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Research Reports

Prevalence of *Salmonella spp.* and *Escherichia coli*, in Rodents and Shrews with Their Associated Risk Factors

Prevalensi *Salmonella* dan *Escherichia coli* pada Hewan Pengerat dan Curut beserta Faktor Risiko Terkaitnya

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ABSTRACT

Background: Rodents are known to be a source of foodborne diseases; however, few researchers have examined rodent faeces. **Purpose:** This study aimed to determine the prevalence of foodborne *Salmonella spp.* and *Escherichia coli (E. coli)* and the risk factors associated with rodents and shrews in five wards from Morogoro Municipality, Tanzania. **Method:** A total of 148 rodents and shrews were captured from domestic, peri-domestic, and marketplace settings. This study isolated bacteria from faeces samples collected from rodents and shrews using a culture test, and identified them using biochemical tests. Molecular tests were used to screen out bacteria-targeted genes. Questionnaires were also used to assess the risk factors of foodborne *Salmonella spp.* and *E. coli* associated with house rodents and shrews. **Results:** *Salmonella enterica* was detected in 3/148 (2%), and *E. coli* was found in 54/148 (36.5%) of the samples. Regarding habitat, a high prevalence of *E. coli* was observed in open markets, at 16.9%, while Salmonella enterica was high inside households, at 1.3%. The results show that 83% of respondents found rodents feces in uncooked or cooked food, 30.4% found rodents feces in the water storage, 93.2% found food eaten by a rodent, and 66.9% of households used food contaminated with feces or eaten by rats. **Conclusion:** The study shows that rodents and shrews carry foodborne pathogens like *Salmonella enterica* and *Escherichia coli* in Morogoro municipality, Tanzania, posing serious public health risks. The rodents had high rates of *E. coli* and low *Salmonella enterica*, with risk factors linked to food and water contamination.

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ABSTRAK

Latar Belakang: Hewan pengerat diketahui sebagai sumber penyakit *foodborne diseases*; namun, hanya sedikit peneliti yang meneliti feses hewan pengerat tersebut. **Tujuan:** Penelitian ini bertujuan untuk menentukan prevalensi *Salmonella spp.* dan *Escherichia coli (E. coli)* bawaan makanan dan faktor risikonya yang terkait dengan hewan pengerat dan tikus tanah. **Metode:** Penelitian ini mengisolasi bakteri dari sampel feses yang dikumpulkan dari hewan pengerat dan tikus tanah menggunakan uji kultur dan mengidentifikasinya dengan uji biokimia. Uji molekuler digunakan untuk menyaring gen yang ditargetkan pada bakteri. Kuesioner juga digunakan untuk menilai faktor risiko *Salmonella spp.*dan *E. coli* bawaan makanan yang terkait dengan hewan pengerat dan tikus tanah rumah. **Hasil:** *Salmonella enterica* terdeteksi pada 2% sampel dan *E. coli* ditemukan pada 36,5% sampel. Mengenai habitat, prevalensi *E. coli* yang tinggi ditemukan di pasar terbuka, sebesar 16,9%, sedangkan *Salmonella enterica* tinggi di dalam rumah tangga, sebesar 1,3%. Sebanyak 83,7% responden menemukan feses hewan pengerat pada makanan mentah atau matang, 30,4% menemukan feses hewan pengerat pada tempat penyimpanan air, 93,2% menemukan makanan yang dimakan oleh hewan pengerat, sedangkan 66,9% rumah tangga menggunakan makanan yang terkontaminasi feses atau dimakan oleh tikus. **Kesimpulan:** Penelitian menunjukkan bahwa tikus dan curut membawa patogen bawaan makanan seperti Salmonella enterica dan *Escherichia coli* di Kotamadya Morogoro, Tanzania, yang menimbulkan risiko kesehatan masyarakat yang serius. Rodensia memiliki *E. coli* yang tinggi dan *Salmonella enterica* yang rendah, dengan faktor risiko yang terkait dengan kontaminasi makanan dan air.

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Kata kunci: *Salmonella spp.; E. coli;* Faktor Risiko; Prevalensi

INTRODUCTION

Rodents comprise more species than any other mammal order (Zhang *et al.*, 2022). Most rodents are considered keystone species in their ecological communities; hence, the survival of many different species in the ecosystem depends on them (Delibes-Mateos *et al.*, 2011). When it comes to public health, this is particularly important for rodent-borne diseases. In the particular case of foodborne illnesses, rodents are the reservoirs and transmitters of various bacterial zoonoses, including *Salmonella spp.* and *Escherichia coli (E. coli)* pathogens, which are responsible for foodborne diseases (Jahan *et al.*, 2021).

