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A Longitudinal Study: Microbiological Quality of Raw Beef from Halal and Non-Halal Meat Markets in the United States

Omar A. Al-Mahmood [1,*], Xiuping Jiang [2], William C. Bridges [3] and Angela M. Fraser [2]

[1] Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Mosul 41002, Iraq
[2] Department of Food, Nutrition, and Packaging Sciences, Clemson University, Clemson, SC 29634, USA; xiuping@clemson.edu (X.J.); afraser@clemson.edu (A.M.F.)
[3] Department of Mathematical Sciences, Clemson University, Clemson, SC 29634, USA; wbrdgs@clemson.edu
* Correspondence: omar.a.almoula@uomosul.edu.iq

Abstract: Halal means permissible according to Islamic law. Halal meat is obtained by hand slaughtering an animal that is not stunned and that is blessed by a Muslim individual immediately before slaughter. The purpose of this study was to determine the microbiological quality of raw meat from halal meat markets. A total of 138 beef samples were purchased from three halal (n = 72 samples) and three non-halal markets (n = 66 samples) between November 2016 and October 2017. All samples were analyzed for the presence of indicator organisms—aerobic plate counts (APCs), *Enterobacteriaceae* counts (ECs), total coliform counts (TCCs), and generic *Escherichia coli* (ECCs). The levels of APCs, ECs, TCCs, and ECCs (mean log CFU/g) in halal samples were 4.93 (100%), 2.89 (91.7%), 2.87 (94.4%), and 1.09 (18.1%), respectively, and those in non-halal samples were 4.92 (100%), 3.07 (95.5%), 3.02 (89.4%), and 1.15 (16.7%), respectively. The levels of TCCs and ECs were higher in halal samples during the summer compared to the other three seasons, whereas the highest ECCs in halal samples were found during autumn. In non-halal samples, significant differences were observed in the ECCs, TCCs, and ECs across seasons, with the highest level of contamination during autumn. Samples having higher levels of indicator organisms (APCs, ECs, TCCs, and ECCs) were more likely to be positive for pathogenic bacteria. The high levels of indicator organisms in both halal and non-halal retail meat samples suggest that the operation size, and not halal or non-halal meat classification, is associated with the microbiological quality. These findings can inform food safety interventions targeting small meat markets in the United States.

Keywords: halal beef; beef retail cut; indicator microorganisms; halal markets

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1. Introduction

In 2023, the U.S. halal food market was valued at approximately USD 43.27 billion and is estimated to grow at a compound annual growth rate (CAGR) of 7.42% between 2023 and 2028 [1]. Its growth is reflected in the number of pure halal food markets in the United States, increasing from 200 stores in 1998 to over 2100 in 2018, with most (85%) selling halal meat (based on data collected by Zabihah.com) [2]. According to Zabihah.com, the states with the highest number of halal food markets are California (262), New York (213), Florida (142), Illinois (119), Texas (114), Michigan (104), and Virginia (102), accounting for nearly 50% of the total number of halal markets in the United States (Figure 1) [2]. Several U.S.-based supermarkets in areas with large Muslim populations also have dedicated space to sell halal foods and meat products [3]. Most halal markets in the United States are independently owned and operated small businesses [4].

Small retailers face more challenges (i.e., financial constraints and limited technical support) than large retailers in ensuring the food safety of their products [5]. The food safety risks reported at small retailers include improper handling, temperature abuse, contaminated equipment, and poor personal hygiene [6,7]. Most halal meat in the United

States is sold through these small halal markets, which are classified as ethnic markets [8]. Within this subsector of the food industry, there is scant good-quality data available about food safety issues [9].

