Full title: Staphylococcal and Non-typhoidal *Salmonella* infection statuses in non-human mammals: A potential source of zoonoses in the Greater Accra region of Ghana.

Short title: Animal-related bacterial infections in Ghana

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Abstract

Background

Bacterial zoonoses are readily transmitted from animals to humans and are thrice more likely to lead to emerging or re-emerging diseases. In Ghana, there is a paucity of animal-related bacterial infection surveillance data, significantly affecting how such diseases are accurately targeted for prevention or control. This study sought to investigate the prevalence of two important bacterial infections in some common animals found in two human-dominated landscapes and ascertain if their prevalence was of imminent public health concern. In most Ghanaian communities, dogs, cats and rodents are non-human mammals that are frequently in contact with humans. As such, they were targeted during this cross-sectional study.

Methods

Biological samples collected from animals in households and veterinary institutions were processed using molecular techniques targeting *Staphylococcus* and Non-typhoidal *Salmonella* species. Additionally, medical records were sourced from three (3) major health institutions to determine if cases of bacterial zoonoses were of imminent concern.

Results

Overall, the prevalence of staphylococcal and Non-typhoidal *Salmonella* infections were 72.5% and 22.8%, respectively. More animals from the urban areas tested positive for Staphylococcal (χ^2=5.721; p=0.017) and Non-typhoidal *Salmonella* (χ^2=16.151; $p < 0.001$) infections compared to those from the peri-urban areas. The medical records also revealed that relatively higher cases of staphylococcal infections were reported within three years (2018-2020), although no significant differences were observed between the urban and peri-urban areas.

Conclusion

The high prevalence of staphylococcal infections in animals and the high number of hospital cases suggest increased exposure to this bacteria and a higher risk of persons residing in these areas to bacterial zoonoses. Data from the study also suggest that rodents are actively and inactively maintaining the cycle of these two bacterial species and as such, a source of concern. Findings underscore the need for active surveillance of bacterial species with zoonotic potential in non-human mammals regularly found in communities, which is fundamental to developing appropriate disease control strategies.

Keywords: Zoonoses, Non-typhoidal *Salmonella* infections, Staphylococcal infections, Surveillance, bacterial infections

Introduction

Nearly all the pandemics that occurred over the last decade originated from animals causing a tremendous impact on the health and economies of nations [1, 2]. Therefore, any pandemic to occur in the future is predicted to have its origin in an animal. Several animal species are reservoirs of zoonoses: they maintain infections in nature and are crucially involved in several zoonotic disease emergence [3,4]. Recently, a more significant part of emerging infectious pathogens has been identified as bacterial [5,6], with zoonotic bacteria thrice more likely to lead to emerging diseases than non-zoonotic bacteria [7].

Compared to animal-related bacterial zoonoses, foodborne bacterial zoonoses attracted much attention in the past [8], similar to the situation in Ghana. However, this trend appears to change as bacterial zoonoses caused by animals are also recognised to impact health systems significantly[9]. For instance, companion animals and rodents transmit important bacteria like *Staphylococcus* and non-typhoidal *Salmonella* species to humans through direct and indirect routes [6,10-11], leading to mild or severe disease outcomes.

Dogs and cats are mostly colonised with *Staphylococcus pseudointermedius,* although many cases have shown that they play vital roles in households and veterinary clinic transmissions

of *Staphylococcus aureus* [10, 12-13]. For *Salmonella*, all species are zoonotic except for *Salmonella typhi* and the paratyphoid serotypes [14]. Two key species, *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium, are transferred from animals to humans [15]. Many serotypes have been detected in dogs and cats who may be asymptomatic or symptomatic [16]. Rodents are usually infected with serotypes most common in their environment [14].

The close association of humans with companion animals has resulted in animals like dogs and cats sharing more than 60 parasites [17]. Rodents who live near human settlements offer opportunities for cross-species transmission of pathogens they harbour [18]. Hence, they play significant roles in disease transmission in endemic areas and are a significant risk to sustainable health. In addition, they are a source of food for dogs and cats, a situation that sustains the enzootic transmission cycle of infectious diseases among them [9]. In Ghana, there is a paucity of data on animal-related bacterial infections focusing on mammals which share the same environment with humans. Recently, surveillance has been directed at natural reservoirs of zoonotic infections to enhance disease detection [19], which is fundamental to developing disease control strategies hinged on the one health approach to avert any potential epidemic or pandemic.

Materials and methods

Study design

This study was cross-sectional. Primary and secondary data were collected from two human-dominated landscapes (landscapes heavily dominated by people) in the Greater Accra Region. The urban and peri-urban areas were the two human-dominated landscapes selected for this study, with data collection spanning from October 2019 to October 2020.

Two non-probability sampling approaches, purposive and convenient sampling techniques, were used in the selection of the study populations. This was because there existed no reliable data on the population of targeted animals in the Greater Accra Region to serve as a sampling frame at the time of this study. Both techniques were used to obtain an eligible and representative sample size of animal subjects for this research.

Study setting

There is no universally accepted definition of 'urban' as most definitions are based on national definitions or characterizations [20] In Ghana, communities with about five thousand (5,000) persons or more are categorised as urban [21]. However, for this research, the use of the minimum population size alone seemed simplistic as over 87% of the population of the Greater Accra region, per this definition is in urban areas [21]. Therefore, the minimum population size and the density of socio-economic activities in such areas were considered in selecting the urban and peri-urban areas from the 29 Metropolitan, Municipal and District assemblies (MMDAs) in the Greater Accra region [22]. Legon and Madina were purposively selected from the Accra Metropolitan Assembly and La-Nkwatanang Madina Municipality, respectively, under the urban category as both study sites are completely urbanised. For this study, Legon comprised the University of Ghana campus (5.6506°N, 0.187°W) and East Legon (5.6358°N, 0.1614°W).

Legon shares close boundaries with Madina (5.6731°N, 0.0980°W), the administrative capital of the La Nkwantanang-Madina Municipal [21]. Both communities are characterised by higher educational institutions and good health facilities. Economically, these two communities are very vibrant, and most of the populations are engaged in commerce.

Dodowa (5.8829° N, 0.00980W) in the Shai-Osudoku District was selected for the peri-urban category because this town was formed due to urban sprawl. Additionally, it exhibits both urban and rural characteristics, as peri-urbanisation connotes.