Foodborne diseases (FBD) result from consuming contaminated or naturally harmful food or drink with pathogenic microorganisms present (Rakshna *et al.*, 2020). Foodborne diseases are mainly spread to people by eating contaminated food that has come into contact with rodent excrement and urine, pests (including rodents), human hands, or water and soil infested by rodents (Torgerson *et al.*, 2015). Many FBDs such as *Salmonella* and *E. coli* infections are zoonotic since they can be spread from animals to humans (Chlebicz, 2018); some are also novel diseases or illnesses that are abl to change in their hosts, locations, or effects (Cissé, 2019). Rodents have been linked to the spread of some of the foodborne diseases commonly found in humans, as stated by the World Health Organization (Kadariya *et al.*, 2014). Different rodent species are highly significant to public health because they are reservoirs of pathogens, such as *Salmonella spp.* and *E. coli* (Hardgrove *et al.*, 2021). According to Jemilehin *et al.*, (2016), rodents feed on stored food and contaminate it with urine and faeces that may contain harmful pathogens. They can also be the carriers of resistant strains of *Salmonella spp.* and *E. coli.* These bacteria are excreted in faeces and are known to cause significant illnesses in humans, like salmonellosis and gastroenteritis (Ma *et al.*, 2019; Sobrinho *et al.*, 2020).

Salmonella spp. foodborne poisoning is one of the most common and widely distributed diseases in the world, estimated to cause 1.3 billion cases of gastroenteritis and three million deaths worldwide (Al-Harthi, 2012). *E. coli* frequently contaminates animals and humans compared to other microbes, and is a reliable indicator of faeces contamination (Samet-Bali *et al.*, 2013). Also, *E. coli* is mainly abundant in the intestinal tract of most mammalian species, including humans and rodents (Fairbrother, 2006). Most *E. coli* are commensal but some are known to be harmful or pathogenic bacteria, causing severe intestinal and extra-intestinal diseases in humans (Wasiński, 2019).

Studies conducted by different researchers in Tanzania show that rodents harbor numerous bacteria species. In Karatu District, 79.2% of *E. coli* isolated from 101 rodents revealed that the intense interaction between humans, animals, and rodents in particular can cause epidemics (Sonola *et al.*, 2021). The study conducted in Ngorongoro District reported a high prevalence of *Salmonella enterica* bacteria, at 43.75% in wild rodents compared to dogs and humans (Issae *et al.*, 2023). The same study reported a lack of knowledge of rodent-borne zoonosis; only 28.13% of participants were aware, while 77.27% of them believed that rodents are animals that destroy crops and do not spread disease. This attitude may be a risk factor of foodborne *Salmonella spp.* and *E. coli* (Issae *et al.*, 2023). In Morogoro, the most common diseases are typhoid, diarrhea, dysentery, and cholera, which are caused by eating contaminated food. Ndunguru found that 67.1% of foods ready-to-eat were contaminated with bacteria associated with rodents and shrews (Ndossi, 2020). However, in the studies conducted in Morogoro, the available information needed to show the source and transmission of bacteria responsible for foodborne diseases is lacking.

Therefore, this study aimed to establish the prevalence of foodborne *Salmonella spp.* and *E. coli* from house rodents and shrews in Morogoro municipality, Tanzania, by (1) determining the prevalence of *Salmonella spp.* and *E. coli* bacteria in rodents captured from house and food markets, and (2) evaluating the risk factors of *Salmonella spp.* and *E. coli* associated with rodents in Morogoro's urban areas. The findings of this study will be helpful to the decision-makers in the public health sector, enabling them to determine the strategies that must be used to reduce the transmission of foodborne pathogens through rodent and shrew management.

MATERIALS and METHODS

Study Area

The study was conducted in five wards of Morogoro municipality: Magadu, Mzinga, Bigwa, Kiwanja cha Ndege/ Mawenzi market, and Mji mpya. Kiwanja cha Ndege/ Mawenzi market and Mji mpya were selected to represent food markets. Magadu, Mzinga, and Bigwa were chosen to represent urban farming in Morogoro, as shown on the map below (Figure 1).

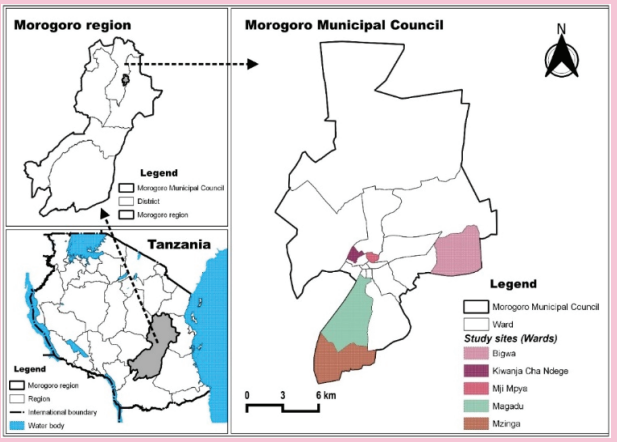

Figure 1. Map of the Morogoro Region and Morogoro Municipal Council showing the wards where the study was conducted. The map was developed using QGIS software version 3.26.1 and the shapefiles are from DIVA-GIS (https://www.diva-gis.org/Data), which was freely accessible by the researcher.

