good manufacturing

practices (GMPs) and good hygienic practices (GHPs) of the lairage, status of the animal welfare, slaughterhouse facilities, and operations. The Point Assignment Guidelines for the checklist were used following [14].

The percentages given to non-conformance for every parameter in the checklist were summed as zero when calculating the totals.

For the evaluation of the checklist, passing a parameter is to score at least 50% of its full conformance. Whereas for the microbiological quality, the values attained should be within the permissible limits.

To this end, the compliance of beef with halal criteria is determined by its ability to pass the various slaughterhouse checklist parameters as well as the meat microbiological quality.

## 2.4 Sample Size

A total of 80 male beef cattle of different age groups were investigated after arrival in the lairages of the designated slaughterhouses and after slaughtering. Forty carcasses (final product) from each conventional and modern slaughterhouse were randomly chosen and swabbed.

## 2.5 Sampling Methods

By using the non-destructive dry swab method described by [15,16], swabs were taken from brisket and flank sites. Swab samples were collected according to [17]. Post to the collection, the swab samples were inserted into labeled sterile plastic containers, kept, and transported in an ice box (0-4 °C) to the laboratory of the College of Veterinary Medicine of Sudan University for Science and Technology within one hour from sampling for microbiological analysis.

## 2.6 Microbiological Analysis

The microbiological safety of meat was assessed on the basis of Total Bacterial Count (TBC) using aerobic plate count and Total Coliform Count (TCC) using the most probable number (MPN) according to [18,19].

Both TBC and TCC were evaluated against the permissible limits mentioned in [19] as a guide to indicate meat wholesomeness. Permissible limit values used are  $1 \times 10^5$  cfu/ml for TBC and  $1 \times 10^2$  cfu/ml for TCC.

## 2.7 Statistical Analysis

The collected data were coded and analyzed by using Statistical Packaging for the Social Sciences (SPSS/PC version 23.0 for windows). One sample t. test, Independent t-test, and correlation were performed. Significant level  $P=0.05$ .

## 3. RESULTS

Assessment of hygiene and management practices for the investigated slaughterhouses using a standardized scoring system checklist was presented in the following tables.

The results of the evaluation of the transport trucks showed that slaughterhouse (A) scored 100% full conformance while slaughterhouse (B) scored a total of 33.3% of full conformance with 18.3% major deficiency in carrying capacity and sanitary status of the trucks (Table 1).

The evaluation of lairage biosecurity and animal welfare measures showed that slaughterhouse (A) scored 86.6% out of the full conformance weight and had a major deficiency of 4% because reasonable distance from the place of slaughter was not satisfied. While slaughterhouse (B) scored 46.6% out of the full conformance weight and with 4% major deficiency due to the lairage floor found slippery and had cracks, not easy to disinfect, and solid waste was not probably managed.

Table 1 also showed that the evaluation of the GMP of slaughterhouse (A) scored 60% of the full conformance weight and a minor deficiency of 32.1% due to toilet facilities were not adequate in number, while slaughterhouse (B) scored a total of 49.0%, with 40% minor deficiency due to floor drainage was not suitable and toilet facilities were not in compliance with the regulations and 9% major deficiency due to floor, walls, ceilings non-compliance. Water supply and pest control were also investigated in Table 1.

Table 2 evaluated halal criteria in operation processes and revealed that slaughterhouse (A) scored 80% of the full conformance weight because there were no signs of identifying dirty and clean areas and 10% minor deficiency due to crisscrossing of personnel, while slaughterhouse (B) scored only 20% of the full conformance weight and 10% minor deficiency due to crisscrossing of personnel and 40 % major deficiency because dirty and clean areas separated by color code only.

