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ASSESSMENT OF PARASITES AND NANOPARTICLES IN WATER AND COW MILK IN SELECTED COMMUNITIES OF ZAMFARA WEST, ZAMFARA STATE, NIGERIA

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ABSTRACT

Background: Water used by cattle and milk produced in the same livestock environment are connected through animal husbandry, environmental hygiene and milking practices. Microbial contamination of either matrix may affect animal health and the safety of milk entering the food chain. Parasites and nanoscale particles also require targeted investigation because their detection depends on appropriate sampling and matrixspecific analytical methods.

Aim: This Research assesses parasitic contamination and nanoparticle occurrence in drinking water and cow milk from selected livestock rearing communities in Maradun, Talata Mafara and Bakura Local Government Areas of Zamfara State, Nigeria.

Methods: The study uses a crosssectional field and laboratory design. The updated laboratory summary covers 41 settlements and 82 environmental samples (41 water and 41 milk). Questionnaire records supplied for the current draft comprise 180 respondents. Descriptive statistics, one-way analysis of variance, Pearson correlation and simple linear regression were reported for selected microbiological and physicochemical indicators. The individual laboratory dataset and full model diagnostics were not included with the summary.

Results: All 41 water samples were reported positive for E. coli and total coliforms. Mean water E. coli was 144.8 ± 169.8 CFU/100 mL. Lead exceeded the stated 0.01 mg/L comparison value in 51.2% of samples. In milk, E. coli was detected in 82.9% of samples,

Staphylococcus counts exceeded the stated 100 CFU/mL comparison value in all samples, and 40 of 41 samples (97.6%) were reported above the stated aflatoxin M1 comparison value. Water-source category was associated with water E. coli and milk E. coli in the reported ANOVA (both $p < 0.001$). Water E. coli and milk E. coli were strongly correlated ($r = 0.84$, $p < 0.01$). A simple regression model reported $R^2 = 0.704$.

Conclusion: The reported results indicate substantial microbiological concerns in the sampled water and milk and identify water-source category as an important correlate of contamination indicators. The observational findings do not, however, prove that water was the direct source of milk contamination. Regulatory comparisons, statistical assumptions and linkage between the 41 laboratory settlements and the 180 questionnaire records require final verification. Parasite and nanoparticle conclusions are reserved until their specific analytical results are available.

KEYWORDS: Cattle; Cow Milk; Drinking Water; Escherichia coli; aflatoxin M1; lead; parasites; nanoparticles; Zamfara State; One Health.

1. INTRODUCTION

Water quality is an important part of livestock health and food production. Cattle may obtain drinking water from boreholes, handdug wells, rivers, earth dams and communal troughs. These sources differ in their exposure to animal waste, surface runoff, sediment and other environmental contaminants. A source that appears clear may still contain faecal indicator organisms, so visual inspection alone is not enough to establish microbiological quality. The World Health Organization (WHO) identifies Escherichia coli as an important indicator of faecal contamination in drinking water intended for human consumption (WHO, 2022, 2026). Where livestock sources are also used by households, contamination may have implications beyond animal production.

Milk can become contaminated at several stages, including on the animal, during milking, through water used for cleaning, from containers and equipment, and during storage or transport. Hygiene practices such as cleaning the udder and teats, using clean utensils, protecting milk from environmental contamination and maintaining suitable storage conditions are therefore important. The Codex Code of Hygienic Practice for Milk and Milk Products provides a framework for managing hygiene throughout milk production and handling (Codex Alimentarius Commission, 2004, amended 2009).

Parasites may contaminate water or food through faecal material, infected animals, contaminated soil or poor hygiene. Their detection requires methods designed for the organism and sample matrix. For example, validated concentration and confirmation procedures are needed for waterborne protozoa such as Giardia and Cryptosporidium. A negative test result should be interpreted in the context of sample volume, recovery efficiency and method detection limits. EPA Method 1623.1 provides one established approach for detecting Cryptosporidium and Giardia in water by filtration, immunomagnetic separation and fluorescence microscopy (U.S. EPA, 2012).

Nanoparticle assessment presents a separate analytical challenge. The term covers materials that differ in composition, size, morphology and origin. Naturally occurring colloids, food constituents and laboratory contamination can complicate measurements. Total elemental concentration measured by conventional elemental analysis is not, by itself, proof that an element is present as nanoparticles. Particle-specific techniques, appropriate sample preparation and contamination controls are needed. EFSA guidance emphasises that analytical methods should be selected according to the material, food or feed matrix and risk-assessment question (EFSA, 2021).

In livestock settlements, drinking-water conditions and milk hygiene may share environmental and management influences. Studying water and milk collected from the same settlements can reveal whether their contamination indicators vary together. Such an association is useful for identifying possible risk patterns, but it does not establish the direction or mechanism of contamination. This study therefore combines questionnaire information with laboratory assessment of water and cow milk, with the broader objective of generating evidence relevant to animal health, food safety and environmental monitoring in selected communities of Zamfara State.

