Investigating the meat pathway as a source of human nontyphoidal *Salmonella* bloodstream infections and diarrhea in East Africa

John A. Crump, MB ChB, MD, DTM&H\textsuperscript{1,2,3*}

Kate M. Thomas, PhD\textsuperscript{1*}

Jackie Benschop, BVSc, PhD\textsuperscript{4}

Matthew A. Knox, PhD\textsuperscript{4}

David A. Wilkinson, PhD\textsuperscript{4}

Anne C. Midwinter, PhD\textsuperscript{4}

Peninah Munyua, BVM, PhD\textsuperscript{5}

John B. Ochieng, PhD\textsuperscript{5}

Godfrey M. Bigogo, PhD\textsuperscript{6}

Jennifer R. Verani, MD, MPH\textsuperscript{5}

Marc-Alain Widdowson, VetMB, MSc, MA\textsuperscript{5,7}

Gerard Prinsen, PhD\textsuperscript{8}

Sarah Cleaveland, VetMB, PhD\textsuperscript{9}

Esron D. Karimuribo, BVM, MVM, PhD\textsuperscript{10}

Rudovick R. Kazwala, BVSc, MVM, PhD\textsuperscript{10}

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Blandina T. Mmbaga, MD, MMed, PhD2,3

Emanuel S. Swai, BVM, PhD11

Nigel P. French, BVSc, MSc, PhD4¶

Ruth N. Zadoks, DVM, MSc, MRes, PhD6,12¶

1Centre for International Health, University of Otago, Dunedin, New Zealand

2Kilimanjaro Clinical Research Institute, Kilimanjaro Christian Medical Centre, Moshi, Tanzania

3Kilimanjaro Christian Medical University College, Moshi, Tanzania

4School of Veterinary Science, Massey University, Palmerston North, New Zealand

5Division of Global Health Protection, US Centers for Disease Control and Prevention, Nairobi, Kenya

6Centre for Global Health Research, Kenya Medical Research Institute, Kisumu, Kenya

7Institute of Tropical Medicine, Antwerp, Belgium

8School of People, Environment and Planning, Massey University, Palmerston North, New Zealand

9Institute of Biodiversity, Animal Health, and Comparative Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland, United Kingdom

10College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania

11Department of Veterinary Services, Ministry of Livestock and Fisheries, Dodoma, Tanzania

12Sydney School of Veterinary Science, University of Sydney, Sydney, Australia
*John A. Crump and Kate M. Thomas contributed equally to this work.

¶Nigel P. French and Ruth N. Zadoks contributed equally to this work.

**Corresponding author:** John A. Crump, MB ChB, MD, DTM&H, McKinlay Professor of Global Health and Co-Director, Centre for International Health, University of Otago, PO Box 56, Dunedin 9054, New Zealand. Tel +64-3-479-9460, Fax +64-3-479-7298, Email john.crump@otago.ac.nz
Summary: The meat pathway may be an important source of human invasive Salmonella Enteritidis ST11 infections in East Africa, but not of Salmonella Typhimurium ST313. Improvements to meat safety are warranted while research on sources for other nontyphoidal Salmonella infections continues.
ABSTRACT

Background. *Salmonella* Enteritidis and *Salmonella* Typhimurium are major causes of bloodstream infection and diarrheal disease in East Africa. Sources of human infection, including the role of the meat pathway, are poorly understood.

Methods. We collected cattle, goat, and poultry meat pathway samples from December 2015 through August 2017 in Tanzania and isolated *Salmonella* using standard methods. Meat pathway isolates were compared with nontyphoidal *Salmonella* (NTS) isolated from persons with bloodstream infection and diarrheal disease from 2007 through 2017 from Kenya by core genome multi-locus sequence typing (cgMLST). Isolates were characterized for antimicrobial resistance, virulence genes, and diversity.

