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Public Health Aspect of Manure Management in Urban and Peri-Urban Livestock Farming in Developing Countries

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Additional information is available at the end of the chapter

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Abstract

Urban and peri-urban livestock farming has been expanding in recent decades due to high demand for animal proteins to feed the growing urban population. The increase in number of livestock and livestock keepers has led to increased manure production in a shrinking space. This chapter evaluates the risks of transmission of manure-borne pathogen between cattle, humans and the environment in urban and peri-urban areas. Cattle and manure management practices, government directives, the presence of zoonotic pathogens and risk of bacteria transmission were assessed by observations, interviews, bacteria isolation and characterization and statistical modeling. Cattle are kept under intensive and extensive systems. Different techniques are used to collect, convey, store and dispose manure, all of which lead to direct contact with humans. The prevalence of diarrheagenic *Escherichia coli* in cattle and water was 2.2% (95% CI: 0.99–3.67) and 0.5% (95% CI: 0.025–2.44), respectively. There was transmission of bacteria between cattle, humans and the environment in 52% of clusters. Cattle and manure management practices expose humans, livestock and the environment to risk of infection or contamination. Holistic approach can be adopted in this scenario to attain one health status and improve urban and peri-urban livestock contribution to community livelihood simultaneously.

Keywords: manure management, peri-urban, pathogen transmission, system thinking, one health

1. Introduction

Urban areas are city areas characterized by a dense human population of mixed age, sex, family and household structure, ethnic, cultural, religious diversity, educational and income levels,

and high built-up area with technological and economic advancement. Rural areas, on the other hand, are open broad areas of land located far from towns and cities, which are composed of extensive bushes between large crop fields and livestock herds and sparse housing and population density. Between the urban and rural settings lies peri-urban zone whose population, livestock, crops and land use features are influenced by the proximate interface. Part of peri-urban area adjacent to urban area has features resembling urban features, while its other side assumes the rural characteristics. There is no distinct line separating the peri-urban from urban and rural settings, but a slow zone of change [1]. The gradual transition from peri-urban to urban setup is moving constantly away from city center toward the rural direction due to persistent urbanization pressure, especially in developing countries.

Urban and peri-urban livestock farming is expanding in developing countries primarily due to high demand for protein of animal origin to feed the rapidly growing urban populations, but also to generate income of livestock keeping households [2]. It is also a diversification tactic to spread livelihood risks in adverse situations [3]. Some urban and peri-urban dwellers continue to keep livestock to maintain their rural cultural values [4]. The expansion of urban and peri-urban livestock farming, which is reflected as an increase in number of both the livestock and households involved in keeping livestock, and rapid urban human population growth has increased the chance of contact between humans, animals and manure. Urban areas of Morogoro in Tanzania, for example, had a cattle population of 2618 in 1996 [5], which almost doubly increased to 4170 in 2006 [6]. By 2008, the cattle population in Morogoro urban was 19,099, and among them, 4425 were dairy cattle [7]. This cattle population hiked up to 49,625 in 2012 [8]. Rapid urban population growth is primarily caused by influx of people from rural areas either as migrants or as commuters [1, 9]. For instance, Tanzania's annual population growth rate between 1988 and 2002 was 3% with the urban population size increasing from 18% in 1988 to 23% in 2002 [10]. Moreover, population size and growth in rural and urban areas of Morogoro region from 2002 to 2012 show that the rural population grew by 23.7% from 1,279,513 in 2002 to 1,582,434 compared to 34.2% growth in urban population from 473,849 in 2002 to 636,058 in 2012 [8]. In this region, the general population density changed from 24 persons per square kilometer in 2002 to 31 persons per square kilometer in 2012 [11]. As a result of increased human and animal density, the chance of contact between humans and livestock has increased. The growth in animal population and concomitant increase in manure production, in shrinking space separating humans, livestock and manure, require appropriate livestock and manure management practices taking into account that livestock harbors zoonotic pathogens [12].

Four decades ago, before the expansion of urban and peri-urban livestock farming, free open communal cattle grazing system required minimal effort to manage manure [3, 13]. Cattle freely grazed during daytime and were confined during the night for security. Most manure was left scattered everywhere except for a small amount which was applied on crop fields [3, 14]. To date, the manure management practices have changed to adapt to densely populated areas where the space separating humans from animals and their wastes has decreased. A question arises: does this change consider prevention of animal and human from pathogen exposure as well as environmental contamination? This chapter describes assessment of

manure management practices and risks of contact and transmission of cattle manure-related zoonotic pathogens between cattle, humans and the environment in urban and peri-urban areas of Morogoro region of Tanzania. This report forms a basis for developing strategies to improve urban and peri-urban livestock farming practices in order to safeguard human, animal and ecosystem health in settings similar to study area.

