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To Evaluate The Microbiology Quality Of Salmonella And Shigella Species Of Row Cow Milk From Sikar

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Abstract

The present study investigated the occurrence of *Salmonella* and *Shigella* species in raw milk samples collected from 20 areas of Sikar district across three seasons—summer, rainy, and winter—to assess seasonal and geographical variations in microbial contamination. A total of 300 milk samples were analyzed using Salmonella–Shigella (SS) agar. Out of all samples examined, 262 (87.33%) showed no bacterial growth, whereas 38 samples (12.67%) exhibited the presence of *Salmonella*, *Shigella*, or both. The occurrence of these pathogens varied seasonally, with the highest contamination observed during the rainy season. During summer, 6% of samples were contaminated—2% with *Salmonella*, 2% with *Shigella*, and 2% with both species. In contrast, the rainy season recorded 19% contamination—5% with *Salmonella*, 5% with *Shigella*, and 9% with both. Winter samples showed 13% contamination—5% with *Salmonella*, 5% with *Shigella*, and 3% with both. Spatial analysis revealed that contaminated samples were distributed across multiple areas, indicating the influence of local hygiene and environmental conditions on milk quality. The detection of these enteric pathogens, prohibited under the Prevention of Food Adulteration Rules (1956) and the Food Safety and Standards Act (2011), highlights lapses in hygiene during milking, handling, and storage. Factors such as poor washing practices, inadequate utensil sanitation, and lack of milk testing facilities likely contributed to contamination. The findings emphasize that raw milk collected during the rainy season, followed by winter, carries a higher microbial load, posing potential public health risks. Seasonal variation and regional differences play a crucial role in shaping the microbial composition of milk, underscoring the need for improved hygienic practices and monitoring to ensure milk safety throughout the year.

Keywords: Raw milk contamination; *Salmonella*; *Shigella*; seasonal variation; microbial load; Sikar district; food safety; hygienic practices; milk quality rainy season.

Introduction

The microbiological quality of raw cow milk is an important aspect of milk safety, as it can affect the shelf life and potential health risks associated with milk consumption. Raw milk can contain a wide range of microorganisms, including beneficial bacteria, potentially harmful bacteria, yeasts, and molds. The microbiological quality of milk can be influenced by various factors, including the cleanliness of milking equipment, hygiene practices during milking, health status of the cows, and environmental conditions. A knowledge of seasonal changes in microbial load of milk is essential to educate milk handlers and common consumers. Raw milk can also contain potentially harmful bacteria, such as Escherichia coli, Salmonella, and Listeria monocytogenes, which can cause foodborne illnesses. These bacteria can originate from various sources, including the udder of cows, the environment, and contaminated milking equipment.

Microbes in milk can serve as disease causing agents when present in milk (Brock and Madigan, 1991). Gunasekera *et al.* (2002) discussed that psychrotrophic microorganisms are the most imperative group of microbes present in milk and dairy products. Milk pasteurization was introduced as a public health measure in order to destroy human pathogens and to eliminate or reduce the activities of spoilage microorganisms (Gunasekera *et al*., 2002). Bacteria can be introduced into milk from a wide variety of sources such as workers, infected cows udder, faeces, dust in barns, milk containers or other equipment. Some microbes can serve as disease causing agents when present in milk (Brock and Madigan, 1991). Milk can be polluted by *Mycobacterium bovis*, *Brucella species*, *Streptococci* and *Coxiella burnetti* from infected cattle. Agents from human sources such as *Salmonella* species, *Shigella* species, *Corynebacterium diphtheria* and *Streptococcus* species can also be presented in milk.

. In this study, raw milk samples from cows belonging to different areas of Sikar district, Rajasthan were collected in varying seasons to investigate microbial load. Seasonal influence was explored on the milk quality, microbial load and nutritional contents.

Material and Methods

All milk samples were collected for microbiological work following standard protocol. To achieve the goals of this objective, raw milk samples of cow collected from different 20 areas of Sikar district, Rajasthan during different seasons i.e. summer, rainy and winter were evaluated for growth of Salmonella and Shigella on SS agar.

