Original Article

Risk Factors Associated with Shiga-toxin *Escherichia coli* Contamination of Raw Milk in Pastoralist Farms in Narok County, Kenya

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Abstract

Background: Narok County has recorded the highest global isolation of the foodborne Shiga toxin-producing *Escherichia coli* (STEC). Nevertheless, information regarding transmission risk in the food systems is limited. **Objective:** This study aimed to investigate risk factors associated with isolation of STEC from bulked farm milk in Narok County. **Materials and Methods:** STEC strains were isolated and identified using standard bacteriological and molecular methods. Management practices data were collected through face-to-face interviews. Logistic regression analyses determined the relevant predictors and model performance. **Results:** *E. coli* was isolated from 15.1% (confidence interval [CI]: 9.2–21.0, n = 139) of the raw milk samples; the majority (85.7%, CI: 70.8–100.0, n = 21) of the isolates were confirmed to be STEC through multiplex polymerase chain reaction and sequencing targeting *gadA*, *stx 1, stx 2, eaeA,* and *hlyA* genes. Informal education (odds ratio [OR] = 30.7; 95% CI: 6.6–142.5; $P < 0.001$) and housing livestock (OR = 19.2; 95% CI: 5.2–71.5; $P < 0.001$) emerged as significant predictors of STEC isolation. Informal education and indoor housing of dairy animals were associated with increased likelihood of isolation of STEC from raw milk (adjusted OR [AOR] = 13.9, $P < 0.001$) and (AOR = 9.0, $P < 0.001$), respectively. **Conclusions:** Our findings provide evidence of poor milk handling practices on farms. The prevalence of STEC in milk may pose a risk to public health and food safety. Therefore, proper hygienic practices in dairy farms are recommended.

Keywords: *Milk, Narok County, risk factors, Shiga toxin Escherichia coli*

Introduction

Milk is a nutrient-rich food that provides significant health benefits, particularly to children. Nonetheless, milk and milk products are prone to contamination with numerous pathogens,[1] including Shiga toxin-producing *Escherichia coli* (STEC). The bacterium may cause life-threatening complications in humans, including hemolytic uremic syndrome (HUS) and potentially fatal outcomes in vulnerable individuals.[2] STEC is responsible for approximately two million instances of acute illness worldwide each year.[3] Importantly, Narok and the adjacent Kajiado Counties record the highest documented human STEC incidences worldwide.[4]

Over 1000 STEC serotypes have been identified, with 400 linked to human disease.[5] The pathogenicity of STEC is primarily due to the production of Shiga toxins (*stx*), categorized into two principal types: *stx 1* and *stx* 2. These toxins are characterized by sequence similarities, antigenic variations, and key biochemical properties.[2] Intimin, a protein encoded by the *eaeA* gene, is another essential virulence factor of STEC that promotes its intimate attachment to intestinal epithelial cells.[2] Clinical severity is complex, influenced by host factors, the microbiota, and environmental conditions and is not determined solely by STEC strain characteristics.[6]

Upon ingestion, STEC colonizes the intestinal tract, adheres to the epithelium of the distal ileum and colon, and subsequently produces Shiga toxin *(stx),* which disrupts epithelial barrier function and ion transport, resulting in diarrhea.[7] Clinical illness, including HUS, is typically linked with the concurrent presence of the *stx 2* and *eaeA* or *aggR* virulence factors.[8] STEC colonizes the gastrointestinal tracts of ruminants asymptomatically and is excreted in their feces.[9] The bacterium enters milk from various sources, including direct contact with the animal (teats, skins, and feces), milking equipment surfaces,

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housing, bedding, feed, air, and water contaminated with excrement from infected carriers.[10]

Possible pathways of infection to humans include direct contact with cattle,[11] ingestion of raw milk,[12] contamination of water sources by agricultural runoff,[13] and contamination of food products.[14] The nomadic lifestyle and water scarcity reported in Narok County[15] also pose a risk of STEC contamination of milk and milk products. The risk is higher with the consumption of raw milk. It is particularly high in communities that rely on fermented raw milk, which plays a crucial role in the nutrition of infants, pregnant women, young children, and the elderly, who are particularly vulnerable to STEC infection.[16] In the Maasai community, fermented milk (*kule naoto*) is widely consumed.[17] Furthermore, strains of pathogenic organisms such as *S. aureus*, Salmonella, and Listeria monocytogenes have been reported to survive fermentation.[18] Therefore, fermented milk poses a risk of foodborne infections with potentially pathogenic organisms, including STEC and many others.

