

## A Look at *E. coli* Contamination in Homemade Cheeses across Kabul's Districts

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### ABSTRACT

Foodborne illness remains a major public health concern, and dairy products—especially homemade cheeses—are frequently linked to pathogen transmission. In Kabul, the safety of such locally produced items has received little research attention. This study offers the first initial look at *E. coli* contamination in the city's homemade cheese supply, using it as a reliable marker of fecal pollution and poor hygienic handling. The work aimed to estimate how widespread the contamination is and whether it varies across different districts. To explore this, a cross-sectional design and collected 51 cheese samples from producers spread across seven districts. All samples underwent standard microbiological testing to isolate and confirm *E. coli* presence. Out of the 51 samples, 4 tested positive, giving an overall prevalence of 7.84%. Yet the numbers weren't uniform across the city. District 17 stood out clearly, with a much higher rate of 28.57%. This uneven pattern suggests that certain areas face greater hygiene challenges than others what these results make clear is that while sanitation issues exist in the local production system, the risk isn't spread evenly—it clusters in specific neighbourhoods. That's an important clue for public health planning. Rather than applying generic measures everywhere, it would be wiser to concentrate monitoring and awareness campaigns on high-burden districts like District 17. Such focused action would do more to cut down foodborne risks and make this cherished staple safer for Kabul's population.

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## INTRODUCTION

Foodborne diseases remain a major global public health concern, causing significant morbidity, mortality, and economic burden worldwide. Contaminated foods of animal origin, particularly milk and dairy products, are recognized as important vehicles for the transmission of pathogenic microorganisms to humans (De Buyser et al., 2001; Havelaar et al., 2015). Among foodborne pathogens, *Escherichia coli* (*E. coli*) is considered one of the most important indicators of fecal contamination and poor hygienic practices during food production, handling, processing, and distribution (Jay et al., 2005; ICMSF, 2011). Certain

pathogenic strains, especially Shiga toxin-producing *E. coli* (STEC), are capable of causing severe gastrointestinal disease, hemorrhagic colitis, and hemolytic uremic syndrome, particularly in children, elderly individuals, and immunocompromised populations (Kaper et al., 2004; WHO, 2025).

Traditional and homemade dairy products prepared from raw or insufficiently pasteurized milk have frequently been associated with microbial contamination and foodborne outbreaks in many developing countries (De Buyser et al., 2001; Grace, 2015). Several international studies have demonstrated the occurrence of *E. coli* contamination in cheese and other dairy products. In Kazakhstan, (Aitkaliyeva et al., 2024) reported a contamination rate of 31.4% in commercial cheese samples collected from different regions. Similarly, studies conducted in Romania, Serbia, Slovakia, and Turkey confirmed the widespread occurrence of *E. coli* contamination and antimicrobial resistance among isolates recovered from traditional cheese products (Imre et al., 2022; Mladenović et al., 2018; Vrabec et al., 2015; UeNLUe et al., 2011). These findings indicate that traditional dairy products can serve as important reservoirs for pathogenic and antimicrobial-resistant bacteria, especially where hygienic standards and regulatory monitoring are inadequate.

In Afghanistan, homemade cheese is widely consumed and represents an important source of nutrition and household income. However, production and marketing are commonly carried out under informal conditions, often involving raw milk, inadequate sanitation, insufficient refrigeration, and limited microbiological quality control. These conditions create favorable opportunities for contamination by pathogenic bacteria, including *E. coli* (Grace, 2015; UNICEF, n.d.). Recent reports have further emphasized the burden of unsafe food consumption in Afghanistan, linking contaminated food and water to a substantial number of illnesses and deaths annually (DID Press, 2025; Salih et al., 2025 ). Despite these public health concerns, there is currently very limited published scientific information regarding the microbiological safety of homemade cheese in Kabul city.

The absence of localized epidemiological data represents a major challenge for evidence-based food safety planning and public health interventions. International organizations such as the World Health Organization have emphasized the importance of foodborne disease surveillance and microbiological monitoring to improve food safety systems and reduce the burden of foodborne illnesses (WHO, 2012). Furthermore, standardized microbiological methods, including ISO protocols for *E. coli* isolation and enumeration, are essential for generating reliable and comparable scientific data (ISO, 2017; Baron et al., 1994). Therefore, this study was designed to provide the first baseline assessment of *E. coli* contamination in homemade cheese marketed in Kabul city, Afghanistan.

