

## Isolation of salmonella from Raw milk and Cheese

Ziaulislam Nowbahar\*<sup>1</sup>, Ahmad Farid Rawan<sup>2</sup>, Hajigul Hamat<sup>3</sup>, Noori Gul Nabi<sup>4</sup> and Hayatullah Takal<sup>5</sup>

<sup>1</sup>Department of Animal Science, Faculty of Agriculture, Zabul Institute of Higher Education, Qalat, Afghanistan.

<sup>2</sup>Pre-Clinic Department, Veterinary Science Faculty, Nangarhar University, Jalalabad, Afghanistan.

<sup>3</sup>Department Animal Science, Faculty of Agriculture, Zabul University, Qalat, Afghanistan.

<sup>4</sup>Department of Animal Science, Faculty of Agriculture, Zabul University, Qalat, Afghanistan.

<sup>5</sup>Department of Animal Science, Faculty of Agriculture, Zabul University, Qalat, Afghanistan.

\*Corresponding author email: [dr.nowbahar21@gmail.com](mailto:dr.nowbahar21@gmail.com)

### ABSTRACT

Milk and dairy products are considered among the most valuable nutritional sources for humans because they provide all the essential nutrients, including proteins, fats, carbohydrates, vitamins, and minerals, the current study aimed to isolation of salmonella from raw milk and cheese In this study, 15 samples of raw milk and 15 samples of cheese, making a total of 30, were collected from different milk and cheese vendors in Jalalabad city. from November 22, 2017, to April 19, 2018, The samples were placed in sterilized bottles and transferred within 24 hours to the Microbiology Laboratory of the Faculty of Veterinary Science. The samples were cultured, subjected to Gram staining, and examined under a microscope for the identification of *Salmonella* bacteria .The results showed that , among cheese samples, 1(7%) were positive for salmonella and 14 (93%) were negative. In raw milk samples, 0(0%) were positive for salmonella and 15(100%) were negative. It can therefore be concluded that Salmonella was detected at a very low prevalence in raw milk and cheese, whereas other microorganisms, such as *Shigella*, were present at a considerably higher rate.

**Keywords:** Cheese, Jalalabad, Raw milk, Salmonella and S.S Agar

### INTRODUCTION

Milk and dairy products are widely consumed due to their high nutritional value, providing essential proteins, fats, carbohydrates, vitamins, and minerals necessary for human health (FAO, 2019; Walstra et al., 2018). However, milk is a highly perishable food that can serve as a medium for the growth of pathogenic microorganisms when hygienic practices are inadequate during production, handling, or storage (EFSA, 2020). Among foodborne pathogens, Salmonella is one of the most significant zoonotic bacteria associated with raw milk and dairy products, causing gastroenteritis and systemic infections in humans worldwide (WHO, 2018; Majowicz et al., 2010). Contamination can occur internally through infected mammary glands or externally from the environment during milking, transportation, processing, and marketing. Raw milk provides favorable conditions for the survival and multiplication of Salmonella and other pathogens due to its rich nutrient composition and near-neutral pH (Claeys et al., 2013; Verraes et al., 2014). Previous studies have reported the presence

of Salmonella in raw milk and dairy products in various regions, with prevalence rates depending on production systems and hygienic conditions (Grace et al., 2015; EFSA, 2020). Globally, milk and dairy products are widely consumed, with consumption patterns influenced by cultural, economic, and climatic factors (Torres et al., 2012; FAO, 2006). Cheese, a major dairy product, is produced through the coagulation of milk proteins facilitated by enzymes, lactic acid bacteria, and optimal temperature and humidity (Raheem et al., 2009; Sanoyomi et al., 2010). The nutritional quality and safety of milk and its derivatives, however, depend on proper handling, hygiene, and storage (Stanton et al., 2013; Claeys et al., 2013). Raw milk is particularly susceptible to microbial contamination due to its nutrient richness and near-neutral pH, which support the growth of pathogenic microorganisms, including Salmonella, Escherichia coli, and Shigella species (Verraes et al., 2014; EFSA, 2020). Salmonella is a gram-negative, facultative anaerobic bacterium that causes salmonellosis in humans and animals (WHO, 2018; Bhunia et al., 2008). Infection can lead to gastroenteritis, typhoid-like symptoms, dehydration, and occasionally systemic infections,