While the significance of STEC is extensively documented in developed countries, the situation in developing nations remains unclear. Numerous countries lack the data on the prevalence and drivers of contamination at critical points, hindering outbreak tracking and source identification. This study aimed to address this deficiency by documenting the risk factors and prevalence of STEC in Narok County, Kenya.

Materials and Methods

Study area

This study was conducted in Narok County, Kenya, which lies between latitudes 0° 50' and 1° 50' South and longitudes 35° 28' and 36° 25' East at an average altitude of 1296 m above the sea level. The county lies in southwestern

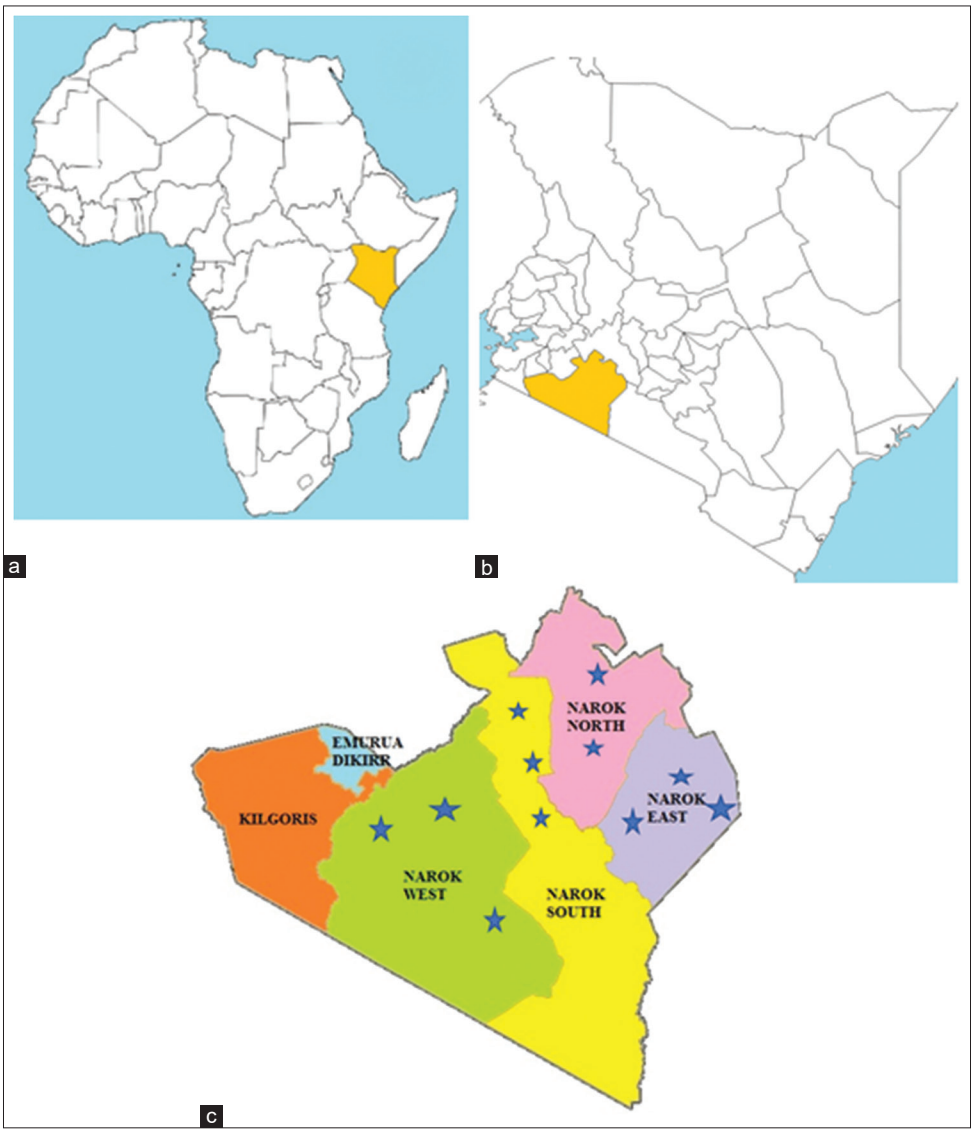


Figure 1: Maps illustrating the study areas. (a) Map of Africa showing the location of Kenya. (b) Map of Kenya showing the location of Narok County. (c) Map of Narok County showing sub-county boundaries in the study area

Kenya and borders Tanzania to the south. The county is subdivided into six sub-counties, namely, Narok North, Narok South, Narok East, Narok West, Emurua Dikkir, and Kilgoris [Figure 1]. Livestock husbandry (pastoralism and transhumance), wildlife tourism/conservation, and crop farming are the key economic activities practiced in the county. Milk is the primary product[19] from approximately 2,000,000 heads of cattle in the county. It is also predominantly ASAL, characterized by low annual rainfall.[20]

Study design

A cross-sectional study to assess risk factors for the occurrence of STEC in raw milk on pastoralist farms was conducted between July 2022 and March 2023.