Results. We isolated NTS from 164 meat pathway samples. Of 172 human NTS isolates, 90 (52.3%) from stool and 82 (47.7%) from blood, 53 (30.8%) were *Salmonella* Enteritidis ST11 and 62 (36.0%) *Salmonella* Typhimurium ST313. We identified cgMLST clusters within *Salmonella* Enteritidis ST11, *Salmonella* Heidelberg ST15, *Salmonella* Typhimurium ST 19, and *Salmonella* II 42:r:- ST1208 that included both human and meat pathway isolates. *Salmonella* Typhimurium ST313 was isolated exclusively from human samples. Human and poultry isolates bore more antimicrobial resistance and virulence genes and were less diverse than isolates from other sources.
Conclusions. Our findings suggest that the meat pathway may be an important source of human infection by some clades of *Salmonella* Enteritidis ST11 in East Africa, but not of human *Salmonella* Typhimurium ST313 infection. Research is needed to systematically examine the contribution of other types of meat, animal products, produce, water, and environmental exposures to nontyphoidal *Salmonella* disease in East Africa.

Keywords: Africa, Eastern; bacteremia; diarrhea; food; *Salmonella*
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INTRODUCTION

Nontyphoidal serovars of *Salmonella enterica* (NTS) were associated with >153 million illnesses and >56,000 deaths worldwide in 2010 [1]. NTS are a leading cause of bloodstream infection in sub-Saharan Africa [2], occurring often in the absence of diarrhea and carrying a case fatality ratio of approximately 20% [3]. NTS bacteremia may be associated with HIV-infection, recent or current malaria, and malnutrition [3]. Even after accounting for HIV-associated disease, the burden of NTS bacteremia in sub-Saharan Africa is substantial [4, 5]. The role of NTS in diarrheal disease in African countries is less clear. While approximately 9% of diarrheal illnesses and 11% of diarrheal deaths in the World Health Organization African region were attributed to NTS [1], NTS have been isolated from <1% of stool samples from infants and children with diarrhea at African sites in one large study of diarrheal disease [6]. Furthermore, NTS have been isolated from the stool of infants and children with diarrhea no more often than from community controls [6, 7]. Nonetheless, acquisition of NTS in stool is likely to precede the development of invasive disease. While there has been relatively little work to characterize NTS causing diarrheal disease in sub-Saharan Africa [8], *Salmonella Typhimurium* sequence type (ST) 313 [9] and several distinct clades of *Salmonella Enteritidis* ST11 [10, 11] strains predominate among bloodstream isolates from the region.

Whereas the typhoidal *Salmonella* serovars Typhi and Paratyphi A are human host-restricted, NTS are generally considered to have their reservoirs in non-human animals. Approximately half of global NTS infections are thought to be transmitted by food [1], with meat being a major food vehicle in high-income countries [12]. Unlike the situation in high-income countries, where foodborne disease surveillance is well developed and epidemiologic investigations inform control measures, there are few data on major reservoirs, sources, and modes of transmission of NTS in Africa. The lack of
epidemiologic data hampers control efforts. NTS may be host generalists such as *Salmonella Typhimurium*, or exhibit degrees of host adaptation such as *Salmonella Dublin* to cattle, or host restriction such as *Salmonella Gallinarum* to poultry [3]. Whole genome sequencing analysis of *Salmonella Typhimurium* ST313 and *Salmonella Enteritidis* ST11 strains has demonstrated inactivation of some genes [9, 11] that have been speculated to indicate adaptation towards a narrower ecologic niche, such as the human host [9]. However, other evidence such as the ability *Salmonella Typhimurium* ST313 to infect and cause disease in poultry [13], is a counterpoint to human host restriction. Furthermore, from a food safety perspective NTS from both healthy animals as well as those with disease have the potential to enter the food chain directly on meat or through contamination of produce and water. Several livestock species in Africa carry *Salmonella Typhimurium* and other NTS serovars, including cattle and poultry [14-16].