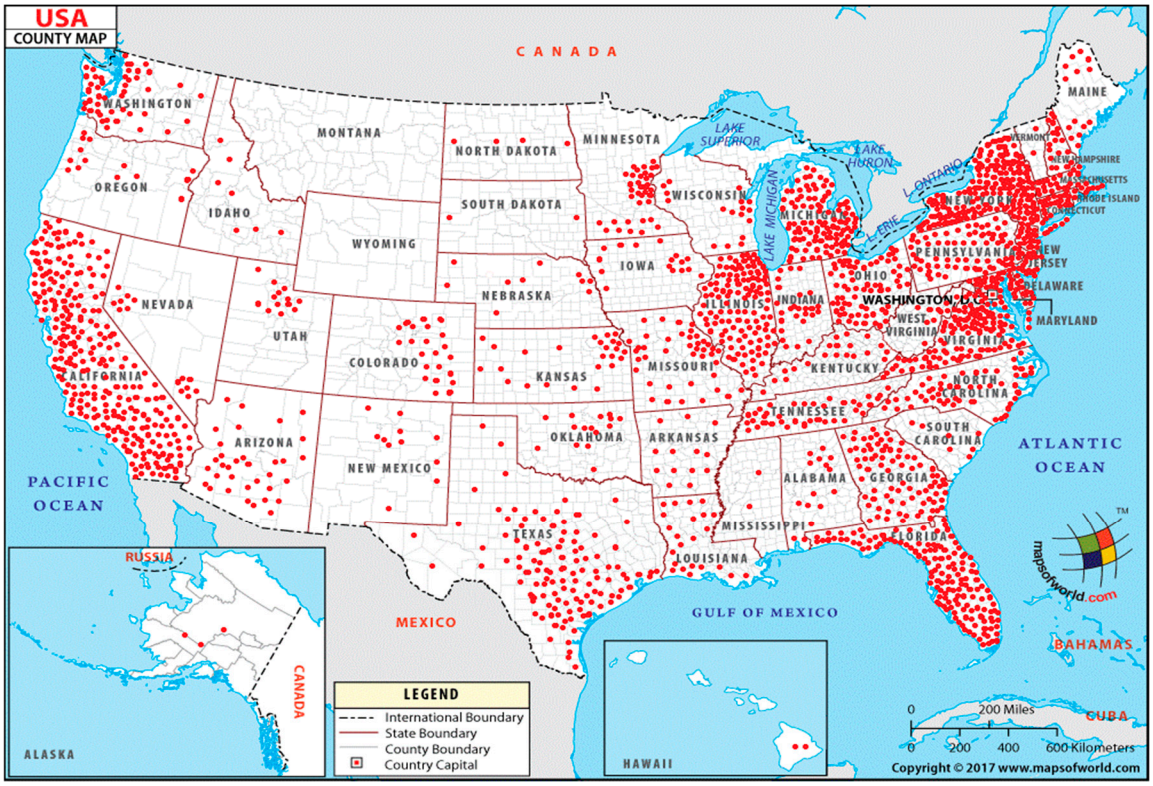

Figure 1. Geographic distribution of the halal food markets in the United States [2].

In small halal markets, there are many opportunities for microorganisms to be introduced into meat. For example, contamination can occur during handling, such as when the meat comes into contact with equipment grinders and knives; food handlers (bare hand contact); and/or by exposure to the environment (unpackaged foods in a refrigerator) [10,11]. One way to determine the microbiological safety of meat is to collect and then analyze samples [12]. This is difficult for two reasons. First, in U.S. small markets, the operations are only inspected by the regulatory authority one to four times/year and samples are not collected during the inspection. Secondly, these markets may not have the resources (i.e., technical knowledge and/or money) to do so. In addition, there is a perception that ethnic markets are "less sanitary". We believed that it was important to compare halal and non-halal meat markets to determine whether food safety issues are due to the size of the business or its classification (halal or not).

At present, limited data are available about the microbiological status of meat sold through halal markets, particularly in non-Muslim-majority countries. In fact, a literature search yielded only one microbiological study conducted in a halal butcher shop in the United Kingdom [13]. Given this, we aimed to determine the microbiological quality of retail cuts of meat in a convenience sample of three halal markets and three non-halal markets in the United States over one year. Two hypotheses informed our research: (1) indicator organisms would be above the maximum acceptable levels from samples collected from small halal and non-halal markets and (2) indicator organisms would be above the maximum acceptable levels in both halal and non-halal meat samples during the summer season compared with other seasons (spring, autumn, and winter).