1.1 Aim and objectives

The aim of the study is to assess parasitic contamination and nanoparticle occurrence in drinking water and cow milk from selected livestockrearing communities in Zamfara State, Nigeria.

1. To determine the occurrence of selected parasitic organisms in drinking water and cow milk from the study communities.
2. To screen for and characterise relevant nanoscale particles in water and cow milk using suitable analytical methods.
3. To assess selected microbiological and physicochemical characteristics of water and cow milk.

4. To examine the relationships between watersource characteristics, livestockmanagement practices, milkhandling practices and laboratory findings.

2. MATERIALS AND METHODS

2.1 Study design and setting

The project is a crosssectional field and laboratory investigation in selected livestockrearing communities in Maradun, Talata Mafara and Bakura Local Government Areas of Zamfara State, northwestern Nigeria. The wards specified in the study plan are Faru/Magami, Gora and Kaya in Maradun; Jangebe, Gwaram and Ruwan Gizo in Talata Mafara; and Damri, Dankadu and Rini in Bakura. The selected locations define the study area and should not be taken as a probability sample of all cattlerearing communities in the state.

2.2 Respondent survey and environmental sampling

The questionnaire captures respondent age, sex, education, years in cattle rearing, herd size, number of lactating cows, water source, milk source, udder washing, milk boiling, water treatment, veterinary attendance and distance to the water source. A total of 180 respondent records have been supplied for the current working manuscript. The updated laboratory summary, however, is based on 41 settlements, with one water and one milk sample reported per settlement, giving 82 environmental samples.

Respondents and environmental samples represent different units of analysis. Where multiple respondents are linked to a shared settlement-level sample, the laboratory result must not be replicated across those respondents and treated as independent observations. Any analysis connecting respondent practices to laboratory results should use a clearly defined settlementlevel aggregation or a multilevel approach that accounts for clustering.

A reconciliation remains necessary: the questionnaire records previously supplied in the conversation identify 36 settlement codes, whereas the updated laboratory summary covers 41 settlements. The final master register must identify the five additional laboratory settlements and confirm whether respondent records are available for them. The LGA sample sizes in the laboratory summary are Maradun n = 13, Talata Mafara n = 14 and Bakura n = 14; these should be distinguished from the respondent counts.

2.3 Sample identification and collection

Water and milk samples are linked through settlement identifiers, with W denoting water and M denoting milk. The final dataset should retain the collection date and time, GPS coordinates, source type, sample collector, sample condition and laboratory receipt information. Water should be collected from the source actually used by cattle, using a

source-appropriate technique. Pre-sterilised microbiological containers must not be rinsed before collection. For particle analysis, containers and equipment should be selected to minimise contamination from plastic, glass, metals or other materials relevant to the target analytes.

Milk records should identify whether the sample was collected directly from an animal or from a collection point and whether it represents an individual or composite sample. A composite sample represents the pooled milk of contributing animals and cannot be interpreted as an individualcow prevalence estimate. Separate aliquots may be required for microbiology, parasitology, elemental analysis and particle characterisation. Storage temperature and holding time should follow the validated procedure for each analyte rather than applying one condition to all tests.

2.4 Laboratory procedures and quality assurance

The supplied summary includes E. coli, total coliforms, total viable count (TVC), pH, total dissolved solids (TDS), lead, Staphylococcus and aflatoxin M1. The final Methods section must state the laboratory procedure used for each parameter, including culture media or analytical platform, incubation conditions, units, detection and quantification limits, calibration and qualitycontrol results. Field blanks, procedural blanks, positive and negative controls, matrix spikes, recovery experiments and reference materials should be documented where relevant.

Parasite testing should use matrixspecific concentration, detection and confirmation procedures. Water and milk differ substantially in composition, and milk fat and protein can affect recovery. The report should identify each target organism, the volume or mass tested, recovery efficiency and detection limit. Nanoparticle analysis should use particle-specific methods suitable for the intended material. Conventional totalelement measurements must not be described as nanoparticle identification unless supported by appropriate particle characterisation.

2.5 Statistical analysis

Categorical questionnaire variables should be summarised using frequencies and percentages. Continuous variables should be reported as means and standard deviations when distributions support this summary, or as medians and interquartile ranges when they do not. Laboratory results should be described separately by matrix and analyte. Oneway ANOVA may be used to compare means across groups when independence, residual distribution and variance assumptions are adequately met. Where assumptions are not met, transformations or suitable nonparametric procedures should be considered.

Pearson correlation describes linear association between continuous variables; it does not establish causation. Simple linear regression estimates the association between a predictor and an outcome under the model assumptions. Regression results should include coefficient estimates, confidence intervals, p-values, residual diagnostics and an assessment of influential observations. Because water and milk samples are paired by settlement, the settlement is the relevant paired unit. The final analysis should confirm that all 41 paired observations were used once and only once.