with higher severity observed in children, elderly, and immunocompromised individuals (De Valk et al., 2000; Fontaine et al., 1980). Milk can become contaminated internally through infected mammary glands, mastitis, or bacteremia, and externally from environmental sources, unhygienic milking practices, or contaminated containers and equipment (Claeys et al., 2013; EFSA, 2015; Scicon, 2011). The prevalence of Salmonella in raw milk varies globally, with higher rates in developing regions where traditional dairy production and informal marketing dominate (Grace et al., 2015; Girmavd et al., 2009). Studies have documented isolation of Salmonella Typhimurium and other serotypes from cow milk and cheese, highlighting the need for improved monitoring and food safety interventions (Wray et al., 1974; Gran et al., 2003). Contamination not only poses public health risks but also affects the economic value of dairy products, as microbial quality often determines market acceptance (Weaver et al., 2013; Ralph et al., 1998). In Afghanistan, particularly in Jalalabad city of Nangarhar Province, raw milk and traditionally produced cheese are widely consumed. The region experiences a warm climate, informal dairy supply chains, and limited regulatory oversight, increasing the likelihood of microbial contamination (Shresth et al., 2011). Despite the critical role of dairy in human nutrition and local economy, recent data on the prevalence of Salmonella in dairy products from this region remain scarce (FAO, 2008; Mourad et al., 2014). Understanding the microbiological quality of milk and cheese in such settings is vital for public health, informing policies for safe production, handling, and distribution of dairy products (Torres et al., 2012; Stanton et al., 2013). Milk is an energy-rich food and a good source of high-quality protein (Mourad et al., 2014). In general, microorganisms can enter milk in two ways: (1) internally, through the bloodstream directly into the udder, a condition known as mastitis, and (2) externally, through contamination from the environment (Claeys et al., 2013, EFSA, 2015, Scicon, 2011, Verraes et al., 2014). The microbiological quality of milk decreases due to contamination, as it provides an ideal medium for the growth and survival of various microorganisms because it is fresh and nutrient-rich (Stanton et al., 2013). The primary function of milk as food is to nourish the young of mammals. Global demand for milk is increasing, and in 2015 its market value rose by 25% (FAO et al., 2008). Milk and its products provide significant nutritional value for people and animals of all ages (Torres et al., 2012). However, when milk is obtained from healthy animals but is improperly handled, stored, or transported, it can cause infectious and toxic conditions in humans (Shresth et al., 2011). Therefore, this study was designed to

isolate Salmonella from raw milk and cheese sold in Jalalabad city, Afghanistan, aiming to provide baseline data that can inform future food safety strategies and public health interventions.

## MATERIALS AND METHODS

### *Study Area*

This study was conducted in Jalalabad, Nangarhar Province, Afghanistan, from November 22, 2017, to April 19, 2018, with the objective of isolating and identifying *Salmonella* from raw milk and cheese. Jalalabad is located in eastern Afghanistan, approximately 150 km from the capital city, Kabul. The city lies at a latitude of 34.43°N and a longitude of 70.45°E, with an elevation of approximately 575 meters above sea level. Jalalabad has a hot desert climate (Köppen: BWh), characterized by very hot summers and mild winters. The mean annual temperature is 21.5°C, with the hottest month being July (up to 45°C) and the coldest month January (around 2°C). The average annual rainfall is 247 mm, occurring over approximately 61 days per year, mainly concentrated in winter and early spring. These environmental and climatic conditions are important factors influencing dairy production, microbial contamination, and the prevalence of *Salmonella* in raw milk and cheese in the region (Wikipedia, 2024, Weatherspark, 2024; NomadSeason, 2024). For this research, milk and cheese samples were collected from various vendors in different parts of Jalalabad city. All collected samples were analyzed in the Microbiology Laboratory of the Faculty of Veterinary Science.

### *Samples Collection*

A total of 15 raw milk samples and 15 cheese samples were randomly collected from various milk and cheese vendors across Jalalabad city. The sample size was selected based on previous regional studies and laboratory feasibility, ensuring sufficient data for preliminary estimation of Salmonella prevalence. Random sampling was employed to provide a representative overview of the milk and cheese available in different parts of the city.