2. Materials and Methods

The Clemson University Institutional Review Board (IRB) approved the research protocol of this study in August 2016. Data collection began in November 2016.

2.1. Sampling Frame and Sample Selection

As the United States covers a large geographic area, we selected three states close in proximity from which to collect samples. All small retail meat markets (halal and non-halal) in three states (North Carolina, South Carolina, and Georgia) were identified. A retail meat market was defined as a small grocery store with an in-store butcher shop. Each state was divided into ten sections. The section in each state nearest to Clemson University (location of sample processing) was selected (Figure 2). One halal meat market (hereafter labeled A, B, and C) and one non-halal meat market (hereafter labeled D, E, and F) were randomly selected from a list of markets in each of the three states. The selected markets were all within the same business size (small business) (A, D in North Carolina; B, E in South Carolina; C, F in Georgia). The market managers at each store were not informed of the study, so that the results would not be influenced. During each market visit (one every month for 12 visits), the sanitary condition of the butcher area accessible to the public was observed and noted. In addition, two samples (500 ± 25 g) (special cuts) from each halal and non-halal market (one from a chuck and another from a round) were purchased during the site visit. Notably, halal meat compared to non-halal meat markets typically do not precut meat into standard retail beef cuts. From our observation, when the sample (special cut) was purchased, the butcher took a piece of meat from the primal cut (e.g., chuck or round), holding the remaining primal cut in the refrigerator for the next customer order. None of the meat samples (halal or non-halal) were pre-packaged and labeled before purchase. After purchase, each sample was placed into a sterile Whirl-Pak® bag (55 oz) (Nasco-Wisconsin, Fort Atkinson, WI, USA) using sterile gloves. All sealed bags were labeled with a unique identifying code and transported on chiller packs for microbiological analysis at Clemson University. The samples were processed within 12 h after returning to the laboratory.

