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Research Article

Effects of Some Biosecurity Measures on Milk Contamination in Khartoum and Gezira States, Sudan

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Abstract

The purpose of this research study was to evaluate the effects of some biosecurity measures on milk contamination in Khartoum and Gezira States. Stratified random sampling technique was used to select 99 dairy farms in the study area. By using specific biosecurity scoring system a pre-structured questionnaire was designed to obtain data such as farm location, general hygiene, absence of insects and pests, bedding hygiene, sources of fodder and water, manure disposal methods, antibiotic usage etc. Accordingly, farms were scored as “Good: >70%”; “Fair: 40-70%” and “Poor: <40 %”. Two hundred and twenty-seven of milk and 144 of water samples were collected to evaluate the bacterial load as a means of confirming the effectiveness of biosecurity protocols. The results indicate that from the total of 99 farms surveyed, 13.1% scored "Good," 55.6% "Fair," and 30.3% "Poor." Mixed-species farming was common, with most farms housing animals in separate enclosures by species and age to minimize disease transmission. Disease prevalence, including mastitis and diarrhea, was linked to suboptimal biosecurity measures and antibiotic misuse. Most (97.00%) of the investigated dairy farms sold milk from cows treated with antibiotics for human consumption indicating extensive use of antibiotics. River water and wells were common in rural and peri-urban farms, but they are frequently contaminated, as shown by high E. coli positivity rates in Bahri and Omdurman. The study found high Coliform count and Total Phosphorus (TPC) in water and milk samples from "Fair" and "Poor" farms, indicating contamination due to neglect of water cleanliness, poor conditions, and hygiene. The average coliform count in milk samples was 187.21 ± 14.19, indicating potential contamination in some areas. Despite no significant differences in coliform counts, contamination underscores the need for improved hygiene and quality control measures. It could be concluded that milk hygiene was significantly impacted by dairy farms' inability to maintain appropriate biosecurity controls.

Keywords: Dairy Farms; Biosecurity; Antibiotic Misuse; Manure Management; TPC; Coliform Count

Abbreviations

The management of farm environments plays a critical role in determining the overall health, productivity, and welfare of livestock. Several criteria are pivotal in assessing the general condition of such environments, encompassing hygiene, available space for animals, absence of pests, floor dryness, sources of fodder, manure removal and disposal methods, disease frequency, antibiotic usage, and the cleanliness of feeding and drinking troughs. Proper evaluation and maintenance of these factors contribute to optimizing animal welfare, reducing disease outbreaks, and ensuring sustainable livestock production systems [1].

Hygiene within animal farming systems is fundamental for preventing the proliferation of pathogens, including bacteria, viruses, and parasites that can compromise animal health and lead to zoonotic transmission risks [2]. Available space for animals sig-

nificantly impacts their physical and psychological well-being, as overcrowding has been associated with stress-induced behavioral issues and increased disease susceptibility [3]. Additionally, the absence of pests, such as rats, flies, cockroaches and other insects, is essential, as these vectors are known to transmit diseases and exacerbate hygiene concerns in farm environments [4]. Another critical aspect of farm management is maintaining floor dryness, which helps minimize the growth of harmful microorganisms and reduces foot-related health problems in livestock [5]. Furthermore, the sources of fodder and their quality significantly influence animal nutrition and health outcomes, emphasizing the importance of proper feed sourcing and handling practices [6].

Moreover, the removal and disposal of manure, often a breeding ground for pathogens, require meticulous attention to prevent environmental contamination and the spread of infections [6]. Frequent disease monitoring and management, including prudent antibiotic usage, are integral to mitigating the emergence of antimicrobial resistance, a growing concern in both veterinary and human medicine [7]. Finally, maintaining clean feeding and drinking troughs ensures that animals have access to uncontaminated food and water, reducing their exposure to foodborne illnesses and enhancing overall productivity [8].

The purpose of this research study was to evaluate the effects of some biosecurity measures on milk contamination in Khartoum and Gezira States.

