

The prevalence of *Escherichia coli* O157:H7 and *Salmonella* in Frozen and Fresh Goat Meat

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ABSTRACT

The demand for goat meat is on the rise in the U.S. A common concern in the production and harvest of animals for human consumption is the presence of foodborne pathogens, *Escherichia coli* O157:H7 and *Salmonella*. As an “under-researched food vehicle”, there are minimal strategies for the control and prevention of foodborne pathogens with goat meat. Freezing can be an effective way of preventing microbial growth in meat, but it does not eradicate bacteria. Five samples of fresh and frozen goat meat, respectively, were collected and utilized in prevalence determination and microbial quality comparison. Using selective and differential media, 25 grams from each sample were tested for both *E. coli* O157:H7 and *Salmonella*. Three of five frozen samples contained *E. coli* O157:H7 suspects ($p = 0.08377$); whereas no *E. coli* O157:H7 suspects were detected with the fresh samples. Two fresh samples and one frozen sample were indicative of the presence of *Salmonella*. Frozen samples were more heavily contaminated than fresh samples, but overall, there was a low prevalence of these bacteria in the two meat types. These findings could be further substantiated with the inclusion of larger sample size and more confirmative tests.

Introduction

There has been an influx to the United States of different cultures that have goat as an important food source. In addition, there is an increase in the consumption of “ethnic” foods as people explore different cuisines. This has made the national market for goats to grow in popularity. According to the National Agricultural Statistics Service (NASS), the total meat goat inventory of the U.S. in 1992 totaled 591,543 head (USDA-NASS, 1992) and grew to 2,075,000 head in 2018 (USDA-NASS, 2019).

These meat products are subjected to process contamination and temperature regulation. A foodborne illness can result, and bacteria such as *Salmonella* and *Escherichia coli* O157: H7 could be the cause (Barkocy-Gallagher et al., 2003). *Escherichia coli* O157: H7 is a zoonotic, enterohemorrhagic bacterial strain that is responsible for hemolytic uremic syndrome, diarrhea, and hemorrhagic colitis in people (Larzabal et al., 2020).

Salmonella is a group of gram-negative, facultative-anaerobic bacilli. There are more than 2000 known serovars, but a proportion are primarily associated with causing symptoms such as mild gastroenteritis to severe illness or death in humans (Healy & Bruce, 2019). These bacteria are introduced via fecal-oral route of transmission (Gerba, 2009) and can further contaminate at the meat market through indecorous practices (i.e., improper handwashing and mishandling with processing).

Refrigeration for fresh meat products sold at retail is usually between 2-5° C (Mohamed, 2017), and freezing temperature is -18 °C. Refrigeration and freezing both affect bacteria, but these microorganisms are not completely eliminated as the temperature is lowered with freezing. There are limited studies and control strategies on the control and prevention of *Salmonella* and *E. coli* O157:H7 in small ruminants at the retail level. Covered under the U.S. Federal Meat Inspection Act of 1906, harvest of goat meat falls under federal or state inspection (USDA-FSIS, 2013). The objectives of this study were to establish the prevalence of *Salmonella* and *E. coli* O157:H7 in goat meat once it

has reached the consumer and to compare the microbial quality between fresh and frozen goat meat. These findings could serve as fundamental information and establish an estimated baseline of the national prevalence of *Salmonella* and *E. coli* O157:H7 in goat meat products for Food Safety and Inspection Services (FSIS) of the United States Department of Agriculture (USDA) to regulate the fast-growing goat products market.

Materials and Methods

Sample Collection

Frozen and fresh raw goat meat samples (n=5, respectively) were bought from three retail markets in Baton Rouge, LA. (The stores were designated as K, G, and L). One to two pounds were obtained for each of the ten samples during February and March of 2021. Samples were kept cool in a cooler with ice packs and taken to Southern University and A&M College in Baton Rouge, LA. Fresh raw meat samples were processed immediately; while frozen goat meat samples were stored at -20 °C before use.

Detection and enumeration of Escherichia coli O157:H7

Enterohemorrhagic *Escherichia coli* (EHEC) enrichment broth (EEB) was prepared by suspending 33 g of modified tryptic soy broth (mTSB) in 1 L of distilled and supplemented with 1.8 mg of vancomycin, 0.01125 mg of cefixime, and 2.25 mg of cefsulodin (VCC; Sigma-Aldrich, St. Louis, MO). After a high-speed homogenization (Interscience BagMixer® 400, Topac Inc., Cohasset, MA) of 25 g of meat in a volume of 225 ml of EEB for 2 min, the suspension was incubated at 35 °C for 24 h.

