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## Assessment of Bacteria and Parasite Contamination of Dried Sliced Beef (Kilishi) Sold within Birnin Kebbi Metropolis, Kebbi State, Northern Nigeria

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#### Authors' contributions

This research work was carried out in collaboration among all authors. Authors RDJ, QO and ADD designed the study, wrote the protocol, manage the analysis of the study and wrote the first draft of the manuscript. Authors UDN, EJD, RJJ and GKT manage the literature reviews and performed the statistical analysis. All authors read and approved the final manuscript.

#### Article Information

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## ABSTRACT

Kilishi is a version of jerky that originated in Hausa land Nigeria. It is made from deboned cow, sheep or goat meat. The dried sliced beef (kilishi) is often left open in a basin thereby exposing it to flies, dust and other effects of the environment by so doing the product can be contaminated. This study was carried out to investigate the bacteria and parasite contaminants of dried slice beef (kilishi) sold in different locations within Birnin Kebbi metropolis. The samples were analyzed using pour plate method. The bacterial species were characterized and identified on the basis of their colonial morphology; gram's staining reaction and biochemical characteristics. The protozoans and helminthes cyst/eggs morphology were identified using microscopy techniques. The total bacteria

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plate count for each sample ranges from the highest (8x10<sup>9</sup>) to the lowest (3.5x10<sup>3</sup>) CFU/g from Birnin Kebbi Kalgo, Jega and Aliero samples respectively. The organisms isolated include; *Staphylococcus aureus* (35.2%), *Escherichia coli* showed 30(21.1%) percentage of occurrence, *Bacillus* species occurred 17(12%) *Klebsiella* spp 16(11.3%), *Pseudomonas spp* 13(9.2%), *Shigella* spp 10(7.04%) and the least was *P. vulgaris* 6(4.2%). The kilishi meat product was also contaminated with some Protozoans and helminthes contaminants which include; *Acaris lumbricoides* (14.2%), *Entamoeba histolytica* (35%), *Girdia lambila* (42.8%), and *Taenia spp*. (7.1%). It was concluded that the high bacteria count and frequency of isolates from the kilishi samples tested is an indication of high contamination of the meat by potential pathogens due to poor handling and sanitary conditions which may pose a potential source of food borne diseases.

Keywords: Kilishi; bacteria; protozoan; helminthes; pathogen; diseases.

#### **1. INTRODUCTION**

Meat is an animal product which comprises of the muscle tissue and also includes internal organ called viscera such as heart, kidney, liver, intestine and bladder. There are different types of meat from different types of animals; examples are pork meat (pig), mutton (sheep) and beef (cow) [1]. According to the chemical composition of meat, it varies considerably based on the age, species, degree of fatness of animal, the part of carcass involved etc. According to the food safety and inspection service (FSIS) people eat meat for the muscle. Meat is one of the most perishable foods and its composition is ideal for the growth of a wide range of microorganisms [2]. Meat water content is approximately 75% (depending on the cut) and 20% protein with the remaining 5% representing a combination of fats, carbohydrates and minerals. Meat can be served as prepared meat product example corn beef, fried meat, cooked meat and kilishi (dried sliced beef) [3].

Kilishi is a version of jerky that originated in Hausa land. It is a dried form of suya, made from deboned cow, sheep or goat meat. The meat can be kept for months without much change to its taste [4]. Each of the selected muscles is sliced into sheets of one meter or less for easy drying. The dried sheets of meat are then collected and kept for the next process [5,6].

Kilishi meat product is prepared by ingredient of paste made from groundnut, called labu in Hausa language, is diluted with enough water, spices, salt, grounded onions, and sometimes sweeteners such as honey, to add sweetness [7]. A more natural way to add sweetness is by adding date palm. The dried "sheets" of meat are then immersed one by one in the labu paste to coat them, before being left to dry for hours before roasting on a wire mesh [8]. The dried sliced beef (kilishi) is often left open in a basin thereby exposing it to flies, dust and other effects of the environment by so doing the product can be contaminated with infective cyst due to flies patch and harmful organisms made their ways into the meat [9,10].