Material and Methods

Stratified random sampling technique was used to select 99 dairy farms in Khartoum and Gezira States.

A pre-structured questionnaire was prepared to obtain data to evaluate some of the biosecurity measures implemented in these farms using specific biosecurity scoring system; these measures include: farm location, general hygiene, available space for animals, absence of insects and pests, floor dryness (bedding hygiene), sources of fodder, manure removal and disposal methods, disease frequency, antibiotic usage, and the cleanliness of feeding and drinking troughs. Accordingly, dairy farms were scored as "Good: >70%"; "Fair: 40–70%" and "Poor: <40 %".

Two hundred and twenty-seven of milk and 144 of water samples were collected from different areas in Khartoum and Gezira States (Table 1). No milking machines were used in all farms. Most of the farms used the municipal water network, some farms used the river water directly while other farms had their own wells in the farm. Different animal breeds were found in every farm. Holstein-Friesian cows, cross breeds, and local breeds all were kept together. Samples were collected in sterile containers, preserved under cold conditions till transferred to the lab for analysis.

Area	Milk	Water	Number of farms
Khartoum	37	18	14
Bahri	76	57	21
Omdurman	36	19	11
Gezira State	76	50	53
Total number of samples	227	144	99

Table 1: Distribution of the total number of samples.

Preparation of serial dilutions

Ten ml from each sample of water were transferred to 90 mL sterile normal saline and thoroughly mixed to give 1:10 dilution 'first dilution'; serial dilutions were prepared by transferring one ml from first dilution (10-1) to 9 ml normal saline, (10-2) and so on (10-3, 10-4, 10-5 ...) as described by [9].

Total coliforms count (TCC)

TCC presumptive test was done according to [10,11]. The Lauryl tryptose broth was used as the media for the presumptive test for total Coliforms count. Peptone water was used as a diluent; this result in a dilution of 101, 102, 103, 104 and 105. A Durham tube was inserted into each Lauryl Tryptose tube. 1 ml of each dilution was pipetted into 3 Lauryl Tryptose tubes. All tubes were incubated at 35 to 37°C for 48h and then examined for gas formation in the Durham tubes.

Confirmed test for coliforms

Each positive (gassing) Lauryl Tryptose tube was gently agitated and a loopful of suspension was transferred to tube of "brilliant green" bile broth. All the tubes were incubated at 35 to 37°C; any gas formation in Durham's tubes with slight turbidity in the media was regarded as positive confirmed test. Results were interpreted using the MPN tables based on combination of confirmed gassing of Lauryl Tryptose broth tubes for three consecutive dilutions [10]. EMB agar was used for the isolation of *E. coli*, where the appearance of a green metallic sheen served as a positive indicator.

Data analysis

SPSS ver. 20 was used for descriptive (the mean standard error of the mean) and analytical statistics (ANOVA and chi square test).

Results

General condition of the farms

Overall, 13(13.1%) of farms were classified as "Good," 56 (55.6%) as "Fair," and 30 (30.3%) as "Poor." In Khartoum State, 19 (41.3%) of farms were rated as "Fair," while in Gezira State, 11 (20.8%) fell into this category. A statistically significant difference was observed between farm locations and their classification (Table 2).

Area		Classification of farm condition according to biosecurity scoring system			Total	Sig.
		Good	Fair	Poor		
Khartoum* State	KH	7 (50%)	6 (42.9%)	1 (7.1%)	14	
Sub total	BA	1 (4.8%)	10 (47.6%)	10 (47.6%)	21	
Gezira*	OM	0 (0%)	3 (27.3%)	8 (72.7%)	11	.000
		8 (17.4 %)	19(41.3 %)	19 (41.3%)	46	
	GZ	5 (9.4%)	37 (69.8%)	11 (20.8%)	53	
Total		13(13.1%)	56(55.6%)	30(30.3%)	99	

Table 2: Association between farm locations and farm classification.

*KH: Khartoum; BA: Bahri; OM: Omdurman; GZ: Gezira

Association between farm location and animal species raised in farms.