3. RESULTS

The results below reproduce the updated summary statistics supplied by the research team for 41 settlements (41 water and 41 milk samples). They should be checked against the individual laboratory worksheets before submission. The respondent questionnaire dataset and the 41settlement laboratory dataset must also be reconciled as described in Section 2.2.

3.1 Physicochemical and microbiological quality of water and milk

Analytical interpretation: The pattern indicates widespread faecal-indicator contamination in the sampled water and a high occurrence of E. coli in milk. The 100% water positivity and 82.9% milk positivity are occurrence proportions, not estimates for all communities in Zamfara State. The reported comparison-value exceedances also indicate that lead and aflatoxin M1 warrant follow-up, but the aflatoxin unit and the precise regulatory basis must be confirmed before a definitive compliance statement is made.

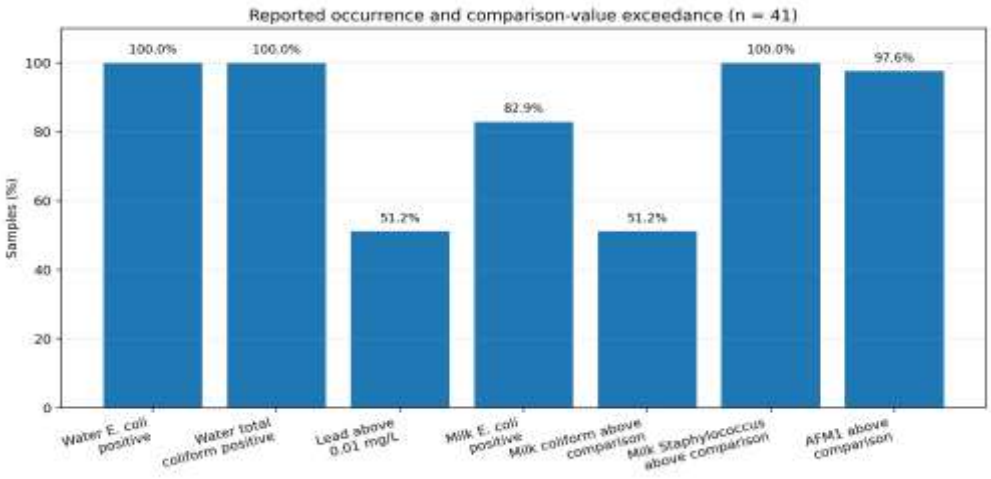


Figure 1. Reported percentage of samples positive or above the stated comparison values. The labels "above comparison" reproduce the supplied summary and should not be interpreted as verified regulatory non-compliance until the applicable standards and units are confirmed.

The updated summary showed that all 41 water samples were positive for E. coli and total coliforms. Mean water E. coli was 144.8 ± 169.8 CFU/100 mL, while mean total coliform count was 479.5 ± 509.4 CFU/100 mL. Mean water pH was 7.05 ± 0.34 and mean TDS was 466.0 ± 112.3 mg/L. Lead concentration averaged 0.02 ± 0.01 mg/L.

For milk, mean TVC was 68,315 ± 30,989 CFU/mL. E. coli was detected in 34 of 41 samples (82.9%), while the reported Staphylococcus count exceeded the comparison value in all 41 samples. Aflatoxin M1 averaged 0.37 ± 0.21 µg/L, and 40 samples (97.6%) were reported above the 0.05 comparison value. The laboratory report also states that 31.7% exceeded 0.5 µg/L; this figure should be checked against the raw results because the reported maximum is 0.76 µg/L.

Parameter	**Mean ± SD**	**Min–Max**	**Standard / comparison value**	**Reported exceeding**
Water E. coli (CFU/100 mL)	144.8 ± 169.8	4–587	No WHO zerocoliform criterion; verify applicable standard	100% (41/41)
Water total coliform (CFU/100 mL)	479.5 ± 509.4	57–1,806	No WHO zero-coliform criterion; verify applicable standard	100% (41/41)
Water TVC (CFU/mL)	22,255 ± 20,373	799–72,224	Not specified	—
Water pH	7.05 ± 0.34	6.31–7.80	6.5–8.5 (comparison range; source/status to verify)	7.3% (3/41)
Water TDS (mg/L)	466.0 ± 112.3	212–662	500 (SON, as supplied)	39.0% (16/41)
Water lead, Pb (mg/L)	0.02 ± 0.01	0.001–0.04	0.01 (WHO, as supplied)	51.2% (21/41)
Milk TVC (CFU/mL)	68,315 ± 30,989	32,844–158,658	100,000 (comparison value)	19.5% (8/41)
Milk total coliform (CFU/mL)	138.1 ± 137.2	10–487	100 (comparison value)	51.2% (21/41)
Milk E. coli (CFU/mL)	78.5 ± 85.9	0–310	0 (as supplied)	82.9% (34/41)
Milk Staphylococcus (CFU/mL)	825.3 ± 701.6	141–3,600	100 (comparison value)	100% (41/41)
Milk aflatoxin M1 (µg/L)	0.37 ± 0.21	0.02–0.76	0.05 (EU value commonly expressed in µg/kg; verify unit)	97.6% (40/41)
Milk pH	6.62 ± 0.21	6.08–7.02	6.6–6.8 (as supplied)	—