Research Design

Within the framework of a cross-sectional study design, purposive sampling was used to select households and food markets. Only households who voluntarily decided to participate in this study were contacted when collecting the samples, and they were given a consent form to sign before conducting any sampling. The representatives of each household were interviewed to obtain information on the risk factors associated with foodborne diseases caused by rodents (Zheng, 2015; Mweshi, 2020). The trapping sites included areas such as the surroundings of restaurants, other eating establishments, and food markets; the rodents were trapped using locally-made wire cages, and Havarhart and Sherman traps were randomly placed inside and outside the selected premises. The traps were baited with peanut butter mixed with maize bran, tomatoes, sweet potatoes, avocado, green maize, and ripened bananas. A minimum number of three traps were used in each premise. The traps were kept for three consecutive nights, and checked and re-baited every morning. All rodents caught were taken to the laboratory at the SUA so then they could be identified, and samples collected (Hoffmann *et al.,* 2005; Thomas *et al.,* 2022).

Collection of Rodent Faeces

Each captured rodent was anesthetized using Diethyl Ether. The gastrointestinal tracts were sliced and opened to remove all contents from the small intestine to the caeca of the rodents. According to the method of Nkogwe *et al.*, (2011), the faeces were collected and stored in a sterile container at 40ºC and transported to the Microbiology Laboratory of the Department of Microbiology, Parasitology, and Biotechnology at Sokoine University of Agriculture (SUA) for further analysis using the Cary Blair Transport Medium (Nagata *et al.,* 2019).

Isolation and Identification of *Salmonella spp.* and *E. coli*

The collected faeces samples were pre-enriched using buffered peptone water (BPW) and were incubated at 37°C for 18–24 h. Using aseptic techniques, 100 µL of the enriched sample was transferred to 10 mL of warmed Rappaport Vassiliadis Soya (RVS) broth (Oxoid) for the selective enrichment of *Salmonella spp.*, then incubated at 41.5°C for a 21-27h preferred water bath. Following incubation, the RVS broth was inoculated onto XLD plate agar and incubated at 37°C for 21–27 h. All XLD plates were observed for *Salmonella spp.*–like colonies, and the positive colonies, which showed as red with or without black centers, were sub-cultured to obtain pure colonies. The enriched samples with buffered peptone water in aseptic procedures were also inoculated onto MacConkey agar and incubated for 18–24 h at 37°C. After incubation, the plates were observed for colonies typical of *E. coli*. The positive colonies, which showed as pink, were sub-cultured to obtain pure colonies. Further biochemical confirmation was then undertaken (Himsworth *et al.,* 2015).

All bacterial colonies were morphologically studied and suspected *Salmonella spp.* and *E. coli* were biochemically confirmed using the Triple Sugar Iron (TSI) test, the (IMViC) test, the SIM test, and the Urea test (Iyer *et al.,* 2013; Shahryari *et al.,* 2017). Isolates that passed all biochemical tests for *Salmonella spp.* and *E. coli* were transferred and cultivated on nutrient agar (N.A.) for molecular confirmation (Nkogwe *et al.,* 2011).

Molecular Analysis of *Salmonella spp.* and *E. coli*

DNA Extraction

The genomic DNA was isolated from the overnight growth bacterial colony using the boiling method. Briefly, the colonies were taken using sterile swabs from a petri dish containing pure *Salmonella spp.* and *E. coli* cultures, and were then transferred in an eppendorf tube containing 100 µl of the nuclease-free water and boiled in a water bath at 95°C for 5 min. This was followed by an immediate transfer to a -20°C freezer for 10 min to purposely stress the bacterial cells to prompt them to release their internal components. This procedure was repeated, and the suspension was centrifuged at 12,000 rpm for 1 minute. Eighty microliters of the supernatant was taken using a micropipette for further processing. The concentration and quality of the extracted DNA was checked by gel electrophoresis (1.5% agarose gel) in order to look at the intactness of the band, and what resulted was spectrophotometrically quantified using th Nano Drop Spectrophotometer to examine the quality and quantity of the extracted DNA. All extracted DNA was stored at -20°C for further analysis (Dashti, 2009; Khosravi *et al.,* 2012; Bagus *et al.,* 2017).

Molecular Identification of Bacterial Species

All bacterial colonies presumptively identified based on biochemical and phenotypic characteristics were subjected to molecular identification using a thermal cycler. The primers (forward and reverse primers) were designed to give a product of approximately 585 base pairs targeting *Escherichia coli*, and 796 base pairs targeting *Salmonella spp.* for Polymerase chain reaction (PCR) amplification. PCR was performed using a master mix (Bioneer premix-Korea). The primers used are described in Table 1 (James, 2010; Silva *et al.,* 2011; Nanteza *et al.,* 2020). The PCR amplification for *Salmonella spp. and E. coli* was done under the following conditions respectively: Initial denaturation steps at 95°C for 5 minutes, final denaturation at 94°C for the 30s, annealing at 58°C, 55°C for 30 seconds, and extension at 72°C for 30 seconds followed by final extension at 72°C for 10 min. The reaction was run for 35 cycles and final cooling was maintained at 40°C. The agarose gel (1.5%) stained with ethidium bromide was used to analyze the PCR products (Amplicons) through gel electrophoresis. The ultraviolet trans-illumination machine visualized positive bands (Rahayuningtyas *et al.,* 2020; Alshaheeb *et al.,* 2023).