The present study was designed to address the following research questions:

- How do different concentrations of ginger extract affect the chemical and sensory quality of apple nectar during storage?
- What level of ginger extract provides the best balance between physicochemical stability and overall consumer acceptability?

## METHODS AND MATERIALS

### Research site

A cross-sectional design was used to estimate the prevalence of *E. coli* in homemade cheeses sold across Kabul and to assess whether contamination levels varied by district. The study was conducted within municipal boundaries, and all procedures were documented in detail to support reproducibility.

### Sample Collection

A purposive sampling strategy was applied, focusing on unpackaged, locally produced cheeses available from small-scale vendors and neighborhood shops. Samples were drawn from seven districts to ensure broad geographic coverage within the city. Each purchased sample was immediately placed into a labeled sterile bag, transferred to a portable cooler maintained at approximately 4°C, and transported to the laboratory within two hours to preserve bacterial integrity prior to analysis.

### Lab Work and Bacterial Detection

For finding and confirming *E. coli*, established international guidelines were followed—specifically, the protocols outlined in ISO 16649 (2017) for food and feed testing. In the laboratory, each cheese sample was first mixed with Buffered Peptone Water and allowed to sit for pre-enrichment. After that incubation period, the enriched broth was taken and spread onto two types of selective agar plates: Eosin Methylene Blue Agar and Violet Red Bile Agar. These media help separate the target bacteria from other microorganisms (Feng et al., 2020).

Any colonies that looked like they might be *E. coli* were picked and re-streaked to obtain pure cultures. For definitive confirmation, a set of biochemical tests known as the IMViC series—Indole, Methyl Red, Voges-Proskauer, and Citrate—was performed. A result of ++-- was considered indicative of *E. coli* (Baron et al., 1994). Figure 1 provides a visual overview of the entire process.

### Data Analysis

Every sample was tested twice to ensure consistency of the results. For the primary finding, the presence or absence of *E. coli* was recorded for each case. From there, the overall contamination rate was calculated as a percentage of positive samples out of the total 51. The data were also broken down by district to identify whether certain parts of Kabul stood out, and those numbers have been presented in a table and descriptive format for easy reading.

## FINDINGS

The laboratory confirmation tests identified *Escherichia coli* in four of the fifty-one samples analyzed. This resulted in an overall contamination prevalence of 7.84% for the total sample set from Kabul. A key finding of this study, however, is that this contamination was not uniformly distributed geographically. The analysis revealed distinct variation in prevalence

rates between the sampled districts. Several districts, including Districts 1<sup>st</sup>, 5<sup>th</sup>, 12<sup>th</sup>, and 13<sup>th</sup>, showed no detectable *E. coli* in the collected samples, indicating effective hygiene practices among those producers. In contrast, contamination was isolated to three specific areas: District 11, District 8, and most notably, District 17. This clustering suggests localized factors influencing food safety standards, as opposed to a city-wide uniform risk.

The complete results of the sample analysis, organized by collection district, are presented in Table 1. The table details the number of samples collected in each location, the count of positive and negative results, and the calculated district-specific prevalence rate.

**Table 1.** Prevalence of *Escherichia coli* in Homemade Cheese Samples from Districts of Kabul City

| Collection District | Total Samples Collected (n) | Positive Samples (n) | Negative Samples (n) | Prevalence (%) |
|---------------------|-----------------------------|----------------------|----------------------|----------------|
| District 1          | 7                           | 0                    | 7                    | 0.00           |
| District 5          | 10                          | 0                    | 10                   | 0.00           |
| District 8          | 8                           | 1                    | 7                    | 12.50          |
| District 11         | 7                           | 1                    | 6                    | 14.29          |
| District 12         | 5                           | 0                    | 5                    | 0.00           |
| District 13         | 7                           | 0                    | 7                    | 0.00           |
| District 17         | 7                           | 2                    | 5                    | 28.57          |
| Total               | 51                          | 4                    | 47                   | 7.84           |

As illustrated in Table 1, District 17 exhibited the highest level of contamination, with two out of seven samples testing positive for *E. coli*, yielding a prevalence rate of 28.57%. This rate is substantially higher than the city's average. The other positive results were found in District 11 (one positive out of seven samples, 14.29%) and District 8 (one positive out of eight samples, 12.50%). The results from District 17 represent a significant concentration of risk, marking it as a potential hotspot for foodborne pathogen contamination within Kabul's informal dairy sector. These district-level variations highlight that the risk to consumers is geographically specific and not evenly distributed across the city.