The pH interval and the TDS and lead comparison values should be tied to the exact WHO or Nigerian standard edition and water-use category. WHO's E. coli criterion should not be automatically extended to total coliforms; the appropriate totalcoliform benchmark must be identified from the applicable national or study protocol. The aflatoxin M1 comparison is supplied in µg/L, whereas the EU maximum level is commonly expressed in µg/kg for milk;

the analytical reporting basis and any conversion must be checked before exceedance is claimed. The TVC and coliform comparison values should also be identified by the applicable milk standard and product category.

3.2 Water and milk quality by LGA

Analytical interpretation: Talata Mafara recorded the highest mean water E. coli (193.1 CFU/100 mL) and milk E. coli (88.2 CFU/mL), while Bakura recorded the lowest mean for both indicators (104.9 and 71.5, respectively). However, the reported ANOVA results were not statistically significant for water E. coli ($F = 0.97$, $p = 0.388$), milk TVC ($F = 0.64$, $p = 0.535$), milk E. coli ($F = 0.14$, $p = 0.872$) and aflatoxin M1 ($F = 0.08$, $p = 0.926$). Thus, the observed differences are descriptive and do not provide statistical evidence of LGA-level differences at $\alpha = 0.05$.

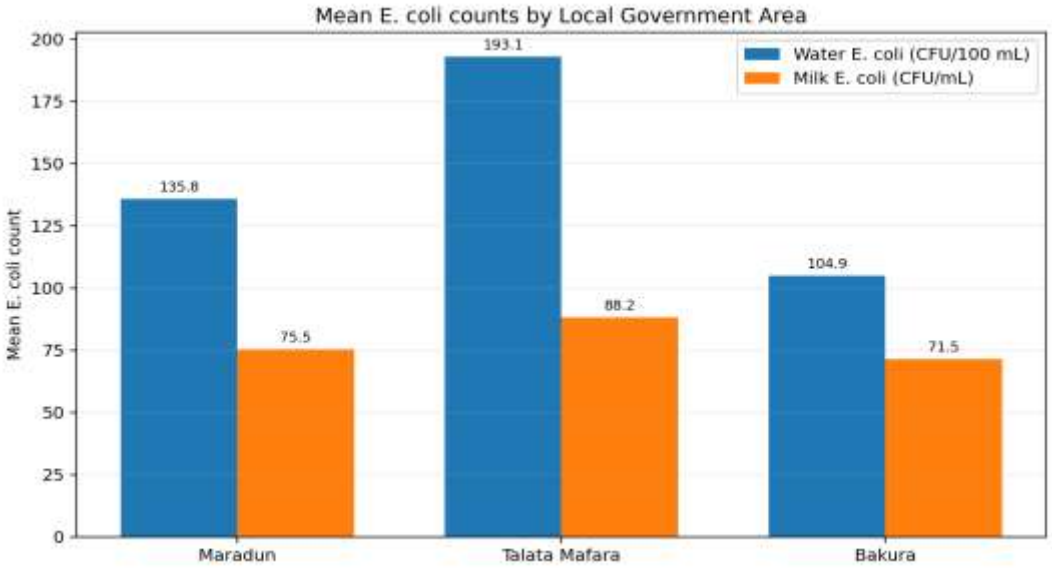


Figure 2. Mean E. coli counts by LGA. Water and milk measurements are displayed together for visual comparison, but their units differ and their bar heights should not be compared directly across matrices.

The reported mean water E. coli count was highest in Talata Mafara (193.1 ± 220.1 CFU/100 mL), followed by Maradun (135.8 ± 161.2) and Bakura (104.9 ± 109.5). The reported one-way ANOVA did not show a statistically significant difference among LGAs ($F = 0.97$, $p = 0.388$). Milk TVC, milk E. coli and aflatoxin M1 also showed no statistically significant LGA differences in the supplied analyses (all $p > 0.05$).

Parameter	Maradun (n=13)	TalataMafara (n=14)	Bakura (n=14)	F	p-value
Water E. coli	135.8 ± 161.2	193.1 ± 220.1	104.9 ± 109.5	0.97	0.388
Water TVC	20,641 ± 21,365	27,471 ± 25,599	18,538 ± 12,394	—	—
Water TDS	484.0 ± 86.3	458.2 ± 119.8	457.1 ± 130.8	—	—
Milk TVC	73,722 ± 24,770	70,761 ± 40,805	60,850 ± 25,056	0.64	0.535
Milk E. coli	75.5 ± 70.4	88.2 ± 115.4	71.5 ± 68.2	0.14	0.872
Milk aflatoxin M1	0.38 ± 0.22	0.37 ± 0.21	0.35 ± 0.20	0.08	0.926

The absence of statistically significant differences does not demonstrate that the LGAs are identical. It indicates that the reported analyses did not detect differences at the 0.05 significance level. The estimates and their uncertainty should be considered alongside the relatively small number of settlement samples per LGA.