Study population and sample size determination

Rodents and companion animals (dogs and cats) were targeted for this study because of the increased interaction or contact of these animals with humans in most Ghanaian communities. Since there is no published data on the prevalence of these two bacterial infections among the targeted animal species, a minimum sample size of 384 was obtained at a 95% confidence interval, assuming a minimum prevalence of 50% using the formula below. This formula, according to Naing et al. [23], is used in calculating sample size which seeks to estimate population prevalence with good precision.

$$n = \frac{Z^2 * p(1-p)}{e^2}$$ [24]

Where n is the sample size, Z represents the Z score at a 95% confidence interval of 1.96; *p* is the minimum estimated prevalence (50%); e=margin of error/absolute error (5%) at a 95% confidence interval.

In all, a minimum of 384 animals (dogs, cats and rodents) were to be sampled using a purposive sampling technique due to the absence of a sampling frame as previously explained.

Sources of data

Biological samples were collected from targeted animals from households, veterinary institutions, and around human settlements in selected study settings after ethical clearance and informed consent were given. In the urban areas, two veterinary institutions were selected: the University of Ghana Small Animal Teaching Hospital (UG-SATH) and the East Legon Veterinary Centre (ELVC). Four (4) criteria were used for the selection of these veterinary institutions: location, accessibility, range of services provided and patronage. Unfortunately, there was no functional veterinary clinic in Dodowa at the time of data collection.

Medical records from 2018-2020 on *Staphylococcus* and Non-typhoidal *Salmonella* infections were sourced from three (3) major health institutions: the University of Ghana and Pentecost Hospitals (urban category) and the Shai-Osudoku District Hospital (peri-urban category). The purpose of these secondary data was to corroborate or contradict the possibility of bacterial zoonotic disease transmission in the study areas.

These three hospitals were also selected using four (4) criteria: accessibility, range of services provided, availability of requisite facilities and patronage. Even though there are a few other health institutions in the study areas, most could not meet these criteria.

The University of Ghana Hospital, referred to as the Legon Hospital is a quasi-government hospital that offers both general services and specialist clinics. It has a 130-bed capacity which consists of a paediatric unit, maternity, casualty and emergency ward. The Pentecost Hospital, situated in Madina is a mission hospital. It is the municipal hospital for the La-Nkwantanang Madina municipality and provides services in obstetrics and Gynaecology, child health, accident and emergency services as well as other relevant services [25]. Comparatively, this hospital has relatively higher patronage. The third hospital, the Shai-Osudoku District Hospital located in Dodowa, is the only major hospital in the Shai-Osudoku district with advanced medical facilities. The hospital offers a variety of clinical services to people from all spheres of life in the district and beyond.

Sample collection

Rodent sampling

Four sampling sites in each human-dominated landscape were used for trapping rodents. The sampling areas had an average of 50 trap lines at least 10 metres apart. A small amount of bait (a mixture of peanut butter, palm nut and maise flour) was placed in each trap which was then labelled and placed under logs near burrows, homes, farmlands, and dumpsites. The traps were left overnight and inspected daily for three consecutive days. After each daily inspection, the

177 traps were re-baited when necessary. Active traps (traps containing rodents) were recorded and
178 carefully handled according to standard protocols [26]. The rodents were described using
179 morphometric keys. Any trap found missing, sprung, or not triggered was regarded as a misfire
180 (capture of non-target species) and was also recorded.

181 Companion animals sampling

182 Dogs and cats were sampled from households and veterinary institutions at Legon-Madina and
183 Dodowa after their owners consented to participate in the study.

184 Collection of blood samples from animals

185 Less than 1 ml of venous blood was collected from the animals into heparinised tubes adhering
186 to standard protocol and procedures for blood collection and transportation. Blood was
187 collected from the tail region of rodents and the cephalic veins of dogs and cats by a veterinary
188 officer adhering to sterile safety measures.
189 On some occasions, swabbing the cloaca with sterile cotton was employed when animals
190 exhibited aggression during blood collection. The swabs were placed into appropriately
191 labelled tubes and transported to the laboratory under cold chain conditions.

192 Processing of biological samples

193 Deoxyribonucleic acid (DNA) of pathogens was extracted from blood and swabs using the
194 protocol described by Soumet et al. [27] with modifications. A total of 100 µL blood was
195 inoculated in 900 µL Luria Bertani broth (Invitrogen 12780-052), and the culture was incubated
196 at 37℃ for 48 h in a shaking incubator (9.44 x 10^{-2} xg). Afterwards, 1 ml of individual culture
197 was picked and centrifuged for three (3) min at 13000 xg (4℃), after which the resultant pellets
198 were washed twice in 500 µL 1X TAE buffer (Tris, Acetic Acid, and EDTA; pH 8.0), and
199 centrifuged at 13000 xg for 3 min (4℃). The pellets were re-suspended in 200 µL sterile water,
200 boiled for 8 min, and then stored at -20℃.

The swabs were eluted in 1000 µL of Phosphate Buffered Saline (PBS) after vortexing for 2 min before extracting the bacterial DNA, as explained earlier.

DNA Amplification by Polymerase Chain Reaction

Direct Polymerase Chain Reaction (PCR) was used to amplify numerous copies of diminutive DNA fragments using genus-specific primers. Further details of the primer set used for the PCR are listed in Table 1.