Cows were the primary type of animals raised on 68 (68.7%) farms, while the remaining farms housed a mix of animals, including cows, goats, sheep, camels, poultry, and donkeys. In 79 (78.8%) of the farms, animals were housed in separate enclosures based on species, whereas in the remaining farms, all animals were kept together. There were no significant differences between locations regarding the rearing of different animal species on the same farm (Table 3). Additionally, 71.7% (71) of farmers reported separating animals based on their age.

Area		Duration of manure removal				Total	Sig.
		Daily	Once a week	Once a month	More than once monthly		
Area*	KH	7 (50%)	7 (50%)	0.0	0.0	14	
	BA	3 (14.3)	4 (19%)	14 (66.7%)	0.0	21	
	OM	0.0	9 (81.8%	2 (18.2%)	0.0	11	.000
	GZ	4 (7.5%)	45 (84.9%)	2 (3.8%)	2(3.8%)	53	
Total		14 (14.1%)	65 (65.7%)	18 (18.2%)	2	99	

Table 3: Association between farm location and frequency of manure removal.

*KH: Khartoum; BA: Bahri; OM: Omdurman; GZ: Gezira

Association between farm location and frequency of manure removal

The frequency of manure removal varied across farms. It was observed that 65 (65.7%) of farmers cleaned their farms weekly, 14 (14.1%) cleaned daily, and 18(18.2%) cleaned once a month. A statistically significant association was found between the frequency of manure removal and the location of the farm (Table 4).

Methods of manure disposal on farms.

Following farm cleaning, it was observed that cow manure was sold in (93) 93% of the farms. In (2) 2.1% of the farms, manure was either burned on-site, reused, or others (Figure 1).

Area		Animals		Total	Sig.
		Cows	Mixed		
Area*	KH	10 (71.4%)	4 (28.6%)	14	
	BA	15 (71.4 %)	6 (28.6%)	21	.300
	OM	10 (90.9%)	1 (9.1%)	11	.237
	GZ	33(62.3%)	20 (37.7)	53	
Total		68	31	99	

Table 4: Association between farm locations and animal species raised.

*KH: Khartoum; BA: Bahri; OM: Omdurman; GZ: Gezira

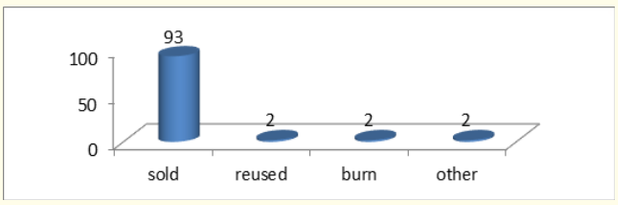

Figure 1: Methods of manure disposal on farms.

Water and fodder sources

The sources of water across farms varied: 61 (60.6%) of farms relied on the municipal water system 12 (12.1%) used river water, and 27 (27.3%) depended on wells inside the farm (Figure 2). Regarding fodder, green fodder grown on-site was found in only 3 (3%) of the farms. The majority 69 (68.7%) purchased fodder from markets, while 28 (28.3%) utilized agricultural byproducts as a primary feeding source.

Association between farm location and reported diseases

The most reported symptoms of diseases on farms were mastitis and diarrhea, affecting 70 (70.1%) of the investigated farms. Fever and respiratory diseases were noted in 29 (29.9%) of farms (Table 5). A highly significant association was found between farm location and the types of diseases reported.

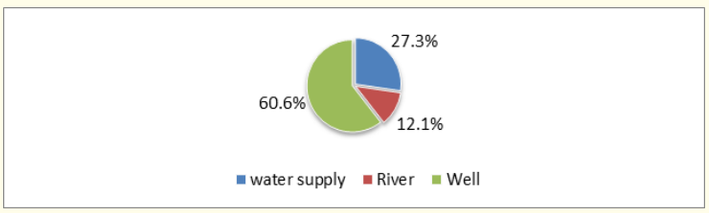

Figure 2: Water sources utilized by farms.

