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Research article

Agroecology influences *Salmonella* food contamination with high exposure risk among children in Karamoja sub-region: A high diarrhoea prevalent locality in Uganda

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ABSTRACT

Uganda is among low-income countries where *Salmonella* induced diarrhoea still contributes significantly to children under five years (CUF) mortality. Karamoja, a sub-region characterised by three agroecologies (pastoral: PE; agro-pastoral: APE; agricultural: AE), is a geographical location that has consistently experienced a high prevalence of diarrhoea among CUF over the last decade nationally. This study examined whether agroecology influences *Salmonella* food contamination and exposure risk to CUF. A total of 240 food samples (PE: 78; APE: 73; AE: 89) were examined for *Salmonella* load using Xylose Lysine Deoxycholate Agar and confirmed using polymerase chain reaction targeting *invA* gene. Analysis of variance for nested design was used to compare *Salmonella* counts among food types within and across agroecologies and means were separated using Tukey Honestly Significant Difference test at 5% ($p < 0.05$). Proportion of foods with *Salmonella* load exceeding World Health Organisation (WHO) set-limit was expressed in percentage. Exposure risk of CUF was computed by multiplying quantity of food intake by *Salmonella* load and compared to minimum infectious dose (MID) of the pathogen (3 log CFU) whereas chi-square was used to compare proportions of CUF with exposure above or below MID among agroecologies. *Salmonella* load ranged from 0.00 ± 0.00 to 8.23 ± 1.12 log CFU but varied by food type and agroecology. *Salmonella* aggregated load in foods from PE was significantly higher than from APE or AE ($p < 0.05$). The proportion of *Salmonella* contaminated foods from PE, APE, and AE was 33–90%, 40–67%, and 17–88%, respectively. *Salmonella* exposure risks to CUF were similar ($p > 0.05$) at 96%, 92%, and 91% in PE, APE, and AE, respectively. Sorghum-based foods and leafy vegetables were the predominant high-risk foods. This study has revealed that *Salmonella* contamination of foods with high exposure risk among CUF in Karamoja sub-region varies with agroecology. Efforts to address food contamination in the sub-region should take into account agroecology.

1. Introduction

Foodborne diseases account for over 600 million cases of illnesses and over 420,000 deaths globally and have been on the rise in the last decade (Havelaar et al., 2015). Foodborne diseases are mainly caused by pathogenic bacteria, fungi, protozoa, and viruses (Bari and Yeasmin, 2018; Rajkovic et al., 2020; Wokorach et al., 2021). Among predominant foodborne pathogens, the largest burden is caused by diarrheagenic pathogens such as *E. coli*, campylobacter, norovirus, and non-typhoidal *Salmonella enterica*, with children under five years bearing up to 40% of all foodborne disease burden (Havelaar et al., 2015). Non-typhoidal *Salmonella enterica* in particular accounted for up to 230,000 deaths in the year 2010 globally (Havelaar et al., 2015). A recent study in Uganda showed that 41% of hospitalised children under five years that were diagnosed with *Salmonella* infection had diarrhoea (Appiah et al., 2021). The high burden of foodborne diseases in children under five years makes them one of the most vulnerable groups in a society and hence are at higher risk of exposure to foodborne pathogens and associated disease conditions (Gayawan et al., 2021). This is largely because of their underdeveloped immunity (Kloc et al., 2020).

Design of appropriate intervention responses to foodborne diseases necessitates the availability of empirical data (Aik et al., 2020). Whereas data on foodborne diseases are available for developed countries, this is often not the case across many low-income countries (Pires et al., 2021).

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In most situations, information is largely available in terms of prevalence of diarrhoea. A typical example is the case of Uganda where diarrhoea prevalence in children under five years in the country has been reported to stand at 20% (Uganda Bureau of Statistics (UBOS) and ICF, 2018). However, there is limited information on the causal agents and routes through which diarrhoea causing pathogens such as *Salmonella* spp. enter into the food chain in such countries. As such evaluating microbial quality of foods with respect to pathogens such as *Salmonella* is very vital for understanding the magnitude of occurrence of foodborne diseases in the context of low-income countries (Ortega and Tschirley, 2017). Whereas pathogenic microbial load in a given food is a precondition for foodborne infection, toxico-infection or intoxication, the development of a disease condition requires that the ingested dose for a given pathogen overcomes the humoral immune system (Hockney et al., 2020). However, the dose-response relationship varies with respect to specific pathogen virulence potential (Brouwer et al., 2017). This makes exposure risk assessment among communities with high diarrhoea prevalence critical in averting foodborne disease burden. In particular, risk assessment of foods fed to children under five years is essential for achieving sustainable development goals (SDGs), goal-3 which aims to attain good health and wellbeing by 2030 (Pradhan et al., 2017).