Salmonella-Shigella agar (Hi media) was used for the isolation of *Salmonella* and *Shigella* species. SS Agar (Salmonella Shigella Agar) is a differential selective media used for the isolation of Salmonella and some Shigella species from pathological specimens, and suspected foods. The peptic digest of animal tissue and beef extract provide essential growth nutrients. Lactose is the fermentable carbohydrate.

Salmonella Shigella agar comprises bile salts, sodium citrate, brilliant green, an enzymatic digest of casein, beef extract, an enzymatic digest of animal tissue, thiosulphate, ferric citrate, neutral red, and agar. The inclusion of Bile Salts, Sodium Citrate, and Brilliant Green serve to inhibit gram-positive, coliform organisms and inhibit swarming Proteus spp. while allowing Salmonella spp. to grow. Beef Extract, Enzymatic Digest of Casein, and Enzymatic Digest of Animal Tissue provide sources of nitrogen, carbon, and vitamins required for organism growth. Lactose serves as a carbohydrate source in Salmonella Shigella Agar. Differentiation of enteric organisms is achieved by the incorporation of lactose in the medium. Organisms that ferment lactose produce acid which, in the presence of the neutral red indicator, results in the formation of red/pink colonies. Lactose non-fermenters form colourless colonies. Sodium thiosulfate and Ferric Citrate permit detection of hydrogen sulfide by the production of colonies with black centers. Neutral red turns red in the presence of an acidic pH, thus showing fermentation has occurred.

Preparation of SS agar

It was prepared by suspending 60 g of the medium in one liter of deionized or distilled water with proper mixing. Heating was done with frequent agitation and boiling for one minute. Do not autoclave the media. Then it was poured into plates. The agar was solidify and stored in the refrigerator (avoid freezing). Prepared culture media can be kept for at least a week in refrigeration. Shigella appear as clear, colourless and transparent. Salmonella appear as colourless, transparent with a black centre.

Result & Discussion

Table 1 presented growth of Salmonella and Shigella species on SS agar from milk samples during summer, rainy and winter seasons. Changes are depicted in Fig.1.

A total of 300 samples were screened throughout the study including all the seasons from 20 areas of Sikar district. Out of this about 262 samples making 87.33% did not show the growth of Salmonella/ Shigella. About 12.67 % revealed the growth. This could be due to poor habits of washing teats; custom of washing utensils with only tap water, poor hygienic pre milking procedures, not having a practice of testing milk for bacterial contamination, and not having a separate milk vending environment. can cause bacterial contamination of raw milk.

During summer season, 94 % samples did not show any growth of Salmonella and Shigella species on SS agar. During summer season, 2 samples exhibited Salmonella species growth on SS agar, 2 samples showed Shigella species on SS agar and 2 samples revealed growth of both Salmonella and Shigella species on SS agar.

During rainy season, 81 % samples did not show any growth of Salmonella and Shigella species on SS agar. During summer season, 5 samples exhibited Salmonella species growth on SS agar, 5 samples showed Shigella species on SS agar and 9 samples revealed growth of both Salmonella and Shigella species on SS agar.

During winter season, 87 % samples did not show any growth of Salmonella and Shigella species on SS agar. During summer season, 5 samples exhibited Salmonella species growth on SS agar, 5 samples showed Shigella species on SS agar and 3 samples revealed growth of both Salmonella and Shigella species on SS agar.

Table 24 presented growth of Salmonella and Shigella species on SS agar from milk samples collected from different areas in each season.

During summer season, samples collected from area 2 (1 sample), 14(1 sample), 20(1 sample), 25(1 sample), 7(1 sample) and 16(1 sample) exhibited growth on SS agar. During rainy season, samples collected from area 1 (3 samples),2(2 samples), 3(1 sample), 4(1 sample), 7(1 sample) and 8(1 sample), 9 (1 sample),10(1 sample), 15(1 sample), 17 (3samples),18 (1 sample),19(1 sample),20 (2samples) exhibited growth on SS agar.