**Table 1. The evaluation of prerequisite programs in the slaughterhouses**

Halal criteria in the prerequisite programs	Slaughterhouse	Full conformance	Minor deficiency	Major deficiency	Non-conformance*	Total %
1.Transport trucks	A	100%			0%	100%
	B	33.3%		18.3%	48.4%	51.6%
2.Lairage (construction, location, and unloading facilities)	A	86.6%		4%	9.4%	90.6%
	B	46.6%		4%	49.4%	50.6%
3.Slaughterhouse (GMPs)	A	60%	32.1%		7.9%	92.1%
	B		40%	9%	51%	49%
4. Water Supply	A	100%			0%	100%
	B	80%			20%	80%
5. Pest control	A	100%			0%	100%
	B			25%	75%	25%

\* The percentages given to non-conformance for every parameter were summed as zero when calculating the totals

For the evaluation of the slaughtering methods, the table also showed that slaughterhouse (A) scored full conformance 100%, while slaughterhouse (B) scored 53.3% of the full conformance weight, and scored 13.3% minor deficiency because the bleeding was incomplete and scored 16.6% major deficiency because knives were not frequently sterilized and the head separated from the animal (Table 2).

The evaluation of cleaning and disinfection (SSOPs) in the two slaughterhouses shows that slaughterhouse (A) scored 26.6% of full conformance weight and 54% minor deficiency, while slaughterhouse (B) scored 6.6% of full conformance weight and 13.3% minor deficiency (Table 3).

Equipment and cleanliness evaluation in the slaughterhouses showed that slaughterhouse (A) scored 70% of full conformance weight and 25% minor deficiency, while slaughterhouse (B) scored 25.7% minor deficiency and 21% major deficiency (Table 3).

The evaluation of waste management in slaughterhouses revealed that slaughterhouse (A) scored 100% full conformance and slaughterhouse (B) scored 40% of full conformance and 13.3% minor deficiency (Table 3).

The evaluation of employees' personal hygiene showed that slaughterhouse (A) scored 6.6% of full conformance weight and 46.6% minor deficiency, while slaughterhouse (B) scored only

6.6% of full conformance weight and 26.6% minor deficiency (Table 4).

The evaluation of the halal criteria of the final product specifications and labeling in slaughterhouse (A) had shown 100% of full conformance weight, while slaughterhouse (B) scored 60.8% non-conformance 8.6% major deficiency, and 30.6% minor deficiency (Table 5).

The mean total bacterial count (TBC) was found to be  $4.556 \times 10^5$  cfu/ml and  $5.53275 \times 10^5$  in slaughterhouses A and B, respectively. Statistically, there was a highly significant difference between slaughterhouses and the standard permissible limits ( $1 \times 10^5$  cfu/ml) of the total bacterial count with  $P=0.05$ , while there were no statistically significant differences (0.847) between slaughterhouses themselves with  $P=0.05$  (Table 6).

The results of the mean total coliform count (TCC) in the two slaughterhouses were  $16.4795 \times 10^2$  cfu/ml and  $47.8670 \times 10^2$  cfu/ml in slaughterhouses A and B, respectively.

While there was a highly significant difference (0.000) between the TCC in the two slaughterhouses and the standard permissible limits ( $1 \times 10^2$  cfu/ml) of the total coliform count with  $P=0.05$ , there were also highly significant differences in the TCC between the two slaughterhouses themselves with  $P=0.05$  (Table 7).

**Table 2. The evaluation of operation processes and slaughtering methods in the investigated slaughterhouses**

Halal criteria in operation processes and slaughtering methods	Slaughterhouse	Full conformance	Minor deficiency	Major deficiency	Non-conformance*	Total %
1-Operations: (Separation between clean and dirty area; signs; one-way production line and no crisscrossing of personnel)	A	80%	10%			90%
	B	20%	10%	40%		70%
2-Slaughtering methods: (a. slaughtered in compliance with the rules laid down in the Codex Recommended Code of Hygienic Practice for Fresh Meat; Using modern slaughtering method; Muslim slaughter man, Healthy live animals; Mentioning the name of Allah; Direction of animal toward Qibla; Anti-mortem (Pre-slaughter health check). (b. The slaughtering knife (sharp, enacted after every animal, sanitized each batch. And the animal does not see it). (c. The slaughter act should sever the trachea, esophagus and main arteries and veins of the neck region; Single incision, Animal not tortured, Head not separated from the carcass. (d. Efficient bleeding for enough period (2-3 min), Short period, Hang the animal immediately.	A	100%			0%	100%
	B	53.3%	13.3%	16.6%	16.8%	83.2%