Sequencing and Phylogenetic Analysis

All PCR products were cleaned using the Zymo Kit (Quick-DNATM Miniprep Plus Kit) according to the manufacturer's instructions, and the sequencing was done by the Macrogen company in Korea based on commercial consider-

Table 1. Primers for The Amplification of *Salmonella spp.* and *Escherichia coli*

Bacteria	Primer Name	Primer Sequence	Size of the PCR product	References
E. coli	16s Forward	5' GACCTCGGTTTAGTTCACAGA 3'	585bp	(James, 2010; Moawad *et al.*, 2017)
	16s Reverse	5' CACACGCTGACGCTGACCA 3'		
Salmonella spp.	invA Forward	5' CGGTGGTTTTAAGCGTACTCTT 3'	796bp	(Silva *et al.*, 2011 ; Paião *et al.*, 2013;)
	invA Reverse	5' CGAATATGCTCCACAAGGTTA 3'		

ations. Raw sequences of the forward primer and reverse primer amplicons were imported into the Bioedit software, then trimmed to remove noise, and finally combined to create a consensus sequence for each sample. The similarity of the consensus sequences was compared with the published sequences in the GenBank database using the nucleotide BLAST program. The isolates were identified at the species level based on ≥ 95% for *E. coli* and ≥ 91% for *Salmonella spp.*, with the sequence determining reported strains (Altschup *et al.*, 1990). The ClustalW Program within the Mega11 software version 11.0 was used to carry out multiple alignments (Kumar *et al.*, 2021). The neighbor-joining method was utilized to infer the phylogenetic trees (Gascuel, 2006), and the estimated reliability of the phylogenetic trees was determined using the bootstrap method. It was decided to delete any missing alignment gaps or data, and the tree was rooted in Proteus species for *E. coli* and the Pseudomonas species for *Salmonella enterica* (Hall, 2013).

Questionnaire Survey

A pretested structured questionnaire was administered to the households and shop owners. A total of 148 households from markets, restaurants, and farmers have been interviewed. The questionnaire captured issues related to the risk factors of foodborne diseases associated with house rodents and shrews. The questions were focused on the consumption and management of food eaten by rats or that had come into contact with rat faeces to see whether it was rejected or consumed by humans. All participants signed the consent form before they were interviewed (Länsimies-Antikainen *et al.*, 2010).

Data Analysis

The prevalence of *Salmonella spp.* and *E. coli* in house rodents and shrews was calculated per site, species, and habitat. The data was analyzed using Microsoft Excel (2016) and the Statistical Package for Social Sciences (SPSS) Program Version 29.0 (2022) (https://www.ibm.com). Chi-square was used to assess the significance of the prevalence of *Salmonella spp.* and *E. coli* between the distinct species of rodents and shrews, as well as by site and habitat. Chi-square was also used to compare the prevalence of the observations made at the various sampling sites. Differences were considered significant at <0.05 (Hanson *et al.*, 2002; Hosein *et al.*, 2008).

DISCUSSION

Total Number of Rodents and Shrews Captured

A total of 148 rodents grouped into three species, *Rattus rattus, Mus spp., Cricetomys ansorgei,* and *Shrews spp.*, were trapped in different areas and used to assess *Salmonella spp.* and *E. coli*. *Rattus rattus* (51.4%) and *Mus spp.*(41.2%) were more abundant than the other species captured. The rodents were found to be more prevalent inside households (53.4%) and in the open market (28.4%) than in other habitats; the distribution of captured animals per sex is not too different, as shown in Tables 2 and 3.

Table 2. Total Number of Rodents and Shrews Captured.

Rodent Species & Shrews	Frequency	Percent (%)
Cricetomys ansorgei	8	5.4
Mus spp.	61	41.2
Rattus rattus	76	51.4
Shrew spp.	3	2.0
Total	**148**	**100**

Table 3. Sex of Rodents and Shrews, and Their Distribution.

	Distribution	Frequency	Percent (%)
Sex	Female	76	51.4
	Male	72	48.6
	Total	**148**	**100**
Habitat	Inside household	79	53.4
	Open market	42	28.4
	Outside household	11	7.4
	Shops	16	10.8
	Total	**148**	**100**

Prevalence of *Salmonella spp.* and *E. Coli* Among Rodent Species Captured.