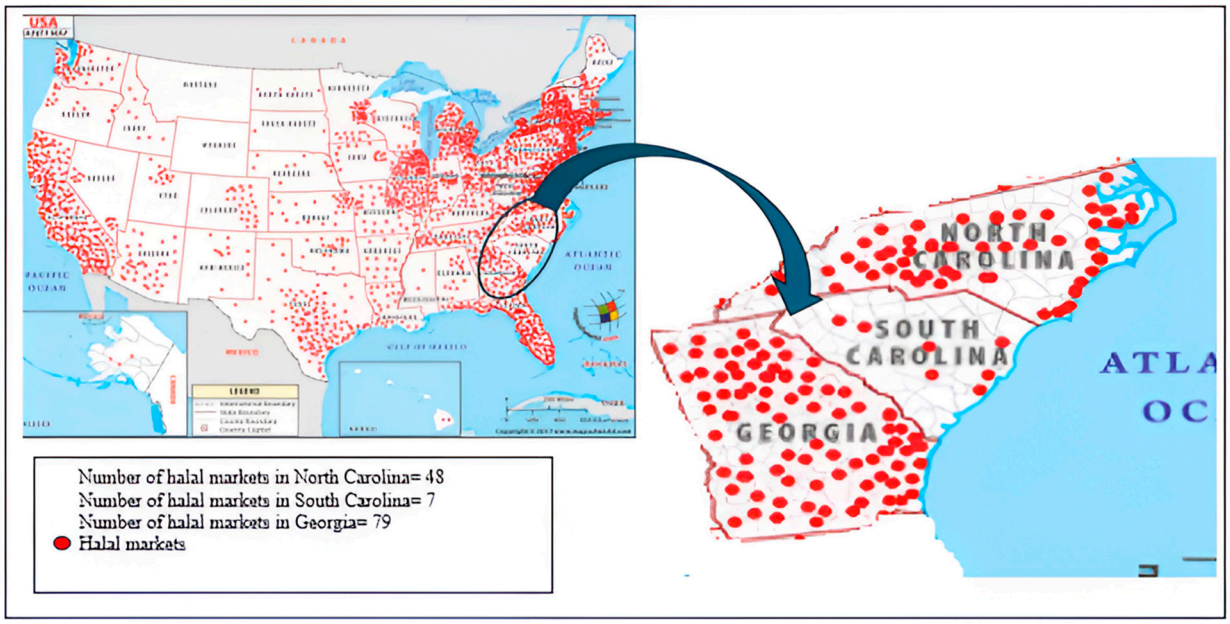

Figure 2. Sampling frame and selection procedure of halal markets [2].

2.2. Subsample Collection and Preparation

The modified N60 method was used to select meat subsamples from the market samples [14]. Five pieces of thinly sliced beef (1 inch long × 1 inch wide) were aseptically

collected using sterile surgical blades. Each was weighed to the equivalent of 10 g and then placed into sterile Whirl-Pak® bags (Nasco-Wisconsin, Fort Atkinson, WI, USA), to which 90 mL of sterile buffered peptone water (NovaLock™, Libertyville, IL, USA) was added. These samples were analyzed for the presence of indicator organisms (aerobic plate counts (APCs), *Enterobacteriaceae* counts (ECs), total coliform counts (TCCs), and generic *Escherichia coli* counts (ECCs)). Each sample bag was homogenized in a stomacher for 2 min at room temperature (Model 400, Seward Stomacher®, Worthing, UK).

2.3. Detection of Indicator Organisms

All samples were analyzed using specific AOAC methods for the detection of APC [15], ECC/TCC [16], and EC [17]. Each sample bag was homogenized in a stomacher for 2 min at room temperature (Model 400, Seward Stomacher®, UK) before serial dilutions were prepared. Serial dilutions for each sample were prepared and homogenized up to 10–5 using Butterfield's Phosphate Buffer (NovaLock, Libertyville, IL, USA). Duplicate petrifilms (3M, Saint Paul, MN, USA) were prepared by inoculating 1 mL test suspension onto the center of the petrifilm. The film was then carefully placed down onto the inoculum. The suspension was distributed with downward pressure into the center using a plastic spreader device. Petrifilms were incubated at 35 °C for 48 h for APC, at 35 °C for 24–48 h for ECC/TCC, and at 35 °C for 24 h for EC. Colonies were counted as per manufacturer instructions. Counts were converted from CFU/mL to CFU/g using the following formulas:

$$\text{Meat sample CFU/g} = \frac{\text{CN} \times 100 \times \text{DF}}{10}$$

where CN is a measure of the colony number of the duplicate petrifilm, 100 is the volume of the diluent, DF is the inverse of the dilution factor, and 10 is the total meat weight sampled (g). The detection limit for the APC, ECC/TCC, and EC methods was 10 CFU/g for beef samples. All positive counts were converted to log10 values.

2.4. Maximum Acceptable Levels for Meat

The mean log values (APCs, ECs, TCCs, and ECCs) were compared to the maximum acceptable limits established by the National Advisory Committee on Microbiological Criteria for Foods [18]. This was used as our reference because the NACMCF provides impartial and scientific advice to U.S. food safety agencies.

2.5. Statistical Analysis

Descriptive and inferential statistics were performed using the JMP Pro12 software (2015 SAS Institute Inc., Carolina, NC, USA). Descriptive statistics included the mean, the standard error, and percentages. To determine the effects of the independent variables (i.e., halal/non-halal beef, seasonal variation) on the APCs, ECs, TCCs, and ECCs, the data were analyzed using analysis of variance (ANOVA) (Hypotheses 1 and 2). Student's *t*-test was used to compare sample means drawn from a normally distributed population. General linear regression models were used for predictive analysis, and multiple linear regression was used to explain the relationship between the indicator microorganisms and two independent variables (i.e., halal/non-halal beef, seasonal variation) (Hypothesis 2). Results were significant with $p < 0.05$.