\* The percentages given to non-conformance for every parameter were summed as zero when calculating the totals

**Table 3. The evaluation of cleaning and disinfection and waste management in the investigated slaughterhouses**

Halal criteria of cleaning and disinfection (SSOPs)	Slaughterhouse	Full conformance	Minor deficiency	Major deficiency	Non-conformance*	Total %
1-Cleaning and disinfection of the slaughterhouse (Chemicals storage; Area designed for storage with a sign secure (locked); legible content labels, in proper shelves, pest proof area, Liquids not stored above powders). Cleaning of equipment: (Adequate in number; Area specific, classification according to purpose; prevent cross contamination; Empty containers stored and disposed of safely; Food grade chemicals substance from halal materials i.e. NO Alcohol; Appropriate concentration; Diluted immediately; Appropriate contact period; Not cause corrosion. Cleaners: well trained (Training certification)	A	26.6%	54%		19.4%	80.6%
	B	6.6%	13.3%		80.1%	19.9%
2- Slaughterhouse equipment and their cleanliness:	A	70%	25%		5%	95%
a. Equipment design and condition: smooth surfaces, free of debris, non- toxic materials, no wood or other absorbent materials, resistant to corrosion, allow access to all areas and no debris, mounted off the floor at least 6 inches.	B		25.7%	21%	53.3%	46.7%
b. There are sufficient number of facilities for cleaning, and for cleaning tools near workstations (adequate in number, appropriate in location, have warm water, wash with water and soap, and sanitizing solution.						
c. Food contact equipment surfaces clean (No food debris, Cleaned and disinfected before and after production, and not using towels to clean surface.						
d. Knives and saws regularly sterilized between carcasses and process steps (by electric sterilizers).						
3- Waste management in the slaughterhouses	A	100%			0%	100%
a. There are suitable dispensers and containers for used towels and disposable gloves.	B	40%	13.3%		46.7%	53.3%
b. Frequent removal of garbage.						
c. Regular cleaning.						
d. Packaged bags.						
e. There are adequate facilities for animal waste material removal and handling (Easy to wash; properly managed).						

\* The percentages given to non-conformance for every parameter were summed as zero when calculating the totals

**Table 4. The evaluation of employees' personal hygiene (good hygiene practices-GHPs) in the investigated slaughterhouses**

Employees' personal hygiene (GHP)	Slaughterhouse	Full conformance	Minor deficiency	Major deficiency	Non-conformance*	Total %
a. Personal hygiene (Dress changing room; Wear protective shoes, gloves, face masks, hair cover;	A	6.6%	46.6%		46.8%	53.2%
	B	6.6%	26.6%		66%.8	33.2%
b. Color coding; Specific area for smoking; Chewing gum; Drinking and eating prevented at all/ permitted at specific area away from production and storage areas; Spitting prohibited in all areas.						
c. All personnel appropriately trained in the requirements of halal slaughtering and basic hygiene; Staff trained with certificates.						
d. Particular emphasis placed on washing hands after using the toilet and following any contamination incident in the slaughterhouse.						
e. Hand washing (stations adequate in number, appropriate in location, have warm water, restricted to hand washing purposes only, efficient employee flow; a minimum of one hand wash station for every 10 persons; Wash hands using water and soap/ Soap and sanitizing solution						

\* The percentages given to non-conformance for every parameter were summed as zero when calculating the totals.

**Table 5. The evaluation of final product specifications and labeling in the investigated slaughterhouses**

Final product specifications and labeling	Slaughterhouse	Full conformance	Minor Deficiency	Major Deficiency	Non-conformance*	Total %
a. Final product and its area specifications (Separated from the production areas; Finished carcasses stored and handled under refrigerator; Products are not being cleaned of debris using cloths and/or towels -rail trim).	A	100%			0%	100%
	B		30.6%	8.6%	60.8%	39.2%
b. Word <i>halal certification</i> or equivalent terms and product specification: (legible label; appropriately labeled; Licensed by the authorities; Date of halal possess lot numbers and/or date coding.						
c. Chilled bone-in storage (+4°C or colder -1 °C).						
d. Thermometers: All non-glass; non-mercury in design; Independent of the thermostat probes present in all coolers and freezers; Calibrated.						