3.3 Water source and contamination indicators

Analytical interpretation: Water E. coli increased across the source categories from borehole (11.3 CFU/100 mL) to well (79.1), dam (283.3) and river (400.8). The reported ANOVA was significant ($F = 52.62$, $p < 0.001$). Milk E. coli was lowest in the well group (25.2 CFU/mL) and highest in the river group (192.0 CFU/mL), also showing a significant source-group difference ($F = 23.51$, $p < 0.001$). These results identify source category as an important correlate in this dataset. They do not establish that water directly caused milk contamination; shared hygiene and environmental factors may contribute. The dam category had only three settlements, so that estimate is comparatively imprecise.

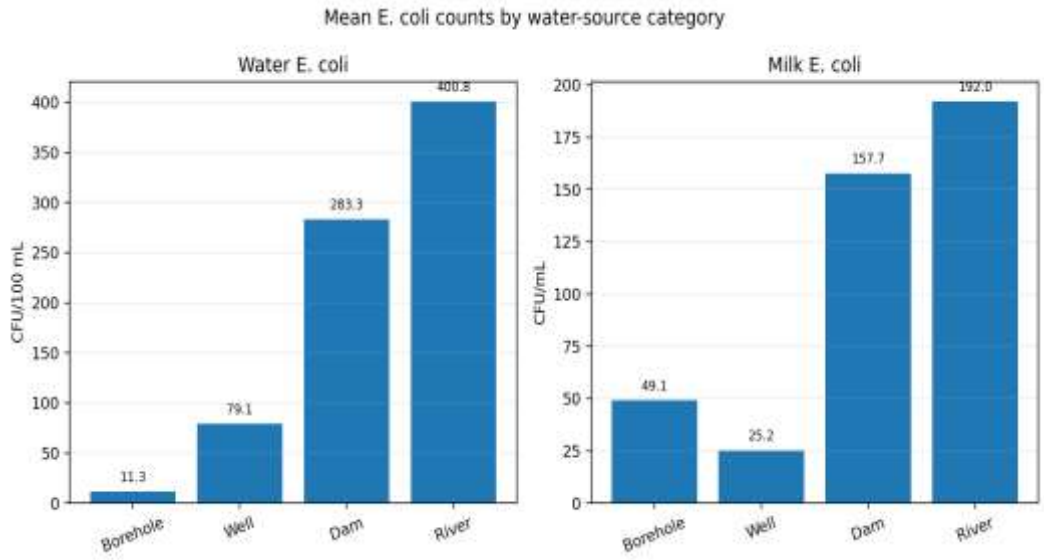


Figure 3. Mean water and milk E. coli counts by reported water-source category. Separate panels are used because the matrices have different measurement units.

Water-source category showed a strong pattern in the supplied results. Riversource settlements had the highest mean water E. coli count (400.8 ± 144.6 CFU/100 mL), while borehole-source settlements had the lowest (11.3 ± 3.4 CFU/100 mL). Mean milk E. coli was also highest in the river group (192.0 ± 87.0 CFU/mL) and lowest in the well group (25.2 ± 26.6 CFU/mL). The reported ANOVA indicated significant differences by water source for water E. coli and milk E. coli, but not for milk TVC or aflatoxin M1.

Water source	**n**	**Water E. coli (Mean ± SD)**	**Milk E. coli (Mean ± SD)**	**Milk TVC (Mean ± SD)**	**Milk aflatoxin M1**
Borehole	12	11.3 ± 3.4	49.1 ± 51.8	63,699 ± 30,525	0.32 ± 0.21
Well	17	79.1 ± 24.3	25.2 ± 26.6	66,728 ± 33,522	0.40 ± 0.23
Dam	3	283.3 ± 145.3	157.7 ± 8.5	55,072 ± 20,137	0.27 ± 0.21
River	9	400.8 ± 144.6	192.0 ± 87.0	81,883 ± 29,351	0.39 ± 0.16
ANOVA	—	$F = 52.62$; $p < 0.001$	$F = 23.51$; $p < 0.001$	$F = 0.85$; $p = 0.475$	$F = 0.61$; $p = 0.612$

The riversource mean water E. coli count was approximately 35.5 times the borehole mean (400.8 compared with 11.3 CFU/100 mL). The mean milk E. coli count in the river group was approximately 7.6 times the well-group mean (192.0 compared with 25.2 CFU/mL). These are descriptive ratios between group means. They should not be interpreted as individual-level risk ratios or proof of transmission. The dam group included only three settlements, so its mean and standard deviation are particularly sensitive to individual observations.