3. Results

APCs were detected in 100% of halal and non-halal samples (Table 1), with (32/72; 44.4%) and (35/66; 53.0%) exceeding the limit of 5 log CFU/g of APCs set by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) in halal and non-halal samples, respectively. ECCs were detected in 18.1% of halal and 16.7% of non-halal samples (Table 1). None exceeded the limit of 2.70 log CFU/g of ECCs set by the NACMCF in halal samples and (2/66; 3%) exceeded the limit in non-halal samples. TCCs were also detected in nearly all samples (94.4% of halal and 95.5% of non-halal samples), with (33/72;

45.8%) and (34/66; 51.5%) exceeding the limit of 3 log CFU/g of TCCs set by the NACMCF in halal and non-halal samples, respectively. ECs were detected in 91.7% of halal and 89.4% of non-halal samples (Table 1), with (12/72; 16.7%) and (16/66; 24.3%) exceeding the limit of 4 log CFU/g of ECs set by the NACMCF in halal and non-halal samples, respectively.

Table 1. Prevalence and mean log values of indicator microorganisms in halal and non-halal meat samples.

Indicators	Halal Meat			Non-Halal Meat		
	No. of Positive	Positive %	Mean Log (SE)	No. of Positive	Positive %	Mean Log (SE)
APCs	72/72	100.0	4.93 (0.14)	66/66	100.0	4.92 (0.15)
ECCs	13/72	18.1	1.09 (0.97)	11/66	16.7	1.15 (1.02)
TCCs	68/72	94.4	2.87 (0.14)	63/66	95.5	3.07 (0.15)
ECs	66/72	91.7	2.89 (0.14)	59/66	89.4	3.02 (0.15)

APCs: Aerobic plate counts, ECCs: *E. coli* counts, TCCs: Total coliform counts, ECs: *Enterobacteriaceae*.

Overall, there were no significant differences in the mean APC, ECCs, TCCs, and ECs between halal and non-halal samples across the 12 months. However, individual comparisons of each visit (n = 12/market) showed a significant difference ($p < 0.001$) in the APCs, TCCs, and ECs between halal and non-halal samples during the October 2017 visit (Figure 3), where non-halal samples yielded higher levels of indicator microorganisms than halal samples.