Sampling and sample size determination

The sample size was calculated using the standard formula for comparing two proportions in a cross-sectional study.[21]

$$n=\frac{\left(Z_{\alpha/2}\sqrt{2p\,(1-\,p)}+Z_{\beta}\,\sqrt{p1\,(1-\,p1)+p2\,(1-\,p2)}\right)}{(p1-\,p2)^{2}}$$

Assuming a baseline prevalence of *E. coli* contamination of 13.8%[22] and an expected prevalence of 35% in farms with the risk factor, with 80% power, 5% alpha, and equal allocation between exposed and unexposed groups, the required sample size was 66 per group (132 total). To strengthen the power of the study, the final sample included 139 households. An additional 7 households were sampled to increase the power of the study. Eligible respondents were adults 18 years and above who were conversant with the handling of milk and animal husbandry. Participants were randomly selected from pastoral communities residing in Narok North, South, East, and West sub-counties. Within the four sub-counties, data were collected from the following villages: Suswa and Nairrekia Enkare, Duka Moja in Narok East sub-county; Motonyi, Eor Enkitok, Olkurto, Tipis, and Limanet in Narok North; Maji Moto, Kishelmoruak, Sekenani, and Ololaimutia in Narok West; Supetai, Ololulung'a, Sogoo, Ewaso Nyiro, Oltikampu, and Kimogoro in Narok South. Data were collected using a pretested structured questionnaire [Appendices 1 and 2], covering demographic and farm practices including housing type, floor type in the animal shed, water source, type of milking equipment, washing udders and teats, washing hands before milking, use of disposable udder towels, use of an exclusive udder towel for each cow, and consumption of raw milk. Approximately 40 mL of raw milk was aseptically collected from the household containers into uniquely labeled sterile Falcon tubes. Overall, 139 raw milk samples and a corresponding questionnaire were collected, including 30 from Narok North, 61 from Narok South, 25 from Narok East, and 23 from Narok West. The samples were processed at the Department of Public Health, Pharmacology, and Toxicology of the University of Nairobi.

Bacteriological isolation and identification

E. coli was obtained through culture methods and biochemical tests outlined by the International Standard Organization.[23] To isolate *E. coli,* raw milk samples were enriched using buffered peptone water (BPW) (Oxoid, UK) and incubated at 37°C for 24 h. Following this, the enriched samples were plated on MacConkey agar (Oxoid, UK) and incubated at 37°C for 24 h. Single pink colonies were then transferred to Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. Colonies with a green metallic sheen on EMB agar, suspected to be *E. coli,* were confirmed by Gram staining and IMViC biochemical tests. The confirmed *E. coli* isolates were subsequently sub-cultured on nutrient agar (Oxoid, UK) and incubated at 37°C for 24 h[24] for DNA extraction and virulence characterization.

Molecular confirmation of *Escherichia coli*

The *E. coli* DNA was extracted using the boiling method as previously described.[25] Briefly, single colonies were suspended in 0.4 ml of Ultra-Pure DNase/RNase-free water (Invitrogen). The suspension was then boiled at 100°C for 25 min to lyse the bacterial cells. Bacterial nucleic material was separated by centrifuging the boiled content at 15,000 revolutions/min for 5 min. The extracted DNA contained in the supernatant was then transferred into a sterile Eppendorf tube and stored at −20°C pending further analysis. The extracted *E. coli* DNA samples were amplified by polymerase chain reaction (PCR) as described previously,[26] using a Veriti Dx 96-Well Thermal Cycler (Applied Biosystems, USA). Primers targeting the glutamate decarboxylase alpha (*gadA*) gene were used for PCR amplification.