Developments in enteric pathogen epidemiology, including cluster-based inference and source attribution models that use microbial subtyping data to assign human infections to animal and environmental sources are being used to understand NTS epidemiology in high-income countries [17]. These approaches have been proposed to investigate sources of NTS disease in Africa [18]. In order to understand the potential contribution of poultry and red meat to human NTS disease in sub-Saharan Africa, we studied NTS from livestock to retail meat along the meat pathway and from human bloodstream and enteric infections in Tanzania and Kenya, East Africa, where movement of livestock, food, and people is common. We used multi-locus sequence typing (MLST) cluster analysis to determine genetic relatedness of isolates to investigate the contribution of meat to human NTS infections.
METHODS

Study setting and sampling

Meat pathway samples were collected from December 2015 through August 2017 in red meat slaughter and butcher facilities and on poultry farms in Arusha Urban, Moshi Municipal, and Moshi Rural Districts of northern Tanzania (Figure 1). As described elsewhere, ten live poultry on each of 80 poultry farms were sampled by cloacal swab and the farm environment by boot socks (Solar Biologicals Inc., Newark, NJ, USA) from 10 wards in Arusha Urban and 10 wards in Moshi Municipal Districts, randomly selecting four farms per ward [19]. Red meat slaughter facilities in Arusha Urban and Moshi Municipal Districts were sampled between two and 25 times. Red meat slaughter and butcher facility environment swabs were taken by swabbing knives and cutting equipment, cutting boards, walls, sinks, hanging rails and other solid surfaces with sterile cellulose sponge swabs predosed with 10 mL buffered peptone water in stomacher bags (TSC Technical Service Consultants, Lancashire, UK). Boot socks were used to sample facility floors. Liquid run-off from open waste drains was also collected, where available, in 60 mL sterile containers. Cloacal swabs were taken from poultry using Amies transport swabs (Sterilin Ltd., Newport, UK). At least 25 g of intestinal samples from cattle and goats were taken post mortem at slaughter. Carcasses of cattle and goats were swabbed at both the rump and the shoulder using cotton tipped swabs moistened with Maximum Recovery Diluent (MRD) (Oxoid) and dry cotton tipped swabs, following the New Zealand Ministry for Primary Industries (MPI) National Microbiological Database (NMD) programme protocol [20]. Metal carcass swab templates (100 cm² for cattle and 25 cm² for goats) were sterilized with 70% ethanol wipes and allowed to air dry between swabbings. Swab heads were snapped off into empty sterile 30 mL universal tubes (Greiner Bio-One Ltd., Gloucester, UK) for transport. Cattle and goat meat was obtained from meat sellers in Arusha Urban, Moshi Municipal, and Moshi Rural Districts whose meat was supplied by study slaughter facilities. Approximately 500 g of the lowest
hanging section of cattle and goat meat on display was purchased and placed in a re-sealable plastic bag. All samples were transported in a cooler box with freezer packs to Kilimanjaro Clinical Research Institute (KCRI) Biotechnology Laboratory in Moshi for testing on the day of sampling.

Equal numbers of human bloodstream and diarrheal disease NTS isolates were sought from Kibera, Nairobi, and Lwak Mission Hospital, western Kenya, Kenya. NTS were sequentially isolated from 2007 through 2017 from an on-going population-based infectious disease surveillance system operated by Kenya Medical Research Institute (KEMRI) in collaboration with the US Centers for Disease Control and Prevention (CDC) [21]. Isolates were identified as described previously [22], frozen and shipped to the Biotechnology Laboratory, KCRI, in tryptone soya broth (TSB) with 20% glycerol, and stored at -80°C.

**Isolation, identification, enumeration, and antimicrobial susceptibility testing of *Salmonella* from the meat pathway**

Isolation and identification of *Salmonella* was performed from meat pathway swabs, from 1g intestinal samples and 25g meat, as described previously [19] to yield 1-5 presumptive *Salmonella* isolates identified per sample. Enumeration of *Salmonella* was performed on the day of sampling using a spiral plater (Wasp, Don Whitley, West Yorkshire, UK) for cloacal swabs, intestinal contents, meat samples, environmental samples, or direct manual spread plate for carcass swabs. Xylose Lysine Deoxycholate agar (Oxoid) with 5 μg/mL novobiocin (Merck KGaA, Darmstadt, Germany) plates were inoculated, in duplicate, with 50 μl of freshly prepared homogenate. Plates were incubated overnight at 37±2°C. Typical *Salmonella* colonies were counted manually, biochemically confirmed [19], and the number of *Salmonella* in the original sample calculated. Routine phenotypic antimicrobial susceptibility testing was performed against amoxicillin/clavulanate, ampicillin,
ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, naladixic acid, and trimethoprim/sulfamethoxazole on environmental, poultry and livestock isolates by disk diffusion and interpreted to contemporary guidelines [23].