Among 148 faeces samples from captured mammals, 54 out of 148 samples tested positive for *E. coli*, representing 36.5% of the rodents. The bacteria were found in the *Mus* species at a prevalenc of 16.2%, followed by *Rattus rattus* at 13.5% (Table 4). Also, the results showed that 3 rodents were positive for *Salmonella enterica*, representing 2% of the total sampled rodents and shrews. The most infected rodents with Salmonella enterica were found in *Mus spp.*, at 1.3% of the total samples (Table 4). The data analysis showed a statistically significant difference in the prevalence of *E. coli* tested under different species ($p<0.05$) using Pearson Chi-Square. There was no statistical significance between the prevalence of Salmonella enterica tested in *Mus spp.* and that found in *Rattus rattus*.

Prevalence of *Salmonella spp.* and *E. Coli* in Rodents in Selected Wards

A greater prevalence of *E. coli* positive was found in the Mji Mpya market, making up 15.5% of 148 samples tested, followed by Mawenzi market with 9.5%. *Salmonella spp.*, with

1.3% of 148 samples tested having been found positive from Mawenzi market and 0.7% from Mzinga. The prevalence of both bacteria was also observed in a different ward of Morogoro Municipality, as shown in Table 5 below. The data analysis showed a statistically significant difference in the prevalence of E. coli tested from the different sites selected ($p<0.05$) using Pearson Chi-Square. However, there was no statistical significance between the prevalence of Salmonella enterica tested in the Kiwanja cha Ndege/Mawenzi and Mzinga wards.

Prevalence of *Salmonella spp.* and *E. coli* per Habitat

A greater prevalence of *E. coli* positive was found in the Mji Mpya market, making up 15.5% of 148 samples tested, followed by Mawenzi market with 9.5%. *Salmonella spp.*, with 1.3% of 148 samples tested having been found positive from Mawenzi market and 0.7% from Mzinga. The prevalence of both bacteria was also observed in a different ward of Morogoro Municipality, as shown in Table 5 below. The data analysis showed a statistically significant difference in the prevalence of E. coli tested from the different sites selected ($p<0.05$) using Pearson Chi-Square. However, there was no statistical significance between the prevalence of Salmonella enterica tested in the Kiwanja cha Ndege/Mawenzi and Mzinga wards.

Prevalence of *Salmonella spp.* and *E. coli* per Habitat

A high prevalence of *E. coli* was observed in the open market at 16.9%, followed by inside households 13.5%, outside households 4.1%, and 2.0% in the shops, while the greatest prevalence of *Salmonella enterica* was observed inside households at 1.3 % and 0.7% in the open market as shown in Table 6. The results showed a statistically significant difference in the prevalence of *E. coli* tested under different habitats for rodents and shrews ($p<0.05$) using the Pearson Chi-Square test. On the contrary, there was no statistical significance between the prevalence of *Salmonella enterica* tested inside households and that found in the open market.

Isolation and Biochemical Identification

A total of 148 feces samples from house rodents and shrews were analyzed; 77.7% (115 samples) of the samples were suspected of being *E. coli* and *Salmonella spp.* positive after using the culture method. A total of 115 bacteria isolates were kept apart for further identification using a biochemical test. The isolates were identified as *Escherichia coli*, at 58.1% (86 samples). In comparison, *Salmonella spp.* made up 2.7% (4 samples) after being confirmed biochemically using the Triple sugar iron (TSI) test, the (IMViC) test, the SIM test, and the Urea test. Accordingly, the data for *Escherichia coli* has a high prevalence compared with *Salmonella spp*.

Molecular Detection of *Salmonella spp.* and *E. coli*

A total of 90 gram-negative bacterial isolates were amplified using universal primers targeting the 16S rRNA gene. The results showed that 36.5% (54 samples) of *E. coli* were positive, and 2% (3 samples) were *Salmonella enterica*. All positive amplicons that appeared on the 585 bp marker were *E. coli*, as shown in Figure 2, and the positive isolates for *Salmonella spp.* were located at 796 bp, as shown in Figure 3.

Table 4. Prevalence of *Salmonella spp*. and *E. coli* Per Rodent and Shrew Species.

Pathogen	Result	Species				Total
		Cricetomys ansorgei	*Mus spp.*	*Rattus rattus*	*Shrew spp.*	
Total Sample Tested		**8**	**61**	**76**	**3**	**148**
E. coli	Mammals infected	7(4.7%)	24(16.3%)	20(13.5%)	3(2.0%)	54(36.5%)
	% within species	7(87.5%)	24(39.3%)	20(26.3%)	3(100.0%)	
Salmonella spp.	Mammals infected	0(0%)	2(1.3%)	1(0.7%)	0(0%)	3(2%)
	% within species	0(0%)	2(3.3%)	1(1.3%)	0(0%)	

Table 5. Prevalence of *Salmonella spp.* and *E. coli.* Per Ward.

Pathogen	Result	Species					Total
		Bigwa	Magadu	Mawenzi	Mji Mpya	Mzinga	
Total Sample Tested		**11**	**16**	**55**	**36**	**30**	**148**
E. coli	Mammals infected	6(4.1%)	5(3.4%)	14(9.5%)	23(15.5%)	6(4.0%)	54(36.5%)
	% within wards	6(54.5%)	5(31.2%)	14(25.4%)	23(66.6%)	6(20 %)	
Salmonella spp.	Mammals infected	0(%)	0(%)	2(1.3%)	0(%)	1(0.7%)	3(2 %)
	% within wards	0(%)	0(%)	2 (66.7%)	0(%)	1(33.3%)	

Table 6. Prevalence of *Salmonella Spp.* and *E. coli* Per Habitat.