Table 1. Primer sets for the polymerase chain reaction (PCR)

Bacteria targeted	Primers	Primer sequence	Reference
Salmonella spp. (Non-typhoidal)	ttr-6	F: 5′-CTC ACC AGG AGA TTA CAA CATGG-3′	Malorny et al. (2004)
	ttr-4	R: 5′-AGC TCA GAC CAA AAG TGA CCATC-3′	
Staphylococcus spp.	TStaG422	F: 5′-GGC CGT GTT GAA CGT GGT CAA ATCA-3′	Martineau et al. (2001)
	TStag765	R: 5′-TIA CCA TTT CAG TAC CTT CTG GTAA-3′ NB. I=nucleotide analog inosine	

All PCR reactions were performed in final volumes of 25.0µL containing 7.50µL sterile nuclease-free PCR water, 12.50µL OneTaq® Quick-Load® 2X Master Mix with Standard Buffer (New England Biolabs Inc.), 0.5µL each of forward and reverse primers and 4.0 µL DNA template. This was done at initial and final denaturation of 95℃ for 2min and 94℃ for 40s, respectively. The annealing temperature was set at 53℃ for 1 min, followed by an initial extension at 72℃ for 3 min. The final extension was at 72℃ for 7 min with a holding temperature of 4℃. The PCR products were examined by electrophoresis on 1.2% agarose gels stained with 4.0 µL Ethidium bromide. The wells were each loaded with 6.0 µL PCR product and run at 80V for 90 min. Fluorescence from the DNA-bound Ethidium bromide was then visualised under Ultra Violet (UV) Trans-illuminator (SYNGENE Inc.). The amplified products were measured using a 100 base pair (bp) DNA ladder (New England Biolabs Inc.) at specific bands corresponding to their base pairs for the two bacterial species.

Analysis of data

Data were entered into Microsoft Excel (2006) and subsequently analysed with IBM Statistical Package for Social Sciences (SPSS version 20). Comparisons were made using the Mann-Whitney U test, Student's t-test, Pearson Chi-square or Analysis of variance (ANOVA) depending on the nature of the distribution of the data (normal or non-normal distribution). The prevalence of infection was also calculated using the formula below

$$\text{Prevalence} = \frac{\text{Number of positive samples}}{\text{Number of samples}} * 100$$

At a 95% confidence level, $p<0.05$ was considered statistically significant.

Ethical approval and informed consent

This study (UG-IACUC 009/18-19) was approved by the University of Ghana Institutional Animal Care and Use Committee (UG-IACUC). Ethical clearance or approval was also obtained from selected veterinary and health institutions as well as the Ghana Health Service. Informed consent was also sought from owners of companion animals before their animals were sampled.

Results

Distribution of animal species

A total of 404 biological samples were collected from dogs (n=185, 45.8%), cats (n=15,3.7%) and rodents (n=204, 50.5%) (Table 2). In all, fewer cats were sampled than dogs from households and veterinary institutions as shown in Table 2.

Table 2. Distribution of sampled animals in the two sampling locations

	Sampling location			**Total**
Studied animals	Households	Veterinary Institutions		
		ELVC	UG SATH	

Dog	74	56	55	**185**
Cat	11	0	4	**15**
Rodent	204	0	0	**204**
Grand Total	**289**	**56**	**59**	**404**

A total of one-hundred and fifteen (115) companion animals consisting of 111 (96.52%) dogs and 4(3.48%) cats were sampled from the two veterinary institutions in the urban areas.

For the rodents, 204 individuals belonging to seven (7) species were trapped. There were variations in the distribution of trapped rodents in the two human-dominated landscapes (Table 3). Statistically, significant differences were found between their distributions in the urban and peri-urban areas using the Mann-Whitney U test (U=2770.00, Z=-4.349, p<0.001) (S1 Table). *Arvicanthis niloticus* was the dominant species trapped in the urban communities in contrast to *Praomys tullbergi,* the dominant species from the peri-urban areas. However, overall, *A. niloticus* was the dominant rodent, and the least was *Crocidura olivieri* (Table 3).

Table 3. Distribution of rodents in the human-dominated landscape

Rodent	**Human-Dominated Landscape**		**Total**
	Urban	Peri-Urban	
Praomys tullbergi	0 (0.0%)	38 (100.0%)	38 (100.0%)
Arvicanthis niloticus	113 (100.0%)	0 (0.0%)	113 (100.0%)
Rattus rattus	9 (47.4%)	10 (52.6%)	19 (100.0%)
Cricetomys gambianus	5 (100.0%)	0 (0.0%)	5 (100.0%)
Rattus norvegicus	18 (100.0%)	0 (0.0%)	18 (100.0%)
Mus musculus	0 (0.0%)	10 (100.0%)	10 (100.0%)
Crocidura olivieri	0 (0.0%)	1 (100.0%)	1 (100.0%)
			U=2770.00,* p<0.001

The asterisk (*) represents a statistically significant result.

Distribution of animals in the two human-dominated landscapes

Out of the total number sampled, 81.08% of the dogs, 86.67% of cats and 71.08% of rodents were from urban areas (S2 Table). More animals were sampled from the urban areas compared to the peri-urban areas. This was confirmed using the Mann-Whitney *U* Test, which was statistically significant (U=12619.500, p=0.019)(S3 Table).

The distribution of the sampled animals from the two human-dominated landscapes is illustrated in Fig 1.

Fig 1. Distribution of sampled animals in the two human-dominated landscapes

Prevalence of bacterial infections

Results from the PCR showed more animals, 72.5% (95% CI= 67.8%-76.8%) were positive for *Staphylococcus* spp. compared to Non-typhoidal *Salmonella* spp., 22.8% (95% CI=18.8%-27.2%). Fig 2 presents the prevalence of Staphylococcal and Non-typhoidal *Salmonella* infections in the targeted animals.

Fig 2. Prevalence of bacterial infections among sampled animal species

Bacterial infections in the two human-dominated landscapes

Human-dominated landscapes are critical loci for disease transmission, and therefore prevalence was analysed in relation to the urban and peri-urban areas. A Pearson Chi-square analysis showed a high number of the sampled animals tested positive for both bacteria species in the urban areas in contrast to the peri-urban areas (Table 4).

Table 4. Prevalence of bacterial infections in the two human-dominated landscapes

Bacteria		Human-dominated landscape		Total	Pearson Chi-square
		Urban	Peri-urban		
Salmonella spp. (Non-typhoidal)	Negative	253 (81.1%)	59 (18.9%)	312 (100.0%)	χ^2=16.151 *p < 0.001
	Positive	56 (60.9%)	36 (39.1%)	92 (100.0%)	

Staphyloco-ccus spp.	Negative	94 (84.7%)	17 (15.3%)	111 (100.0%)	χ^2=5.721 *p=0.017
	Positive	215 (73.4%)	78 (26.6%)	293 (100.0%)	

The asterisk (*) represents a statistically significant result.