2. Exploration of animals-humans-environment interaction

Most of the people who keep cattle in urban and peri-urban areas also keep other livestock such as goats and chicken. Members of livestock keeping households share premises with livestock. In this community, livestock keeping households are randomly mixed with households which do not keep livestock. There is no tangible demarcation between livestock keeping households and non-livestock keeping households, and hence, the two types of households are in close contact. A total of 119 households keeping cattle, randomly selected, were willingly enrolled for the study in urban and peri-urban areas of Morogoro, Tanzania. Each cattle keeping household was paired to a non-cattle keeping household selected from any direction within a radius of 100 m for purpose of comparison. This pair was regarded as a cluster. Assessment of the interaction between cattle, humans and the environment within and between clusters involved field visits in order to make observations and interview household representatives about livestock and manure management practices. Questionnaire to cattle keeping households inquired about herd characteristics and management, manure management practices, awareness on zoonotic health risks and constraints to livestock farming in urban and peri-urban areas. Observations were made to top up and confirm the information gathered from the questionnaire. Details of labor division, herd composition and size, animal housing and feeding, herd health management, means and frequency of manure collection, storage and disposal were obtained at household level. Questionnaires to non-cattle keeping households enquired about attributes which may contribute to contact between humans, cattle and manure. Moreover, District Livestock Officers were interviewed about monitoring of manure handling practices in their respective areas of jurisdiction and were asked to present documents guiding livestock and manure management. This cross-sectional study was carried out from February 2010 to February 2012.

Cattle feces, human stool, soil and water samples were collected from each participating household for isolation and characterization of bacteria to check for the presence of pathogens and evidence of transmission between cattle, humans and the environment. In this particular study, *Escherichia coli* and non-typhoidal *Salmonella* spp. were target bacteria. Individual 100–150 g cattle fecal samples were collected by a gloved hand. A 100 g pooled soil sample from each household (cattle keeping and non-cattle keeping households) was obtained by taking 2–5 cm of top soil from five different areas within household premise. From each participating household, 100 ml water sample was collected in 250 ml container from stored water or sources such as boreholes, ponds or river which are used by humans and livestock. Stool sample from one household member was requested. For cattle keeping households, a member involved in cattle and/or manure management was eligible to give stool sample, while

for non-cattle keeping households, any member was eligible. On the evening before sample collection day, a stool collection container was given to an appropriate person for collection of stool in the following morning. All samples for a cluster were collected on the same day and immediately placed in an insulated box with cooling elements and transported to the laboratory where bacteriological analysis was initiated.

Ethical clearance was approved by Sokoine University of Agriculture Ethical Committee to handle animals and animal samples. Approval was also obtained from the Tanzania National Institute for Medical Research (NIMR) Ethical Board (NIMR/HQ/R8a/Vol. IX/927) to handle and process human sample. All conditions for research approval were observed throughout the study.

3. Cattle and manure management practices

Observations and face-to-face interviews conducted during field visits to 119 households keeping cattle generated data which were analyzed by descriptive statistics such as means, frequencies and cross-tabulations by using SPSS 15.0. Information about herd characteristics and management, manure management practices and awareness on cattle manure-related zoonotic pathogens was obtained from the cattle keepers and Livestock Officers.

From observations and interviews, a total of 806 cattle were kept by study participants (minimum = 1, maximum = 36, mean = 7, median = 5, SD = 5.85), 95.8% of whom also kept animal species other than cattle in same residential premises. These animal species, with percentage of participants keeping these species in brackets, include chicken (80.7%), dogs (62.2%), goats (50.4%), pigs (27.7%), ducks (23.5%), cats (21.9%), sheep (10.9%), guinea fowls (9.2%), turkeys (5.9%), guinea pigs (1.7%), rabbits (1.7%) and monkey (0.8%). Cattle and manure management practices were carried out either by family members (46.2%) or by hired laborers (53.8%). Most cattle houses (71.4%) had concrete floor and the rest (28.6%) had floor made of earth. It was observed that majority of cattle houses (84%) had roofs and 16% were open cattle "boma." Cattle kept in earth floor houses with open or broken roof stayed on mud during rainy season. Three out of 119 respondents (2.5%) put grass on the floor of cattle house as bedding material, one of them had a house with earthed floor. All respondents kept their cattle in a confinement near to their residence for security reasons. Cattle were fed by "cut and carry" method under intensive system (47%) or were allowed to go around foraging (53%) where they mixed with livestock from other herds. There was sharing of water sources between cattle and humans. Free range cattle (40.3%) used surface water such as rivers, ponds and wells, while intensively kept cattle (59.7%) were provided water from taps also serving the people [15].