During winter season, samples collected from area 1(2samples), 2(1sample), 3(2samples), 5(1sample),6 (1sample), 17(1sample),18 (2samples), 19(2samples) and 20 (1sample) exhibited growth on SS agar.

According to the Prevention of Food Adulteration Rules of 1956 and Food Safety and Standard Act 2011,none of the milk sample should contain *Salmonella* or *Shigella* species. The presence of pathogens in raw as well as pasteurized milk samples is the matter of health concern (Agarwal *et al.*, 2012). Tusa *et al.* (2024) discussed that Coliform, *Shigella* and *Salmonella* are not only regarded as gauge of faecal contamination but are more likely a guide of pitiable hygiene and sanitary practices during milking and handling. They reported that 43 (20.5%) milk samples were contaminated with coliform.

Important factors like health of cow, farm management techniques, environmental hygiene and proper temperature control affect the microbiological status of raw milk. Till consumption, milk takes up many microbes and few of them include *Shigella*, coliforms, *Salmonella, Bacillus cereus*, *Escherichia coli* , *Mycobacterium* and *Staphylococcus aureus*. These are linked to food borne outbreaks upon consumption of raw milk.

It can be deduced that differences in seasons and geographical areas can produce differences in the bacterial composition of raw milk.

It can be stated that bacterial composition of raw milk collected in different seasons throughout the year varied. Our results clearly showed that raw milk collected during different months has highly variable microbes. Raw milk collected during rainy season followed by winter had a higher probability of microbial contamination. The microbial composition of raw milk did not show correlation with milk fat, lactose, SNF and protein content. This study may help to control the risk of microbial contamination at different times of year.

Table 1: Growth of Salmonella and Shigella species on SS agar from milk samples during summer, rainy and winter seasons

(N=100)

Growth on SS agar		Seasons		
		Summer	Rainy	Winter
Growth of Salmonella and Shigella Species from milk samples on SS agar	Salmonella species	2	5	5
	Shigella species	2	5	5
	Salmonella and Shigella species both	2	9	3
	No Growth	94	81	87

N= Number of samples

SS agar= Salmonella Shigella agar

Fig.1: Changes in growth of Salmonella and Shigella species on SS agar from milk samples during summer, rainy and winter seasons