The possible source of contaminants for kilishi includes using contaminated water, contaminated utensil, (knives, spoons etc) and food handlers. Food borne illnesses resulting from the consumption of foods contaminated with pathogens or toxins accounts for a significant cause of morbidity globally. In Nigeria, the incidence of food borne pathogens is guiet alarming and represents a significant source of public health concern [11,12]. More so, the risk of emerging infection which may occur through the transfer of zoonotic diseases to man is quite high [13]. The muscle tissue of healthy living animals is usually free from microorganism. However, during the slaughtering process, this meat gets contamination on external surface, such as hair and skin, the gastrointestinal and respiratory tract. The equipment used in the slaughtering and dressing operations (knives, saws, cleavers and hooks) make significant contribute to the overall contamination through direct contact with hides and hair as well as by contact with steels, knifes scabbards and the clothing of operatives [14].

Kilishi (dried sliced beef) is consume in different countries/locality because of its nutritional content. Safety of these products is therefore essential for public health. The processing lacks standard therefore there is increase in health risk. It is processed under unhygienic conditions, which involve; using contaminated water to wash the meat, contaminated utensil (knives, spoon, etc) and food handlers, drying under the sun where flies (which is a vector carrier of many pathogenic organism) parch on it thereby depositing protozoa cyst. Some of the kilishi unlike suya is not heated with much temperature as such is likely to be contaminated. Moreover the product is sold in an open tray on the streets, bus stop etc, exposing it to dust and other environmental contamination [15,16].

### 2. METHODOLOGY

## 2.1 Study Area

This research work was carried out in Aliero local government Area of Kebbi State, Nigeria. The area is located between latitude 12°27'13"N and longitude 4°12'01"E. It is a city located in northwestern part of Nigeria, having an estimated population of 125, 594 as of 2007 census. It has a tropical weather conditions characterized by wet, dry and harmattan season; the average annual temperature is 28.4°C and average temperature of 32.7°C at the hottest month in the year. The wet season is hot oppressive, and mostly cloudy and the dry season is sweltering and partly cloudy, the cold harmattan periods is characterized by dust and wind [17].

#### 2.2 Sample Collection

Freshly prepared dried meat and unprepared slim dry meat samples were collected from Birnin Kebbi, Kalgo, Jega and Aliero Local government of Kebbi State. The samples were transported to microbiology laboratory in a clean, polyethylene bags for microbiological analysis. The samples were refrigerated at 4°C and were all analyse within 24 hours of collection following the methods of Harris [1]. All experimental determinations were made in triplicate.

#### 2.3 Media Preparation

All media were prepared according to the manufacturer's instruction as contain in the label of the medium. The following media were used for this analysis: - Nutrient Agar (NA) and Peptone Water (PW), MacConkey agar (MCA), Eosin Methylene Blue (EMB)), Citrate Agar (CA), Christensen's Urea Agar (CUA) and Mannitol Salt Agar (MSA).

#### 2.4 Sample Processing

Ten (10) gram of each dried slice beef meat sample was weighed and dissolved in 100ml of sterile distilled water using waring blender and left to homogenized for 1minutes. Serial dilution of the homogenates was made using sterile pipettes, where 1ml of the homogenize sample was aseptically transferred into 9ml of sterile distilled water in first tube  $10^{-1}$  the tube was shaked and 1 ml was removed from the first tube  $(10^{-1})$  to the second tube  $(10^{-2})$  Subsequently, serial dilutions of up to  $10^{-7}$  was made as described by the methods of Pelezar [18].

#### 2.4.1 Inoculation

Aliquots (0.1ml) of each dilution was transferred in replicate into corresponding differential and selective media, and then spread uniformly using a bent glass rod. The plates were then incubated aerobically at 37°C for 18 to 24 hours (for bacteria). Discrete colonies were subcultured into fresh agar plates aseptically and a pure culture of each isolate was obtained. Pure isolates of resulting growth was stored for further identification [19].

#### 2.4.2 Enumeration of bacteria

From the  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-7}$  dilutions of the homogenates; 0.1 ml of  $10^{-1}$ ,  $10^{-2}$  and  $10^{-7}$ dilutions of the homogenate was inoculated on different media using surface plating method. Mac Conkey agar and EMB agar was used for coliform enumeration while Mannitol salt agar was use for the isolation of S. aureus. Total viable aerobic bacteria count was performed on Nutrient Agar. At end of the incubation periods, colonies on the nutrient agar plates were counted on each plate for the different dilutions using the colony counter. The counts on each plate were expressed as colony forming unit per gram of the suspension (CFU/g) [20].