Area		External Parasites		Total	Sig.
		Present	Absent		
Area*	KH	4 (28.6%)	10 (71.4%)	14	
	BA	9 (42.9%)	12 (57.1%)	21	0.000
	OM	11 (100%)	0	11	
	GZ	16 (30.2%)	37 (69.8%)	53	
Total		40	59	99	

Table 5: Association between farm location and prevalence of external parasites.

*KH: Khartoum; BA: Bahri; OM: Omdurman; GZ: Gezira

External parasites were reported in 40 (40%) of the farms in the investigated areas. In Gezira, 16 (30.2%) of farms reported issues with external parasites, compared to 24 (72.2%) in Khartoum State. This difference in prevalence was statistically significant (p-value .001) (Table 6).

Types of medications used

Dairy farmers used different types of medications including only antibiotics 24 (24.2%) and antibiotics and anti-parasitic medicines were used in75 (74.7%) of the farms (Figure 3).

Disposal of milk from treated animals

Table 7 showed that milk treated with antibiotics and anti-parasitic was discarded in one farm 1 (1%), was sold for human consumption in 96 (97.00%) farms, while it was given to calves in two farms 2 (2%).

Association between farm location and coliform count (MPN) in milk samples

The mean coliform count (MPN) varied among the areas, with the highest mean value observed in OM (259.23 ± 51.39) and the lowest in BA (146.00 ± 29.29). The total average coliform count

Usage of contaminated milk	Frequency	%	Valid %	Cumulative %
Discarded	1	1.0	1.0	1.0
Sold	96	97.0	97.0	98.0
Fed to calves	2	2.0	2.0	100.0

Table 6: Usages of milk within the withdrawal period.

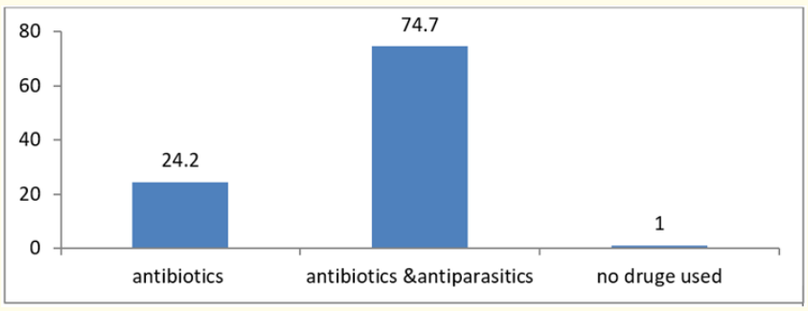

Figure 3: Different medication used in the investigated farms.

Area*		N	Mean			
				Min.	Max.	Sig.
Milk MPN	KH	14	236.49 ± 55.15	.00	281.50	0.164
	BA	21	146.00 ± 29.29	.00	320.00	
	OM	11	259.23 ± 51.39	.00	235.00	
	GZ	53	175.57 ± 14.96	.00	260.00	
	Total	99	187.21 ± 14.19.	.00	580.00	

Table 7: Association between farm location and Coliform count (MPN) in milk samples.

*KH: Khartoum; BA: Bahri; OM: Omdurman; GZ: Gezira

reached 187.21 ±14.19. in the total samples, indicating potential contamination in some areas. Statistical analysis (Sig. = 0.164) indicates no significant difference in coliform counts between the areas, but the presence of contamination highlights the need for improved hygiene and quality control measures in milk production and handling processes (Table 8).

Association between farm location and MPN in water samples

The coliform count (MPN) in water samples from the investigated farms showed significant variability. For coliform count, KH had a mean value of 77.2 ± 23.5, with a range of 0.0 to 251.5. BA exhibited the highest mean coliform count at 194.7 ± 48.1, ranging from 0.0 to 780.0. OM had a mean of 105.4 ± 35.4, with values

Area*		N	Mean			Sig.
				Min.	Max.	
Water MPN	KH	14	77.2 ± 23.5	.00	251.50	.029
	BA	21	194.7 ± 48.1	.00	780.00	
	OM	11	105.4 ± 35.4	.00	335.00	
	GZ	53	104.8 ± 11.8	.00	460.00	
	Total	99	120 ± 13.4	.00	780.00	

Table 8: Coliform count (MPN) in water samples in the investigated farms.