Overnight confinement of cattle resulted into manure accumulation which necessitated collection and storage/discard. Various methods were employed to collect, convey and store or discard manure. These included uses of utensils like spade, bucket or plastic bags, use of water splash and use of bare hands. Manure was collected by bare hands by a few respondents where there was direct contact with the manure. However, the majority of respondents used

utensils such as spades, hand hoes and rakes to collect manure into a pile within the cattle house. Some respondents used a water hose to collect manure (**Table 1**). Manure was removed from cattle house at different rates per day, week or month to storage or disposal site by using utensils (plastic bags, buckets, raw hides, spades and hand hoe and wheelbarrow), bare hands or water. The use of rubber boots was an observed practice by less than a half of the respondents, while the remaining fraction wore ordinary shoes, e.g., sandals or were barefooted while handling manure (**Table 1**). In all these different manure collection or conveyance methods, people did not use any protective measures such as special clothes or gloves and were observed to have direct skin contact with manure. A large proportion of respondents stored manure into piles before disposal as fertilizer or waste, whereas a few respondents threw fresh manure from cattle house direct into the surroundings. Most cattle keepers disposed manure within a radius of 10 m from their residential houses, especially those with land area of more than 1000 m². Respondents who did not spread manure on land opted for burning or giving it away to friends in plastic bags. Allowing effluent from cattle house to leach into immediate land was a common practice among cattle keepers although a few cattle keepers directed the effluent into a pit (**Table 1**).

Variable	Category	Frequency (%)
Manure disposal method	Spread on land	108 (90.8)
	Not spread on land	11 (9.2)
Means of manure collection	Hand picking	5 (4.2)
	Use of utensils	112 (94.1)
	Water splash	2 (1.7)
Frequency of manure collection	Once a day	72 (60.5)
	More than once a day	19 (16.0)
	Weekly	28 (23.5)
Means of manure conveyance	Hand picking	3 (2.6)
	Use of utensils	115 (96.6)
	Water splash	1 (0.8)
Use of rubber boots	Yes	70 (58.8)
	No	49 (41.2)
Manure treatment	Heaping	99 (83.2)
	Direct spread on land	20 (16.8)
Manure disposal distance	Within 10 m from residence	83 (69.7)
	Outside 10 m from residence	36 (30.3)
Effluent treatment	Direct spread on land	95 (79.8)
	Use of pit	24 (20.2)
Household area	>1000 m ²	87 (73.1)
	≤1000 m ²	32 (26.9)

Table 1. Manure management practices among 119 Morogoro urban and peri-urban cattle keepers.

Out of 119 respondents, 5% reported to have heard about manure-associated pathogens which can infect human. There were 125 responses to problems related to manure management which respondents encounter. Out of these 125 responses, 77 (61.6%) said they encounter no problem, while 15 (12%) responses reported that poor infrastructure impedes manure management practices. Lack of working facilities such as utensils and transport was reported in 13 (10.4%) responses as one of the problems cattle keepers face, whereas land scarcity appeared in 6 (4.8%) responses. Health problems related to respiratory tract, injuries and foot rot to manure handlers were mentioned in 5 (4%) responses, same as for the presence of poor cattle housing facilities. Odor and water scarcity were each mentioned in 2 (1.6%) responses as among problems of manure management practices in urban and peri-urban areas of Morogoro.

During the interview, Livestock Officers presented documents such as “Environmental Sanitation By-Laws” and “Animals in Urban areas By-Laws” which give directives on animal keep in the area and how to deal with wastes including manure. From interviews and the documents, the guideline which allows maximum herd size of four cattle per herd in urban area does not give area requirement specification and is not observed, and cattle manure is regarded by the by-laws and treated like any solid household waste [15]. It was observed that cattle keeping households are randomly distributed among non-cattle keeping households and there are no preconditions for a household to start keeping cattle. Anybody can start a herd of cattle anywhere in urban and peri-urban areas of Morogoro at any time.

The current manure management practices differ from those methods used a few decades ago in both the actual practices and resource base available which is shared between humans, animals and manure. Increased manure production in populated urban and peri-urban areas has resulted into the problems mentioned by cattle keepers. Some of these problems such as land scarcity odor and increased flies population have been previously reported to be due to exclusion of livestock farming during urban and peri-urban land use planning [4]. Increased manure production in a shrinking space has forced cattle keepers to collect, convey, store and finally dispose manure. Diverse cattle and manure management practices are determined by customs, convenience and availability of resources including land and equipment. Some farmers said that they keep cattle and handled the manure by the same methods since childhood; others opted for a particular cattle and manure management practice because it was easy to execute. Generally, there was direct contact between humans, cattle and manure and there was environmental contamination by fresh manure. In this scenario, humans, animals and environment are exposed to manure-related pathogens.

4. Pathogens in cattle, humans and the environment

Sample size of 100 clusters was calculated as previously described [16]. Face-to-face interviews were conducted to cattle keeping household members about cattle and manure management practices. Interview was also conducted to cattle keeping household neighbors who do not keep cattle about possible contact with cattle and manure. Individual fecal sam-

ples from 446 cattle, 100 stool samples from individuals keep cattle and 100 who do not keep cattle, 200 soil and 200 water samples from sources within homesteads were collected for bacteria isolation.