\* The percentages given to non-conformance for every parameter were summed as zero when calculating the totals

**Table 6. The mean of TBC in the investigated slaughterhouses**

Slaughterhouse	Mean TBC (cfu /ml)	N*	Std. Deviation
A	455,600.000	40	8925.134
B	553,275.000	40	8679.739
Level of significance compared to the standard permissible limits		78 df*	0.000*
Level of significance between the two slaughterhouses		78df	0.847

\* N: number of Sample; df: degree of freedom; cfu/ml: colony forming unit per milliliter

**Table 7. The mean of TCC in the investigated slaughterhouses**

Slaughterhouses	Mean TCC (cfu/ml)	N*	Std. Deviation
A	1,647.95	40	45.42598
B	4,786.70	40	83.09145
Level of significance compared to the standard permissible limits		78 df*	0.000*
Level of significance in between the two slaughterhouses		78df	0.000

\*N: number of Sample; df: degree of freedom; cfu/ml: colony forming unit per milliliter

### 3.1 Confidence Interval 95%

High counts in total bacteria do not necessarily produce high counts in coliform when applying Pearson Correlation. There was an insignificant correlation (0.154) between the TCC and TBC with  $P=0.05$  as shown in Table 8.

## 4. DISCUSSION

As far as we are aware, this is the first study to be done in Sudan that evaluates beef halal requirements. Hence, this study attempted to assess the halal standards for beef's microbiological quality intended for export in Khartoum State's slaughterhouses.

In this study, the results of the evaluation of the proper application of halal criteria in transport trucks, lairage, biosecurity and animal welfare and GMPs were evident in slaughterhouse A. This agreed with the rules stated by [20,21] for proper application of halal slaughter.

Slaughtered animals in slaughterhouse (A) scored full conformance weight regarding the slaughtering method. This finding is in line with the rules laid down by the Codex Recommended Code of Hygienic Practice for Fresh Meat, halal criteria set in [10,8].

In the present study the application of mechanical restraint tools in slaughterhouse A, comparatively less contamination of meat was

observed than in slaughterhouse B. This is consistent with the statement of [22] who mentioned that meat from animals slaughtered without prior stunning would be of superior quality or wholesomeness. This also conforms with [7] who mentioned that mechanical restraint tools like a full inversion rotary casting box are accepted Halal- and Kosher-compliant techniques.

Slaughterhouse A was in full conformance with the Sudan national animal welfare law with regard to hygiene and management practices for trucks loading animals from feedlots [23]. This finding also agreed with that reported by [24,25] who stated that animal welfare should be considered during transport from feedlots to slaughterhouses.

Additionally, slaughterhouse A was found to have met the requirements for handling animals gently when they are unloaded from trucks into lairages and for allowing them enough time to relax and access potable water. This coincides with that stated by [24,4].

The evaluation of lairage biosecurity and animal welfare measures in this study showed that slaughterhouse (B) failed the evaluation. This was due to the lairage floor being found slippery and had cracks and not easy to disinfect, and that solid waste was not probably managed and disposed of. This is contrary to what is stipulated in the guidelines of [24].

**Table 8. The correlation between TBC and TCC in the slaughterhouses**

TBC	TBC	TCC
Pearson Correlation	1	0.154
Sig. (2-tailed)		0.172

GMPs resemble both manufacturing and quality control procedures that lead products to be consistently manufactured to their specifications [26]. In the present study, both slaughterhouses failed to obtain a high score in the evaluation of GMPs. This was due to toilet facilities not being adequate in number, drainage and conditions of floors, walls, and ceilings not in compliance with the regulations of slaughterhouses.

Also, slaughterhouse (B) failed to attain a reasonable conformance weight for slaughtering methods. This was due to using the conventional slaughtering method for stunning purposes.