Bacterial infections among the sampled animals

In comparing infection prevalence among the three sampled animals, the highest prevalence of Staphylococcal and Non-typhoidal *Salmonella* infections were found in rodents. However, in dogs, the prevalence was also high. A Pearson Chi-square analysis revealed that these differences were not statistically significant (Table 5).

Table 5. Prevalence of bacterial infections among the sampled animal species

Bacteria	Infection status	Sampled animals			Total	Pearson Chi-square
		Dog	Cat	Rodent		
Staphylococcus spp.	Negative	48 43.2%	5 4.5%	58 52.3%	111 100.0%	χ^2=0.569 p=0.752
	Positive	137 46.8%	10 3.4%	146 49.8%	293 100.0%	
Total		185 45.8%	15 3.7%	204 50.5%	404 100.0%	
Salmonella spp. (Non-typhoidal)	Negative	144 46.2%	9 2.9%	159 51.0%	312 100.0%	χ^2=2.630 p=0.269
	Positive	41 44.6%	6 6.5%	45 48.9%	92 100.0%	
Total		185 45.8%	15 3.7%	204 50.5%	404 100.0%	

Although higher infection prevalence was recorded from animals sampled from the households compared to the veterinary institutions, no statistically significant differences were observed (Table 6).

Table 6. Prevalence of bacterial infections in animals from households and veterinary institutions

Bacteria		Sampling location		Total	Pearson Chi-square
		Household	Veterinary Institutions		
Salmonella spp. (Non-typhoidal)	Negative	225 72.1%	87 27.9%	312 100.0%	χ^2=0.227 p=0.634

	Positive	64 69.6%	28 30.4%	92 100.0%	
Total		289 71.5%	115 28.5%	404 100.0%	
Staphylococcus spp.	Negative	83 74.8%	28 25.2%	111 100.0%	
	Positive	206 70.3%	87 29.7%	293 100.0%	χ^2=0.789 *p*=0.374
Total		289 71.5%	115 28.5%	404 100.0%	

Images of gel electrophoresis

PCR products were analysed using agarose gel electrophoreses, and the images for Non-typhoidal *Salmonella* and *Staphylococcus* spp. are shown in Figs 3 and 4. The molecular weight marker, or the DNA ladder denoted by M (Lane M), was used to detect the approximate sizes of the amplicons.

Fig 3. Amplified PCR product of *Salmonella* spp. (Non-typhoidal)

(Lane M=100 bp, Lane 1-15, 17-26=positive, Lane 16=negative)
Lanes 1-26 contain DNA from the blood of sampled animals after preparation with DNAzol and PCR. Gel depicting 350 bp PCR product.

Fig 4. Amplified PCR product of *Staphylococcus* spp.

Lane M=100 bp, Lane NC= negative control, Lanes 10, 11, 13, 16 and 18= positive, Lane 1-9, 12,14-15, 17, 19=negative)
Lanes 1-19 contain DNA from the blood of sampled animals after preparation with DNAzol and PCR. Gel depicting a 550 bp product.

Medical records from hospitals in the two human-dominated landscapes

Secondary data from two out of the three hospitals revealed cases of bacterial zoonotic infections. In scrutinising bacterial zoonoses presented at the hospitals, bacterial infections that were non-zoonotic (S4 Table) were excluded. Therefore, cases of zoonotic bacterial infections and bacterial infections with zoonotic potential presented by clients at the two hospitals are shown in Table 7.

320 **Table 7. Bacterial infections presented by clients at the hospitals.**

Hospital	Zoonotic Agent/ bacterial infection	Frequency of Infection/Year			Total
		2018	2019	2020	
SODH	*Salmonella enteritidis*	4	5	15	24
	S. aureus/other Staphylococcal infections	39	5	1	45
	Total	**43**	**10**	**16**	**69**
UGH	*Salmonella enteritidis*	5	0	2	7
	Staphylococcal infections	146	148	47	341
	Total	**151**	**148**	**49**	**347**

321 SODH: Shai-Osudoku District Hospital UGH: University of Ghana Hospital

322 In comparing cases of Non-typhoidal *Salmonella* to staphylococcal infections, both hospitals
323 reported relatively higher cases of staphylococcal infections. Nevertheless, there were no
324 statistically significant differences (p>0.05) in these two bacterial infections between the urban
325 and peri-urban areas(S7 Table).
326 In addition, records of staphylococcal infections from SODH showed a decline from 2018 to
327 2020, whereas that of *Salmonella enteritidis* increased. On the other hand, Staphylococcal
328 infections were very high in UGH until the number of cases decreased considerably in 2020.
329 On the whole, cases of bacterial zoonoses recorded in UGH were the lowest in 2020 compared
330 to SODH. However, there were no significant differences between the two bacterial infections
331 recorded over the three years (p>0.05)(S8 Table).
332
333 Gender-based records of the two bacterial infections presented at the hospitals were also
334 examined and the results shown in Table 7.

335 **Table 7. Gender differences in bacterial infections presented at the hospitals**

Hospital	Zoonotic agent/ bacterial infection	2018		2019		2020	
		Male	Female	Male	Female	Male	Female
SODH	*S. enteritidis*	3	1	4	1	6	9
	S. aureus, other staphylococcal infection	21	18	2	3	1	0
U. G	*S. enteritidis*	3	2	0	0	2	0
	Staphylococcal	72	74	63	85	20	27

	infection						
Total		99	95	69	89	29	36

SODH: Shai-Osudoku District Hospital UGH: University of Ghana Hospital

Records of these infections between males and females were also compared and the results showed that on average, more females (M=17.416, SD=27.73) than males (M=16.42, SD=24.93) were diagnosed with these bacterial infections (S9 Table). Even so, an independent t-test showed that the difference was not statistically significant, t (22) =-0.093, p=0.927 (S9 Table).

Again, a cursory look at the total cases of these two bacterial infections reported at the hospitals appeared to differ between the urban and peri-urban areas as illustrated in Fig 5.

Fig 5. Bacterial infections in the two human-dominated landscapes

An independent t-test confirmed there existed a significant difference in the total cases of *S. enteritidis* and Staphylococcal infections reported in the urban (M=28.08, SD=32.73) and peri-urban areas (M=5.75, SD=6.92).