Escherichia coli was isolated and characterized as described earlier [16]. In summary, non-sorbitol fermenting (NSF) *E. coli* were isolated by using sorbitol MacConkey agar, and suspect colonies were characterized biochemically by use of MacConkey agar, Brilliance *E. coli* agar and indole test. Confirmed NSF *E. coli* isolates were assessed for the presence of virulence genes: intimin gene (*eae*), verocytotoxin 1 (*vtx1*), verocytotoxin 2 (*vtx2*), heat-stable enterotoxin, human variant (*estA*-human), heat-stable enterotoxin, porcine variant (*estA*-porcine), heat-labile enterotoxin (*eltA*) and invasive plasmid antigen (*ipaH*) by multiplex diarrheagenic *E. coli* (DEC) PCR. Dot-blot DNA hybridization was done by using *vtx1*, *vtx2*, *eae*, *ehxA*, *EAF*, *bfpA*, *saa*, *astA* and *vtx2f* DNA probes to confirm the presence of virulence genes in isolates positive by DEC PCR. The colonies were lysed, denatured and neutralized using standard conditions and then hybridized as formerly described [17].

Somatic antigen O and flagella antigen H on diarrheagenic *E. coli* were typed by using specific antisera at Statens Serum Institut, Denmark, using a standard protocol [18]. In summary, both somatic O and flagella H antigens were tested by agglutination method against both pooled and specific antisera. For somatic O antigen, a boiled culture of *E. coli* isolate was tested against a pooled O antisera and culture with positive agglutination test was further tested against single specific O antisera. Somatic O antigen was assigned a number according to positive agglutination on a specific single O antigen. For flagella H antigen, an *E. coli* culture was tested for motility in semi-solid medium and fixed with formaldehyde 0.5%. Fixed culture was tested against pooled H antisera, and positive culture was further tested against single specific H antisera. Fluffy reaction indicated positive result, and the isolates were assigned a number.

Phenotypic activity of virulence genes was assessed on Vero cell monolayers to test for cytopathic effects using protocol formerly described [17].

For non-typhoidal *Salmonella* spp. isolation, 1 ml of the sample suspension was enriched by overnight incubation in selenite fecal broth at 37°C. The bacteria growth was subcultured on *Salmonella*-*Shigella* agar at 37°C for 24 h. Colorless colonies with a black center were biochemically tested by urease and lysine carboxylase tests. Urease-negative and lysine carboxylase-positive colonies were tested against *Salmonella* polyvalent agglutinating sera (REMEL30858201 ZC02—LOT 820883) and serotyped by Kauffmann-White M03-03-001 method at Danish Institute for Technology (DTU).

Vero cytotoxin-producing *E. coli* (VTEC) from cattle, enteropathogenic *E. coli* (EPEC) from cattle and water and attaching and effacing *E. coli* (A/EEC) from cattle were isolated (**Figure 1**). Overall prevalence of diarrheagenic *E. coli* in cattle (n = 446) was 2.2% (95% CI 0.99–3.67) and in water (n = 200) was 0.5% (95% CI 0.025–2.44). The prevalence of VTEC in cattle was 1.6% (95% CI 0.69–3.08), (**Table 2**) [16].

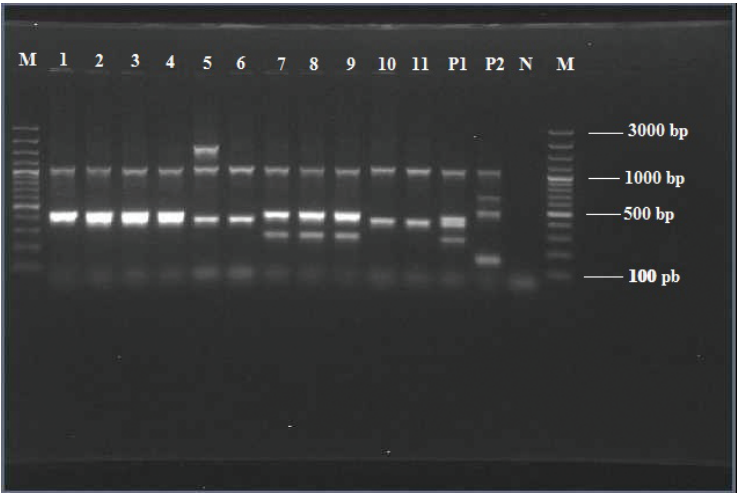


Figure 1. Multiplex DEC PCR for NSF *E. coli* isolates: lanes M: molecular weight size marker (100-bp plus DNA ladder); lane 1: *vtx2* and *eae*; lane 2: *vtx2* and *eae*; lane 3: *vtx2* and *eae*; lane 4: *vtx2* and *eae*; lane 5: *eae*; lane 6: *eae*; lane 7: *vtx1* and *vtx2*; lane 8: *vtx1* and *vtx2*; lane 9: *vtx1* and *vtx2*; lane 10: *eae*; lane 11: *eae*; lane P1: positive control for *vtx2*, *eae* and *astA*; lane P2: positive control for *ipaH*, *eltA* and *estA*; lane N: negative control.