*KH: Khartoum; BA: Bahri; OM: Omdurman; GZ: Gezira

between 0.0 and 335.0, while GZ showed a mean of 104.8 ± 11.8, ranging from 0.0 to 460.0. Overall, the total mean coliform count was 120.0 ± 13.4, with a maximum value of 780.0 (Table 9).

Table 9 presents the association between farm location and the presence of *E. coli* in water samples. The data reveal significant variations in *E. coli* contamination across different areas. In Khartoum (KH), 42.9% of samples tested positive, while Bahri (BA) had the highest contamination rate at 76.2%. Omdurman (OM) showed 63.6% positivity, and Gezira (GZ) had the lowest rate at 26.4%. The overall significance level (Sig.) of .001 indicates a statistically significant association between farm location and *E. coli* presence in water samples. Out of 99 total samples, 43 tested positive for *E. coli*, while 56 were negative.

Area		E. coli in water		Total	Sig.
		Positive	Negative		
Area*	KH	6 (42.9%)	8	14	
	BA	16 (76.2%)	5	21	
	OM	7 (63.6%)	4	11	.001
	GZ	14 (26.4%)	39	53	
Total		43	56	99	

Table 9: Association between farm location and *E. coli* in water samples.

*KH: Khartoum; BA: Bahri; OM: Omdurman; GZ: Gezira

Discussion

The purpose of this research study was to evaluate the effects of some biosecurity measures on milk contamination in Khartoum and Gezira States.

In this study, cows were the predominant livestock, raised on 68.7% of farms, consistent with findings of [12] in Sudan. Farms also housed other animals, such as goats, sheep, and camels, reflecting reliance on mixed farming systems. Similar patterns have been observed in East Africa, where studies like [13] in Ethiopia documented mixed-species farming as a common strategy for optimizing resource use and risk management.

This study also highlights that more than three quarters of farms kept animals in separate enclosures by species, a practice that minimizes interspecies disease transmission and supports effective farm management. This practice aligns with recommendations set by [14], linking mixed-species housing to outbreaks of bacterial and viral diseases. Furthermore, 71.7% of farmers reported separating animals by age, a critical practice for protecting younger animals from diseases and ensuring proper nutrition. This aligns with findings reported from Kenya [5]. However, the absence of significant differences between locations regarding species and age segregation suggests a relatively uniform adoption of these practices across Sudanese farms.

The evaluation of farm conditions in the investigated areas in this study revealed that the Khartoum area scored the highest percentage in farm conditions classified as "Good" and "Fair." This showed a highly significant association between areas and farm condition (p-value .000). The reason behind this may be attributed to the awareness of farms owners gained from proximity to veterinary guidance centers as well as competition between farms in reflecting a positive image to consumers [1]. Moreover, this high classification explains why Khartoum area farms scored the lowest TPC in milk (2.157 x10^5 CFU/ml).

This research revealed high Coliform count in water samples from "Fair" and "Poor" farms, with significant association (p- value .000) between areas and Coliform count suggesting contamination due to neglecting water cleanliness. Similar findings in milk samples suggest poor conditions and hygiene [15-17].

Referring to specific areas in this study, the high percentage of contamination of milk with MPN in farms classified "Good" and "Fair" in Bahri area was evident from the fact that this area scored the highest percentage (76.2%) of positive *E. coli* in water samples, the highest mean of coliform count in water (194.7 ± 48.1 CFU/ml). These results may be due to poor knowledge of workers regarding proper sanitation of milking equipment and the use of unhygienic practices such as rubbing hands with grease to facilitate milk letdown.