**Table 2.** IMViC Pattern and Gram Staining Results of Confirmed *E. coli* Isolates from Homemade Cheese in Kabul Districts

| Collection District            | Total Positive <i>E. coli</i> Isolates (n) | IMViC Pattern of Isolates             | Gram Staining Result             | Confirmation Status      |
|--------------------------------|--------------------------------------------|---------------------------------------|----------------------------------|--------------------------|
| District 8                     | 1                                          | + + - - (Indole+, MR+, VP-, Citrate-) | Gram-negative rods               | Confirmed <i>E. coli</i> |
| District 11                    | 1                                          | + + - - (Indole+, MR+, VP-, Citrate-) | Gram-negative rods               | Confirmed <i>E. coli</i> |
| District 17                    | 2                                          | + + - - (Indole+, MR+, VP-, Citrate-) | Gram-negative rods               | Confirmed <i>E. coli</i> |
| Total Confirmed <i>E. coli</i> | 4                                          | All isolates: + + - -                 | All isolates: Gram-negative rods | 100% confirmation        |

This table confirms the microbiological identity of the bacterial isolates obtained from contaminated cheese samples. All four positive isolates from Districts 8<sup>th</sup>, 11<sup>th</sup>, and 17<sup>th</sup> demonstrated an identical and definitive biochemical fingerprint. The IMViC pattern of "+ + - -" (Indole positive, Methyl Red positive, Voges-Proskauer negative, Citrate negative) is the gold standard for confirming *Escherichia coli*. Furthermore, the consistent result of Gram-negative rods from the staining procedure aligns perfectly with the expected cellular morphology of *E. coli*. This uniformity is crucial as it confirms that the contamination issue

across different districts is caused by the same indicator organism, ruling out contamination by other types of bacteria. The 100% confirmation rate validates the initial laboratory screening and provides a solid foundation for public health conclusions, as there is certainty that the isolates are *E. coli*.

Table 3 synthesizes the geographical distribution of the confirmed contamination with the microbiological results. It highlights a distinct spatial clustering of risk. While the overall city-wide prevalence is moderate at 7.84%, this figure masks severe localized problems. District 17 is a clear hotspot, with both the highest prevalence (28.57%) and the highest absolute number of confirmed *E. coli* isolates. Districts 8 and 11 represent areas of moderate but confirmed risk. Most importantly, the data reveals significant "cold spots" Districts 1, 5, 12, and 13 showed no contamination whatsoever. This stark geographical disparity strongly suggests that the risk factors are localized and not systemic across Kabul's entire homemade cheese production chain. The contamination is not random; it is concentrated in specific areas, pointing to local failures in hygiene, water quality, or handling practices that require targeted investigation.

**Table 3.** Summary of District-wise *E. coli* Contamination and Confirmation

| Collection District | Samples (n) | Positive Samples (n) | Prevalence (%) | Biochemical Confirmation (IMViC)           |
|---------------------|-------------|----------------------|----------------|--------------------------------------------|
| District 1          | 7           | 0                    | 0.00           | No isolates for testing                    |
| District 5          | 10          | 0                    | 0.00           | No isolates for testing                    |
| District 8          | 8           | 1                    | 12.50          | Confirmed <i>E. coli</i> (+ + - -)         |
| District 11         | 7           | 1                    | 14.29          | Confirmed <i>E. coli</i> (+ + - -)         |
| District 12         | 5           | 0                    | 0.00           | No isolates for testing                    |
| District 13         | 7           | 0                    | 0.00           | No isolates for testing                    |
| District 17         | 7           | 2                    | 28.57          | Both confirmed <i>E. coli</i> (+ + - -)    |
| Total               | 51          | 4                    | 7.84           | All 4 isolates confirmed as <i>E. coli</i> |

**All 4 positive isolates** from Districts 8, 11, and 17 showed the definitive *E. coli* IMViC pattern (+ + - -)\* **All isolates were Gram-negative rods**, consistent with *E. coli* morphology\***District 17** had the highest number of confirmed *E. coli* isolates (2 out of 4 total)\***No *E. coli* contamination** was detected in Districts 1, 5, 12, and 13 (0% prevalence)**Interpretation:** The uniform + + - - IMViC pattern across all positive samples confirms that the contamination in Kabul's homemade cheese is specifically due to *Escherichia coli*, with District 17 representing the highest-risk area for this specific pathogen.