Molecular confirmation and DNA preparation of Salmonella isolates

Salmonella isolates from livestock and poultry, and their environments, from northern Tanzania, and from human blood and stool from Kenya were shipped to EpiLab, Hopkirk Research Institute, Massey University, Palmerston North, New Zealand. Following subculture, DNA was extracted from each isolate using the QiaAmp DNA minikit (Qiagen, Hilden, Germany). Salmonella isolates were confirmed by PCR targeting the Salmonella enterotoxin (stn) gene [24]. Libraries were prepared using an Illumina Nextera XT library preparation kit (Illumina, San Diego, CA, USA) following the manufacturer’s instructions and submitted to New Zealand Genomics Limited, University of Otago, Dunedin, New Zealand, for whole genome sequencing using a HiSeq 2 x 125-bp PE v4 instrument (Illumina).

Data analysis

Illumina read data were cleaned using Trimmomatic version 0.38 [25]. Draft genomes were assembled using SPAdes version 3.11 [26]. Processed reads are publicly available on the National Center for Biotechnology Information Sequence Read Archive under BioProject ID PRJNA602741. Metadata are stored under BioSample accession nos. SAMN13905911-SAMN13906457 (Supplementary Table 1). Resistome and virulome profiles were assessed using ABRicate (https://github.com/tseemann/abricate) to query the ResFinder and the Virulence Factors (VFDB) databases [27, 28]. Annotation of antimicrobial resistance genes and resistance mechanisms was performed using the Resistance Gene Identifier algorithm web portal (RGI 5.0.0) and the
comprehensive antibiotic resistance database (CARD 3.0.2) [29]. We employed criteria of ‘high quality/coverage,’ ‘perfect and strict’ hits only, and excluded ‘nudging of ≥95% identity loose hits to strict.’ Assembly statistics were compiled using seqkit [30] as part of the Nullarbor pipeline [31]. Seven gene ST was identified using multi-locus sequence typing (MLST) (https://github.com/tseemann/mlst) [32]. Core genome MLST (cgMLST) types and serovar information was predicted using SISTR [33]. Allelic profiles were clustered using globally optimal eBURST (goeBURST) [34] in PHYLOViZ [35]. A distance threshold (T), expressed as the number of allelic differences for which isolates form the same cluster, was applied and used to generate goeBURST clusters at all possible similarity thresholds. The Neighbourhood Adjusted Wallace Coefficient (nAWC) [36], which examines the congruence of partitions between adjacent similarity thresholds (T), was used for cluster definition (https://github.com/theInnuendoProject/nAWC), was calculated to assess cluster grouping dynamics. We identified cgMLST clusters reflecting basic units in overall Salmonella population structure, defined as the earliest point at which five or more consecutive thresholds yielded nAWC values >0.99. To visualize Salmonella population structure, we generated a minimum spanning tree (MST) using R packages ‘igraph’ [37], ‘MLSTar’ [38], ‘RCColorBrewer’ [39], ‘gplots’ [40], and ‘ape’ [41]. A tree displaying the relationship between ST type, resistome, and genotype was generated using the Interactive Tree of Life [42]. The circular dendrogram was generated by calculating a distance matrix based on the pairwise number of core genome allele differences between isolates, and clustering using Ward’s method [43]. ST diversity was estimated for each source by calculating the Simpson (1-D) and Shannon indices and plotting rarefaction curves. Both indices compare the diversity allowing for sample size, but the Shannon index places more emphasis on the richness or number of different lineages than the evenness or how evenly distributed the different lineages are. The diversity indices with bootstrapped confidence intervals were calculated using the R package ‘vegetarian’ version 1.2 [44] and the rarefaction curves were plotted using the R package ‘vegan’ version 2.5-3 [45]. Human and meat
pathway Salmonella Enteritidis strains were compared with previously described African and global lineages by cgMLST [11].