In Uganda, Karamoja sub-region is a geographical area with the highest prevalence of diarrhoea among children under five years. The latest demographic health statistics show that the sub-region has a diarrhoea prevalence of 24% which is above the national rate of 20% (Uganda Bureau of Statistics (UBOS) and ICF, 2018). The high prevalence of diarrhoea among children under five years in Karamoja sub-region suggests occurrence of high prevalence of contamination of foods with diarrheagenic pathogenic organisms such as *Salmonella* spp. in the sub-region. Contamination of food or water is likely to occur due to the high non-adherence to hygienic practices of food and water handling or environmental contamination such as open defecation that stood at 65% in the sub-region (UNICEF & WFP, 2016). Agroecology is a likely factor that affects *Salmonella spp*. contamination level in food and exposure risk. This is largely because agroecology presents unique food types whose inherent attributes can influence the extent to which *Salmonella* spp. proliferates and hence their contamination level (Emeana et al., 2019). This suggests that agroecology could play an important role in food contamination and the extent of exposure risk. Karamoja sub-region is characterised by three agro-ecological types. i.e pastoral, agro-pastoral and agricultural zones. The pastoral zone is a semi-arid zone where household food needs mainly depend on livestock production (cattle, goats, and sheep) with crop cultivation only in years of adequate rainfall focused on millet, cowpeas, and groundnuts. The average annual rainfall in pastoral zone is 300–500 mm (United States Agency for International Development (USAID), 2017). The agro-pastoral zone receives an annual rainfall of 500–800 mm, though the rains are erratically distributed. The agro-pastoral zone support crops such as sorghum, millet, maize, beans, cowpeas and groundnuts and livestock reared mainly include steers, bulls, sheep, and goats. The agricultural is a wetter zone with average rainfall ranging from 800 to 1200 mm annually that supports a wide variety of crops (USAID, 2017). However, the influence of agroecology on contamination of food with diarrheagenic pathogens such as *Salmonella* and exposure risk has largely remained unexplored.

Despite the high prevalence of diarrhoea among children under five years in Karamoja sub-region with agroecology-based disparity, it remains largely unknown whether *Salmonella* contamination level and exposure risk among children under five years vary among pastoral, agro-pastoral, and agricultural ecologies. This information is necessary for targeting interventions to address pathogen contamination of food in such localities. This study, therefore, investigated the magnitude of contamination and dietary exposure risk to *Salmonella* spp. among children under five years among pastoral, agro-pastoral, and agricultural agro-ecological zones of Karamoja sub-region.

2. Materials and methods

2.1. Study location and sampling

The study was conducted in Karamoja sub-region, located in the north Eastern part of Uganda. Karamoja sub-region is bordered by Kenya to the East and South Sudan to the North, Acholi and Lango sub-regions to the West and Teso sub-region to the South. The sub-region is comprised of three agro-ecological zones (pastoral, agro-pastoral, and agricultural) (USAID, 2017). A sample size of 240 food samples was used in this study. It was determined using the sample size equation according to Thrusfield (2007) for a known population attribute as presented in Eq. (1).

$$n = \frac{z^2 p(1-p)}{e^2} \quad (1)$$

where n is the required sample size, z is the confidence level at 95% (standard value = 1.96), p is the prevalence of diarrhoea among children under five years admitted in health facilities which stands at 14% (0.14) (Appiah et al., 2021) and e is the margin of error set at 5% (standard value = 0.05). Using Eq. (1), the sample size was found to be 185. However, the sample size was adjusted to 240 to reduce sampling error. In each agro-ecological zone, one district was randomly selected, followed by random selection of two sub-counties in each district corresponding to the respective agro-ecological zone. The selected sub-counties that took part in the study were: Rupa and Tapac in pastoral; Nakapelimoru and Rengen in agro-pastoral and; Lolelia and Napore (Karenga) in agricultural zone as per the map according to Okidi et al. (2022). As such, 78, 73, and 89 food samples were collected from pastoral, agro-pastoral, and agricultural zones to make a total of 240 samples as described under sub-section 2.2.

2.2. Sample collection and transportation

A total of 240 food samples consisting of 154 different types of cooked food samples (cowpea leaves: n = 18; meat: n = 6; sorghum bread: n = 31; beans: n = 36; pumpkin: n = 6; pumpkin leaves: n = 26; sorghum porridge: n = 10 and; maize bread: n = 21), fresh and fermented milk (n = 35), and cereal flours (n = 51) were collected from households in the three agro-ecological zones that characterise Karamoja sub-region of Uganda. The distribution of the samples by agroecology was 78, 73, and 89 for pastoral, agro-pastoral, and agricultural zones, respectively. For each agro-ecological zone, approximately 300–500 g or mL of each food sample from a household was aseptically mixed using a sterile disposable spoon and transferred into sterile screw-capped sampling bottles, labelled, and placed under ice packs at 4 °C in a cool box. All samples were immediately transported in the cool box to the laboratory, stored at 4 °C, and analysed within 12–24 h.