Pathogen	Result	Species				Total
		Inside Household	Open Market	Outside Household	Shop	
Total Sample Tested		**79**	**42**	**11**	**16**	**148**
E. coli	Mammals infected	20(13.5%)	25(16.9%)	6(4.1%)	3(2%)	54(36.5%)
	% within habitat	20(25.3%)	25(59.5%)	6(54.5%)	3(18.7%)	
Salmonella spp.	Mammals infected	2(1.3%)	1(0.7%)	0(0%)	0(0%)	3(2%)
	% within habitat	2(2.5%)	1(2.4%)	0(0%)	0(0%)	

Sequencing Analysis

The obtained nucleotide sequences of the 16S r RNA gene of all bacterial isolates were analyzed using BLAST (Basic Local Alignment Search Tool, http://www.ncbi.nlm.nih.gov-/BLAST/Blast.cgi) for identification according to the close reference sequences available in GenBank (Figure 4). The BLAST analysis showed the similarity of *Salmonella spp.* and *E. coli* from this study to samples from the GenBank database. The results of the sequenced PCR product confirm the results obtained in previous tests (Culture and Biochemical) in the current study; 3 (2%) were *Salmonella enterica*, and 54 (36.5%) were *E. coli*.

The neighbor-joining phylogenetic trees based on 16S rRNA gene partial sequences of *Escherichia coli* (Bolded) obtained from this study are clustered with the closely-related genera of the family Enterobacteriaceae retrieved from the GenBank database. The bootstrap values (expressed as percentages of 1000 replications) are shown at the branch points. The Proteus species was used as an outgroup to root the tree.

The neighbor-joining phylogenetic trees based on InvA gene partial sequences of Salmonella enterica (Bolded) obtained from this study are clustered together with the closely-related genera of the family Enterobacteriaceae retrieved from the GenBank database. The bootstrap values (expressed as percentages of 1000 replications) are shown at the branch points. The Pseudomonas species was used as an outgroup to root the tree.

Risk Factors of the Foodborne Diseases Associated with House Rodents.

A total of 148 participants were interviewed during the survey of this study; each participant represented one household. The results of the questionnaire used during the data collection showed that rodents were present in 94.6% of selected households. This survey showed that rodents caused problems, including eating food in storage facilities, as well as the fruits and maize bran used to feed livestock. This study also revealed the risk factors associated with foodborne *Salmonella spp.* and *E. coli* from rodents and shrews, as shown in Table 7. In some households, 24.3% did not have food stores; this increased the likelihood of rodent invasion and the transmission of pathogens harbored by them. Similarly, 10.8% of households did not cover food before and after cooking, and 83.70% of respondents found the faeces of rodents in uncooked or cooked food. Additionally, 22.3% removed the faeces with their bare hands, 12.8% of them did not wash their hand after handling either the rodents and their faeces, 30.4% found the faeces of rodents in the water storage, 93.2% of households found food eaten by rats, 66.9 % of households had used the food contaminated with faeces or eaten by rodents, and 27% within the households selected were diagnosed with diarrhea, which may have a link with the pathogens from house rodents. This study revealed that the main risk factors of foodborne diseases were using food contaminated by rodent faeces and urine, or what has been eaten by them and otherwise spoiled foods.

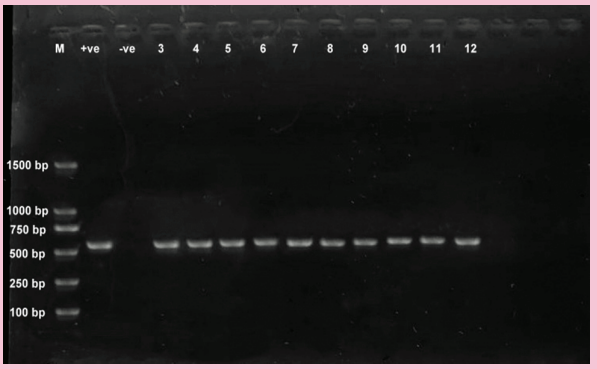

Figure 2. PCR amplification of *E. coli* species, Where M is a DNA molecular marker and lanes 3-12 are samples, where lanes 3-12 are positive samples located at 585bp and lanes 1 - 2 are positive and negative controls, respectively. For all isolates confirmed as *E. coli* positive, seven isolates were from *Cricetomys ansorgei*, 24 were *Mus spp.*, 20 were *Rattus rattus*, and three isolates were from shrews.

Figure 3. PCR amplification of *Salmonella spp.*; Where M is a DNA molecular marker and lanes 3-5 are samples, lanes 3-5 are positive samples located at 796bp, and lanes 1 and 2 are positive and negative controls, respectively. These three isolates confirm *Salmonella enterica;* 2 isolates were from *Mus spp.*, and one was from *Rattus rattus*.