The key takeaway from interpreting both tables together is that Kabul faces a specific, localized *E. coli* contamination problem in its homemade cheese supply. Not merely general bacterial contamination has been detected, but a fecal indicator organism has been specifically identified in pinpointed locations.

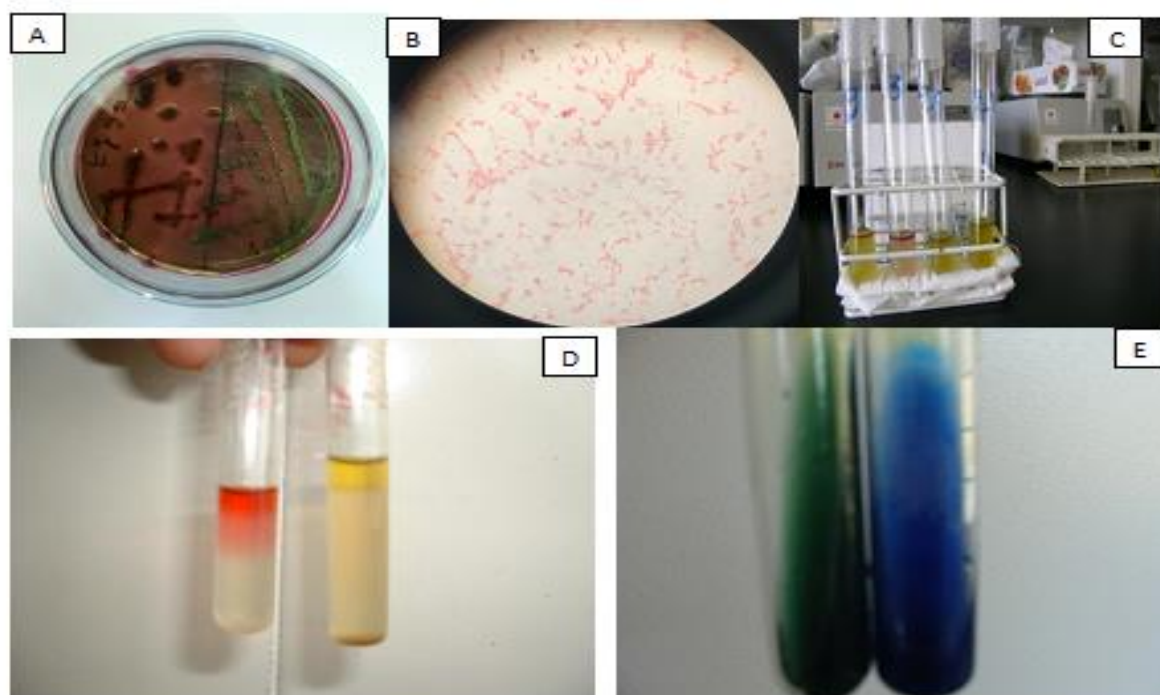
The convergence of the highest prevalence and multiple confirmed isolates in District 17 marks it as the priority area for immediate public health intervention. The evidence argues against a broad, city-wide awareness campaign. Instead, resources for food safety training, vendor inspections, and environmental sampling (especially water sources) should be strategically concentrated in District 17, followed by Districts 11 and 8, to achieve the greatest impact on reducing foodborne illness.

## DISCUSSION

This study provides the first empirical assessment of *Escherichia coli* infection contamination in homemade cheeses across Kabul. The findings revealed an overall contamination rate of 7.84%, with a pronounced and uneven geographical distribution, identifying District 17 as a significant risk hotspot with a prevalence of 28.57%. These results highlight important concerns regarding hygienic practices and food safety standards in locally produced dairy products. This discussion compares the findings with existing regional and international literature and explores their scientific and public health implications within the broader context of food safety in Afghanistan (Ahmadi et al., 2023; Ministry of Public Health, 2021; World Health Organization [WHO], 2020).