Bacteria species	Source	Serotype	Pathotype	Virulence genes
<i>Escherichia coli</i>	Cattle	O157:H7	VTEC	<i>vtx2</i> , <i>eae</i> , <i>ehxA</i> and <i>astA</i>
	Cattle	O157:H7	VTEC	<i>vtx2</i> , <i>eae</i> , <i>ehxA</i> and <i>astA</i>
	Cattle	O157:H7	VTEC	<i>vtx2</i> , <i>eae</i> , <i>ehxA</i> and <i>astA</i>
	Cattle	O157:H7	VTEC	<i>vtx2</i> , <i>eae</i> , <i>ehxA</i> and <i>astA</i>
	Cattle	O113:H2	VTEC	<i>vtx2</i>
	Cattle	O+:H16	VTEC	<i>vtx1</i> and <i>vtx2</i>
	Cattle	O113:H21	VTEC	<i>vtx1</i> and <i>vtx2</i>
	Cattle	O142:H34	EPEC	<i>eae</i> , <i>EAF</i> and <i>bfpA</i>
	Water	O142:H34	EPEC	<i>eae</i> , <i>EAF</i> and <i>bfpA</i>
<i>Salmonella kentucky</i>	Cattle	O+:H-	A/EEC	<i>eae</i> <i>ehxA</i> and <i>astA</i>
	Cattle			
	Cattle			
<i>Salmonella weltevreden</i>	Human			
<i>Salmonella amager</i>	Human			

Table 2. Zoonotic bacteria isolated from cattle, humans and environment in urban and peri-urban areas of Morogoro, Tanzania.

The prevalence of *Salmonella kentucky* in cattle was 0.45% (95% CI 0.001–0.016), while one *Salmonella weltevreden* and one *Salmonella amager* were isolated from different apparent healthy humans (Table 2).

The VTEC strains contained *vtx1a*, *vtx2b*, *vtx2c* and *vtx2d* subtypes either singly or in combinations, and phenotypic expression of virulence was confirmed by the cytopathic effect they caused to Vero cell monolayers (Table 3) [16].

Sample ID	Serotype	Source	VCA	<i>vtx1</i>	<i>vtx2</i>	<i>vtxsubtypes</i>
BKIH101	O4:H16	Bovine	+	+	+	<i>vtx1a</i> ; <i>vtx2c</i>
BKIN069	O157:H7	Bovine	–	–	+	<i>vtx2c</i>
BMKB070	O157:H7	Bovine	+	–	+	<i>vtx2c</i>
BMKB068	O157:H7	Bovine	+	–	+	<i>vtx2c</i>
BMKB069	O157:H7	Bovine	+	–	+	<i>vtx2c</i>
BMZU001	O113:H21	Bovine	+	–	+	<i>vtx2b</i> + <i>vtx2d</i>
BBIG020(1)	O113:H21	Bovine	+	+	+	<i>vtx1a</i> ; <i>vtx2b</i> + <i>vtx2d</i>

Table 3. Vero cell assay (VCA) and *vtx* subtyping for non-sorbitol fermenting diarrheagenic *E. coli* isolates.

Isolation of diarrheagenic *E. coli* and *Salmonella* species from cattle feces is an evidence of risk of infection to humans and environmental contamination. There was also isolation of diarrheagenic *E. coli* from water in the study area. The risk in this scenario is due to direct contact between cattle, humans and manure as well as direct spread of fresh manure onto land within residence. This risk can cross between cattle keeping households because different cattle herds come into contact during grazing, and the spread can reach the non-cattle keeping neighbors. Sharing of water sources between humans and cattle, at some instances during dry season, poses another threat to public health. It is fortunate that these highly pathogenic and fatal diarrheagenic *E. coli* were not detected in humans because only apparent healthy subjects were sampled. Isolation of *Salmonella amager* and *S. weltevreden* in human stool calls for an attention on pathogen transmission route because humans can also act as a source of pathogens to livestock and the environment.

5. Transmission of bacteria between cattle, humans and environment

The study on transmission of bacteria involved 100 clusters, and each cluster was formed by a pair of a cattle keeping household and a neighboring non-cattle keeping household. Each cluster contributed two stool samples, two water samples and two soil samples, one of the samples from cattle keeping household and another from a non-cattle keeping household. Isolation, characterization and quantification of the risk of transfer of *E. coli* were done as earlier reported [19]. In summary, isolation of *E. coli* was carried out by inoculating a loopful suspension of cattle feces and stool from cattle keepers and non-cattle keepers, soil and water on

MacConkey agar followed by 24-h incubation at 37°C. *E. coli* suspected isolates were confirmed and screened for double antimicrobial resistance to ampicillin and tetracycline on antimicrobial embedded Petrifilm™ Select *E. coli* count (SEC) plate. Preparation of antimicrobial stock solution and screening procedure was done according to Ref. [20]. Ampicillin-tetracycline-resistant *E. coli* isolates were genetically assessed by pulsed-field gel electrophoresis (PFGE) according to Ref. [21]. Analysis and comparison of PFGE gel pictures were done by using GelCompar II software (Applied Maths, St-Martens-Latem, Belgium) as previously reported [18]. Isolates from cattle, humans, soil and water with 100% band pattern homology were considered genetically identical. A face-to-face interview was conducted to each household in the cluster. Semi-structured questionnaire which aimed at gathering information related to cattle and manure management (for cattle keeping households) and events or scenario leading to contact with cattle and manure (for non-cattle keeping households) was administered.