Further comparison was also done between the type of bacterial infection and the total cases recorded. The medical records showed that more staphylococcal infections (M=31.25, SD=30.59) were reported than *S. enteritidis* (M=2.583, SD=2.712) (S11 Table). Using the independent t-test, the difference was found to be statistically significant, t (11.173) =-3.233, p=0.008 (S11 Table).

Discussion

Bacterial zoonoses are readily transmitted from animals to humans, and reports suggest that a higher number of zoonoses are caused by bacterial agents [6]. Therefore, this study aimed to ascertain if the prevalence of these two important bacterial species was of imminent concern in the Greater Accra region. As dogs, cats and rodents are non-human mammals frequently

361 found in most communities in Ghana [28] they were targeted during this study. The prevalence
362 of *Staphylococcu*s and Non-typhoidal *Salmonella* infections were 72.5% and 22.8 %,
363 respectively.
364 Of the three animals examined, rodents recorded the highest infection prevalence. Rodents are
365 known natural hosts of *Staphylococcus* spp. [29]. Thus, many rodents that tested positive for
366 *Staphylococcus* spp. were not out of place. However, this data indicates that rodents around
367 human settlements significantly increase the risk of zoonotic bacterial infections due to high
368 exposure. Besides, transmission may be indirect when companion animals (dogs and cats) feed
369 on these rodents, get infected and consequently transmit these infections to their owners [30].
370 This high prevalence of *Staphylococcus* spp. in rodents corroborates that of Ribas et al. [12] in
371 Thailand. In comparison to other works, the prevalence of *Staphylococcus* spp. among dogs
372 was higher (46.8%) than what was reported by Han et al. [31] in South Korea and Qekwana et
373 al. [32] in South Africa, where a prevalence of 37% and 27% respectively, were recorded.
374 Additionally, the most dominant rodent trapped, *Arvicanthis niloticus* is identified as an
375 important agricultural pest with extensive distributions in Ghana and Africa. This implies that
376 high populations of these rodents could cause significant pre- and post-harvest losses of
377 agricultural products and increase the probability of human exposure to the diseases they
378 transmit [28].
379 Research has shown that *Salmonellae* are commonly distributed by domestic and wild animals
380 that maintain the animal-to-animal cycle through the usual faecal-oral route [15]. The high
381 prevalence of dogs and rodents being positive suggests the environment may be contaminated
382 with this bacterium because these animals get infected by ingesting infected faeces from the
383 environment. Subsequently, the shedding of the parasite by dogs, cats and rodents may lead to
384 the potential transmission of zoonotic *Salmonella* infections to humans in the same household.
385 Though dogs and cats are usually subclinical, Marks et al. [33] believed that infections could

386 progress from mild to fatal conditions of gastroenteritis and septicaemia. Therefore, the
387 importance of this relatively high infection prevalence, particularly in dogs, cannot be
388 overemphasised. In this study, the prevalence of *Salmonella* spp. in dogs was relatively higher
389 than what was reported by Núñez Castro et al. [34] (6.27%) and Bataller et al. [35] (1.85%).
390 The differences could be because both studies sampled healthy dogs, whereas, in this study,
391 both healthy and unhealthy dogs were sampled.
392
393 Even though positive cases of Staphylococcal and *Salmonella* infections from hospitals within
394 the study areas were low, there is an indication of possible zoonotic bacterial transmissions in
395 urban and peri-urban communities. Data from the urban and peri-urban categories revealed a
396 high record of staphylococcal infections, consistent with prevalence from the sampled animals.
397 The high cases of staphylococcal infections from rodents and dogs suggest that persons living
398 in such areas are at a higher risk of zoonotic staphylococcal diseases due to the high exposure.
399
400 Findings from the study demonstrated that the distribution of the various rodent genera between
401 the two human-dominated landscapes was statistically significant, indicating that some rodents
402 thrived better in one area than the other. This data corroborates the study by Assefa & Chelmala
403 [36], where the distribution and diversity of rodents were found to differ across habitats. The
404 two dominant rodents, *Arvicanthis niloticus* and *Praomys tulbergi,* found in the urban and peri-
405 urban areas, respectively, suggest that they might be the most typical species found in these
406 areas which merit further surveillance in terms of zoonotic disease transmission.
407 Furthermore, the high abundance of rodents recorded from the urban areas in contrast to the
408 peri-urban areas which have a combination of rural and urban characteristics and good
409 vegetation is worth investigating. This could probably be due to poor sanitary conditions such
410 as the irregular collection of garbage and open sewers that often characterise urban spaces in

411 the Greater Accra Region. In the view of Panti-May et al. [37], such conditions create
412 favourable conditions for rodents to thrive. On the other hand, one reason for the low numbers
413 of rodents in the peri-urban areas may be the continuous rodent-hunting activities in these areas,
414 as observed during the data collection.

415 Conclusion

416
417 The high prevalence of staphylococcal infections in animals and the high number of hospital
418 cases suggest increased exposure to this bacteria and a higher risk of persons residing in these
419 areas, especially urban communities, to bacterial zoonoses. Results from this study indicate
420 that rodents are actively and inactively maintaining the cycle of these two bacterial species
421 which could threaten the sustainable health of persons found in those areas. Furthermore, the
422 data shows that these two bacteria merit continued investigations in our urban and peri-urban
423 areas. Lastly, the findings underscore the need for active surveillance of zoonotic and possible
424 zoonotic bacterial diseases in non-human mammals regularly found in our communities, which
425 is fundamental to developing zoonotic disease control programmes using the one health
426 approach.