2.3. Culture and enumeration of presumptive colonies of Salmonella

Food samples were analysed for *Salmonella* counts by pour plate technique using Xylose Lysine Deoxycholate Agar (OXOID, Basingstoke, UK) following aerobic incubation at 37 °C for 24 h (Loynachan and Harris, 2005). In the case of solid or liquid food samples, each sample was mixed, followed by weighing 25 g using the MS403S/01 weighing balance (Mettler Toledo, Zürich, Switzerland) or measuring 25 mL using an autoclaved measuring cylinder, respectively. For solid food, the sample was crushed using a sterile mortar and a pestle under Mini-V/PCR Laminar flow cabinet (Telstar, Catalonia, Spain). Each measured food sample was then added to 225 mL of sterile bacteriological peptone (Condalab, Madrid, Spain), homogenised in FB505 stomacher (Fischer Scientific, Pittsburgh, United States) and serially diluted six-fold. Exactly, 1 mL of the samples from appropriate dilutions were pour plated. Each determination was performed in triplicate. The colonies were counted using a digital colony counter (Scan^R 100 Interscience, Paris, France) at a

limit of detection of 2 log colony forming units (CFU)/g or mL (Ongeng et al., 2011). Colonies that were phenotypically pink/red with black centres typical of *Salmonella* spp. as previously applied by Fernández et al. (2021) were counted and converted to CFU/g or mL for solid and liquid food samples, respectively. For each sample, a few colonies phenotypically depicting *Salmonella* spp. were randomly selected from the plates, gram stained and confirmed using Polymerase Chain Reaction (PCR) as described under sub-section 2.4.

2.4. PCR confirmation of Salmonella

Prior to PCR, the genomic DNA (gDNA) was extracted using Quick-DNA Fungal/Bacterial Miniprep Kit (Zymoresearch, California, United States) following the manufacturer's instructions. The DNA extracts were stored at −20 °C until used. PCR was carried out to confirm the isolates as *Salmonella* using primers that target the *invA* virulence gene (invA-F: 5′-GCTCTTTCGTCTGGCATTATC-3′ and invA-R: 5′-GCATCAAATCAAAA-TAGACCG-3′) (Priya et al., 2020). The *invA*, the gene that is particularly involved in host recognition and invasion of the epithelial cells of intestinal mucosa is believed to occur in all *Salmonella* serovars. As such, the *invA* has previously been used in several studies to confirm isolates belonging to the genus *Salmonella* (Villamil et al., 2020; Dantas et al., 2020; Yin et al., 2020). PCR was conducted according to the procedure optimised by Babu et al. (2013) with modifications. Briefly, the PCR reaction was standardised in a final volume of 25 μL consisting of 12.5 μL Taq 2X master mix, 0.5 μL each of the forward and reverse *invA* primer, 9.5 μL PCR water, and 2 μL of gDNA template. The following PCR conditions were used: 1 cycle of initial denaturation at 95 °C for 2 min followed by 35 cycles of actual denaturation at 95 °C for 35 s, annealing at 50 °C for 35 s, extension at 72 °C for 45 s, and a final extension cycle at 72 °C for 5 min. The reaction was performed in an Applied Biosystems Thermal cycler (SimpliAmp™ Thermal Cycler, Singapore, Singapore). PCR amplicons were separated using 1% Agarose gel stained with Ethidium bromide dye at 120 V for 90 min and visualised under the Biorad UV gel documentation system (ChemiDoc™ Imaging System, Singapore, Singapore). Non-typhoidal *Salmonella enterica subsp. enterica serovar Typhimurium* (ATCC 14028) was used as positive control whereas PCR reaction without DNA template (with PCR water instead) was used as a negative control (Figure 1 and Supplementary materials: Figure 2, A–C). All the presumptive isolates randomly selected for PCR confirmation were positive for *Salmonella*.