Logistic regression was run to quantify risk factors for the presence of isolates from cattle, humans, water or soil which are genetically identical to at least one other isolate from same or different clusters by using PROC GENMOD in SAS as earlier described [19]. The response variable was the occurrence of identical PFGE band pattern of *E. coli* isolates (yes or no), while the independent variables comprised of factors focusing on cattle herd characteristics and management (the presence of species other than cattle and labor division), cattle housing infrastructure (roof, floor and beddings), feeding and water system and manure management issues (collection and disposal). Univariable analysis was performed to all explanatory variables and those with an arbitrary *p*-value of equal or less to 0.25 were included in a multivariable model. A final model was obtained by a backward stepwise strategy. Chi-square test was used to check for association between different cattle and manure management factors at 5% significance level.

From 1046 samples, 118 (11.28%) samples produced ampicillin-tetracycline-resistant *E. coli*. Forty samples with resistant *E. coli* isolates (34%) were human stool, 50 (42%) were cattle feces, 21 (18%) were soil and 7 (6%) were water. One ampicillin-tetracycline-resistant *E. coli* isolate per sample was taken for further analyses. The 118 ampicillin-tetracycline-resistant *E. coli* isolates came from 44 out of the total 100 clusters. Twenty-three out of 44 clusters showing ampicillin-tetracycline-resistant isolates (52.3%) yielded at least one isolate with identical PFGE band pattern to another isolate from another source, suggesting that transfer of *E. coli* was a common event. Eight distinct PFGE band patterns designated arbitrary letters A, B, D, E, F, G, H and I for distinguishing purposes were identified. Inclusion of *Salmonella enterica* serovar *Braenderup* in all the gels showed a band pattern reproducibility of 100% (type C) (Figure 2) [19]. These PFGE band patterns cut across different clusters and were from cattle, humans, soil and water. Sixteen clusters out of 44 (36%) yielded at least one *E. coli* isolate which was identical to another isolate from another source by 100%. Seven clusters (16%) had isolate with similarity between 95 and 99.1% (Figure 2). PFGE band pattern A was comprised of five clusters, pattern B had two clusters, pattern D had three clusters, pattern E had six clusters, pattern F had two clusters, pattern G had one cluster, pattern H had two clusters and pattern I had also two clusters. Twelve isolates from cattle, human and soil constituted PFGE band pattern A, while pattern E was made up of eight isolates from cattle, soil and water (Table 4).

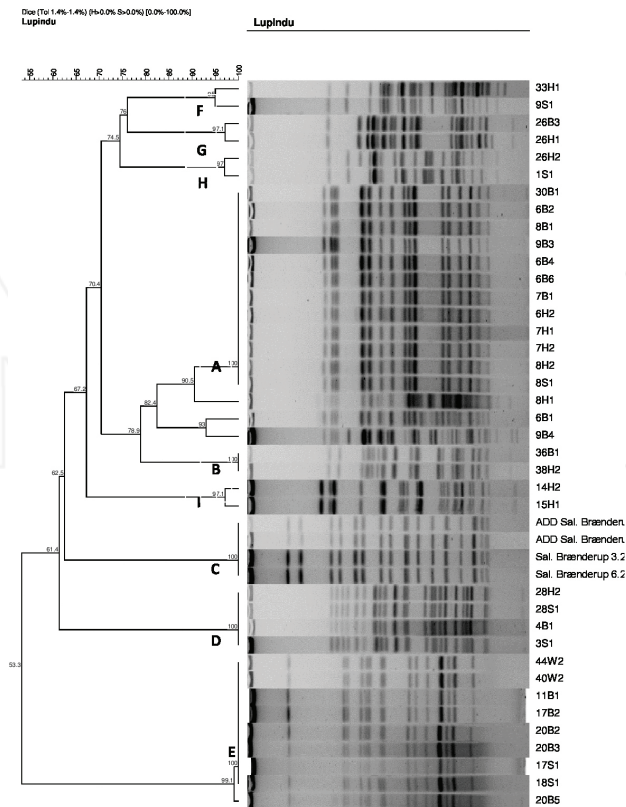


Figure 2. PFGE band pattern for ampicillin- and tetracycline-resistant *E. coli* isolates from humans, cattle, soil and water.