The overall prevalence of *Escherichia coli* contamination in the present study was 7.84%, providing an important baseline for evaluating the microbiological quality and hygienic status of milk and dairy products in Kabul. This finding directly addresses the primary objective of the study and reflects the level of fecal contamination associated with milk handling, processing, storage, and marketing practices. Although the detected prevalence was lower than that reported in several international studies, such as the 31.4% contamination rate observed in commercial cheeses in Kazakhstan (Ivanov et al., 2022), the presence of *E. coli* at any detectable level remains a significant public health concern because it indicates inadequate sanitary conditions and the possible presence of pathogenic enteric microorganisms (WHO, 2020). In developing countries, including Afghanistan, contaminated dairy products continue to represent an important source of foodborne infections and gastrointestinal diseases. Recent global health reports have further highlighted the substantial burden of unsafe food consumption on morbidity and mortality in the region (Global Burden of Disease Collaborative Network, 2022).

The accompanying figure (1) systematically demonstrates the standard microbiological identification and confirmation procedures for *E. coli* according to internationally accepted laboratory methods. Figure A shows the characteristic metallic green sheen colonies grown on Eosin Methylene Blue (EMB) agar, which is considered a selective and differential medium for the isolation of *E. coli*. Figure B illustrates the Gram-negative morphology of the bacterial isolates observed after Gram staining. Figure C presents the positive Indole test reaction, confirming the organism's ability to produce indole from tryptophan metabolism. Figure D demonstrates the biochemical confirmation tests, including positive Methyl Red and negative Simmons Citrate reactions, which are essential for the definitive identification of *E. coli*. Collectively, these microbiological and biochemical findings validate the accuracy and reliability of the laboratory diagnosis and support the authenticity of the reported prevalence in this study.



**Figure (1).** Microbiological Isolation and Biochemical Identification of *Escherichia coli* from homemade cheese Samples

***E. coli*** A: the metallic sheen colonies of *E. coli* on Eosin methylene blue agar; B: Gram Negative colonies of *E. coli*; C: results of Indole test test; D: results Methyl Red Simons Citrate test

The most critical and novel finding of this study is the significant geographical clustering of contamination. The concentration of positive samples in Districts 8, 11, and particularly 17 (28.57%), while other districts showed 0% prevalence, strongly suggests that risk factors are highly localized rather than city-wide. This pattern of uneven distribution aligns with findings from Kazakhstan, where contamination levels also varied significantly by region and cheese type (Ivanov et al., 2022). Studies in neighboring regions show similar patterns; for example, Al-Dulaimy and Al-Mayah (2019) documented contamination clusters in dairy products from specific governorates in Iraq, and research in Iran has isolated various *E. coli* serotypes from regional dairy samples (Farhadkhani et al., 2018). The high prevalence in District 17 indicates potential localized issues such as compromised water sources—a severe and widespread problem in Afghanistan where two-thirds of the population is impacted by drought and access to clean water is limited (United Nations Children's Fund [UNICEF], 2023). Other factors could include common milking or processing practices among a cluster of producers or specific market-level handling problems. This finding provides a clear, evidence-based target for targeted public health intervention, moving beyond generic city-wide advisories.

When interpreting the presence of *E. coli* as an indicator, it is essential to consider the potential for more severe pathogens like Shiga toxin-producing *E. coli* (STEC), which cause serious foodborne disease (WHO, 2020). While this study confirmed the presence of the indicator, the absence of virulence gene testing means the pathogenic potential remains unknown. This gap in characterization is common in preliminary surveys. However, studies in

comparable settings underscore the risk. For example, in Kazakhstan, the enter pathogenic strain *E. coli* O157:H7 was detected in 0.5% of cheese samples, and isolates showed high levels of antibiotic resistance (Ivanov et al., 2022). Furthermore, a review highlights that food handlers can be key contributors to transmitting antimicrobial-resistant *E. coli*, with colonization rates among handlers varying widely (2.8% to 89.3%) globally (Smith et al., 2021). The detection of generic *E. coli* in Kabul's cheese, therefore, serves as a valid sentinel for these higher risks, especially in a context where consumer knowledge of food safety is only moderate (Ahmadi et al., 2023) and the healthcare system is fragile (Global Burden of Disease Collaborative Network, 2022& Khafaji et al 2025).