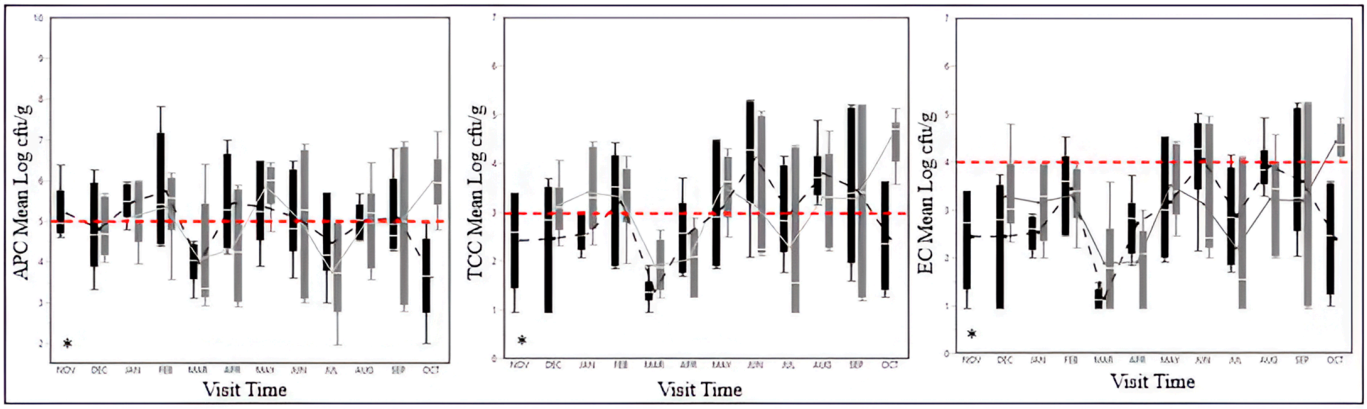

Figure 3. Mean log values of APCs, TCCs, and ECs obtained from halal and non-halal markets throughout the study (12 visits). * Non-halal meat samples of November visit were missed. - · - · -: Maximum acceptable level—National Advisory Committee on Microbiological Criteria for Foods (CFU/g) (NACMCF), 2015. — · — · —: Mean log connection of halal samples (each month, six samples). ————: Mean log connection of non-halal samples (each month, six samples). ▮: Halal value. ▮: Non-halal value.

The indicator microorganisms of halal and non-halal market samples were categorized by season (Tables 2 and 3, respectively). The APCs were not significantly different across the four seasons in either halal or non-halal samples. The levels of TCCs and ECs were higher in halal samples during the summer compared with the other three seasons, whereas the highest ECCs in halal samples were found during autumn. In non-halal market samples, significant differences were observed in the ECCs, TCCs, and ECs across seasons, with the highest level of contamination during autumn.

Table 2. Mean log values of indicator microorganisms from halal samples during four seasons.

Season	Halal Meat			
	APCs	ECCs	TCCs	ECs
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Spring	4.91 ^{a} ± 0.25	0.98 ^{a} ± 0.08	2.34 ^{a} ± 0.25	2.39 ^{a} ± 0.25
Summer	4.84 ^{a} ± 0.25	1.11 ^{ab} ± 0.08	3.63 ^{b} ± 0.25	3.71 ^{b} ± 0.25
Autumn	4.62 ^{a} ± 0.25	1.24 ^{b} ± 0.08	2.77 ^{a} ± 0.25	2.80 ^{a} ± 0.25
Winter	5.33 ^{a} ± 0.25	1.04 ^{ab} ± 0.08	2.74 ^{a} ± 0.25	2.80 ^{a} ± 0.25

Within the same column of each indicator, levels not connected by the same letter are significantly different. Seasons: Spring (March–May), Summer (June–August), Autumn (September–November), and Winter (December–February).

Table 3. Mean log values of indicator microorganisms from non-halal samples across four seasons.

Season	Non-Halal Meat			
	APCs	ECCs	TCCs	ECs
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Spring	4.75 ^{a} ± 0.30	0.95 ^{a} ± 0.14	2.48 ^{c} ± 0.27	2.57 ^{c} ± 0.27
Summer	4.58 ^{a} ± 0.30	1.10 ^{a} ± 0.14	2.90 ^{ac} ± 0.27	2.98 ^{ac} ± 0.27
Autumn	5.44 ^{a} ± 0.37	1.60 ^{b} ± 0.17	3.89 ^{b} ± 0.33	3.95 ^{b} ± 0.34
Winter	5.08 ^{a} ± 0.30	1.09 ^{a} ± 0.14	3.27 ^{ab} ± 0.27	3.38 ^{ab} ± 0.27

Within the same column of each indicator, levels not connected by the same letter are significantly different. Seasons: Spring (March–May), Summer (June–August), Autumn (September–November), and Winter (December–February).

Significant correlations between all pairs of indicators are shown in Figure 4. The highest positive correlations were found between the log CFU/g of EC and TCC (r = 0.96), APC and EC (r = 0.76), and APC and TCC (r = 0.73). The presence or absence of generic *E. coli* in the samples was best predicted by the level of total coliforms, followed by the level of *Enterobacteriaceae*.