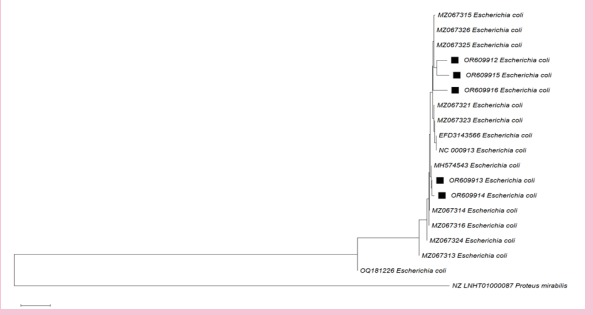

Figure 4. Phylogenetic tree of *E. coli.*

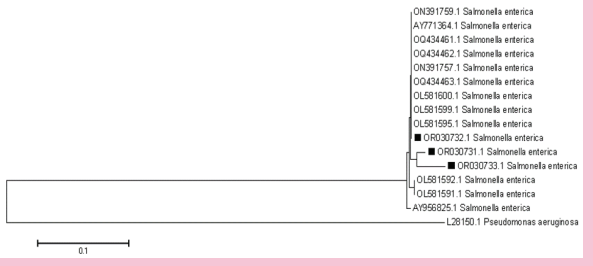

Figure 5. Phylogenetic tree of *Salmonella enterica.*

Table 7. Risk Factors of Foodborne *Salmonella spp.* and *E. coli* Associated with House Rodents.

Variables	Household and Shop Interviewees (n= 148), % = Percentage		
	Yes	No	Percentage of Risk Factors
Presence of rodents at home	140 (94.6%)	8(5.4%)	94.60%
Problems of rodents at home (eating food in storage, clothes, maize bran, fruits, flour, papers)	138(93.2%)	10(6.8%)	93.20%
If they have a food store	112(75.7%)	36(24.3%)	24.30%
Cover food before and after cooking	132(89.2%)	16(10.8%)	10.80%
Faeces of rodents in uncooked or cooked food	124(83.7%)	24(16.3%)	83.70%
Faeces removed by hand	33(22.3%)	115(77.7%)	22.30%
Washing hands after handling rodents or their faeces	129(87.2%)	19(12.8%)	12.80%
Faeces of rodents in the water storage	45(30.4%)	103(69.6%)	30.40%
Found food eaten by rodents	138(93.2%)	10(6.8%)	93.20%
Consummation of food in contact with faeces or eaten by rodents	99(66.9%)	49(33.1%)	66.90%
Diarrhea in the last three months	40(27%)	108(73%)	27%

DISCUSSION

Sequencing Analysis

The trapping activities conducted in each selected premises in Morogoro municipality captured 148 mammals, whereby 145 were rodents grouped into three species, specifically *Rattus rattus, Mus spp.*, and *Cricetomys ansorgei*, and 3 were shrew spp. Out of the 148 mammals, 54 were confirmed positive for *E. coli*, representing 36.5% of all captured rodents and shrews, while only three were confirmed positive for *Salmonella spp*. representing 2% of all captured rodents and shrews. The study results indicate that the main prevalence of *E. coli* and *Salmonella spp.* bacteria is in *Mus spp.* and *Rattus rattus*. The results are similar to what was observed in the study conducted by Kimwaga et al. (2022) in Tanzania, Kilosa district, which reported a higher prevalence of *E. coli* and *Salmonella spp.* in Rattus rattus than other species.

Similarly, the study conducted by Sonola *et al.* (2021) in Karatu District reported a high prevalence of *E. coli* in rodents captured indoors and per domestic habitat, and the study by Issae *et al.* (2023) in Ngorongoro District reported a high prevalence of *Salmonella spp.* in wild rodents. *Mus spp.* and Rattus rattus are known to be more abundant where there is food, meaning that the transmission of these pathogens to humans is high when they consume contaminated food and water (Meerburg *et al.*, 2009). These species have been researched and been found to serve as carriers for numerous human diseases (Hill, 2011; Murray *et al.*, 2020). According to Jahan *et al.* (2021), it has been observed that *Mus spp.* and *Rattus rattus* can be effectively infected by *E. coli* and *Salmonella spp.*, which can persist for a duration exceeding ten months. In the current study, *E. coli* exhibited a high prevalence, accounting for approximately 36.5% of the total species composition in the feces samples. However, the low prevalence of *Salmonella enterica* does not mean that it cannot have a negative impact on human health (Jahan *et al.*, 2021). According to Jahan *et al.* (2021), just one rodent in a house or food-producing facility can introduce around 23 million *Salmonella* pathogens into production pipelines in 24 hours.