# 2.4.3 Identification and characterization of the isolates

Colonies identify as discrete on nutrient agar was carefully examined macroscopically for cultural characteristics such as the shape, color, size and consistency. Bacterial isolates were characterized based on microscopic appearance, colonial morphology and Gram staining reactions as well as appropriate biochemical tests such as; Catalase test, Coagulase test, Citrate utilization test, Motility test, Urease test, Carbohydrate fermentation test as described by [20]. The isolates were identified by comparing their characteristics with those of known taxa, as described by [18].

#### 2.5 Biochemical Test

#### 2.5.1 Gram staining

A dried smear was fixed appropriately, smear was covered with crystal violet stain for 1minute, and stained was washed under slow running tap water, lugols iodine was then added for 1minute, and washed with slow running tap water, it was then decolorized with 75% ethanol for few seconds to remove excess color of the crystal violet stain and was washed immediately before safranin was added to it for 30 seconds and washed under slow running tap water, the back of the slide was wiped with cotton wool and the slide was left to air dried on slide rack. The slides were viewed using x100 oil immersion lens [21].

#### 2.5.2 Motility test

A sterile needle was used to pick a colony from a 24 h old culture and was stabbed onto nutrient agar in test tube. The tubes were incubated at 37°C for 24-48 hours. Non-motile bacteria had growth confined to the stab line with definite margins without spreading to surroundings area while motile bacteria gave diffused growth extending from the surface [22].

#### 2.5.3 Catalase test

A small quantity of 24 hour culture was transferred into a drop of 3% Hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Gas was seen as a bubble which indicates the presence of catalase enzyme according to the method of [19].

#### 2.5.4 Coagulase test

The test was carried out using 18-24 hours culture. A loopful of isolated bacterium was emulsified with normal saline solution on a microscope slide. A drop of undiluted plasma was added to the suspension and stirred for 5 seconds. A coagulase-positive result was indicated by clumping of colonies together [22].

#### 2.5.5 Urease test

About 100 ml of sterile urease agar was prepared, 10 ml of urease medium was dispensed into each tubes and place undisturbed in a slanting position and allowed to cool and solidify. The tubes were inoculated by streaking the organism once across the surface. The presence of pink color indicated a positive result [22].

#### 2.5.6 Mannitol fermentation test

Sterile mannitol salt agar was aseptically dispensed into sterile petri-dishes, after which a loopful of the 24 hours pure culture of the bacteria was inoculated on the surface the each plates using streaking method. All the plates were incubated at 37°C for 24 hours and results were determined. A positive test consists of a color change from red to yellow, indicating a pH change to acidic [16].

#### 2.5.7 Citrate test

This test detects the ability of an organism to use citrate as a sole source of carbon and energy. 2.4 g of citrate agar was dissolve in 100ml of distilled water, 10ml of citrate medium was dispensed into each tubes and covered, then sterilized and allowed to cool in a slanted position. The tubes were inoculated by streaking the organisms once across the surface. A change from green to blue indicates utilization of the citrate.

#### 2.5.8 Oxidase test

A piece of filter paper was soaked with few drops of oxidase reagent. Sterile inoculating loop was used to pick a colony of the test organism and smeared on the filter paper. The phenylenediamine in the reagent were oxidized to a deep purple colour, for positive result [19].

#### 2.6 Protozoan Isolation

Ten (10) gram of dry slim meat of the product was weighed aseptically and dissolved in 100ml of sterile saline-peptone-water and was mixed by shaking vigorously with hands for 5 minutes in a sterile 250ml screw-capped bottle. Thereafter, it was then left untouched for 30 minutes. The soaked sample was filtered. After filtration the solid part of the sample was discarded and the liquid part was centrifuged. A drop of the suspension was placed on a glass slide and was covered with a cover slip and viewed under a microscope using xl0 and x40 objective lenses in order to identify possible cyst or egg of protozoans as describe by the method of [23] Ash et al. [23].