Selective, differential media such as sorbitol MacConkey agar (SMAC; March & Ratnam, 1986) and sorbitol MacConkey medium with added cefixime and tellurite (CT-SMAC) have been formulated for the detection of *E. coli* O157:H7 (Nataro & Kaper, 1998; Müller et al., 2002). After samples were homogenized, ten-fold serial dilutions were prepared with buffered peptone water (BPW; Sigma-Aldrich, St. Louis, MO), and 0.1 ml of diluents was spread plated onto CT-SMAC (Sigma-Aldrich, St. Louis, MO). The plates were allowed to incubate at 37 °C for 24 h. Following incubation, colorless colonies (*E. coli* O157:H7 suspects) on plates containing 30-300 colony-forming units (CFU) were enumerated and multiplied by the dilution factor to produce the CFU/gram of meat.

Proportion tests were used in the statistical analysis of CFU data. The detection of the presence of *E. coli* served as the proportions of successes compared with the tests. The R Core Team (2020), R Foundation for Statistical Computing, was used in conducting analysis.

Detection of Salmonella

The current horizontal culture method (ISO 22964, 2017) with modifications was utilized as the detection procedure for *Salmonella*. The first step included a pre-enrichment culture in a non-selective liquid medium, such as BPW. Approximately 225 ml of BPW were added to 25 g of raw meat samples, and using a stomacher, samples were homogenized for 2 min at high speed and then incubated at 35 ± 2 °C for 20-24 h. Rappaport Vasiliadis broth (RV) was employed after the 24-h non-selective enrichment with the addition of 0.1 ml of the BPW mixture to 9.9 ml of RV (Millipore Sigma, Burlington, MA) in Pyrex glass tubes. The samples were incubated at 42 ± 0.5 °C for 22-24 h.

After incubation, bacterial growth was observed by development of a cloudy appearance or turbidity. *Salmonella* spp. are selected due to the presence of malachite green and the low pH with a high osmotic pressure (Peterz et al., 1989). Confirmation of a presumptive negative (clear medium) could be made through subculture onto solid media, e.g., Brilliant Green Agar, Xylose Lysine Deoxycholate agar, or other appropriate *Salmonella* media.

Results

Tests for *Escherichia coli* O157:H7 and *Salmonella* species were conducted with 5 fresh and 5 frozen raw goat meat samples from 3 local retail stores in Baton Rouge, LA. Samples with colorless colonies were positive suspects for *E. coli* O157:H7. A display of strong turbidity amongst the samples was considered a positive suspect for *Salmonella* species.

After a serial dilution of 1:10 to 10^{-7} , the five frozen goat meat samples were spread plated onto sorbitol MacConkey medium supplemented with cefixime and tellurite (CT-SMAC). Plates with 30-300 colonies were used in enumeration. Three of the five frozen samples had shown colonies that were *E. coli* O157:H7 suspects, and this included a sample from every retail meat market. An amount of 3×10^8 CFU/ml was calculated for the sample 2 from retail market G (G2) and the sample 1 from store K (K1), and this amount was the highest (Table 1). An observation of 13×10^7 CFU/ml was made with sample 1 from L (Table 1). At a significance level of 10%, there was no significance, $p = 0.1621$, in the proportion of successes in samples G2, K1, and L1.

Table 1. Results of *Escherichia coli* O157:H7 and *Salmonella* species detection and enumeration in frozen goat meat samples.

Sample	Type	<i>E. coli</i> O157:H7	<i>Salmonella</i> species
G1	Frozen	Negative	Negative
G2	Frozen	3×10^8 CFU/ml	Negative
L1	Frozen	13×10^7 CFU/ml	Negative
L2	Frozen	Negative	Negative
K1	Frozen	3×10^8 CFU/ml	Positive

After a display of a strong turbidity, there was one frozen sample, K1, that was considered a *Salmonella* spp. positive suspect (Table 1). During the enrichment step there was a greenish-yellow color change observed with sample 1 from G (G1). However, there was no turbidity after incubation in the Rappaport-Vassiliadis (RV) broth, and this sample was considered as negative for *Salmonella* species.

A serial dilution from 1:10 to 10^{-7} was performed with the five fresh goat meat samples. With *E. coli* O157:H7, no colonies were observed for sample f2 from L (fL2). Reddish colonies could be seen with samples f1 of G (fG1), f1 of L (fL1), and f1 of K. *Escherichia coli* O157:H7 would have colorless colonies; therefore, these samples were considered as negative for this strain (Table 2). A single colorless colony was seen with sample f2 of G (fG2), and this amount was not enough to enumerate. The serial dilution was repeated for samples fG2 and fL2, and a more concentrated dilution was spread plated onto CT-SMAC. Colonies not indicative of *E. coli* O157:H7 resulted, and these samples were considered as negative (Table 2).

Table 2. Results of *Escherichia coli* O157:H7 and *Salmonella* species detection and enumeration in fresh goat meat samples.