Research ethics

This study was approved by the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee, the Kenya Medical Research Institute Scientific and Ethics Review Unit, the University of Otago Human Ethics Committee, and the University of Glasgow College of Medical, Veterinary, and Life Sciences Ethics Committee.

RESULTS

Salmonella from poultry and red meat pathways, and humans

Of 164 meat pathway samples yielding Salmonella, 33 (20.1%) were from poultry farms, 32 (19.5%) from ruminant slaughter or butcher environments, 62 (37.8%) from cattle or their meat, and 37 (22.6%) from goats or their meat. Detailed information on specific sources and locations and enumeration among positive samples is shown in Table 1. The 164 meat pathway samples yielded 367 NTS isolates. Of 172 Salmonella isolates selected from humans, 90 (52.3%) were from the bloodstream and 82 (47.7%) were from the stool of patients with diarrhea.
Salmonella sequence types, serovars, and diversity

Of 539 NTS isolates, 91 (16.9%) were Salmonella Typhimurium and 78 (14.5%) were Salmonella Enteritidis (Supplementary Table 2). Of 72 allelic profiles identified among all isolates, 17 (23.6%) were of previously undescribed STs. The predominant STs were Salmonella Enteritidis ST11 (n=78) and Salmonella Typhimurium ST313 (n=62) (Table 2, Supplementary Table 2). Of the eight sample types, Salmonella Enteritidis ST11 was found in seven, being absent only from the slaughter and butcher environment, whereas Salmonella Typhimurium ST313 was found only from human stool and blood. Salmonella Orion ST639 was found in four non-human sources.

A comparison of the diversity of 7-gene MLSTs between different sample types, using rarefaction curves and the Simpson and Shannon indices, showed the highest diversity was associated with isolates from cattle and goat meat followed by isolates from the red meat slaughter and butcher environment (Supplementary Table 3, Supplementary Figure 1). In contrast the lowest diversity was associated with isolates from human blood. Blood isolates were significantly less diverse than the population isolated from human stool (Supplementary Table 3).

Core genome multi-locus sequence types

cgMLST analyses using nAWC resulted in a cut-off of 38 allelic differences and 157 separate clusters, ranging in size from one to 65 isolates. The Salmonella population structure and cgMLST sequence types is shown in Figure 2. Similar to 7-gene MLST findings, sources of cgMLST clusters exhibited contrasting cluster-related patterns. Some cgMLSTs were found in all sample types, while others were restricted to human samples or non-human samples only. Within Salmonella STs 11, 16, 19, 27, and 1208 we identified cgMLST clusters that included both human and meat pathway isolates. When compared with previously described clades [11], Salmonella Enteritidis ST11 cgMLST clusters of
similar human and meat pathway isolates belonged to the so-called global epidemic clade, rather than to Africa-restricted clades.

**Antimicrobial resistance and resistome**

Antimicrobial susceptibility results and resistome profiles for 49 resistance genes from nine antimicrobial classes are shown in Supplementary Table 4. Phenotypic antimicrobial susceptibility results of human isolates are reported elsewhere [46]. Of 539 isolates, resistance genes to aminoglycosides were found in 133 (24.7%), beta-lactams in 105 (19.5%), chloramphenicol in 136 (25.2%), trimethoprim in 97 (18.0%), sulphonamides in 131 (24.3%), and tetracycline in 102 (18.9%). Resistance genes to fosfomycin, macrolides, and quinolones were present in ≤11 isolates. Human blood isolates had the widest range of resistance genes, with 69 (84.1%) of 82 isolates having genes for five or more resistance classes (Supplementary Figure 2). *Salmonella* bearing genes for resistance to two or more antimicrobial classes were found in 14 (35.9%) of 39 poultry cloacae, 13 (38.2%) of 34 poultry farm environment, 29 (32.2%) of 90 human stool samples, and <10% of other sample sources (Supplementary Figure 2, Supplementary Figure 3, Supplementary Figure 4). The median (interquartile range) number of resistance gene classes found in isolates from human, poultry, and ruminant samples was 5 (0-6), 0 (0-3), and 0 (0-0), respectively.