This shows that there was sharing of genetic characteristics between bacteria isolates from different sources. There was also genetic relatedness in cluster seven between isolates from cattle keeping human (7H1), cattle (7B2) and non-cattle keeping human (7H2). This scenario suggests that sharing of bacteria go beyond cattle keeping households to their non-cattle keeping neighbors. In some instance, like in cluster six, isolates from cattle (6B2, 6B4 and 6B6) did not resemble humans in the same household, but had PFGE band pattern identical to neighboring non-cattle keeping human (6H2). Sharing of genetic features was also observed in isolates from cattle, humans and the environment. For instance, isolate from cattle in cluster eight (8B1) was identical to isolate from non-cattle keeping human (8H2) and isolate from soil collected from cattle keeping household (8S1) in the same cluster eight. In PFGE band pattern E, isolates from water sources of non-cattle keeping households (40W2 and 44W2) had identical PFGE patterns to isolates from cattle (11B1, 17B2, 20B2 and 20B3) and soil (17S1 and 18S1) from cattle keeping households (**Table 4**). Some isolates with identical PFGE band patterns from cattle, e.g., in PFGE band pattern A, came from different households/herds, signifying the role of communal grazing in sharing of bacteria between cattle.

Isolates with distinct PFGE band patterns within clusters had a good temporal relationship in terms of sampling and isolation. Most of them came from samples collected on one day or within a week (**Table 4**) [19].

Clonal group	Cluster	Isolate ID ^a				Sample date
A	6	6B2	6B4	6B6	6H2	16 July 2011
	7	7B2	7H1	7H2		16 July 2011
	8	8B1	8H2	8S1		20 July 2011
	9	9B3				20 July 2011
	30	30B1				20 July 2011
B	36	36B1				21 September 2011
	38	38H2				21 September 2011
D	28	28H2	28S1			15 September 2011
	4	4B1				15 September 2011
	3	3S1				22 July 2011
E	11	11B1				24 September 2011
	17	17B2	17S1			15 January 2012
	18	18S1				15 January 2012
	40	40W2				15 January 2012
	20	20B2	20B3			18 January 2012
	44	44W2				11 January 2012
F	33	33H1				20 July 2011
	9	9S1				20 July 2011

^a*E. coli* isolates from humans (H), water (W) and soil (S) with odd last digit originated from cattle keeping households while those with even last digit were obtained from non-cattle keeping neighbors.

Table 4. Identical PFGE patterns of ampicillin- and tetracycline-resistant *E. coli* isolated from cattle keeping and non-cattle keeping neighbor households in peri-urban areas of Morogoro, Tanzania.

Escherichia coli isolates from cattle were found in all clusters with identical PFGE bands patterns (**Figure 2**), proposing that cattle are the focal point of bacteria sharing and manure is the center of contact between cattle, humans and the environment. These roles of cattle and manure in bacteria sharing between cattle, humans and the environment lead to a hypothetical bacteria transmission pathways presented in **Figure 3**. The bacteria sharing pathways can be used to set up strategies to break the contact and transmission pathways. However, there is a need to

develop procedures which can be used to determine the donor-recipient bacteria transmission relationship, something that was not done in the current study.

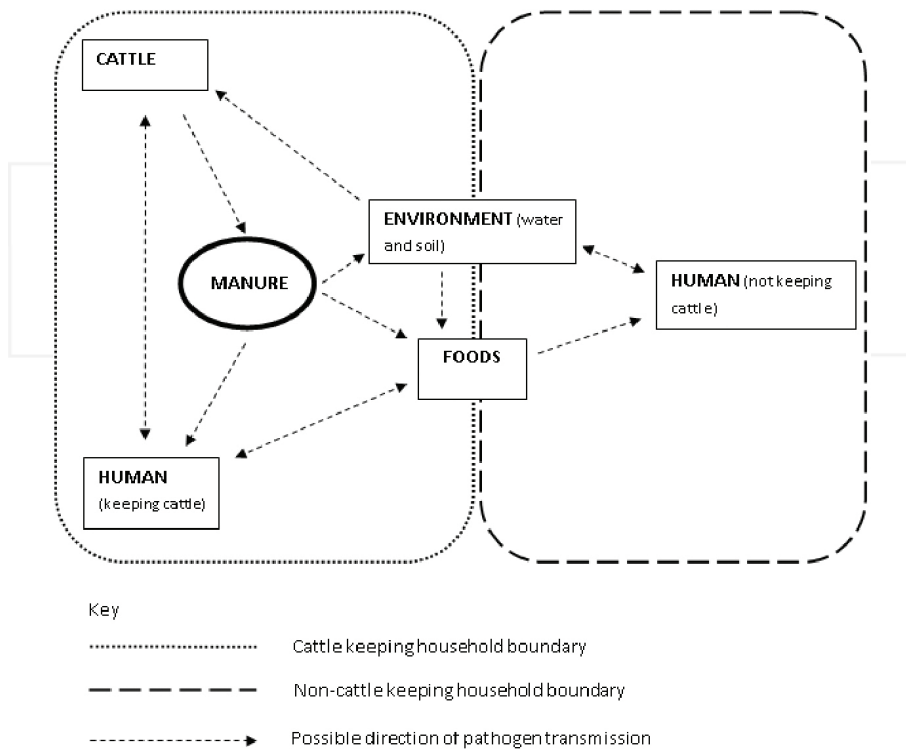


Figure 3. Hypothetical transmission pathways of enteric bacteria in urban and peri-urban livestock farming systems in Morogoro, Tanzania.