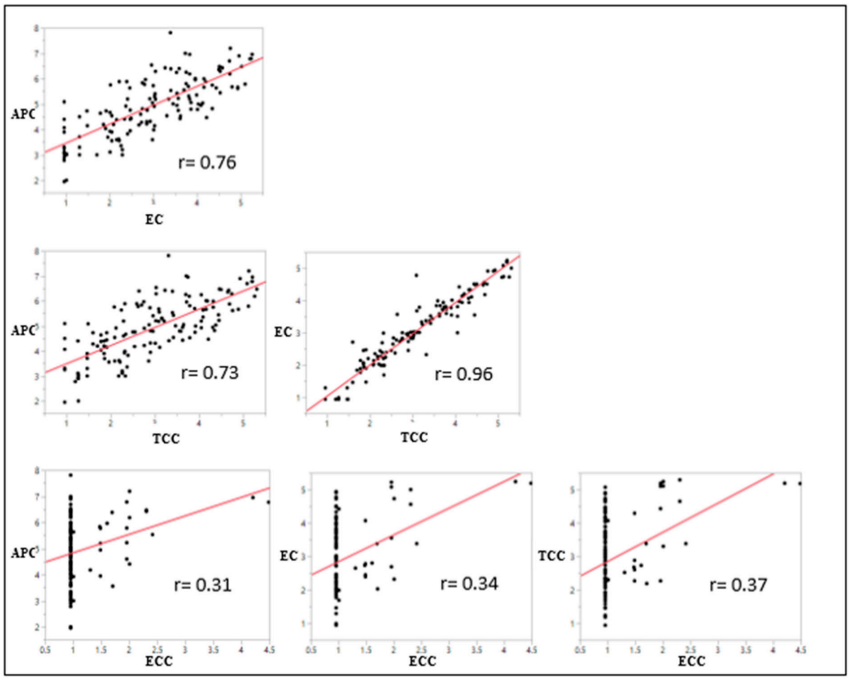

Figure 4. Correlations of pairings of indicator microorganisms (APCs, ECCs, TCCs, and ECs) in beef samples (halal and non-halal combined).

4. Discussion

This study was informed by two hypotheses. First, we hypothesized that the size of the operation would influence the microbiological quality, rather than whether it was a halal or non-halal meat market. In both types of markets (halal and non-halal), specialty (unpackaged) cuts of meat were purchased. The multiple handling steps and cutting of the primal cut can increase microbial contamination as a result of exposure to the environment, hand contact, equipment, and utensils. In addition, poor handling and unhygienic practices were observed during data collection, which also might explain the high microbial contamination at both types of markets. For example, workers were observed using unclean tools and working tables, as well as having dirty hands and apparel, during data collection. All of these issues have been reported by other investigators [19–22] and could increase the microbial load of the meat.

Pre-packaged meats might be safer than those packaged to order [23–25]. Furthermore, all halal retailers included in the study had a walk-in cooler, which was also used to store other perishable food and vegetables, so cross-contamination was possible. However, meat display chillers at non-halal retailers (open-air cooler) may not be suitable for displaying non-packaged beef cuts, potentially increasing the contamination by workers and the environment [26].

Similar findings of high APCs, ECs, and ECCs/TCCs (above the maximum acceptable levels) in raw beef have been reported in halal markets in Morrocco [27], Egypt [28], Malaysia [29], Pakistan [30–32], and Saudi Arabia [33], as well as in non-halal markets in Korea [34], Australia [35], and India [36]. However, it is difficult to compare the results across published studies because of differences in the study methodology and food safety regulations.

Secondly, we hypothesized that the indicator organisms would be higher in halal meat samples during the summer season compared with other seasons (spring, autumn, and winter), where the TCCs and ECs of halal samples were higher in summer. Significant seasonal variations were detected in which the TCC and EC levels were highest during the summer. Several published studies suggest that the rise in temperature and humidity levels during summer may constitute optimum conditions for the microflora present in meat to grow and proliferate during meat processing [25,37,38]. Our results are also similar to those of five studies that showed that the microbial contamination of beef in the summer is the highest compared with other seasons [33,38–41]. The process of selling and displaying meat in halal markets may affect the maintenance of the required temperature (41 °F) inside the cooler, particularly during the summer, creating ideal conditions for bacterial growth. The microflora of the meat can also be affected during meat preparation (butcher cutting), chilling, and handling conditions during storage (chiller hygiene) at markets [27,33]. Butcher fabrication rooms at halal and non-halal markets should be maintained at 41 °F (temperature control room) during meat processing [42]. However, any temperature abuse can increase the risk for the microbial contamination of meat at markets [43].