Oligonucleotide primers targeting the *gadA* gene included: forward primer 5' GATGAAATGGCGTTGGCGCAAG-3' and reverse primer 5'-GGCGGAAGTCCCAGACGATATCC-3' (GenBank accession number M84024) as described by.[27] The PCR was done using a 25 µl mixture containing 0.25 µl of each primer, 5 µl of sample DNA, 12.5 µl PCR Master Mix, and nuclease-free water (Invitrogen). PCR cycling conditions were as follows: initial denaturation at 94°C for 4 min, then 30 cycles of denaturation at 94°C for 30 s, annealing at

Table 1: Distribution of *Escherichia coli* isolated from household milk in the sub-counties

Sub-county	Number of milk samples	Number of milk samples with STEC	Proportion of STEC detection (%)
Narok North	30	5	16.7
Narok South	61	7	11.5
Narok East	25	2	8.0
Narok West	23	4	17.4
Total	139	18	15.1

STEC: Shiga toxin-producing *Escherichia coli*

Table 2: Farm demographics and management practices

Variable	Category	STEC isolation		Prevalence (%)	95% CI
		Yes	No		
Gender	Male (50)	10	40	20.0	10.2–33.0
	Female (89)	8	81	9.0	4.0–17.0
Education level	Informal (41)	16	25	39.0	24.2–55.5
	Formal (98)	2	96	2.0	0.2–7.0
Housing of livestock	Yes (40)	15	25	37.5	22.7–54.2
	No (99)	3	96	3.0	0.6–8.5
Type of floor in an animal shelter	Earthen (125)	16	109	12.8	7.5–20.1
	Concrete (14)	2	12	14.3	1.8–42.8
Water source	Potable (51)	4	47	9.1	2.2–18.9
	River/Stream/Dam (88)	14	74	14.7	9.0–25.0
Clean the animal shed before milking	Yes (11)	3	8	27.3	6.0–61.0
	No (128)	15	113	11.7	6.7–18.5
Wash udders and teats before milking	Yes (39)	8	31	20.5	9.3–36.5
	No (100)	10	90	10.0	4.9–17.6
The milker washes their hands before milking	Yes (44)	4	81	9.1	2.5–21.4
	No (95)	14	40	14.7	8.3–23.5
Use non-reusable udder towels	Yes (0)	0	0	0	
	No (139)	18	121	12.9	
Use a separate towel for each cow	Yes (34)	5	29	14.7	5.0–31.1
	No (105)	13	92	12.4	6.8–20.2
Type of milking	Hand (139)	18	121	12.9	
	Machine (0)	0	0	0	
Type of milk storage container	Plastic (126)	17	109	13.5	8.1–20.7
	Stainless steel (13)	1	12	7.7	0.2–36.0
Wash equipment with hot water and soap	Yes (102)	9	93	8.8	4.1–16.0
	No (37)	9	28	24.3	11.8–41.2
Teat dipping	Yes	11	7	61.1	38.6–83.6
	No	10	111	8.3	3.4–13.2

STEC: Shiga toxin-producing *Escherichia coli*, CI: Confidence interval

65°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min. Amplified products were analyzed by electrophoresis on a 2% agarose gel stained with ethidium bromide (1 mg/ml) at 100V for 45 min, and the image was captured using gel documentation imaging (Bio-Rad, England). A 100 bp ladder (Biolabs, New England) was used as a molecular size marker. *E. coli* strain (ATCC 25922) and nuclease-free water were used as positive and negative PCR controls, respectively. The PCR products were purified using the QIA-quick gel purification kit (QIAGEN GmbH, Hilden, Germany). The purified *E. coli* DNA was sequenced with the same primers used for PCR. The isolates were confirmed by BLAST analysis accessed through the GenBank database of the National Center for Biotechnology Information (NCBI).

Multiplex polymerase chain reaction for Shiga toxin-producing *Escherichia coli (stx 1, stx 2, eaeA,* and *hlyA)* virulence genes

The *E. coli* isolates were screened for *stx 1, stx 2, eaeA,* and *hlyA* genes responsible for STEC virulence phenotypes. Oligonucleotide primer sets for the genes used were as described previously[28] [Table 1]. All *E. coli* isolates were screened by multiplex PCR for virulence genes (*stx 1, stx 2, eaeA,* and *hlyA*) as described previously[24] with slight modifications. PCR was performed in 25 µL reaction mixtures in 200 µL thin-walled PCR tubes. Cycling conditions included: initial denaturation at 94°C for 5 min, 30 cycles of denaturation (94°C, 45 s), annealing (65°C, 45 s), elongation (72°C, 45 s), and a final extension at 72°C for 5 min [Table 2]. For each sample, duplicate PCR experiments were performed. The amplified PCR products were stained with ethidium bromide (0.5 µg/mL) and subjected to agarose gel electrophoresis (1.5% agarose in 1X TAE buffer) at 80 V/cm for 45 min. Each sample's PCR product (5–10 µL) and a positive control were added to separate wells. For each run, a 100-base pair ladder was used as the molecular weight marker to determine the amplicon size. The products were recorded using a gel documentation system (Bio-Rad, England) and observed under a UV transilluminator. Positive and negative controls were used for quality control in PCR identification of virulence genes. Each PCR run employed the *E. coli* reference strain ATCC 25922, which contained all the genes under investigation, as a positive control, and nuclease-free water without DNA template as a negative control.