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428
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552

553 Supporting information

554

555 S1 Table. Comparison of the distribution of rodents 556 between the two human-dominated landscapes

557 S2 Table. Distribution of animals in the two human-558 dominated landscapes

559 S3 Table. Comparison of the distribution of animals in the 560 two human-dominated landscapes

561 **S4 Table. Summary of the two bacterial infections**
562 **documented by the hospitals from 2018 to 2020**

563 **S5 Table. Comparison of staphylococcal infections between**
564 **the two hospitals**

565 **S6 Table. Comparison of *Salmonella* (non-typhoidal)**
566 **infections between the two hospitals**

567 **S7 Table. Comparison of bacterial infections recorded by**
568 **the hospitals from 2018-2020**

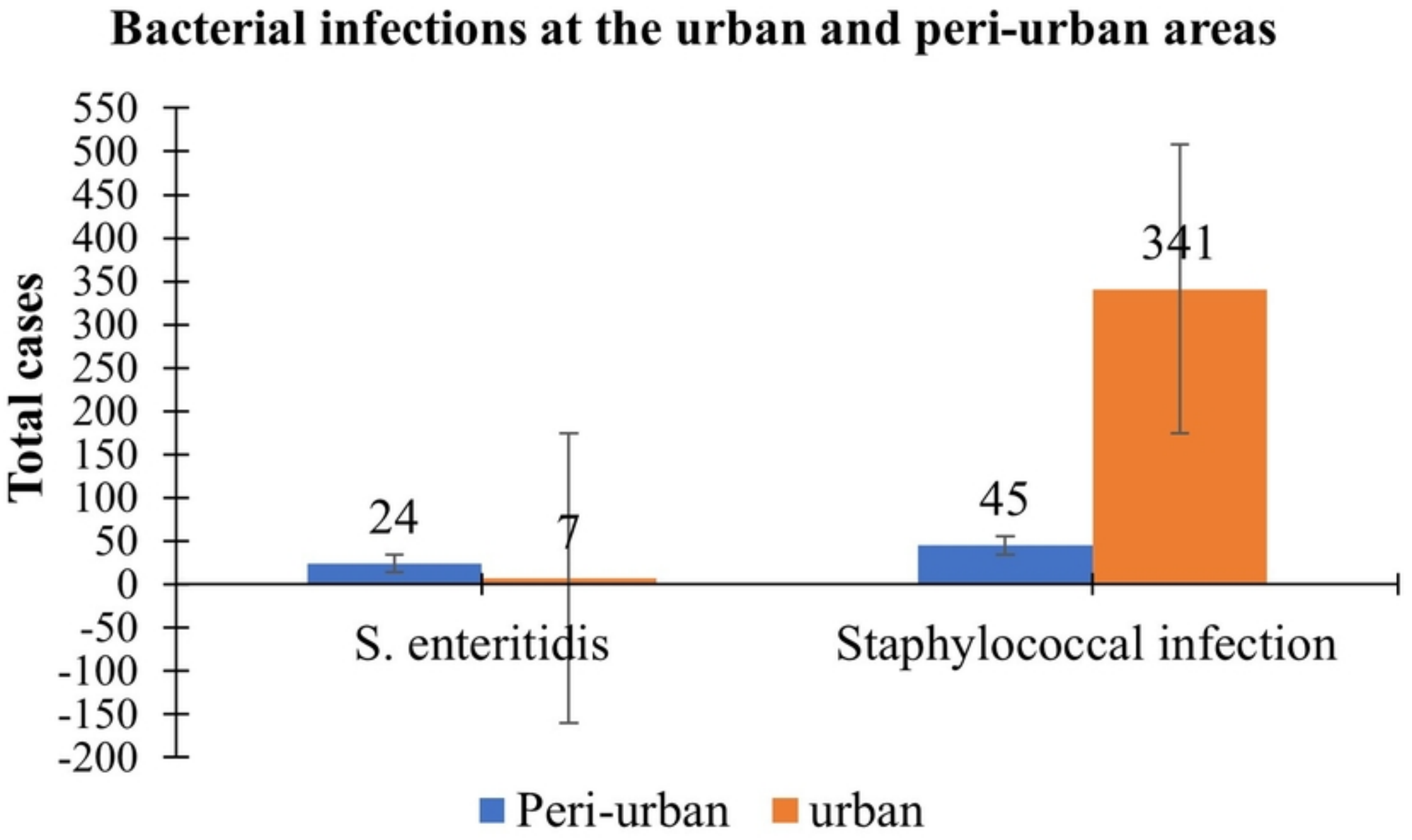
569 **S8 Table. Comparison of bacterial infections recorded by**
570 **the hospitals in the human-dominated landscapes.**

571 **S9 Table. Comparison of total cases of bacterial infections**
572 **between males and females**

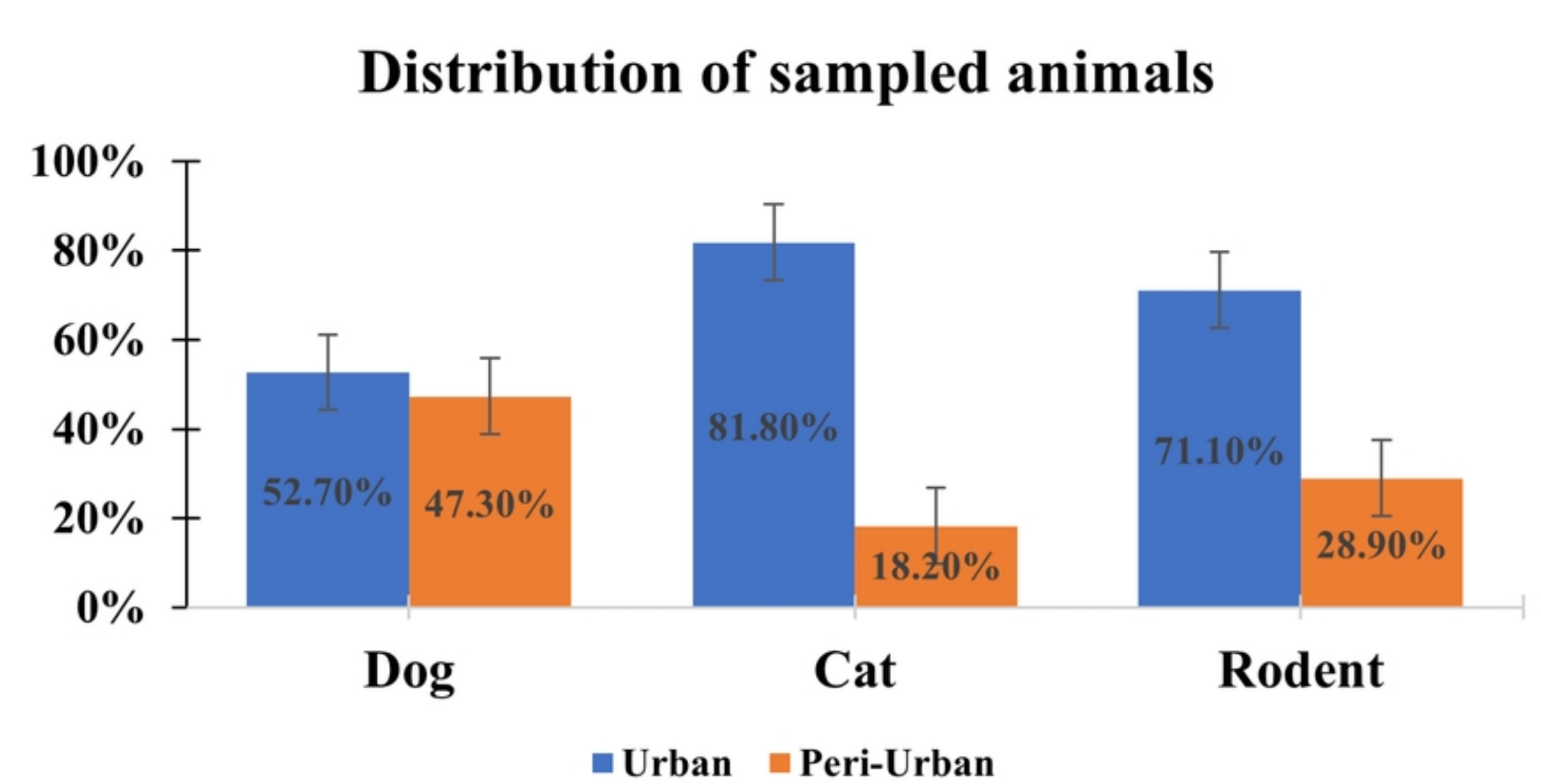
573 **S10 Table. Comparing total cases between the two human-**
574 **dominated landscapes**