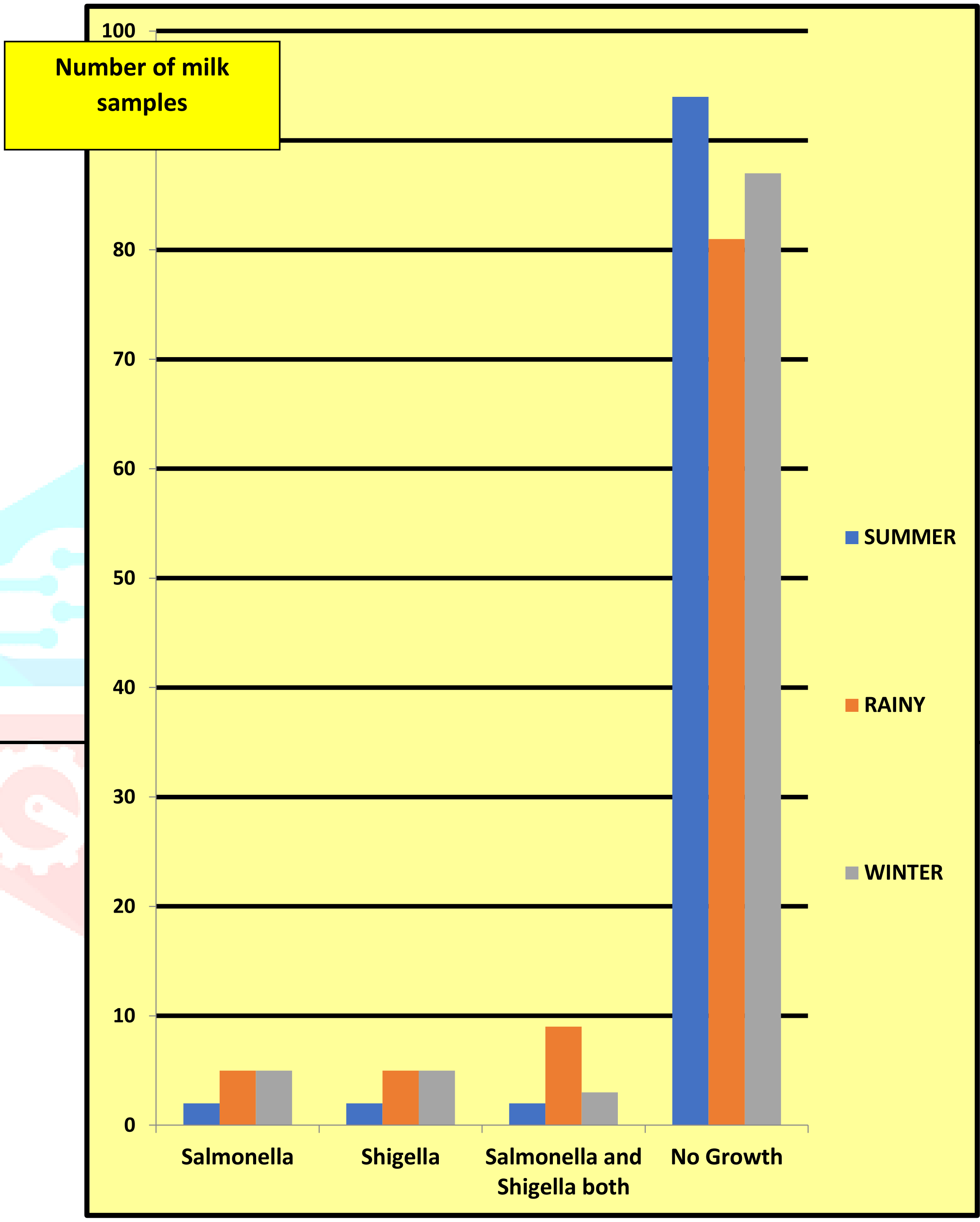

Table 2: Presence of Salmonella and Shigella species in milk samples (1-100) during summer, rainy and winter seasons

Milk Sample	Area / Dairy	Parameters					
		Summer		Rainy		Winter	
		Salmonella	Shigella	Salmonella	Shigella	Salmonella	Shigella
1.	1	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
2.		No Growth	No Growth		Growth observed	No Growth	No Growth
3.		No Growth	No Growth	No Growth	No Growth	Growth observed	No Growth
4.		No Growth	No Growth	No Growth	Growth observed	No Growth	No Growth
5.		No Growth	No Growth	No Growth	Growth observed	No Growth	Growth observed
6.	2	Growth observed	No Growth	No Growth	No Growth	No Growth	No Growth
7.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
8.		No Growth	No Growth	No Growth	Growth observed	Growth observed	No Growth
9.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
10.		No Growth	No Growth	Growth observed	No Growth	No Growth	No Growth

11.	3	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
12.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
13.		No Growth	No Growth	No Growth	No Growth	No Growth	Growth observed
14.		No Growth	Growth observed	No Growth	No Growth	No Growth	No Growth
15.		No Growth	No Growth	Growth observed	Growth observed	Growth observed	Growth observed
16.	4	No Growth	No Growth	Growth observed	No Growth	No Growth	No Growth
17.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
18.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
19.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
20.		Growth observed	No Growth	No Growth	No Growth	No Growth	No Growth
21.	5	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
22.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
23.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
24.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth

25.		No Growth	Growth observed	No Growth	No Growth	Growth observed	Growth observed
26.	6	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
27.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
28.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
29.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
30.		No Growth	No Growth	No Growth	No Growth	Growth observed	Growth observed
31.	7	Growth observed	Growth observed	No Growth	No Growth	No Growth	No Growth
32.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
33.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
34.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
35.		No Growth	No Growth	Growth observed	Growth observed	No Growth	No Growth
36.	8	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
37.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
38.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth

39.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
40.		No Growth	No Growth	No Growth	Growth observed	No Growth	No Growth
41.	9	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
42.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
43.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
44.		No Growth	No Growth	Growth observed	Growth observed	No Growth	No Growth
45.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
46.	10	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
47.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
48.		No Growth	No Growth	Growth observed	Growth observed	No Growth	No Growth
49.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
50.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
51.	11	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
52.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth

53.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
54.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
55.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
56.	12	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
57.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
58.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
59.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
60.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
61.	13	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
62.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
63.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
64.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
65.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
66.	14	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
67.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth

68.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
69.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
70.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
71.	15	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
72.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
73.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
74.		No Growth	No Growth	Growth observed	No Growth	No Growth	No Growth
75.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
76.	16	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
77.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
78.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
79.		Growth observed	Growth observed	No Growth	No Growth	No Growth	No Growth
80.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
81.	17	No Growth	No Growth	Growth observed	No Growth	Growth observed	No Growth

82.		No Growth	No Growth	Growth observed	No Growth	No Growth	No Growth
83.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
84.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
85.		No Growth	No Growth	Growth observed	Growth observed	No Growth	No Growth
86.	18	No Growth	No Growth	No Growth	No Growth	No Growth	Growth observed
87.		No Growth	No Growth	No Growth	No Growth	No Growth	No growth
88.		No Growth	No Growth	Growth observed	Growth observed	No Growth	Growth observed
89.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
90.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
91.	19	No Growth	No Growth	No Growth	No Growth	Growth observed	No Growth
92.		No Growth	No Growth	Growth observed	Growth observed	No Growth	No Growth
93.		No Growth	No Growth	No Growth	No Growth	No Growth	Growth observed
94.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
95.		No Growth	No Growth	No Growth	No Growth	No Growth	No Growth

96.	20	No Growth	No Growth	No Growth	No Growth	No Growth	No Growth
97.		No Growth	No Growth	No growth	No Growth	No Growth	No Growth

Discussion

The microbiological examination of raw milk samples from Sikar district revealed seasonal and regional variations in the occurrence of *Salmonella* and *Shigella* species. Out of 300 milk samples analyzed, 12.67% exhibited contamination, while the majority (87.33%) showed no growth on SS agar. The presence of these enteric pathogens in raw milk is an indication of fecal contamination and poor hygienic practices during milking, handling, and transportation. The results align with the findings of Agarwal et al. (2012) and Tusa et al. (2024), who reported that *Salmonella* and *Shigella* are key indicators of inadequate hygiene and improper sanitation in dairy management.

A distinct seasonal pattern was observed in this study. The highest rate of contamination occurred during the rainy season, followed by winter and summer. Rainy season samples showed a 19% contamination rate, which could be attributed to increased environmental humidity, water stagnation, and higher microbial load in the surroundings. These conditions favor the survival and proliferation of enteric bacteria. Similar seasonal variations have been reported in studies by Kumar et al. (2018) and Singh & Yadav (2020), emphasizing that warm and humid climates enhance bacterial growth and persistence in milk and dairy environments.

The lower contamination rate during summer may be due to higher ambient temperatures, which can reduce bacterial survival in open environments, although inadequate cooling facilities may still allow for bacterial multiplication post-milking. The moderate contamination in winter may result from improper storage practices and prolonged transportation time without temperature regulation.

Spatial variation across different areas further indicates that local management practices, water quality, and utensil hygiene play an important role in determining milk safety. Areas with poor infrastructure and lack of awareness regarding clean milking procedures showed higher contamination. The absence of regular microbial testing of milk also contributes to the unnoticed circulation of contaminated milk in the local market.

The presence of *Salmonella* and *Shigella* in milk poses serious public health risks as both pathogens are associated with foodborne illnesses, including gastroenteritis and dysentery. Contaminated milk can act as a vehicle for disease transmission, especially in rural and semi-urban regions where pasteurization and proper refrigeration are not consistently practiced. According to the Food Safety and Standards Act (2011), milk should be free from enteric pathogens, and hence, the detection of these bacteria indicates non-compliance with safety regulations.