2.5. Determination of exposure risk to Salmonella among children under five years

Exposure risk was determined using data from food intake that was collected using 24-hour dietary recall (Pastoral: 55; Agro-pastoral: 96 and; agricultural: 89) and *Salmonella* load as determined under sub-section 2.3. The weight of food consumed by each child was established using a digital food weighing scale (Uniweigh, Kerala, India). Using *Salmonella* load (CFU/g or CFU/mL) of a given food determined under sub-section 2.3, the probable ingested number of *Salmonella* was computed for the quantity of a given food consumed by the child within 24 h preceding the survey (Park et al., 2011). The probable *Salmonella* ingested from the different foods by each child were aggregated to obtain an aggregate probable ingested number of *Salmonella* (Eq. (2)).

$$ASI = (QFI_1 \ x \ MSL_1) + (QFI_2 \ x \ MSL_2) + \ldots (QFI_n \times MSL_n) \quad (2)$$

where *ASI* is aggregate *Salmonella* ingested, *QFI* refers to the quantity of food intake for the first, second, and *n*th food, respectively and *MSL* is the mean *Salmonella* load for the first, second, and *n*th food, respectively. *Salmonella* exposure risk was determined by comparing the aggregate probable number of *Salmonella* ingested to the minimum infectious dose of 3 log CFU (Loynachan and Harris, 2005) as presented in Eq. (3).

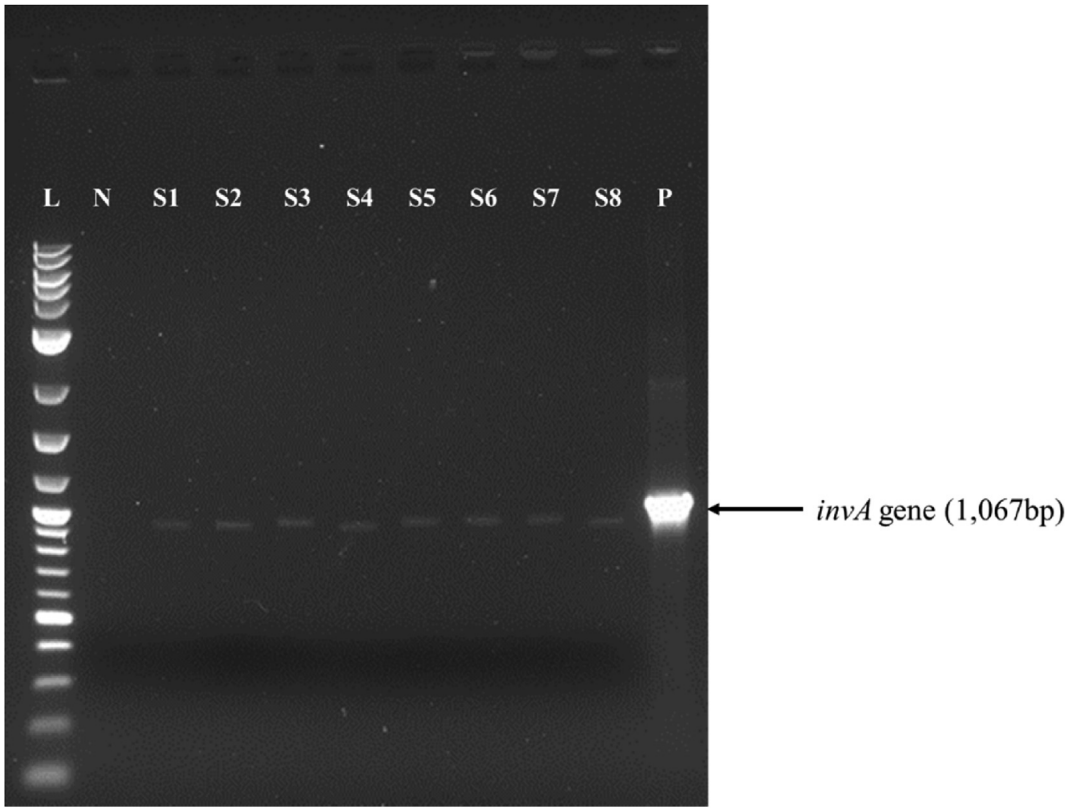

Figure 1. Gel map for confirmation of *Salmonella* isolates. L is 1 kb DNA ladder, N is negative control, S1–S8 are *Salmonella* isolates, and P is positive control (*Salmonella* Typhimurium-ATCC 14028). Additional gel maps are provided as supplementary materials (Figure 2, A–C).

$$SER = \frac{ASI}{MID} \quad (3)$$

where *SER* is the *Salmonella* exposure risk, *ASI* is the aggregate *Salmonella* ingested and *MID* is the minimum infectious dose for *Salmonella*. *SER* was considered to be high or low when the *SER* value was greater or equals/less than one, respectively.

2.6. Ethical considerations

Ethical approval was obtained from Gulu University Research Ethics Committee (Approval number: GUREC-063-19). Chief Administrative Officer (CAO), district nutrition and health inspectors as well as sub-county leaders for the respective districts provided administrative clearance. Each participant (child's mother) was informed of confidentiality and voluntarily signed the consent form before participating in the study.