Aflatoxin M1 did not differ significantly across water-source categories in the reported analysis ($F = 0.61$, $p = 0.612$). This absence of association with watersource category does not, by itself, demonstrate that feed was the source. Aflatoxin M1 is generally associated with the metabolism of aflatoxin B1 ingested in contaminated feed, but confirming the source in this study would require feed sampling or other direct evidence.

3.4 Correlation between water and milk contamination

Analytical interpretation: The strongest reported relationship was between water E. coli and water TVC ($r = 0.99$), followed by water E. coli and milk coliforms ($r = 0.89$), water TVC and milk coliforms ($r = 0.88$), and water E. coli and milk E. coli ($r = 0.84$). These positive coefficients indicate that higher microbial counts tended to co-occur across settlements. In contrast, the correlations between aflatoxin M1 and microbial indicators were weak ($r = -0.01$ to 0.15) and reported as non-significant. Correlation does not establish a transmission pathway or causal relationship.

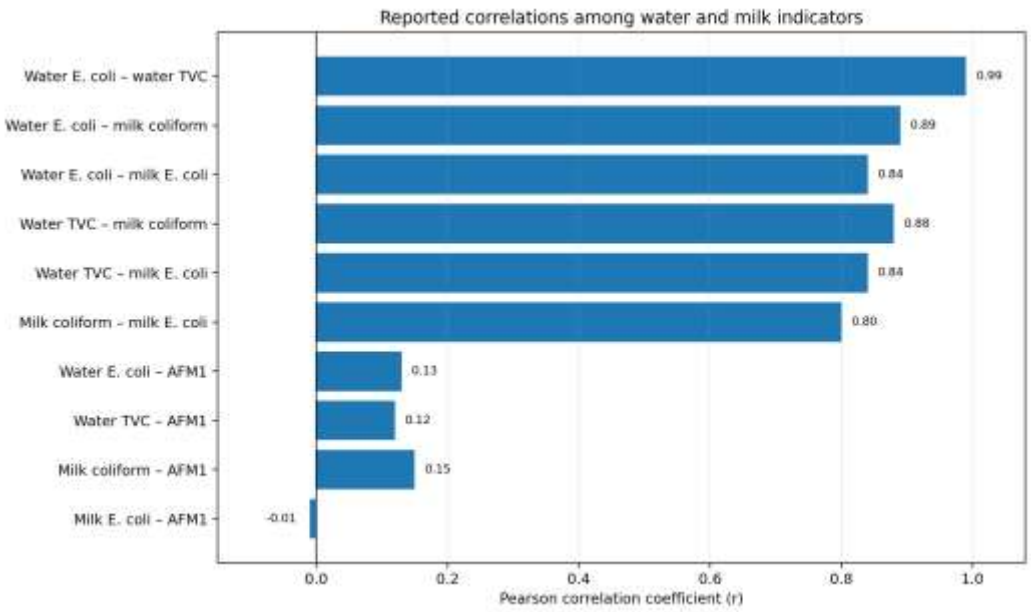


Figure 4. Reported Pearson correlation coefficients. Positive bars indicate positive linear associations; the near-zero AFM1 correlations indicate weak relationships with the listed microbial indicators.

The supplied Pearson correlation matrix showed strong positive associations among several microbial indicators. Water E. coli was strongly correlated with water TVC (r = 0.99), milk coliform count (r = 0.89) and milk E. coli (r = 0.84). Milk coliforms were also correlated with milk E. coli (r = 0.80). Aflatoxin M1 showed weak, nonsignificant correlations with the reported microbial indicators.

Variable pair	Pearson r	Reported significance
Water E. coli – Water TVC	0.99	$p < 0.01$
Water E. coli – Milk coliform	0.89	$p < 0.01$
Water E. coli – Milk E. coli	0.84	$p < 0.01$
Water TVC – Milk coliform	0.88	$p < 0.01$
Water TVC – Milk E. coli	0.84	$p < 0.01$
Milk coliform – Milk E. coli	0.80	$p < 0.01$
Water E. coli – Milk aflatoxin M1	0.13	Not significant
Water TVC – Milk aflatoxin M1	0.12	Not significant
Milk coliform – Milk aflatoxin M1	0.15	Not significant
Milk E. coli – Milk aflatoxin M1	−0.01	Not significant

The correlation between water E. coli and milk E. coli indicates that settlements with higher water E. coli counts tended also to have higher milk E. coli counts. It does not show that water contamination explains milk contamination in a causal sense. Shared environmental

conditions, animal health, udder hygiene, water used during cleaning, equipment sanitation and milk storage may contribute to the observed relationship.