575 **S11 Table. Differences in the total number of cases reported**
576 **for the two bacterial infections**

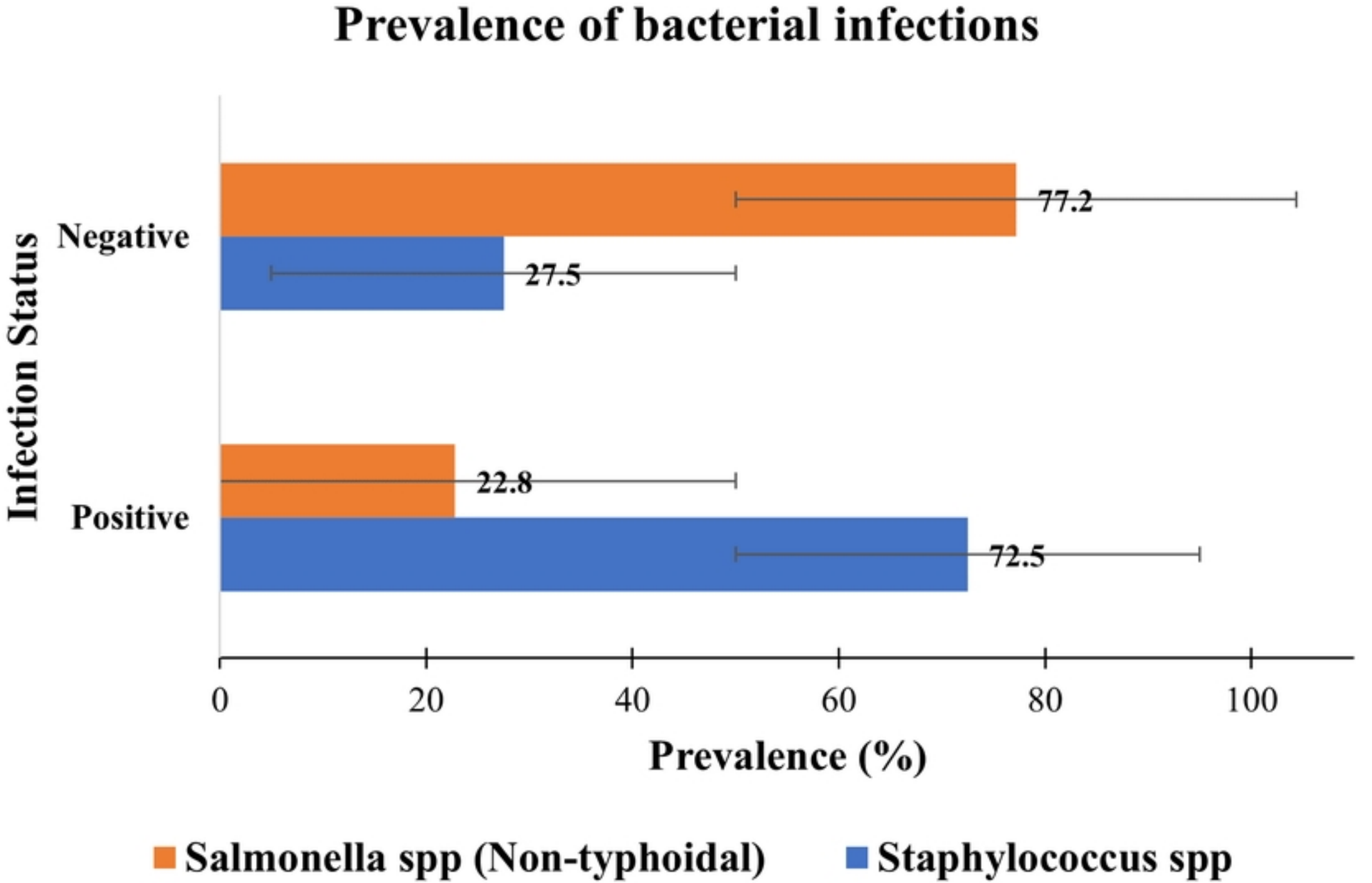
577


Bacterial infections at the urban and peri-urban areas
Total cases
550
500
450
400
350
300
250
200
150
100
50
0
-50
-100
-150
-200
24
7
45
341
S. enteritidis
Staphylococcal infection
Peri-urban
urban