Data handling and analysis

Data were entered into a Microsoft Excel spreadsheet for preprocessing before export to StataCorp. 2013. Stata 13.0 Statistical software. StataCorp LP, for analysis. Summary statistics of variables were produced as frequencies and percentages. The Chi-square/Fisher's exact test for independence was used to evaluate the associations between demographics and farm practice variables and the presence or absence of STEC virulence genes in milk. A binary logistic model was employed to evaluate the impact of all variables on contamination of raw milk with STEC. Preliminary models were created to detect crude associations, followed by adjusted odds ratio (AOR) models for statistical significance. The forward selection method was employed to identify covariates that elucidate variance in the dependent variable. Model assumptions, including multicollinearity and influential values, were evaluated to ascertain goodness of fit. $P < 0.05$ was considered statistically significant.

Results

Farm demographics of dairy farms

In total, 139 milk samples and corresponding questionnaires were collected from dairy farms. Most of the respondents (64%) in this survey were female. The mean age was 38.58 years and ranged between 18 and 72 years. Most (80.6%) respondents had formal education, whereas 19.4% had no formal education. Few (19.4%) respondents had no formal education. In the majority (75.5%) of farms, livestock were sheltered in outdoor *bomas* (enclosures made from thorn hedges or wooden posts), and on a few farms (24.5%), livestock were sheltered indoors. Most (74.8%) farms kept crosses of exotic and indigenous Zebu cattle; another 13.7% kept indigenous cattle, and 11.5% of farmers kept exotic cattle. The average herd size was 17.12 heads of cattle. The daily milk yield on farms was 10.7 L.

Isolation of *Escherichia coli* and Shiga toxin-producing *Escherichia coli* from raw milk

Of the 139 milk samples, *E. coli* was isolated from 21, representing 15.1% (confidence interval [CI]: 29.9%–37.9%). These 21 isolates were identified as *E. coli* by conventional bacterial culture and biochemical profiling. The *E. coli* sequences are accessible in NCBI GenBank accession numbers: CP159152.1, CP147125.1, CP055251.1, and CP147125.1. We further determined that 18 (85.7%) of the isolates harbored one or more virulent genes. Of these, 15 (83.3%) had *stx 1* genes, 2 (11.1%) had *stx 2*, while only 1 (5.6%) had *eaeA* genes. None of the isolates harbored the *hlyA* gene. The highest (17.4%) STEC detection was in Narok West Sub-County, followed by Narok North, while the lowest detection was in Narok East (8.0%) [Table 1].

Prevalence of Shiga toxin-producing *Escherichia coli* in raw milk on farms and dairy management practices

The occurrence of STEC varied markedly across management practices. As shown in Table 2, the prevalence of *E. coli* in milk handled by males was 20.0% (95% CI: 10.2–33.0), while that handled by females was 9.0% (95% CI: 4.0–17.0). Milk from farms in which the handlers had informal education had a prevalence of 39.0% (95% CI: 24.2–55.5), compared to 2.0% (95% CI: 0.2–7.0) among farmers with formal education. The prevalence of *E. coli* in farms in which the livestock were housed was 37.5% (95% CI: 22.7–54.2) and 3.0% (95% CI: 0.6–8.5) on farms where animals were kept outdoors.

The prevalence of *E. coli* in farms in which milking sheds were cleaned before milking was 27.3% (95% CI: 6.0–61.0) and 11.7% (95% CI: 6.7–18.5) on farms where milking sheds were not cleaned. The prevalence of *E. coli* on farms where udders were cleaned before milking was 20.5% (95% CI: 9.3–36.5) and 10.0% (95% CI: 4.9–17.6) on farms where udders were not washed. The prevalence of *E. coli* in farms in which milkers washed their hands before milking was 9.1% (95% CI: 2.5–21.4) and 14.7% (95% CI: 8.3–23.5)

Table 3: Associations between respondent characteristics significant in the final model and Shiga toxin-producing *Escherichia coli* isolation