**Virulome**

Virulome profiles of the 539 isolates tested identified 153 virulence genes in total, of which 26 (17.0%) were considered major [28], including those associated with adherence (*sinH, ratB, pef, ipf, genes associated with Type 1 fimbriae, shdA and misL*); magnesium uptake (*mgtBC*); resistance to an antimicrobial peptide produced by macrophages (*mig-14*)[47]; serum resistance (*rck*); an oxidative stress defense protein (*sodCl*), and other proteins (*spv* and *cdtB*). The median (interquartile range) of
major virulence genes found in isolates derived from human, poultry, and ruminant samples were 26 (19-26), 18 (17-18) and 13 (12-17), respectively (Supplementary Figure 5).

**DISCUSSION**

We demonstrated that NTS from East Africa, while relatively uncommon in cattle, goat, and poultry intestinal samples, were highly prevalent in the slaughter and butcher environment and in cattle and goat meat. *Salmonella* Enteritidis ST11 and *Salmonella* Typhimurium ST313 predominated among isolates from persons with bloodstream infection and diarrhea. By cgMLST, *Salmonella* Enteritidis ST11 from humans belonging to the so-called global epidemic clade [11] and other less common *Salmonella* serovars and sequence types clustered closely with strains isolated from the cattle, goat, and poultry meat pathway. However, *Salmonella* Typhimurium ST313 was not isolated from any meat pathway sample. Taken together, our findings suggest that the meat pathway may be an important source of human *Salmonella* Enteritidis ST11 infection in East Africa, but not of human *Salmonella* Typhimurium ST313 infection.

As in other regions, we confirm that in East Africa *Salmonella enterica* serovars Enteritidis and Typhimurium are leading causes of NTS invasive and diarrheal disease in humans [1]. Consistent with studies from high-income countries [48, 49], our cgMLST data suggests that both poultry and red meat may be sources of human *Salmonella* Enteritidis ST11 infections in East Africa. Notably, *Salmonella* Enteritidis ST11 that were highly similar between meat pathway and human disease isolates belong to the so-called global epidemic clade rather than to Africa-restricted clades [11], leaving open questions about sources of Africa-restricted *Salmonella* Enteritidis ST11 clades. While we found *Salmonella* Typhimurium in all components of the meat pathway tested, no *Salmonella*
Typhimurium ST313 were isolated from the meat pathway. While relatively few studies from African countries have examined *Salmonella* Typhimurium sequence types in non-human sources [50, 51], the existence of a non-human reservoir for *Salmonella* Typhimurium ST313, if any, remains to be established.

NTS was isolated throughout the meat pathway in northern Tanzania, including on a small proportion of caracasses and >10% of retail cattle and goat meat. While the prevalence of NTS was low in livestock intestinal samples and poultry cloacal samples, it was higher in poultry, livestock slaughter, and butcher environments. These findings point to the importance of cooking meat well prior to consumption, and suggest that contamination of meat during slaughter and butchering from a range of sources may contribute substantially to contamination by *Salmonella* of retail meat in this setting.

Our study has a number of limitations. First, our meat pathway research and human disease surveillance were not co-located, and human disease isolates were collected over a period that extended beyond the period of meat pathway data collection. While movement of livestock, food, and people is common in East Africa, location and time differences may have reduced our ability to attribute human infections to meat pathway sources. Second, our research was limited to cattle and goat meat pathways, and the poultry component was restricted to live chickens and their environments. Although cattle, goats, and poultry are the major sources of meat in Tanzania, we cannot exclude roles for pigs, sheep, and other species to the epidemiology of human NTS infections. Finally, rarefaction curves did not plateau suggesting that more diversity likely exists that was not sampled by the study.
In conclusion, we demonstrate that non-typhoidal Salmonella is common in the meat pathway in Tanzania, especially in slaughter and butcher environments, and as a contaminant of retail meat. Multi-locus sequence typing (MLST) cluster analyses suggest that the meat pathway likely contributes to both human bloodstream infections and diarrheal disease due to Salmonella Enteritidis ST11 and to other less common Salmonella serovars and sequence types, including Salmonella Typhimurium other than ST313. However, we did not find evidence of a contribution to Salmonella Typhimurium ST313 infections. In addition to studies in humans, more research is needed to systematically examine the contribution of other types of meat, animal products, produce, water, and environmental exposures in the epidemiology of NTS disease in East Africa.
NOTES