2.7. Data analysis

Salmonella load data (CFU/g or CFU/mL) was first transformed to Log_{10} and checked for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Normality test showed that the log-transformed data were normally distributed. Therefore, Analysis of Variance (ANOVA) that takes into consideration the nesting of food types at agro-ecological zone level was carried out to establish differences in *Salmonella* load among ecological zones, households, and food types. ANOVA was carried out using SAS software (Version 9.1) making use of the model presented in Eq. (4).

$$Y_{ijkl} = \mu + A_i + B_{j(i)} + C_{k(i*j)} + \varepsilon_{ijkl} \quad (4)$$

where; Y_{ijkl} = observations on *Salmonella* count of the food, μ = overall *Salmonella* mean count, A_i= effect of the ith agro-ecological zone on the observations, $B_{j(i)}$ = effect of the jth household within the ith agro-ecological zone on the observations, $C_{k(i*j)}$ = the effect of kth type of food within j^{th} household and i^{th} agro-ecological zone on observations and ε_{ijkl}= random error associated with Y_{ijkl}. The post-hoc test employing Tukey Honestly Significant Difference (HSD) test at 5% confidence level was conducted to compare mean *Salmonella* counts between agro-ecological zones or between food types across agro-ecological zones. The proportions of the different food types that were microbiologically unsafe were determined by comparing the *Salmonella* count to the World Health Organisation (WHO) maximum threshold (0 CFU/g or mL) for dried and instant products for infants and children (Christian, 1998). This threshold was used taking into consideration the fact that standards for microbial safety of the indigenous foods considered in this study have not been established. The proportion of children under five years with exposure risk above or below the minimum infectious dose among the three agroecologies was compared using chi-square test. Lastly, food types for each agroecology were ranked based on the probable number of *Salmonella* ingested.

3. Results

3.1. Level of Salmonella contamination in foods from various agroecologies

Generally, significant variation in *Salmonella* load in foods by agro-ecology and food type across the three agro-ecological zones were observed (Table 1).

At the agro-ecological level, *Salmonella* aggregated load in foods from pastoral zone was significantly higher than that found in foods from the agro-pastoral or agricultural zone. In the case of food types across the agro-ecological zones, *Salmonella* load varied in three clusters. In the first cluster, beans, maize bread, pumpkin, and sorghum porridge from pastoral; sorghum bread from agro-pastoral, and maize bread from agricultural ecology had identical load of *Salmonella*. However, the loads of the pathogen in the aforementioned foods were higher or lower than detected in cowpea leaves and sorghum flour in agricultural; beans and fresh milk in agro-pastoral; and meat in pastoral ecology. In the second cluster, *Salmonella* load of fermented milk, maize flour, pumpkin leaves, sorghum bread, and sorghum flour from the pastoral; beans, maize flour, pumpkin leaves, and sorghum bread in the agricultural ecology was identical to that of cowpea leaves in agricultural or beans in the agro-pastoral ecology. However, it was significantly higher or lower than detected in foods in cluster one. In the last cluster, fermented milk in agricultural; sorghum flour and cowpea leaves in agro-pastoral zone had identical load of *Salmonella* with foods in cluster one or sorghum flour, fresh milk, and meat in the agricultural, agro-pastoral, and pastoral ecological zones, respectively.

Table 1. *Salmonella* contamination of different food types across agro-ecological zones.

Food type	*Salmonella* count (Log_{10} CFU/g or mL)
Pastoral	
Fermented milk (n = 7)	5.68 ± 2.58^{ab}
Maize flour (n = 5)	6.61 ± 4.11^{ab}
Pumpkin leaves (n = 10)	8.23 ± 1.12^{ab}
Sorghum bread (n = 7)	5.67 ± 3.70^{ab}
Sorghum flour (n = 9)	6.95 ± 3.58^{ab}
Beans (n = 9)	4.14 ± 4.32^{c}
Maize bread (n = 9)	3.91 ± 4.52^{c}
Pumpkin (n = 6)	3.61 ± 4.96^{c}
Sorghum porridge (n = 10)	4.32 ± 6.10^{c}
Meat (n = 6)	0.90 ± 0.31^{d}
Overall pastoral ecology (n = 78)	2.85 ± 1.90^{A}
Agro-pastoral	
Beans (n = 15)	4.44 ± 3.44^{b}
Sorghum bread (n = 16)	3.52 ± 2.85^{c}
Sorghum flour (n = 14)	2.92 ± 3.44^{cd}
Cowpea leaves (n = 12)	2.86 ± 3.30^{cd}
Fresh milk (n = 16)	0.52 ± 2.10^{d}
Overall agro-pastoral ecology (n = 73)	1.18 ± 1.50^{B}
Agricultural	
Cowpea leaves (n = 6)	7.87 ± 0.87^{a}
Beans (n = 12)	5.53 ± 3.37^{ab}
Maize flour (n = 10)	6.74 ± 0.37^{ab}
Pumpkin leaves (n = 16)	7.01 ± 2.83^{ab}
Sorghum bread (n = 8)	5.48 ± 3.42^{ab}
Maize bread (n = 12)	4.12 ± 4.58^{c}
Fermented milk (n = 12)	1.00 ± 2.45^{cd}
Sorghum flour (n = 13)	0.00 ± 0.00^{d}
Overall agricultural ecology (n = 89)	2.65 ± 1.92^{C}