This non-conformance is contrary to that stated by [27] who mentioned that animals throughout the slaughtering procedure should be laid on their left side and that the slaughterers must be skilled in order to rapidly and successfully sever all the veins and arteries with a sharp sterilized knife on the first try. Incomplete bleeding will also have a detrimental impact on the meat's appearance, acceptability, cleanliness, and shelf life [11].

The evaluation of cleaning and disinfection in the two slaughterhouses showed low conformance. This is opposite to the ritual requirement procedures stated by several standards [28,8,29,30]. Therefore, failure to comply with this ritual requirement may render meat unacceptable.

The findings of this investigation demonstrated that neither slaughterhouse met the standards for staff personal hygiene that were established. This result was in contrast to what was stated by [31,32], who claimed that failing to follow proper sanitation and hygiene procedures, such as washing hands and wearing protective clothing, can result in microbial contamination, deterioration of meat quality, and post-harvest meat losses.

The evaluation of the final product specifications and labeling of slaughterhouse B contradicts [25] guidelines that call for meat to be stored under refrigeration (+4°C or colder -1 °C). Additionally, both meat cold storage and rail trim (meat free of debris) process steps are considered critical control points under the HACCP system [25].

On the other hand, the poor conformance of slaughterhouse (B) may be attributed to the fact that the meat was not appropriately labeled and labels such as the date of halal lot numbers and/or date coding were not legible. Such a practice may impede halal certificate issuing. This finding is contrary to [33] who stated that in order to ensure the integrity of halal across the supply chain, a globally renowned halal certification authority must be established.

Studying beef slaughterhouse food safety procedures is crucial to safeguarding the public's health and boosting customer trust [34], as microorganism contamination can happen during the slaughtering and processing of animals [35].

TBC and TCC were utilized as microbiological indicators for hygienic and sanitary meat quality in the current investigation since they may offer health risks when present in high concentrations [36,37].

The high level of TBC in this study may be a result of ineffective cleaning and disinfecting techniques, workers' disregard for their assigned locations and intersections in the slaughterhouses, and their non-adherence to health regulations. This outcome supports the claims made by [38,39] that food contamination by foodborne pathogens may occur from greater bacterial counts brought on by this insufficiently controlled processing environment.

These results also are in accord with [40] who revealed that the workers' hands and the equipment were the sources of meat contamination. Also, this would confirm the conclusions of [41] who attributed meat contamination to the unhygienic and poor sanitary condition under which it was handled.

Higher bacterial counts than that recorded in this study were observed by [42] who estimated the total bacterial count samples in beef meat taken from the shoulder, external thoracic, and thigh muscles immediately after evisceration and found that the least count ( $4.8 \times 10^6$  cfu/g) was in the thoracic muscle, compared to  $8.6 \times 10^6$  and  $7.9 \times 10^6$  cfu/g in the shoulder and thigh muscles, respectively.

Higher findings were also recorded by [43] who evaluated the degree of contamination in cattle carcasses in a slaughterhouse in Khartoum State and revealed a high level of total bacterial counts after post-washing of bovine carcasses.

Lower TBC counts (within acceptable limits) were detected by [44] in USA in 100% of pre-evisceration samples in plant A and B, while 99.1 and 76.9% of the post-evisceration samples had detectable levels in plant A and B, respectively.

The highly significant differences in the TCC between the two slaughterhouses in the present study may be attributed to differences in general sanitation conditions and personal hygiene measures applied. Furthermore, there is a chance that the high level of TCC in this study is a result of fecal contamination and unsanitary water conditions, particularly in the conventional slaughterhouse where there was a serious problem with temperature control for knife sterilization. This finding is also supported by [45].

Lower TCC counts (within acceptable limits) were detected by [44] in USA in 83.3% (plant A) and 88.9% (plant B) of pre-evisceration samples, while 82.4 and 47.7% of the post evisceration samples at plant A and B, respectively.

## 5. CONCLUSION

It could be concluded that both modern and conventional slaughterhouses failed to meet the requirements of the various beef halal criteria. It is recommended that quality assurance systems be used at every stage of the meat supply chain.

## DISCLAIMER

The authors declare that they are not associated with or actively involved in any group or organization that has a financial stake in the topics or resources covered in this work.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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