3.5 Simple linear regression

Additional model interpretation: The reported R^2 of 0.704 means that the fitted one-predictor model accounted for 70.4% of the observed sample variation in milk E. coli. The remaining 29.6% was not accounted for by this simple model. The slope (B = 0.424) represents the estimated change in milk E. coli count for a one-unit increase in water E. coli, under the fitted linear model. Because confidence intervals and residual diagnostics were not supplied, the coefficient should be treated as provisional rather than as a fully validated predictive relationship.

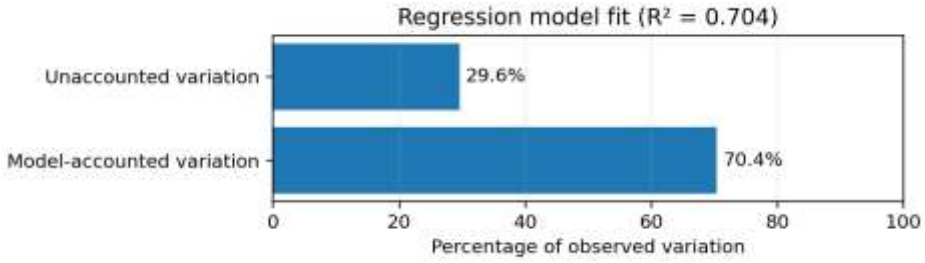


Figure 5. Proportion of observed variation in milk E. coli accounted for by the reported simple linear regression model. This is a statistical fit measure, not evidence that water contamination caused 70.4% of milk contamination.

The supplied simple linear regression model used water E. coli as the predictor and milk E. coli as the outcome. The reported unstandardised slope was 0.424, the intercept was 17.0, and the coefficient of determination was 0.704 ($p < 0.001$). The fitted equation was: Milk E. coli = 17.0 + 0.424 × Water E. coli.

Predictor	Unstandardised coefficient (B)	R^2	p-value
Water E. coli (CFU/100 mL)	0.424	0.704	<0.001
Constant	17.0	—	—

Under this fitted model, an increase of 100 CFU/100 mL in water E. coli corresponds to an estimated increase of 42.4 CFU/mL in milk E. coli. The R^2 value indicates that 70.4% of the observed variation in milk E. coli was accounted for by the fitted linear model in this sample. It does not mean that water contamination caused 70.4% of milk contamination. The coefficient should be accompanied by a 95% confidence interval, and the model should be

checked for linearity, residual normality, constant variance, influential observations and the effect of potential confounders before publication.

3.6 Parasite and nanoparticle outcomes

The updated information supplied for this revision contains no parasitespecific detection results or particle-specific nanoparticle measurements. Accordingly, this manuscript does not report parasite prevalence, parasite species, nanoparticle concentration, particle size, morphology or composition. These outcomes must be added only after the relevant laboratory records, analytical methods, quality-control results and detection limits have been reviewed.

4. DISCUSSION

The updated laboratory summary indicates that microbial contamination was common in the sampled water. All 41 samples were reported positive for E. coli and total coliforms, with mean counts of 144.8 and 479.5 CFU/100 mL, respectively. If these sources are used for human drinking as well as livestock watering, the findings raise a direct public-health concern and warrant prompt risk communication. If they are used only by cattle, the results still indicate a need to assess livestock exposure and the possible contribution of contaminated water to the wider farm environment. The final interpretation should clearly state the intended use of each source.

Lead was reported above the stated 0.01 mg/L comparison value in 21 of 41 water samples. This finding deserves attention because lead is a toxic element, but the final paper should document the analytical method, calibration, detection limit, blank results and the exact standard used. It should also distinguish dissolved lead from particulate lead if the study aims to make claims about nanoparticle occurrence. A conventional total-lead result cannot identify the physical form of lead in the sample.

The milk summary suggests several hygiene concerns. E. coli was reported in 82.9% of samples, total coliform counts exceeded the stated comparison value in 51.2%, and Staphylococcus counts exceeded the supplied reference value in all 41 samples. These patterns support attention to udder and teat preparation, cleanliness of milking equipment, hand hygiene, water quality used for cleaning and prompt cooling of milk. Nevertheless, the organism identification method should be stated precisely. If the laboratory measured presumptive Staphylococcus spp. rather than confirmed Staphylococcus aureus, the manuscript should use the broader term and avoid speciesspecific claims.

The reported aflatoxin M1 result is also important, with 40 of 41 samples stated to exceed the 0.05 comparison value. Before the exceedance percentage is retained in the publication

manuscript, the team should verify the analytical unit, the relevant milk category and the regulatory source. The current table gives µg/L, whereas regulatory limits may be expressed as µg/kg. The reported additional statement that 31.7% exceeded 0.5 µg/L should be checked against the raw dataset and the stated maximum of 0.76 µg/L. Aflatoxin M1 in milk is commonly associated with the transfer into milk of aflatoxin B1 metabolites after animals consume contaminated feed; however, the present water-source comparison does not identify the feed source in these herds.