### *Study Methodology*

A total of 15 raw milk samples and 15 cheese samples were randomly collected from various milk and cheese vendors across Jalalabad city. The sample size was selected based on previous regional studies and laboratory feasibility to

provide sufficient data for preliminary estimation of Salmonella prevalence. Samples were transported to the Microbiology Laboratory within 24 hours under cold chain conditions (4°C) to maintain microbial viability. Initially, nutrient broth medium was prepared by dissolving 13 g of broth powder in 1000 mL of distilled water. Both the serum cups and the broth medium were sterilized in an autoclave at 121°C for 20 minutes. After sterilization, 1–2 mL of broth was transferred into sterile serum cups and incubated at 37.5°C for 12–24 hours to enrich potential Salmonella bacteria. Salmonella-Shigella (S.S.) Agar was prepared by dissolving 63 g of medium in 1000 mL of distilled water with 1–2% agar, boiled, cooled slightly, and poured into sterile Petri plates under aseptic conditions. A small portion of the enriched broth culture was streaked onto the S.S. Agar plates using a sterile inoculation loop. The plates were labeled and incubated at 37.5°C for 24–48 hours, and colonies showing black centers were considered presumptive Salmonella. A small portion of these colonies was picked to prepare a thin smear on a clean glass slide, which was then heat-fixed by passing over a flame. Gram staining was performed by covering the slide with crystal violet for 5 minutes, rinsing with distilled water, applying iodine solution for 3 minutes followed by rinsing, decolorizing with alcohol for 1 minute, rinsing again, and finally staining with safranin for 30 seconds. The slide was air-dried and observed under an oil immersion lens at 100× magnification. All procedures were conducted following standard QA/QC protocols, with sterile media, reagents, and equipment verified prior to use. The study was conducted following ethical guidelines for the collection and handling of animal-derived food samples, and approval was obtained from the Ethical Committee of the Faculty of Veterinary Science, Nangarhar University.

### **Statistical Analysis**

Data from this study were processed and analyzed in MS Excel, with results illustrated through tables and line graphs.

## **RESULTS**

A total of 30 samples, consisting of 15 raw milk and 15 cheese samples, were collected from different vendors in Jalalabad city. These samples were analyzed in the Microbiology Laboratory of the Faculty of Veterinary Science using culture methods, followed by Gram staining.

### **Cheese Samples:**

Out of 15 cheese samples, 1 sample (7%) was positive for Salmonella, while the remaining 14 samples (93%) were negative. Regarding other microorganisms (Shigella), 12 samples (80%) were positive and 2 samples (13%) were negative, indicating the presence of bacteria such as Shigella, which were not further identified. These findings suggest that although Salmonella contamination in cheese was low, other microbial contamination (Shigella) was relatively high, highlighting the importance of proper hygiene, handling, and storage during production and sale.

### **Raw Milk Samples:**

Among 15 raw milk samples, none (0%) were positive for Salmonella, and all 15 samples (100%) were negative. Regarding other microorganisms (Shigella), 7 samples (47%) were positive, while 8 samples (53%) were negative, indicating the presence of bacteria such as Shigella, which were not further identified. These findings suggest that although Salmonella was absent in the analyzed milk samples, other microbial contamination was present in nearly half of the samples, emphasizing the importance of proper hygiene and handling during milk collection, transport, and storage.

### **Findings:**

Out of the total 30 samples (15 milk and 15 cheese), 20 samples (66.6%) were positive for microorganisms (Shigella), while 10 samples (33.3%) were negative. Only 1 sample (3.3%) tested positive for Salmonella, whereas the remaining 19 positive samples (63.3%) contained other microorganisms, such as Shigella, which were not further identified. These results indicate that although Salmonella prevalence was very low, microbial contamination from other bacteria was relatively common across the analyzed milk and cheese samples, emphasizing the need for proper hygiene, handling, and storage practices. From the total 30 samples, 20 (66.6%) were positive for microorganisms and 10 (33.3%) were negative. Only 1 sample (3.3%) of the total tested positive for Salmonella, while 19 positive samples contained other microorganisms. Table 1. Summary of microbiological analysis of milk and cheese samples

Sample Type	Total Samples	Positive Samples for salmonella n (%)	Negative Samples for salmonella n (%)	Other Microorganisms positive for (Shigella) (%)
Cheese	15	1 (7%)	14 (93%)	12 (80%)
Raw Milk	15	0 (0%)	15 (100%)	7 (47%)
Total	30	1 (3.3%)	29 (96.6%)	19 (63%)