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Potential conflicts of interest

N.F. receives financial support as Director of New Zealand Food Safety Science and Research Centre, outside the submitted work. All other No reported conflicts.
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Table 1. Numbers of samples positive for *Salmonella* and enumeration from poultry cloaca, poultry farm environment, cattle and goat intestinal, carcass, meat, and slaughter and butcher environment samples in Arusha Urban, Moshi Municipal, and Moshi Rural Districts, 2015-17

| Sample Type                              | Arusha Urban | Moshi Municipal | Moshi Rural | Salmonella enumeration, log CFU/sample | TOTAL |
|------------------------------------------|--------------|-----------------|-------------|----------------------------------------|-------|
|                                          | n/ n (%)     | n/ n (%)        | n/ n (%)    | Median (range)                         | n/ n  |
| Poultry farm environment                 | 5/ 40 (12.5)| 9/ 40 (22.5)    | -           | 3.6 (3.0-5.1)                         | 14/ 80 (17.5) |
| Poultry cloaca                           | 8/ 393 (2.0)| 11/ 402 (2.7)   | -           | 3.7 (2.3-4.2)                         | 19/ 795 (2.4) |
| Slaughter and butcher environment        | 15/ 108 (13.9)| 10/ 71 (14.1) | 7/ 48 (14.6)| 3.7 (3.0-5.1)                         | 32/ 227 (14.1) |
| Cattle intestinal                        | 1/ 114 (0.9)| 2/ 93 (2.2)     | 0/ 128 (0.0)| 2.3 (2.3-2.3)                         | 3/ 335 (0.9) |
| Goat intestinal                          | 0/ 139 (0.0)| 6/ 84 (7.1)     | 1/ 10 (10.0)| 2.9 (2.6-3.3)                         | 7/ 233 (3.0) |
| Cattle carcass                           | 4/ 105 (3.8)| 0/ 50 (0.0)     | 1/ 119 (0.8)| 0* (0-0)                              | 5/ 274 (1.8) |
| Goat carcass                             | 0/ 134 (0.0)| 6/ 40 (15.0)    | 0/ 12 (0.0)| 0* (0-0)                              | 6/ 186 (3.2) |
| Cattle meat                              | 19/ 180 (10.5)| 21/ 140 (15.0)| 14/ 143 (9.8)| 3.4 (3.4-5.4)                         | 54/ 463 (11.7) |
| Goat meat                                | 13/ 118 (11.0)| 10/ 76 (13.2)| 1/ 11 (9.1)| 3.9 (3.1-4.3)                         | 24/ 205 (11.7) |
| TOTAL                                    | 65/ 1,331 (4.9)| 75/ 996 (7.5)| 24/ 471 (5.1)| -                                    | 164/ 2,798 (5.8) |

CFU, colony forming units; *Below level of enumeration in all samples; -, not part of the study.
Table 2. *Salmonella* sequence types and serovars by sample source for types with at least 15 isolates, East Africa, 2007-17*