The high prevalence of *Salmonella enterica* and *E. coli* inside households and open markets found in this study was influenced by the rodents captured in these premises compared to others. Rodents prefer to live where they can easily find food and leave behind pellets containing *E. coli* and *Salmonella spp.* bacteria. These bacteria can be transmitted from rodents to other animals and humans (Phifer-Rixey, 2015). Therefore, food markets and households are particularly vulnerable to *E. coli* and *Salmonella spp.* (Damborg *et al.*, 2016; Ribas *et al.*, 2016). Hence, rodents and shrews can act as reservoirs of several pathogens, and controlling them effectively leads to the control of these pathogens and the diseases they cause (Kijlstra, 2007).

This study has assessed the possible risk factors associated with foodborne *Salmonella spp.* and *E. coli*, where 27% of the respondents mentioned having diarrhea after eating food contaminated by the feces and urine of rodents or that had been eaten by rodents. These pathogens are a threat to public health as they cause human diseases such as diarrhea, typhoid, urinary tract infection, salmonellosis, and gastroenteritis (Tawyabur *et al.*, 2020). The risk factors identified in this study are related to the results from the study conducted by Chengula *et al.* (2015) in Morogoro municipality, who found several diseases, such as diarrhea, dysentery, cholera, typhoid, worm diseases, and different bacteria, whereby the root causes were *Salmonella typhimurium* (16.7%) and *E. coli spp.* (8.3%) in relation to the frequency of isolation from solid waste disposal in Morogoro municipality, with direct linkage with rodents and shrews.

The results in Table 7 illustrate the risk factors by which foodborne *E. coli* and *Salmonella spp.* are transmitted from rodents and shrews to humans. In brief, human exposure to foodborne *E. coli* and *Salmonella spp*. in rodents and shrews can occur through various pathways. These include the feces and urine of rodents in uncooked or cooked food, feces in water storage, eating food eaten by rodents, etc. (Islam *et al.*, 2022). Additionally, the consumption of edible rodents and associated foods contaminated with foodborne Salmonella spp. and *E. coli*, particularly when not properly cooked, can lead to exposure (Greig *et al.*, 2015; Heredia, 2018). Consequently, individuals who contract *E. coli* and *Salmonella spp.* derived from rodents can contribute to its spread within the environment, among their family members, and throughout the community (Winfield *et al.*, 2003). Nevertheless, there is a lack of empirical evidence about the role and relative contribution of several of the channels through which humans are exposed to aspects of foodborne *E.coli* and *Salmonella spp.* (WHO, 2015). Consequently, until now, most of the literature reviews about foodborne disease and their impact on human health have failed to adequately consider the contribution of rodents and shrews regarding transmission and associated risks (Jahan *et al.*, 2021; Islam *et al.*, 2022).

The problem of identifying and estimating the detrimental human health implications of foodborne diseases caused by house rodents and shrews, such as illness rates and outbreaks, has been acknowledged (Meerburg *et al.*, 2009; Jahan *et al.,* 2021). This phenomenon can be attributed, in part, to a convergence of methodological constraints and a need for more crucial data for a full risk assessment. Strategies for rodent management and foodborne control methods are required to reduce the rate of foodborne diseases that burden public health.

CONCLUSION

The findings of this study indicate that there is a significant difference in the presence of E.coli depending on the habitat, species, and setting. On the contrary, there was no statistically significant prevalence of Salmonella enterica according to rodent species, habitat, and trapped site. Human beings are at a high risk of foodborne pathogens because they live close to rodents and shrews. Nevertheless, these investigations widely acknowledge that rodents and shrews carry human pathogens, leading to various human infections on a global scale following their interaction with food.

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CONFLICT of INTEREST

The authors declare that no conflicts of interest are associated with this research study. No financial, personal, or professional relationships with other people or organizations could potentially influence the work or the interpretation of its results. This includes, but is not limited to, employment, consultancies, honoraria, funding, grants, or other forms of payments. The authors affirm that this research was conducted with the highest integrity and impartiality.

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ETHICAL PPROVAL

The animal-related procedures conducted in this study followed the ethical standards and guidelines for the care and use of animals in research. The research protocol involving animals was reviewed and approved by the Sokoine University of Agriculture under the ethical review board. The Sokoine University of Agriculture gave an approval letter for conducting this study (Ref. No. SUA/ADM/R.1/8/995; 16th January 2023). Also, the local administrative authorities TAMISEMI Dodoma (Ref. No. AB.307/323/01/191; 16th February 2023) and Morogoro Municipality (Ref. No. R.10/MMC-24/32; 10th March 2023) provided permission before starting the data collection in the field.

AUTHOR'S CONTRIBUTION

CMU: Methodology design, data collection, laboratory work, data analysis, and interpretation, as well as the drafting of the manuscript. GGM: Critical review of the manuscript. NEB: Contributed to the statistical analysis, discussion, and critical manuscript review. EM: Collaborated to the analysis of the laboratory data and the critical review of the manuscript. SIK: Supervisor, overall research management, statistical analysis, data interpretation, writing, and critical manuscript review. AASK: Supervisor of the overall research management, statistical analysis, data interpretation and writing, and critical manuscript review.

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