This study has several limitations that must be acknowledged. The sample size, while sufficient for a preliminary survey, is modest, and the cross-sectional design provides only a snapshot in time. Seasonal variations in milk quality and production practices could affect contamination rates. Furthermore, the study did not include detailed surveys of producer practices, which limits the ability to correlate specific behaviors (e.g., use of raw milk, cleaning protocols, water source) with the microbiological results. Despite these limitations, the study successfully achieves its core objectives of establishing a baseline prevalence and identifying risk hotspots, providing a robust foundation for future, more detailed investigations.

The implications of these findings are both practical and significant for public health strategy in Afghanistan. Efforts must shift from general awareness to prioritized, risk-based interventions. Resources for hygiene training and monitoring should be concentrated in identified high-prevalence districts, beginning with District 17. Training should emphasize the WHO's "Five keys to safer food," particularly the use of safe water, a critical challenge in Afghanistan (UNICEF, 2023; WHO, 2020), and thorough cooking. Future research must build on this baseline by: (1) conducting virulence characterization of isolates to assess the direct threat of STEC; (2) implementing case-control studies in hotspots to identify specific behavioral and environmental risk factors; and (3) evaluating the impact of targeted training interventions. Such an evidence-based, focused approach is essential to mitigate the substantial burden of foodborne disease and strengthen the safety of this vital component of the local food system (Global Burden of Disease Collaborative Network, 2022; Ministry of Public Health, 2021).

## CONCLUSION

This study provides the first foundational data on the microbiological safety of homemade cheese in Kabul, Afghanistan. The analysis confirms that *Escherichia coli*, a critical indicator of fecal contamination, is present in Kabul's cheese supply, establishing an overall prevalence of 7.84%. Crucially, the research reveals that this contamination is not uniformly distributed but is concentrated in specific geographical hotspots, most notably in District 17, which exhibited a markedly high prevalence of 28.57%.

The scientific merit of this work lies in its dual contribution. First, it moves the discourse on food safety in Afghanistan from general assumptions to evidence-based assessment, providing a concrete baseline against which future interventions can be measured. Second, by identifying significant district-level variation, it challenges the notion of a uniform risk profile and demonstrates that public health risks are highly localized. This finding is novel for the Kabul context and provides a powerful scientific justification for moving beyond broad awareness campaigns to a targeted, risk-based intervention strategy.

The practical implication of this conclusion is clear and urgent. Public health resources and food safety initiatives should be strategically prioritized in identified high-risk areas, beginning with District 17. Interventions must focus on the critical control points in the local production chain, likely related to water source safety, milking hygiene, and equipment sanitation, as indicated by the international literature and the local context of water scarcity. Producer education based on the WHO's "Five keys to safer food" is essential.

To build upon this foundational study, future research should pursue three key directions: (1) the molecular characterization of isolated *E. coli* to determine the presence and prevalence of pathogenic strains, such as Shiga toxin-producing *E. coli* (STEC); (2) detailed epidemiological surveys within hotspot districts to identify the precise behavioral, environmental, and socio-economic factors driving the elevated contamination; and (3) the design and evaluation of practical, culturally appropriate training programs for producers in these high-risk zones. By following this evidence-based pathway, stakeholders can work effectively to mitigate a tangible public health risk, protect consumers, and strengthen the safety and sustainability of Afghanistan's vital informal dairy sector.

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## Ethics Statement

Ethical clearance for this study was obtained from the Research Ethics Committee of the Faculty of Veterinary Sciences, Kabul University. All sampling and laboratory procedures followed the institution's ethical guidelines, and proper consent was secured from all participating vendors.

## Conflict of Interest Statement

The author declares that there are no known conflicts of interest, financial or personal, that could have influenced the work reported in this paper. No funding was received for conducting this study or for the preparation of this manuscript.

## Author Contributions

Sayed Arif Ahmadi was responsible for the entire study from start to finish. He came up with the original idea, designed the research framework, and oversaw the sample collection process across Kabul's districts. He also carried out all the laboratory work; from preparing samples and running microbiological tests to confirming *E. coli* presence. In addition, he wrote the first draft of the manuscript, handled all revisions, and prepared the final version for submission.

## Data Availability Statement

The data generated during this study are included in this article and its accompanying tables. Raw lab records and detailed microbiological sheets are kept on file with the corresponding author and can be made available upon reasonable request. Because of the nature of the fieldwork and vendor privacy, some location-specific details have been kept general to protect participant identities.

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