These findings align with studies, by [18] which highlight poor condition denoting poor manure disposal, unhygienic feeding practices, and inadequate floor dryness as contributors to milk and water contamination and disease outbreaks, including *E. coli* and Salmonella. Al Mofti., *et al*. [19] stated that 85% of dairy farm managers were illiterate, which may be a challenge for biosecurity improvement. Also, similar results were obtained by [20] who found

that half (50%) of the dairy farms used to remove manure weekly, one-quarter (25%) used to remove it daily, and one-quarter (25%) used to remove it monthly. Similar challenges were documented across Africa, with [13] in Ethiopia reporting that most farms failed to meet hygiene standards, and [5] in Kenya identifying poor water quality and feeding practices as drivers of zoonotic diseases.

The findings on water sources, fodder utilization, and *E. coli* contamination in farms reflect broader trends documented in Sudan and other African countries, highlighting challenges in water quality and resource management. In Sudan, reliance on the municipal water system (60.6%) aligns with studies such as [21] which noted the dominance of centralized water systems in urban areas, though often plagued by inconsistent supply and poor sanitation. The use of river water (12.1%) and wells (27.3%) was common in rural and peri-urban farms, but these sources were frequently contaminated, as evidenced by high *E. coli* positivity rates in Bahri (76.2%) and Omdurman (63.6%). This contamination is consistent with the findings by [12], which reported that open water sources near farms in Sudan are highly susceptible to contamination due to runoff and inadequate waste management.

In the present study, the significant association between farm location and *E. coli* contamination ($p = 0.001$) was in line with studies in Tanzania [14] and [22] who documented similar geographical variations in water contamination, exacerbated by poor waste disposal and the absence of water treatment systems near farms. However, in the present study, Gezira State had the lowest *E. coli* contamination rate (26.4%), which might be attributed to better water management practices compared to Khartoum and Bahri, as previously suggested by [18].

In this study, low percentage (3%) of farms grew green fodder on-site. Similar trends were observed in other African nations; for instance, [13] in Ethiopia highlighted that limited access to land and water often forces farmers to depend on external fodder sources, increasing costs and dependency on market conditions. In Kenya, [5] reported that agricultural byproducts were a common feed option. Depending on external fodder sources may predispose farms to invasion by disease-agents and vectors.

In the present study, most (97.00%) of the investigated dairy farms sold milk from cows treated with antibiotics for human consumption indicating extensive use of antibiotics. A Tanzanian study conducted by [14] also noted frequent antibiotic usage due to poor management, mirroring trends observed in Sudan.

Also, this finding aligns with previous research by [23,24] indicating that 80% of conventional dairy herds use antibiotics to treat mastitis, the leading and most common reason for antibiotic use in cattle. Also, [25] found that factors like using penicillin and lack of knowledge on withdrawal periods contribute to antibiotic residues in milk. Similar concerns were raised in other studies in Khartoum State by [19,26]. The findings of this study support that stated by [27], who reported TBC and TCC may pose health hazards when present at high levels in foodstuffs.

Conclusion

It could be concluded that milk hygiene was significantly impacted by dairy farms' inability to maintain appropriate biosecurity controls. The study highlights critical deficiencies in farm management practices across the investigated areas, particularly in manure handling, water quality, and disease control.

Improvements in biosecurity measures, including regular cleaning, adequate water treatment, and appropriate fodder sourcing, are essential to mitigate production challenges alongside with farmers education, resource allocation, and policy enforcement to address systemic issues.

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Competing Interest

The authors declare that they have no competing interests.

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Authors' contributions

This work was carried out in collaboration between authors. Author (1) collected the research data, and conducted laboratory analysis. Author (2) contributed to drafting the initial manuscript. Author (3) contributed to checklist preparation and edited and reviewed the final manuscript. Author (4) conceptualized the initial idea, performed supervision over the research, drafted the initial manuscript, conducted statistical analysis, and carried out correspondence duties. All authors read and approved the final manuscript.

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