The values are means ± standard deviation. Mean values with different superscripts are significantly different ($p < 0.05$). Superscripts in lower and upper case are for food types and ecological zones, respectively.

The proportion of foods with *Salmonella* counts exceeding the WHO set-limit in foods across agro-ecological zones is presented in Table 2.

Generally, the occurrence of *Salmonella* contamination was above 50% for all foods across the three ecological zones except maize bread in the pastoral zone and fermented milk in the agricultural ecological zone. However, the overall proportion of foods for which contamination exceeded the WHO acceptable limit for *Salmonella* were highest in pastoral ecology and were greater than those observed in the agro-pastoral and agricultural ecologies by 33% and 7.4%, respectively.

Table 2. Proportion of ready to eat foods (%) with *Salmonella* load beyond WHO set-limit across agro-ecological zones.

Food type	*Salmonella* spp.					
	Pastoral agroecology		Agro-pastoral agroecology		Agricultural agroecology	
	n	%	n	%	n	%
Beans	9	55.6	15	66.7	12	75.0
Fermented milk	7	85.7	-	-	12	16.7
Maize bread	9	33.3	-	-	12	50.0
Meat	6	83.3	-	-	-	-
Pumpkin	6	50.0	-	-	-	-
Pumpkin leaves	10	90.0	-	-	16	87.5
Sorghum bread	7	71.4	16	62.5	8	75.0
Sorghum porridge	10	50.0	-	-	-	-
Cowpea leaves	-	-	12	50.0	6	66.7
Overall	64	73.5	43	40.3	66	66.1

3.2. Exposure risk to Salmonella among children under five years

Table 3 shows variation in exposure risk of children under five years to *Salmonella* from different foods consumed across the agro-ecological zones.

Generally, the level of exposure risk to *Salmonella* was high (>90%) across the three ecological zones but were not significantly different in magnitude χ^2 (2, N = 240) =1.564, p > 0.05. However, a higher exposure risk was observed in pastoral ecology where up to 96.4% (53/55) of children under five years were exposed to the pathogen above the minimum infectious dose. A similar magnitude of exposure risk was recorded in the agro-pastoral (91.7%: 88/96), and agricultural ecological zones (91.0%: 81/89). However, the levels determined in the two zones were lower than what was detected in the pastoral ecology by about 5%.

3.3. Ranked Salmonella exposure risk by food type across agroecology

Exposure risk to *Salmonella* varied with food type in each agro-ecological zone (Table 4).

Generally, foods with the highest exposure risk varied with the agro-ecological zone but were dominated by sorghum-based foods and leafy vegetables. For instance, exposure risk from sorghum bread and beans varied from agricultural, pastoral, and agro-pastoral ecology in decreasing order of magnitude. Conversely, exposure risk to the pathogen through consumption of pumpkin leaves and fermented milk was highest in the pastoral than in the agricultural ecology while exposure risk from cowpea leaves was higher in the agricultural than agro-pastoral zone by nearly three-fold. Variations in exposure risk also occurred among foods in each agroecology. In the case of the pastoral ecology, sorghum porridge and bread, as well as pumpkin leaves exposed children under five years to a higher risk than meat by 1047, 483, and 793 $\log_{10}$ CFU, respectively. Minimal variation in exposure risk was observed for foods in the agro-pastoral zone and was highest from beans. The exposure risk from beans in the agro-pastoral zone was higher than from sorghum bread and cowpea leaves by a magnitude ranging from 80 to 128 $\log_{10}$ CFU. In the agricultural ecology, exposure risk from sorghum bread was at least two times that from pumpkin or cowpea leaves and approximately 3 and more than a hundred-fold higher than from beans and fermented milk, respectively. The least risk food for *Salmonella* exposure was fermented milk, cowpea leaves, and meat from the agricultural, agro-pastoral, and pastoral ecological zone, respectively.

Table 3. Proportion of children with high exposure risk to *Salmonella* across agro-ecological zones.