## DISCUSSION

The findings of the present study indicate a low prevalence of *Salmonella* in raw milk and cheese samples collected from vendors in Jalalabad city, which is consistent with reports from several international studies. Similar low detection rates of *Salmonella* in milk and dairy products have been documented in Malaysia, where a large-scale survey involving 930 samples from multiple regions reported an overall prevalence of only 0.2% (MCC, 2003). This suggests that, although *Salmonella* is a recognized foodborne pathogen, its occurrence in milk and dairy products is generally lower compared to other microorganisms. Comparable results were also reported by the (CDC ,2008), where only one out of 107 raw milk samples and three out of 254 cheese samples tested positive for *Salmonella*. Likewise, a market surveillance study conducted by the U.S. Food and Drug Administration detected *Salmonella* in only 3 out of 1,606 cheese samples (FDA, 2014). These findings reinforce the observation that *Salmonella* contamination in milk and cheese is relatively infrequent, particularly when compared with organisms such as *Escherichia coli* and *Staphylococcus spp.*, which are more commonly associated with dairy contamination. In contrast, higher prevalence rates have been reported in some developing countries. For instance, a study conducted in India between 2010 and 2012

detected *Salmonella* in 11 out of 142 milk and cheese samples (Kumar et al., 2012), indicating that regional differences in hygiene practices, milk handling, and processing conditions may significantly influence contamination levels. These variations highlight the importance of local production systems and food safety infrastructure in determining microbiological quality. The low prevalence of *Salmonella* observed in the present study may be attributed to several factors, including limited survival of *Salmonella* in dairy environments and competition with other microorganisms. However, the relatively high occurrence of other microorganisms, such as *Shigella*, suggests deficiencies in hygiene practices during milk handling, cheese preparation, storage, and retail sale. In Afghanistan, particularly in urban markets like Jalalabad, milk and dairy products are often sold under traditional conditions with minimal temperature control and limited regulatory oversight. Such conditions favor contamination by non-*Salmonella* enteric bacteria, posing a potential public health risk. Overall, the findings of this study align with international literature indicating that *Salmonella* is less prevalent in milk and cheese than other microorganisms. Nevertheless, the presence of *Salmonella*, even at low levels, remains a concern due to its pathogenic potential. These results emphasize the need for improved hygiene practices, routine microbiological monitoring, and public awareness programs tailored to Afghanistan's dairy production and marketing system.

## CONCLUSION

Milk and dairy products are a vital component of human nutrition, providing essential nutrients such as proteins, fats, carbohydrates, minerals, and vitamins, and contributing significantly to both human health and economic value. However, these products can also act as vehicles for foodborne pathogens, including *Salmonella*, which poses a public health risk if consumed unpasteurized. The present study demonstrated a low prevalence of *Salmonella* in raw milk and cheese samples from Jalalabad, while other microorganisms, such as *Shigella*, were more frequently detected. These findings are consistent with previous international studies indicating that *Salmonella* contamination in dairy products is generally less common than contamination by other enteric bacteria. The results emphasize the importance of maintaining hygienic

practices during milking, cheese production, handling, storage, and sale to minimize microbial contamination. From a scientific perspective, the study highlights the need for continuous monitoring of microbial quality in milk and dairy products, particularly in regions with traditional handling and retail practices. For public health, the findings reinforce the recommendation to consume pasteurized milk and properly boiled milk products to reduce the risk of foodborne illnesses. Future research should focus on broader surveillance of dairy products, identification of specific microbial strains, and assessment of contamination sources of contamination sources within the local dairy supply chain. Implementing such measures will contribute to improved food safety, protect consumer health, and support evidence-based policy decisions in Afghanistan's dairy sector.

#### CONFLICT OF INTEREST:

All authors declare that there is no conflict of interest associated with any part of this study.

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#### AUTHORS CONTRIBUTIONS:

Ziaulislam Nowbahar and Ahmad Farid Rawan was responsible for designing the research, performing laboratory analyses, collecting data, and preparing the initial draft of the manuscript. Haji Gul Hamat contributed by providing supervision, methodological guidance, and critical revision of the manuscript. Noori Gul Nabi assisted in analyzing the data, interpreting the results, and refining the manuscript academically. Hayatullah Takal contributed through literature review, proofreading, and making minor revisions.

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#### Note For Author(s)

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