## 2.7 Statistical Analysis

The data collected were subjected to statistical analysis using instat Graphpad software version 21 and all the data obtained were analyzed using one-way analysis of variance (ANOVA).

#### 3. RESULTS

Table 1: The total plate count of bacteria isolated from different samples of kilishi

Total plate count of bacterial isolated from dried slice beef "kilishi" samples collected at different sites, Birnin kebbin, Kalgo and Jega showed a higher microbial count of  $4 \times 10^9$ ,  $4 \times 10^9$  and  $8 \times 10^9$  CFU/g while the least was Aliero sample with5 x 10<sup>8</sup> CFU/g.

Table2:Morphologicalandbiochemicalcharacteristics of bacterial isolated from kilishi

The Morphological and biochemical characteristics of bacterial isolates revealed the presence of; *Staphylococcus aureus*, *E. coli*, *Salmonella typhi*, *Pseudomonas aureginosa*, *Klebsiella pneumoniae*, *Bacillus subtilis*, *Shigella*, and *Proteus vulgaris* isolated from kilishi meat product.

Table 3: Protozoan's, Helminthes cyst, ova/egg and trophozoite detected from kilishi

The kilishi meat production was found to contain some protozoan cyst such as *Entamoeba histolytica* and *Girdia lambila* and helminthes; *Taenia saginata* and *Ascaris lumbricoids*.

 Table 4: Percentage occurrence of Parasites

 isolated from kilishi at different site

The percentage occurrence of each parasites include; *Giardia lambila* 42.8%, *Entamoeba histolytica* 35%, *Ascaris lumbricoides* 14.2%, and *Teania saginata*, 7.1%. *Giardia lambila* was found to have the highest percentage of occurrence followed by *E. histolytica* and the least was *A. lumbricoid*.

Table 5: Percentage Occurrence of Bacteria Isolated from Kilishi at different site

The Percentage occurrence of bacteria isolated from kilishi from Birnin Kebbi metropolis revealed that *Staphylococcus aureus* had the highest percentage of occurrence of 50(35.2%), followed by *Escherichia coli* 30(21.2%), *Pseudomonas aureginosa* 13(9.2%), *Bacillus subtilis*, 17(12%), *Klebsiella pneumonie* 16(11.3%), *Shigella*, 10(7. 04%), and the least was *Proteus vulgaris* with 6(4.2%).

## 4. DISCUSSION

The presence of potentially life threatening pathogens in our environment indicates the seriousness of the potential hazards with which we are faced. Reports shows that gram negative bacteria account for approximately 69% of the cases of bacterial food borne disease [24]. This research recorded a high microbial load from kilishi meat product. The total bacteria plate count for each sample ranges from the highest (8x10<sup>9</sup>) to the lowest (3.5x10<sup>3</sup>) CFU/g from Birnin Kalgo, Jega and Aliero samples Kebbi respectively. All these location showed high microbial load but with a higher microbial contamination from Jega sample while the lowest is sample from Birnin Kebbi. The hike in microbial load may be attributed to lack of good hygienic practice and the harsh dusty environmental condition characterized by heavy wind along with dust. The sample of the slime kilishi meat product from Jega was obtained from the road side where vehicles passing by raise and drops dust on the meat. Another source of contamination is mostly by poor handling and storage condition by the meat handlers, exposing the meat to mechanical vectors (flies) during processing, sneezing directly into the meat and touching of the meat by buyers. Similarly, [25] Felanage et al. [25] reported high microbial loads of 2.85 x 10<sup>5</sup> CFU/ml with a least bacterial count of 9.8 x 10<sup>4</sup> from suya in Ado-Ekiti metropolis, it was stated that the high bacteria count is due to poor processing and unhygienic practices by "suya" vendors and that "suya" was prepared and sold under largely unhygienic and unsaved conditions thereby constituting a food safety risk among the consumers [26], stated that the