Agroecology	n	Percentage (%)
Pastoral	55	96.4
Agro-pastoral	96	91.7
Agricultural	89	91.0

4. Discussion

Foodborne diseases have been experienced as one of the major public health burdens in both developed and low-income countries in the last decade with viruses, bacteria, and fungi being the major implicated causal agents (Rajkovic et al., 2020; Wokorach et al., 2021; Banna et al., 2022). However, the greatest mortality has been associated with bacterial infections with non-typhoidal *Salmonella* being one of the main bacterial pathogens implicated in high mortality associated with diarrhoeal diseases among children in low-income countries (Marchello et al., 2022). Owing to the high burden of diarrhoea among children under five years in Karamoja sub-region which stands at 24% (Uganda Bureau of Statistics (UBOS) and ICF, 2018), this study investigated the *Salmonella* contamination of foods for children under five years across the three agro-ecological zones that characterise the sub-region. As shown in Table 1, *Salmonella* counts in foods varied significantly among the three agroecologies. The disparity in *Salmonella* counts with agroecology could be a consequence of the varied extent of adherence to recommended hygiene practices for food handling among households located in the three agroecologies (Ehuwa et al., 2021) as well as the limited diversity of foods consumed in the agro-pastoral ecology. Additionally, *Salmonella*

Table 4. *Salmonella* exposure risk level by food type across agroecology.

Food type	Exposure risk (Mean $\log_{10}$ CFU ± Standard Deviation)	Rank
Pastoral agroecology (n = 55)		
Sorghum porridge	1060.45 ± 438.18	1
Pumpkin leaves	806.33 ± 352.29	2
Sorghum bread	496.13 ± 100.23	3
Fermented milk	397.60 ± 98.38	4
Beans	302.11 ± 215.71	5
Pumpkin	295.13 ± 125.72	6
Meat	13.20 ± 12.30	7
Agro-pastoral agroecology (n = 96)		
Beans	299.81 ± 142.78	1
Sorghum bread	219.63 ± 112.07	2
Cowpea leaves	171.92 ± 88.25	3
Agricultural agroecology (n = 89)		
Sorghum bread	1042.23 ± 129.72	1
Pumpkin leaves	560.42 ± 227.95	2
Cowpea leaves	495.59 ± 218.89	3
Beans	311.10 ± 136.15	4
Fermented milk	5.38 ± 9.00	5

load also varied significantly with food type across the different agro-ecological zones. The high *Salmonella* load in cowpea leaves in agricultural than in cereal-based foods; and in pumpkin, beans, fresh milk, and meat in all the three agroecologies suggest that food handling practices are food type-specific and vary with agroecology (Odeyemi et al., 2020). As observed in the case of cowpea leaves in the agricultural ecology, other leafy vegetables also had higher *Salmonella* load across all the ecological zones. This observation suggests that leafy vegetables could be among the food types associated with high levels of non-adherence to hygienic practices of food handling and consequently making them high-risk foods for children in Karamoja sub-region irrespective of the agroecology. On the other hand, the low *Salmonella* load in sorghum flour in the agricultural zone compared to other zones could be due to the presence of higher polyphenolic content exerting stronger antimicrobial properties than sorghum varieties in other zones (Khoddami et al., 2021). Additionally, the observed lower *Salmonella* load in sorghum flour in agricultural zone could also be due to the lower moisture content (typically below 13%) that hinder the growth of bacteria and fungi as opposed to fresh vegetables that generally have high moisture content (Emeana et al., 2019). On the other hand, the significantly lower *Salmonella* load in meat from pastoral ecology compared to other zones could be attributed to the practice of smoking meat which likely confers antimicrobial effects against *Salmonella* despite the likelihood of post-processing contamination due to poor hygiene that has largely been documented in the pastoral ecology (UNICEF & WFP, 2016).

The proportion of foods exceeding the WHO acceptable limit for *Salmonella* varied with agroecology and was at least 50% for most foods across the three ecological zones (Table 2). The level of occurrence of *Salmonella* in foods observed in the current study is somewhat higher than the 40% reported by Raza et al. (2021) for street vended foods in Pakistan. Despite differences in level of occurrence of the pathogen between the two studies, the reported statistics indicate that the organism is still of public health concern in both commercial and indigenous food chains. The higher occurrence of *Salmonella* in foods from pastoral than agro-pastoral or agricultural agroecologies suggests that non-adherence to hygienic food handling practices is more prevalent in pastoral than in other agro-ecological zones. Indeed, a prior study had reported much lower adherence to hygienic practices among pastoralists than agro-pastoralists of Kenya (Wanjala et al., 2016). Additionally, the high level of *Salmonella* contamination in foods from pastoral than agro-pastoral and agricultural ecological zones could also be due to higher interaction between humans and animals, a practice typical of the pastoral ecological zone (Kamau et al., 2021). This is because livestock are known to harbour *Salmonella* in the gastro-intestinal tract and can shed them in faeces both in clinical situations and in asymptomatic state (Calle et al., 2021).