Non-halal market samples had elevated ECCs, TCCs, and ECs in the autumn season compared with other seasons. These results were in agreement with [39], which found an elevation in microbial contamination in early autumn as compared to the summer (continuing warm weather). The reason for this might be the fact that the temperature drops gradually from the summer to the autumn season and the weather is warm during the early autumn. At this point, further research is recommended to identify the causes of this difference in microbial contamination between halal and non-halal meat in terms of seasonal variation.

The APCs were not significantly different across seasons in both halal and non-halal samples. The reason may be that the APCs of fresh meat include a wide range of different genera that can grow at a wide range of temperatures and humidity levels [44]. The variations across seasons and fresh meat indicator organisms should be interpreted with

some caution, suggesting that further studies are needed to determine the relevance of these implications.

Our study showed positive correlations between the pairs of indicators (log CFU/g) in halal and non-halal samples (combined), which agree with the findings reported by [45]. Typically, the APCs or ECs, consisting of a larger number of bacterial genera, can have low-to-medium levels without any (non-detected) or with few bacteria detected from a smaller indicator group (e.g., generic *E. coli* (ECC)). Figure 4 shows that the presence or absence of ECCs in samples was predicted by the level of TCCs, followed by the level of ECs, followed by the level of APCs. Our results showed the superiority of the level of TCCs over the level of ECs in predicting the presence of ECCs. However, the APC levels were a comparatively poor predictor of the presence of ECCs (r = 0.31). Moreover, the correlations between the levels of the indicator organisms were expected as ECC is a sub-group of TCC, which is a sub-group of the EC family, which is a subset of the APC group. Notwithstanding, the APC remains an appropriate indicator of the overall load of aerobic bacteria in terms of meat quality (spoilage bacteria) rather than meat safety (pathogenic bacteria). Furthermore, the degree of correlation can provide a basis for determining when and whether the results of one test can be replaced by another [45].

5. Limitations

This study had two limitations. The first was the lack of an official list of halal and non-halal markets to be selected from the geographic region of study. We created our own list based on publicly accessible data to determine the number and locations of halal and non-halal markets in the three states, so we might not have included all markets. Another limitation was the difficulty of collecting samples because of the large distances between the three state markets and the study center (Clemson University). Hence, this resulted in a small sample size and non-convergence between market visits.

6. Conclusions

The microbiological quality of the halal and non-halal beef cuts in this study was substantially different from that determined in microbial surveys of large markets in the United States that display a high level of food safety, sanitation, and in-house monitoring. The high levels of indicator organisms in both the halal and non-halal samples indicated the same sanitary conditions between the two types of small retail markets. We concluded that the size of the business (retail meat markets) and not the halal classification is associated with microbial contamination. Both types of markets cut meat to order, which could possibly explain the indicator levels being above the acceptable levels. In addition, the non-use of proper packaging for meat in both types of markets may have decreased the protection against microbial contamination. Meat markets may need to be inspected more frequently by the appropriate regulatory authority, as less frequent inspections (1–4 times/year) may lead to the negligent implementation of food safety regulations. Further research in halal retail markets is needed to extensively assess the microbial status of other halal meats in several U.S. states. Moreover, further research is recommended to identify the causes of the differences in microbial contamination between halal and non-halal meat in terms of seasonal variation.

Author Contributions: O.A.A.-M. and A.M.F.: conceptualization; O.A.A.-M. and X.J.: methodology; O.A.A.-M.: data curation; O.A.A.-M. and W.C.B.: statistical analysis. O.A.A.-M. and A.M.F.: writing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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