# Table 1. Total plate count (cfu/g) for each dried sliced beef "kilishi" samples collected from different site

Total Plate Count (CFU/g)									
Sample site Dilution factor 10 <sup>-1</sup> 10 <sup>-2</sup> 10 <sup>-7</sup>									
Birnin Kebbi	3.5 x 10 <sup>3</sup>	2.2 x 10 <sup>4</sup>	4 x 10 <sup>9</sup>						
Kalgo	$4 \times 10^{4}$	3 x 10⁵	4 x 10 <sup>9</sup>						
Jega	6 x 10 <sup>4</sup>	4 x 10⁵	8 x 10 <sup>9</sup>						
Aliero	5 x 10⁴	3.1 x10⁴	5 x 10 <sup>8</sup>						

Key: CFU/g (colony forming unit per gram)

Gram`s Reaction	Cat	Coa	Man	Cit	Ure	Mot	Oxi	$H_2SO_4$	Gas	Organisms
Positive cocci/grape-like cluster	+	+	+	+	+	-	-	-	-	Staphylococcus aureus
Negative straight rods in singles	+	-	+	+	-	+	+	-	+	Pseudomonas aeruginosa
Negative short rods singles/pairs	+	-	+	-	-	+	-	-	+	Escherichia coli
Negative straight rods paired	+	-	+	-	-	+	-	+	-	Salmonella typhi
Positive rods singles/ Pairs	+	-	+	+	-	+	ND	ND	-	Bacillus subtilis
Negative Short rods/cocci	+	ND	ND	-	-	-	-	ND	-	Shigella spp
Negative rods in singles	+		+	+	+	-	-	-	+	Klebsiella pneumoniae
Negative short straight rods	+	-	-	+	+	+	-	ND	+	Proteus vulgaris

## Table 2. Morphological and biochemical identification of bacteria isolates in Birnin kebbi metropolis

Key: +=positive, - =Negative, Cat=catalase, coa=coagulase, man=manitol, cit=citrate, ure=urease, mot=motility, oxi=oxidase, ND=not determined

## Table 3. Protozoan's, helminthes cyst, ova/egg and trophozoite detected from kilishi

Sample site	Ova/egg	Cyst	Trophozoite	Organism
Birnin Kebbi	Nil	present	Nil	Entamoeba histolytica
Kalgo	Nil	present	Nil	Girdia lambila
Jega	present	Nil	Nil	Ascaris lumbricoides
	Present	Nil	Nil	Teania trichuria
	Nil	Present	Nil	Entamoeba histolytica
	Nil	present	Nil	Girdia lambila
Aliero	Nil	present	Nil	Entamoeba histolytica
	Nil	Present	Nil	Girdia lambila

## Table 4. Percentage occurrence of Parasites isolated from kilishi at different site

Parasites isolated	Birnin Kebbi	Kalgo	Jega	Aliero	Frequency	Percentage
Ascaris lumbricoides	Nil	Nil	2	Nil	2	14.2%
Giardia lambila	Nil	2	3	1	6	42.8%
Entamoeba histolytica	1	Nil	2	2	5	35%
Teania saginata	Nil	Nil	1	Nil	1	7.1%
Total					14	99.1%

Organism isolated	Bk (%)	Kalgo (%)	Jega (%)	Aliero (%)	Total (%)	
Staphylococcus aureus	7(14%)	12(24%)	18(36%)	13(26%)	50(35.2%)	
Escherichia coli	4(13.3%)	7(23.3%)	12(40%)	7(23.3%)	30(21.2%)	
Pseudomonas aeruginosa	1(7.6%)	3(23%)	7(53.8%)	2(15.4%)	13(9.2%)	
Bacillus subtilis	2(11.8%)	4(23.5%)	8(47%)	3(17.6%)	17(12%)	
Klebsiella pneumoniae	1(6.3%)	6(37.5%)	6(37.5)	3(18.7%)	16(11.3%)	
Shigella spp	0(0.0%)	3(30%)	4(40%)	3(30%)	10(7.04%)	
Proteus vulgaris	0(0.0%)	1(16.6%)	3(50%)	2(33.3%)	6(4.2%)	
Total	15(10.6%)	36(25.4%)	58(40.8%)	33(23.2%)	142(100%)	

Key: BK=Birnin Kebbi, % = percentage

colony counts obtained was higher than the acceptable limits (this shows that the product is contaminated to unsafe level and should attract public health attention. On the other hand, [27] Raji [27] also stated that the observed bacteria counts from kilishi samples is a potential source of food infection.