The LGA comparisons did not detect statistically significant differences in the selected water and milk indicators. This suggests that the contamination indicators were not confined to one of the three LGAs in this sample. It should not be interpreted as evidence that the LGAs have identical water and milk quality. The sample size per LGA was modest, and the uncertainty around each mean should be considered. Reporting confidence intervals and effect sizes would help readers judge the magnitude and precision of the differences.

Water-source category showed a strong association with water E. coli and milk E. coli. River-source settlements had the highest reported mean counts, while borehole-source settlements had the lowest mean water E. coli. This pattern is biologically plausible because surface water may be more exposed to animal access, runoff and faecal inputs than protected groundwater sources. However, source categories alone do not capture source protection, water collection practices, trough hygiene, seasonal variation or treatment. The dam group included only three observations, which limits the stability of comparisons involving that category.

The strong correlation between water E. coli and milk E. coli, together with the reported regression R^2 of 0.704, identifies a potentially important relationship for further investigation. However, the phrase 'water contamination explains milk contamination' would overstate the evidence. The model accounts for statistical variation in the observed sample; it does not establish the direction of transfer, exclude confounding or prove that water is the principal source. The study is crosssectional, and both water and milk may be influenced by common environmental and hygiene conditions. A stronger causal assessment would require repeated sampling, detailed exposure measurements and, where appropriate, molecular comparison of isolates.

The absence of a statistically significant association between watersource category and aflatoxin M1 is consistent with the possibility that aflatoxin contamination follows a pathway different from microbial contamination. It does not prove that feed was the source in this

dataset. Testing feed for aflatoxin B1 and recording feed storage and sourcing would be needed to investigate that pathway directly.

The study's parasite and nanoparticle objectives remain important, but the current results do not yet address them. E. coli and coliform counts are indicators of microbial contamination; they are not substitutes for parasite testing. Similarly, lead concentration measured by a bulk elemental method does not establish the presence of lead nanoparticles. The final manuscript should include these objectives only alongside results generated using validated, matrix-appropriate methods.

5. CONCLUSION

The updated results indicate that the sampled cattlewater sources and cow milk had substantial microbiological quality concerns. All 41 water samples were reported positive for E. coli and total coliforms, while E. coli and elevated Staphylococcus counts were reported in a large proportion of milk samples. Lead concentrations exceeded the stated comparison value in 51.2% of water samples, and aflatoxin M1 exceeded the supplied comparison value in 97.6% of milk samples, subject to confirmation of the analytical units and regulatory basis.

Water-source category was significantly associated with water E. coli and milk E. coli in the reported analyses. River-source settlements had the highest mean water E. coli, while boreholesource settlements had the lowest. Water E. coli was also strongly correlated with milk E. coli. These findings identify watersource quality as an important correlate and a practical target for risk reduction. They do not prove that cattle drinking water was the direct or sole source of milk contamination. The final conclusion should therefore describe water source as a significant associated factor, not as a proven causal source.

The present results do not establish the occurrence or absence of parasites or nanoparticles. Those objectives can only be concluded upon after the relevant laboratory analyses and qualitycontrol documentation have been completed.

6. Recommendations

1. Prioritise microbiological assessment and risk management of river and dam sources used by cattle, while maintaining routine monitoring of wells and boreholes.
2. Where water is also used for human consumption, communicate the E. coli findings promptly to relevant public-health and water authorities and advise against consuming untreated contaminated water.

3. Improve protection of water points from direct animal access, runoff and faecal contamination; maintain clean troughs and collection containers.
4. Promote hygienic milking practices, including appropriate teat and udder preparation, clean hands and equipment, and protection of milk from contamination after milking.
5. Improve milk cooling, transport and storage practices and strengthen routine microbiological monitoring at farm and collectionpoint levels.
6. Investigate aflatoxin M1 through parallel assessment of animal feed, particularly feed storage conditions and aflatoxin B1 contamination, rather than attributing the result to water-source category.

7. Limitations and data reconciliation

This revision uses summary statistics supplied by the research team rather than the underlying 41row laboratory dataset. The reported ANOVA, correlation and regression results therefore could not be independently recalculated, and full model diagnostics and confidence intervals were not available. The questionnaire records previously supplied comprise 180 respondents and identify 36 settlement codes, whereas the updated laboratory summary covers 41 settlements. The final report must reconcile the five additional laboratory settlements, their LGA and ward allocation, and any associated questionnaire records. The distribution of the 41 laboratory settlements by LGA (13, 14 and 14) must not be confused with respondent distribution.

The study is cross-sectional and cannot establish causal direction. The number of dam-source settlements is small (n = 3), which limits the precision of that group estimate. Regulatory comparisons require confirmation of the exact standard, sample category, analytical units and applicable averaging rules. Parasite and nanoparticle-specific results were not included in the material used for this revision.

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