Variable	Crude OR (CI)	*P*	AOR	*P*
Informal education	30.72 (6.62–142.5)	<0.001	13.87 (3.72–51.73)	<0.001*
Housing livestock	19.20 (5.15–71.54)	<0.001	8.96 (1.87–42.89)	0.006*
No handwashing	7.1 (2.19–22.93)	0.001	-	-
No hot water/soap	3.3 (1.20–9.17)	0.021	-	-
Teat dipping	17.4 (5.54–54.95)	<0.001	-	-
Gender (male)	2.5 (0.93–6.91)	0.070	-	-
Water source (river)	2.2 (0.69–7.16)	0.181	-	-
Clean shed before milking	2.8 (0.67–11.83)	0.155	-	-
Wash udders before milking	2.3 (0.84–6.41)	0.104	-	-
Separate towel per cow	1.2 (0.40–3.71)	0.726	-	-
Plastic container	1.8 (0.23–15.33)	0.559	-	-
Teat dipping	0.9 (0.18–4.30)	0.875	-	-

*P<0.05. OR: Odds ratio, CI: Confidence interval, AOR: Adjusted OR

in farms where milkers did not wash their hands. None of the respondents reported use of single-use disposable udder towels. On farms where udder towels were maintained exclusively for each cow, the prevalence of *E. coli* was 14.7% (95% CI: 5.0–31.1) and 12.4% (95% CI: 6.8–20.2) on farms where the towels were shared. All (100%) the respondents reported practicing hand milking. Most (90.6%) respondents used plastic containers for handling and storing milk, and another 9.4% used easy-to-clean stainless-steel containers. On farms where milking equipment was sanitized with hot water and soap, the prevalence of *E. coli* was 8.8% (95% CI: 4.1–16.0) and 24.3% (95% CI: 11.8–41.2) on farms where cold water was used.

Associations of risk factors with isolation of Shiga toxin-producing *Escherichia coli*

As shown in Table 3, crude analyses revealed strong associations between STEC isolation and informal education (odds ratio [OR] =30.7; $P < 0.001$), indoor housing of livestock (OR = 19.2; $P < 0.001$), and teat dipping (OR = 17.44; $P < 0.001$). The male gender demonstrated a moderate association (OR = 2.53; $P = 0.07$), while sanitation with hot water and soap was identified as significantly protective (OR = 0.3; $P = 0.02$).

Univariate analyses revealed several factors strongly associated with STEC isolation. Informal education (OR = 30.7; 95% CI: 6.6-142.5; $P < 0.001$) and housing livestock (OR = 19.2; 95% CI: 5.2–71.5; $P < 0.001$) emerged as significant predictors of STEC isolation. Hygiene-related practices also showed significant crude associations: no handwashing before milking (OR = 7.1; 95% CI: 2.2–22.9; $P = 0.001$), no hot water/soap for equipment sanitation (OR = 3.3; 95% CI: 1.2–9.2; $P = 0.021$), and teat dipping (OR = 17.4; 95% CI: 5.5–55.0; $P < 0.001$).

In the final model, informal education (AOR = 13.9; $P < 0.001$) and indoor housing of dairy animals (AOR = 9.0; $P < 0.001$) remained independently significant predictors of STEC isolation. Although lack of hot water was significantly associated with contamination in crude analysis (OR = 0.14, 95% CI: 0.04–0.46, $P = 0.001$), it was not significant in the adjusted model, suggesting that its effect was confounded by education and housing practices.

Discussion

Many studies have examined the epidemiology of STEC in cattle populations; few have investigated its epidemiological picture in pastoralist food systems. This study is among the few, to our knowledge, that have assessed farm management factors associated with STEC contamination of raw milk on pastoralist farms in Kenya.

In this study, the overall isolation rate of *E. coli* was 15.1% (95% CI: 9.2%–21.0%) through standard bacteriological methods. Among these, 85.7% (95% CI: 70.8%–100.0%) were confirmed by PCR to express STEC virulence genes. These findings contrast with other reported estimates, which had comparatively higher *E. coli* isolation rates of 90.67% in Tanzania.[29] However, the detection in our study is higher than that reported by others who found 59.3% in Ethiopia[30] and 8.75% in Malaysia,[31] respectively. It was also higher than 0.9% (95% CI: 0.5%–1.5%) in a meta-analysis.[32] The variation between bacteriological and molecular results in the current study is likely due to genetic differences of *E. coli* strains, which influence the observed phenotypic characteristics.[33] Further, the isolates were processed using multiplex PCR targeting four genes (s*tx 1, stx 2, hlyA,* and *eaeA*). We further detected *stx 1, stx 2, hlyA,* and *eaeA* genes responsible for STEC virulence in 83.3%, 11.1%, 5.6%, and 0% of the isolates, respectively. This contrasted with other reports,[34,35] which reported higher *stx 2* gene detection than *stx 1*.