This research isolated series of bacteria such as: S.aureus, P. auregenosa, E. coli, S. typhi, B. subtilis, Shigella spp, K. pneumoniae and P. vulgaris. Staphylococcus aureus has the highest occurrence which account for 35.2% of all isolates. according to Adesiyun et al. [28] S. aureus is one of the major cause of food born diseases, food handlers could be responsible for S. aureus contamination of this product which indicates a low level of hygienic practice by the handlers. It was also stated that enterotoxin producing strains of S. aureus have been isolated from food handlers and the possibility of their transmission highlighted [28]. Likewise, Escherichia coli showed 30(21.1%) percentage of occurrence, Bacillus spp occurred 17(12%) Klebsiella spp 16(11.3%), Pseudomonas spp 13(9.2%), Shigella spp 10(7.04%) and the least was P. vulgaris 6(4.2%).

The presence of *E. coli* is an indication of faecal contamination and a threat to the health of the consumers. The presence of *Bacillus* spp could be as a result of environmental contamination during processing handling and packaging of the product because this species inhabit the soil in the process of drying, the product is left open.

The occurrence of *Pseudomonas* spp and *Klebsiella* spp could be due to post production contamination of these organisms in kilishi product, although these organisms are expected to have been destroyed during roasting and drying but kilishi unlike suya is not properly heated with high temperature. However, gram negative aerobic rod shaped bacteria especially

*Pseudomonas* have been reported as dominant meat spoilage organisms [29] and [30]. The occurrence of *B. subtilis* from the samples could be as a result of the ubiquitous nature of the organism and its endospores forming ability, which confers resistance to it. The presence of *Bacillus* in some of the kilishi samples can be attributed to the fact that samples may have been contaminated before production at the raw meat stage. *Bacillus* has been reported as the most pathogenic organism that can survive intermediate moisture meat (IMM), thermal processing and osmotic equilibration because it is aerobic together with the fact that no anaerobic have been isolated from IMM.

Microorganisms isolated in this study have been earlier found in foods, environment and other places, and their pattern is similar to previous reports by [31] Nkanga and Uraih [31] Agbeyegbe and Uraih [32]; Enabulele and Uraih [33], Clarence et al. [24] Okonko et al. [15]. The isolate in this study is in line with the report of [13] which reported Bacillus subtilis. Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa and Proteus spp. However, it was reported that the most frequently coliform identified on meat were C. freundii, E coli, En. agglomeram and less frequently strains are of the genera Klebsiella spp, Shigella sonnie and Proteus. E. coli and S. aureus are commensal of the human microbiota, it can also become an opportunistic pathogen to human and animals, their presence in foods are indications of excessive human handling [24]. Members of the gram negative bacteria e.g. E. coli are widely distributed in the environment contaminated food and water are the major sources by which the bacteria are spread [24].

The percentage occurrence of *Girdia lambila* is high with (42.8%), followed by *E. histolytica* (35%), *Acaris lumbricoides* (14.2%) and least was *Taenia saginata*. (7.1%) and are significant

to call for public health concern. The disparity in number of parasites is due to the difference of processors hygiene standard and the sales environment Parasites are responsible for a range of health problems. Mean parasites count of 2.8log<sub>10</sub> CFU/g, is high enough to threaten health this is because they can multiply in human system. Some of the samples showed no detectable eggs or cysts of some parasites while others signify difference in hygienic practice. The contamination of the product with these parasites could be from the processing water, slaughtering of unhealthy animals and during drying of the product where it is left open and flies patch on it. However, unlike suya, kilishi is not heated with enough fire which could reduce the microbial loads of the product.

## **5. CONCLUSION**

This research revealed that the microbial load of kilishi is high and strains of pathogenic bacteria were isolated due to the fact that the meat is not produced under hygienic condition. The meat is therefore consider un-safe for consumption and should be avoided so as to reduce the risk of gastro-intestinal born disease and food disorders. Most of the producers of kilishi meat around Birnin Kebbi metropolis are illiterates who have no much knowledge about hygiene, if kilishi must be taken due to its nutritional content, then, there is need to sensitize and educate the producers, processors and the final meat product handlers on a good sanitary and hygienic method of handling and processing the meat so as to reduce the risk of contamination by microorganisms.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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