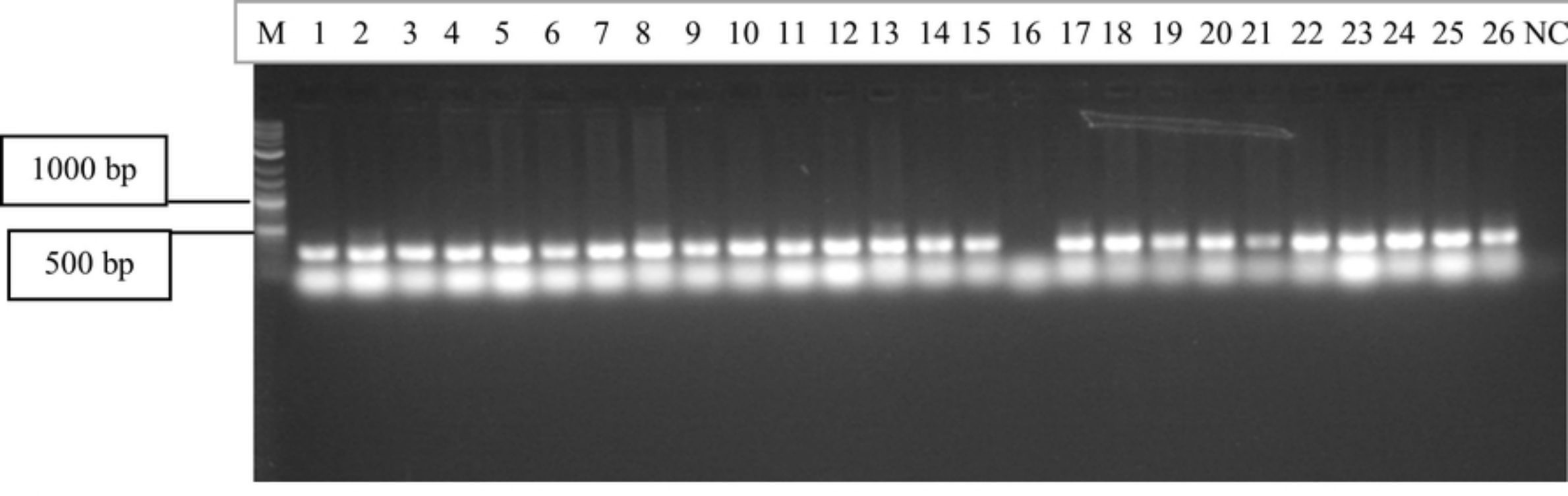
Figure


Distribution of sampled animals
100%
80%
60%
40%
20%
0%
52.70%
47.30%
81.80%
18.20%
71.10%
28.90%
Dog
Cat
Rodent
Urban
Peri-Urban

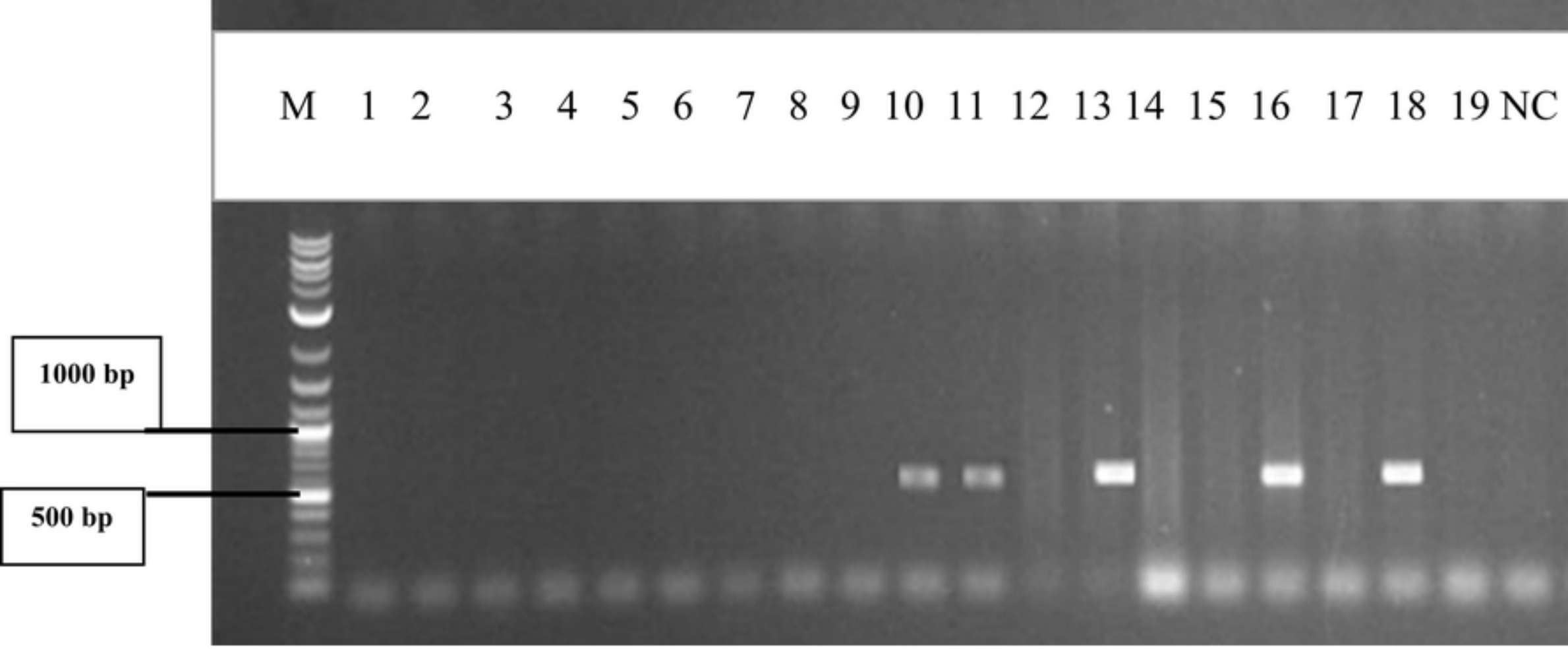
Figure


Prevalence of bacterial infections
Infection Status
Negative
Positive
77.2
27.5
22.8
72.5
0
20
40
60
80
100
Prevalence (%)
Salmonella spp (Non-typhoidal)
Staphylococcus spp

Figure


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 NC
1000 bp
500 bp

Figure



Figure