There was low occurrence of *Salmonella* in fermented milk from the agricultural zone compared to samples taken from the pastoral ecology (Table 4). Fermentation is known to inhibit pathogenic bacteria such as *Salmonella* through the production of lactic acid and bacteriocin by lactic acid bacteria (Nemo and Bacha, 2021). In addition, it has been documented that lactic acid accumulation to levels that can elicit antimicrobial activity occurs after 24 h of spontaneous fermentation (Nemo and Bacha, 2021). However, the antimicrobial potential of fermentation is pathogen dependent on the fermentation stage (Abaci et al., 2022) and initial pathogen load (Desta et al., 2021). Therefore, the differences in *Salmonella* load in fermented milk between the two zones could be attributed to the fermentation stage at which samples were collected or differences in the initial level of *Salmonella* before fermentation in the two agroecologies (Owusu-Kwarteng et al., 2020).

A very high but similar proportion of children (>90%) across all the three ecological zones were exposed to *Salmonella* beyond the minimum infectious dose (Table 3). High *Salmonella* exposure risks in the general population due to unhygienic food handling has previously been reported based on food contamination level (Gargano et al., 2021) but limited information exists for children under five years on the basis of minimum infectious dose. This study, therefore, adds another dimension to the available evidence in literature on food-based *Salmonella* exposure risk focusing on children under five years as a function of agroecology. The high exposure risk to *Salmonella* among children under five years with no observed disparity across the three agro-ecological zones recorded in this study suggests that *Salmonella* contamination of food is a widespread public health challenge in the entire Karamoja sub-region. The exposure risk recorded in this study is very high relative to risk rate reported in previous studies conducted in other countries. For instance, Raza et al. (2021) reported the risk of salmonellosis to be at 40% based on contamination level of street vended foods in Pakistan. The difference between the findings of the current study and that of Raza et al. (2021) could be attributed to differences in context between them. Whereas the current study was conducted on foods from households in a purely rural setting, that of Raza et al. (2021) was performed using ready to eat foods in an urban setting. In addition, methodological disparities used in quantifying exposure risk by the two studies could also account for the disparities. Whereas the current study quantified risk based on probable ingested microbial load relative to the minimum infectious dose, Raza et al. (2021) used the proportion of foods contaminated with *Salmonella* instead.

The observed disparity in the extent of exposure risk of children to *Salmonella* from the same type of food among the three agro-ecological zones (Table 4) suggests differential effect of the prevailing food handling practices on contamination level and differences in dietary practices with respect to frequency and quantity of food intake per meal among children in the respective agroecology (Ehuwa et al., 2021; Wokorach et al., 2021). A similar account can be made for the disparity in exposure risk of children to the pathogen from different foods in each agroecology. This study has highlighted the high-risk foods in each agroecology. These foods should be prioritised and strategies to control *Salmonella* contamination designed if the high exposure risk observed among children under five years in each agroecology is to be addressed. Therefore, efforts to reduce the observed high *Salmonella* exposure risk among children in Karamoja sub-region should take into consideration foods through which higher *Salmonella* exposure risk in each agroecology occur.

4.1. Limitations

A major limitation of this study is that it dealt largely with *Salmonella* at the genus level but did not distinguish between *Salmonella bongori* and *Salmonella enterica* and yet only certain serotypes of *Salmonella enterica* are foodborne pathogenic such as *Salmonella* Typhimurium. An in-depth genomic study is underway to understand food associated pathogenic *Salmonella enterica* serotypes circulating in the sub-region.

5. Conclusion

This study has revealed that agroecology influences *Salmonella* food contamination with high exposure risk among children under five years in high diarrhoea prevalent localities in low-income countries such as Karamoja sub-region of Uganda. This study identified foods that are associated with high exposure risk to *Salmonella* among children under five years in pastoral, agro-pastoral, and agricultural ecologies. Therefore, taking the identified high-risk foods as a priority, a concerted effort is needed to reduce *Salmonella* food contamination in order to control foodborne *Salmonella* exposure risk among children under five years in high diarrhoea prevalent localities in low-income countries such as Karamoja sub-region of Uganda.

Declarations

Author contribution statement

Lawrence Okidi, MSc: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Duncan Ongeng, Ph.D; Patrick Simiyu Muliro, Ph.D; Joseph Wafula Matofari, Ph.D: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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