| Salmonella sequence type | Salmonella serovar | Sample type | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | n (%) | TOTAL |
|--------------------------|--------------------|-------------|-------|-------|-------|-------|-------|-------|-------|--------|
|                          |                    | Poultry farm environment |       |       |       |       |       |       |       |        |
|                          |                    | Poultry cloaca environment |       |       |       |       |       |       |       |        |
|                          |                    | Slaughter and butcher environment |       |       |       |       |       |       |       |        |
|                          |                    | Cattle and goat intestinal samples |       |       |       |       |       |       |       |        |
|                          |                    | Cattle and goat carcass |       |       |       |       |       |       |       |        |
|                          |                    | Cattle and goat meat |       |       |       |       |       |       |       |        |
|                          |                    | Human feces |       |       |       |       |       |       |       |        |
|                          |                    | Human blood |       |       |       |       |       |       |       |        |
|                          |                    | TOTAL |       |       |       |       |       |       |       |        |
| 11                       | Enteritidis         | 6 (26.1) | 9 (27.3) | 0 (0) | 4 (28.6) | 2 (18.2) | 4 (4.9) | 21 (35.6) | 32 (39.5) | 78     |
| 313                      | Typhimurium         | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 17 (28.8) | 45 (55.6) | 62     |
| 639                      | Orion               | 0 (0) | 0 (0) | 7 (14.6) | 2 (14.3) | 2 (18.2) | 22 (26.8) | 0 (0) | 0 (0) | 33     |
| 1208                     | 42·r:-              | 0 (0) | 0 (0) | 5 (10.4) | 0 (0) | 4 (36.4) | 18 (22) | 1 (1.7) | 0 (0) | 28     |
| 19                       | Typhimurium         | 1 (4.3) | 4 (12.1) | 1 (2.1) | 3 (21.4) | 1 (9.1) | 3 (3.7) | 9 (15.3) | 2 (2.5) | 24     |
| 27                       | Saintpaul           | 2 (8.7) | 0 (0) | 2 (4.2) | 0 (0) | 2 (18.2) | 11 (13.4) | 1 (1.7) | 0 (0) | 18     |
| 16                       | Virchow             | 3 (13) | 3 (9.1) | 0 (0) | 0 (0) | 0 (0) | 7 (8.5) | 1 (1.7) | 1 (1.2) | 15     |
| 166                      | Newport             | 5 (21.7) | 6 (18.2) | 1 (2.1) | 0 (0) | 0 (0) | 2 (2.4) | 0 (0) | 0 (0) | 14     |
| 22                       | Braenderup          | 0 (0) | 0 (0) | 14 (29.2) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 14     |
| 15                       | Heidelberg           | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 4 (4.9) | 9 (15.3) | 1 (1.2) | 14     |
| 912                      | Karamoja            | 0 (0) | 0 (0) | 7 (14.6) | 2 (14.3) | 0 (0) | 5 (6.1) | 0 (0) | 0 (0) | 14     |
|      | Kentucky |    |     |    |     |     |     |     |     |     |     |     |     |
|------|----------|----|-----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|      |          | 2  | (8.7)| 7  | (21.2)| 0   | (0) | 0   | (0) | 4   | (4.9)| 0   | (0) | 0   | (0) | 13   |
| 198  |          |    |      |    |      |    |     |    |     |    |     |    |     |    |     |
| 2533 | Durban   |    |      |    |      |    |     |    |     |    |     |    |     |    |     |
|      |          | 4  | (17.4)| 4  | (12.1)| 0   | (0) | 3   | (21.4)| 0   | (0) | 2   | (2.4)| 0   | (0) | 0   | (0) | 13   |
|      |          |    |      |    |      |    |     |    |     |    |     |    |     |    |     |
|      | Unknown  |    |      |    |      |    |     |    |     |    |     |    |     |    |     |
|      |          | 0  | (0) | 0  | (0) | 11  | (22.9)| 0   | (0) | 0   | (0) | 0   | (0) | 0   | (0) | 11   |
|      |          |    |      |    |      |    |     |    |     |    |     |    |     |    |     |
|      | TOTAL    | 23 | (100.0)| 33 | (100.0)| 48  | (100.0)| 14  | (100.0)| 11  | (100.0)| 82  | (100.0)| 59  | (100.0)| 81  | (100.0)| 351 |

*Details of other sequence types and serovars available in Supplementary Table 2*
FIGURE LEGENDS

Figure 1. Map showing data collection sites in Kenya, 2007-17 (panel A) and slaughter slab, butcher, and poultry farm locations sampled for nontyphoidal Salmonella, Arusha and Kilimanjaro Regions, northern Tanzania, 2015-17 (panel B)

A.

B.

Figure 2. Minimum spanning tree based on Salmonella core genome multi-locus sequence type profiles overlaid with common 7-gene multilocus sequence type clusters, East Africa, 2007-17

Human isolates are presented as squares, isolates from non-human sources as circles. Shading represents 7-gene multilocus sequence type clusters. Node color differentiates cgMLST groupings, determined using globally optimal eBURST (goeBURST) [34] in PHYLOViZ [35].