Sample	Type	<i>E. coli</i> O157:H7	<i>Salmonella</i> species
fG1	Fresh	Negative	Positive
fG2	Fresh	Negative	Negative
fL1	Fresh	Negative	Negative
fL2	Fresh	Negative	Negative
fK1	Fresh	Negative	Positive

Out of the two types of meat, there were more frozen goat meat samples that had positive *E. coli* O157:H7 suspects (3/5 or 60%) compared to no *E. coli* O157:H7 suspects detected in the fresh goat meat samples (0/5 or 0%), and there was a significant difference ($p = 0.08377$) at a significance level of 10%. At least one *Salmonella* spp. suspect was found in both meat types, in which frozen meat had 1/5 (20%) and the fresh samples had 2/5 (40%). At one location, K, a *Salmonella* spp. suspect was found in both frozen and fresh meat.

Discussion

With its moniker as the “melting pot,” the U.S. is home to a blend of cultures. With these different cultural backgrounds, there is a diversification of cuisines, which would include consumption of more types of red meat such as goat. Although there is an increase in goat meat popularity, there is limited research with this type of meat, especially with foodborne pathogens.

Exploratory studies could provide the prevalence and other information on foodborne pathogens, such as *Escherichia coli* O157: H7 and *Salmonella* species, in which future studies can expound. Under the U.S. Federal Meat Inspection Act of 1906, goat meat must be slaughtered under inspections at the state or federal level (USDA-FSIS, 2013). The USDA, FDA, or other government agencies would be able to utilize these studies’ findings to more effectively strategize the control and prevention of foodborne pathogens with this animal species.

In this study, three of the five frozen samples were positive *Escherichia coli* O157: H7 suspects. No suspects were detected with the fresh samples. The fresh samples seem to be contaminated with *E. coli* O157: H7. The presence of *E. coli* O157:H7 could be confirmed with molecular testing (polymerase chain reaction with *E. coli* O157:H7 gene specific primers) and serological testing (anti-*E. coli* O157 magnetic beads and latex agglutination).

One of the five frozen samples was a positive suspect for *Salmonella* species. *Salmonella* spp. suspects were detected in two of the five fresh samples. During the enrichment step, one sample (G1) did exhibit a greenish yellow color change. This change is not seen with *Salmonella* spp., but it can be displayed with *Staphylococcus aureus*. The detection of *Salmonella* spp. could be confirmed with a subculture performed onto solid media, e.g., Brilliant Green Agar, Xylose Lysine Deoxycholate (XLD) agar, or on other suitable *Salmonella* media after incubation in the Rapaport-Vassiliadis (RV) broth. Biochemical and serological testing could be used to further identify the suspicious colonies grown on the solid media.

In comparison, the fresh goat meat had no *E. coli* O157:H7 positive suspects; while the frozen meat samples did. With more turbid samples in the RV broth, fresh goat meat produced more positive suspects for *Salmonella*. There was one retail store (K), in which *Salmonella* spp. suspects were detected in both meat types. Since meat was purchased at different times, it is unsure whether it was due to possibly contaminated meat being supplied to the store or by the handling of workers. Overall, there was a low prevalence of these pathogens detected in these meat types. This may have been attributed to the increased sanitization as a result of the heightened awareness with the COVID-19 pandemic.

In order to substantiate these findings, samples from other places outside of East Baton Rouge Parish should be included. This could provide additional prevalence rates. A larger sample size would enhance the statistical power. Also, samples before the retail level should be utilized to provide additional explanation of contamination routes. Additional tests should be used in order to confirm and identify the presence of these foodborne pathogens.

Conclusion

The broad spectrum of foodborne pathogens is evolving. Some pathogens have been controlled or eliminated; while new ones have emerged and spread globally. *Escherichia coli* O157:H7 and *Salmonella* are two of the most important bacterial pathogens of public health, in which both could lead to a foodborne illness. These bacteria have been found contaminating different meats such as beef and poultry worldwide. The uptick in goat meat consumption here in the U.S. may spawn more cases of foodborne illnesses with this species of meat. Research with this red meat, could pave the way for more science-based information for future studies. In terms of regulation, this in turn could be beneficial to federal government agencies. With better prevention and control, contamination would decrease as the demand for goat meat would increase.

Limitations

This study took place during the COVID-19 pandemic, and this posed some challenges. The stock and shipment of supplies were impacted. Some items were out of stock or on backorder, and shipping times were delayed. These issues in conjunction with approaching deadlines and graduation resulted in the tests that were run for this study. With additional tests, more confirmation and identification could have transpired. Also, in order to ensure safety, off-campus travel had limitations, and in some instances, it was restricted. The project was modified from sample collections on-farm with varying producers and processing facilities to the inclusion of only postmortem samples from retail markets. This also caused the fresh and frozen goat meat samples to be collected from stores of East Baton Rouge Parish. Samples collected outside of this parish would have provided more insight into the prevalence of these foodborne pathogens in this species of meat at the retail market level.

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