To mitigate the risks, stringent hygienic measures should be adopted at the farm level, including proper washing of teats and utensils, use of clean water sources, regular equipment sterilization, and awareness programs for dairy farmers. Moreover, implementation of microbial monitoring and testing before milk distribution can significantly reduce contamination risks.

Conclusion

The present study highlights the seasonal and spatial variability in microbial contamination of raw milk in Sikar district, with *Salmonella* and *Shigella* detected in 12.67% of samples. The contamination was most prominent during the rainy season, followed by winter and summer. These findings indicate that environmental conditions, hygienic practices, and farm management strongly influence the microbial quality of milk.

The results underscore the urgent need for improving hygiene standards during milking and handling, ensuring proper sanitation of utensils, and maintaining temperature control during milk storage and transport. Awareness among dairy farmers and vendors regarding the health hazards of contaminated milk and adherence to food safety regulations are crucial to safeguard public health. Regular microbial screening and seasonal surveillance programs should be established to ensure milk quality and reduce the prevalence of foodborne pathogens in the dairy sector.

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References

1. Agarwal, S., Singh, D., & Sharma, R. (2012). Microbial quality of raw and pasteurized milk and their public health significance. *Indian Journal of Dairy Science*, 65(5), 419–425.
2. Boor, K. J., Wiedmann, M., Murphy, S., & Alcaine, S. (2017). A 100-year review: Microbiology and safety of milk handling. *Journal of Dairy Science,* 100(12), 9933–9951. https://doi.org/10.3168/jds.2017-12969
3. Chatterjee, S., Bhattacharjee, I., Chatterjee, S. K., & Chandra, G. (2015). Microbiological examination of milk in Tarakeswar, India, with special reference to coliforms. *African Journal of Biotechnology*, 15(4), 81–87.

4. Kumar, A., Singh, P., & Yadav, S. (2018). Seasonal variation in bacterial contamination of milk and its impact on public health. *International Journal of Food Microbiology*, 275, 45–52. https://doi.org/10.1016/j.ijfoodmicro.2018.03.012
5. Mukherjee, S., Patel, R., & Bhatt, M. (2019). Prevalence of *Salmonella* and *Shigella* species in milk and milk products from local markets. *Journal of Foodborne Pathogens and Disease*, 16(6), 412–419. https://doi.org/10.1089/fpd.2018.2583
6. Nanu, E., Latha, C., Sunil, B., Prejit, N., & Menon, K. (2007). Quality assurance of raw milk in Kerala, India. *Food Control*, 18(4), 408–412. https://doi.org/10.1016/j.foodcont.2005.11.006
7. Patel, V., & Kumar, D. (2020). Assessment of microbial contamination in raw milk from organized and unorganized sectors. *Journal of Animal Research*, 10(2), 285–291.
8. Singh, R., & Gupta, P. (2015). Hygienic quality and microbial safety of raw milk: An Indian perspective. *Journal of Food Safety and Hygiene*, 1(3), 15–22.
9. Singh, R., & Yadav, B. (2020). Impact of seasonal variation on microbial quality of milk in rural areas of Rajasthan. *Indian Journal of Public Health Research and Development*, 11(8), 1132–1138.
10. Tusa, R., Kebede, A., & Hordofa, T. (2024). Microbiological quality assessment of raw milk and associated hygiene practices among dairy farmers. *African Journal of Microbiology Research*, 18(2), 54–63. https://doi.org/10.5897/AJMR2023.9898
11. Vijayalakshmi, P., Ramasamy, D., & Rajendran, P. (2016). Enumeration of pathogenic bacteria from raw milk and their antibiotic resistance pattern. *Veterinary World*, 9(4), 425–430. https://doi.org/10.14202/vetworld.2016.425-430
12. Younus, M., Rahman, M., & Hossain, M. M. (2017). Bacteriological quality of raw milk and its implications for public health in developing countries. *Food Science and Nutrition*, 5(6), 1136–1142. https://doi.org/10.1002/fsn3.504