The study further examined the association between dairy farm management practices and the prevalence of STEC in raw milk, identifying educational background and housing conditions as the most significant predictors. Informal education and indoor housing increased the likelihood of raw milk contamination with STEC (AOR = 13.9, $P < 0.001$) and (AOR = 9.0, $P < 0.001$), respectively.

The strong effect of education was congruent with previous reports showing the impact of farmers' education on public health and safety.[36] In this study, STEC was detected in 39% of farms among informally educated respondents compared with only 2% among those with formal education. Similarly, the association between livestock housing and STEC isolation is consistent with reports associating confinement systems with increased pathogen exposure.[37] Milk from cows housed indoors had a 37.5% prevalence compared to 3% housed outdoors, suggesting that stocking density and bedding hygiene are the critical determinants of contamination of raw milk on farms. Improvements in housing conditions, or transitions to pasture-based systems where feasible could substantially mitigate risk.

Gender showed moderate associations in univariate analysis but lost significance in multivariate models. This suggests that the gender of the milk handler may be correlated with education and housing type, reinforcing the idea that knowledge drives adoption of effective hygiene protocols.

This study estimated the prevalence of STEC in raw milk samples on farms, revealing gaps in sanitary practices, including limited access to potable water for drinking and sanitation. This finding suggests that STEC is endemic in the sampled farms. Current hygiene and sanitary practices may be permitting the contamination.

Limitations of the study

This study is cross-sectional and therefore possesses various limitations. The lack of longitudinal data hinders the evaluation of long-term trends and the efficacy of

long-term interventions. This study's questionnaire-based design introduces the potential for recall bias. This study did not determine total counts or bacterial diversity in milk, which limits the ability to correlate practices with actual contamination. Despite attempts to obtain a diverse sample, some bias may persist. The work is focused, potentially resulting in the loss of clarity within the complexities of the questions and answers.

Conclusions and Recommendation

This study identified significant deficiencies that predispose milk on farms in Narok County to STEC contamination. While most respondents recognized milk-borne zoonoses, especially Brucellosis, none mentioned *E. coli* strains. Risky practices such as the production and consumption of *kule naoto* constitute a significant STEC transmission pathway in Narok. Consequently, educational programs should be customized to the socio-cultural context of pastoralist farmers to create awareness. Moreover, the Arid and Semi-Arid climates, along with inadequate infrastructure, posed significant obstacles. Consequently, enhancing access to potable water on farms may be essential for mitigating STEC contamination in dairy operations.

Data availability statement

The data used during the current study are available from the corresponding author upon request. The sequences of the isolated *E. coli gadA* gene are openly available in GenBank at http://www.ncbi.nlm.nih.gov/genbank with accession numbers CP159152.1, CP147125.1, CP055251.1, and CP147125.1.

Ethics statement

Ethical approval was obtained from the Safety and Bio-Ethics Committee, Faculty of Veterinary Medicine, University of Nairobi (Approval No. FVM/BAUEC/2019/189). In addition, informed verbal consent was obtained from all participating farmers before conducting the interviews.

Author contribution statement

E. E. E., A. G. O., G. P. B. and O. J. O. conceptualization; E. E. E., A. G. O., G. P. B. and O. J. O. designed the study; G. P. B. and O. J. O. defined the intellectual content, E. E. E., M. E. W. and O. J. O. performed literature search; E. E. E., M. A. O. and A. G. O. performed bacteriological and molecular analysis; E. E. E. and O. J. O. gathered data; E. E. E., M. E. W., A. G. O. and O. J. O. performed data analysis; E. E. E, M. E. W., O. J. O. performed statistical analysis; E. E. E. and O. J. O. prepared the manuscript; E. E. E., O. J. O. and G. P. B. edited and reviewed the manuscript; Guarantor: E. E. E.

Declaration of generative AI in scientific writing

The authors declare that they did not use generative artificial intelligence (AI) and AI-assisted technologies in the writing process.

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Conflicts of interest

There are no conflicts of interest.