From univariable analysis, five explanatory variables, namely manure responsible personnel (family member or hired laborer), cattle house roof (present or absent), cattle house floor (concrete or earth), use of bedding (yes or no) and animal water source (tap or surface water) qualified and progressed to multivariable logistic regression analysis. There were no detected confounders during the model building process, and the final logistic regression model was made up of a single explanatory variable, the type of cattle house roof. The cattle house with a roof was at 11 times odds of having isolates with identical PFGE band pattern to another isolate from another source (OR = 11.2, 95% CI 1.1–119.3). Generally, isolates with PFGE band pattern identical to at least one isolate from another source were 33, 86.8% of which were isolated from cattle houses with a roof. The model goodness-of-fit test, expressed as the ratio of deviance to degree of freedom, was 1.2, while the correlation of 0.1344 existed between sample sources from different clusters. This shows that the variables were well explained by the model.

From this study, it seems that there was transmission of bacteria in roofed cattle houses than in cattle houses without roof. This could be due to the effect of direct sun rays in open cattle houses killing the bacteria before the transmission.

Cattle feeding system was statistically associated with cattle water sources ($X^2 = 28.5$, $df = 1$, $p \leq 0.0001$), whereby free range cattle used surface water and cattle under zero grazing used tap water which was also used by humans. On the other hand, distance from residence to manure disposal site was statistically associated with the way manure was handled ($X^2 = 8$, $df = 1$, $p = 0.005$). That is, cattle keeping households which stored manure in heaps disposed manure within residential areas, whereas households which opted to spread fresh manure on land did it outside residential area [19].

6. Conclusion

Cattle and manure management practices in urban and peri-urban livestock farming allow direct contact of cattle manure with humans, cattle and the environment. Humans and cattle are at risk of infection with enteric pathogens and the environment to contamination because enteric pathogens have been isolated from fresh cattle feces in urban and peri-urban areas. Under the current manure management system, there is transmission of commensal enteric bacteria between cattle, humans and the environment (water and soil), in which case, same route can transmit enteric pathogens. The risk of human and livestock infection and environment contamination is potentiated by the fact that cattle keepers are unaware of such manure-related pathogens and majority of them do not perceive that there are public health threats from the current cattle and manure management practices. The risk of enteric pathogen transmission to humans extends beyond cattle keeping households to their non-cattle keeping neighbors. Current cattle and manure management practices in urban and peri-urban areas of Morogoro put the whole community (cattle keepers and non-cattle keepers), cattle and other domestic animals, at risk of infection and the environment (water and soil) to contamination.

7. Recommendations

The reported public health challenges can be alleviated by adopting a system thinking or holistic approach, whereby all stakeholders are identified and involved, at their respective capacities, in planning, execution and monitoring of urban and peri-urban livestock farming. This approach will aim at safeguarding public, livestock and ecosystem health at the same time improving urban and peri-urban livestock contribution toward community livelihood. Some of the key stakeholders, each of whom may have a different key role in ensuring this goal is achieved, include personnel from health section, agriculture, livestock, local government authorities, land use planning, civil engineers, environmental conservation, demography, law enforcing sections, politicians and the general public. For example, local government authorities may put preconditions for starting a cattle herd in urban and peri-urban areas and set

criteria for maintenance of livestock keeping permit. This procedure may facilitate other livestock-related activities such as disease control, surveillance and traceability of animals. Moreover, land use planning and environmental conservation sections may set specific areas for keeping livestock, while medical and veterinary sections may jointly control zoonoses. The law enforcing personnel can facilitate in making sure regulations related to livestock, and livestock products are observed. The general public should be well informed of and participate in control of manure-related zoonotic pathogens. The holistic setup of urban and peri-urban livestock farming should take into account all the features of continuous change from rural to peri-urban to urban setting. This means that planning of livestock farming in peri-urban should suit the urban setup even when the peri-urban area is urbanized.

To reduce human and animal contact with manure and to reduce the risk of human and animal infection and environmental contamination, the following strategies are recommended.

- Urban and peri-urban land use planning should include livestock industry during planning so that specific areas are legally recognized for livestock farming in urban and peri-urban areas.
- There should be strategies to convert manure into a convenient, safe and valuable commodity. This should involve reduction in water content and odor from manure while maintaining its soil fertilizing quality.
- Education to community (livestock keepers and non-livestock keepers) on livestock and manure-related zoonotic risks which are associated with management practices. It should be the responsibility of the whole community to ensure one health status is achieved.
- Appropriate regulations, by-laws and guidelines should be formulated and reinforced to guide safe cattle and manure management practices which safeguard public, livestock and ecosystem health. The guidelines should clearly give directives on personal protection, cattle and manure handling and environmental protection.

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