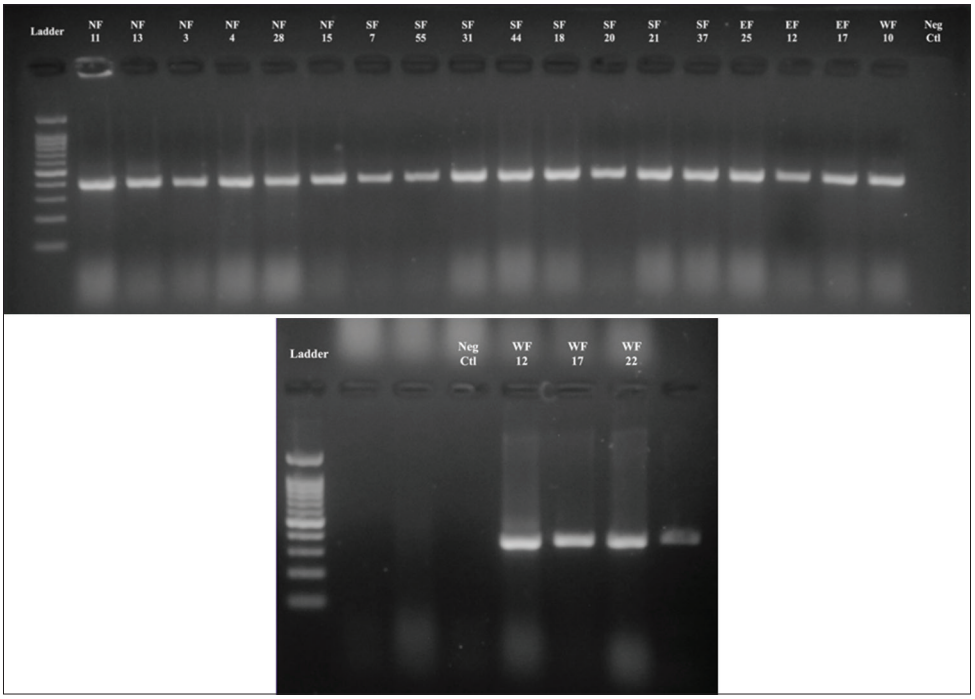
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Appendices



Appendix 1: Images showing polymerase chain reaction gels for *E. coli* isolated from raw milk from farms in Narok County, Kenya

Appendix 2 : STEC Contamination Risk Factor Questionnaire

Questionnaire ID: _____

Greetings,

My name is Eric Eshitera and I am carrying out research on dairy hygiene and safety in the dairy value chains in Narok. I am from the University of Nairobi, Faculty of Veterinary Medicine, Department of Public Health, Pharmacology and Toxicology. Kindly note that your participation is voluntary, and that you may withdraw from the study at any point. I also request about 50 mls of milk for laboratory analysis.

Section A: Socio-demographic data

1. Name of Ward: ……..
2. Name of Village: ……..……..
3. Gender? (A) Male (B) Female
4. Age group: (A) 10–18 (B) 18–25 (C) 25–35 (D) 35–45 (E) 45-above
3. Kind of Education: (A) Formal (B) Informal
4. Education Level: (A) Primary (B) Secondary (C) Tertiary
5. Farming system: (A) Intensive (B) Semi-intensive (C) Extensive
6. Cattle breed kept: (A) Zebu, (B) Zebu-crosses, (C) Exotic
7. Herd size: (A) 1–5 (B) 5–10 (C) 10–15 (D) 15 and above

Section B: Knowledge of milk hygiene in dairy farms

Knowledge attribute	Yes	No
Do you know any diseases that affect your livestock?		
Some diseases can be transmitted through milk.		
Do you know how to control milk-borne infections?		
Microorganisms are involved in milk spoilage		
Avoiding milking after treatment makes milk safe for consumers		
Washing hands and containers reduces milk spoilage		
Do udder infections get transmitted between animals?		
The cleanliness of water used on the farm influences the cleanliness of milk		
Do you know the benefits of good dairy hygiene?		

Section C: Hygiene attitudes of dairy farmers

Attitudes	Yes	No
Storage of milk in a cool place prolongs its shelf life		
Drinking raw milk directly from the udder leads to foodborne diseases		
Suckling of foremilk by the calf decontaminates teats		
Covering milk immediately after milking is the part of hygiene to prevent environmental contaminants (e.g., dust) from entering milk		
Mastitis is a concern on your farm		
Clean milk lasts longer		
Household members can make a good diagnosis and treat sick animals		
Boiled milk lasts longer		
Air-drying milking containers help prolong the shelf life of milk		
Souring the milk makes it more nutritious		

Section D: Dairy farm hygiene practices

Practice	Yes	No
Do you clean the milking area before milking?		
Do you wash your hands with soap before milking?		
Do you use aluminum containers for handling milk?		
Cleaning of milk handling equipment		
Do you drink raw milk?		
Do you store milk in refrigerators?		
Do you use disposable udder towels?		
Do you have individual udder towels?		
Do you dip teats after milking?		
Do you process milk before sale?		

Thank you very much.