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Salmonella species in piglets and weaners from Uganda: Prevalence, antimicrobial resistance and herd-level risk factors

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ABSTRACT

Non-typhoidal salmonellosis is of concern in humans in sub-Saharan Africa, and this is partly due to the high number of immunocompromised persons. Pork and pork products could be among the sources of these non-typhi *Salmonella* spp. The aim of this study was to identify *Salmonella* spp. in piglets and weaners in northern and eastern Uganda, characterize their antimicrobial resistance patterns and determine herd-level risk factors. Fecal samples were collected from 465 piglets and weaners from 93 herds (49 and 44 from northern and eastern Uganda, respectively). In addition, information about the herd management and potential risk factors were collected. The fecal samples were cultured for the identification of *Salmonella* spp. The *Salmonella* spp. confirmed by serotyping were further characterized by determination of minimum inhibitory concentration (MIC) to 12 antimicrobials by broth microdilution. At individual level, the total prevalence of *Salmonella* spp. was 12% (12.2% in northern and 11.9% in eastern Uganda). At herd level, the total prevalence was 39% (43% in northern and 34% in eastern Uganda). From 56 samples with *Salmonella* spp., 20 serovars were identified including two serovars identified only by their antigenic formulae. The predominant serovars were *S.* Zanzibar, *S.* Heidelberg, *S.* Infantis, *S.* Typhimurium, *S.* Stanleyville, *S.* Aberdeen and *S.* Kampala. In total, 57% of the 53 *Salmonella* spp. analyzed, originating from 27% of the herds, were resistant to at least one antimicrobial agent. The majority of drug-resistant isolates (60%) were from northern Uganda. Eight multidrug-resistant (MDR) isolates were from northern Uganda and three MDR isolates were from eastern Uganda. Increased prevalence of *Salmonella* spp. was associated with feeding the young and adults separately as compared to feeding the young and adults together ($p = 0.043$, OR = 4.3; 95% CI 1.1, 17.38). Protective factors were "intensive" method of keeping the pigs *versus* "tethering and roaming" ($p = 0.016$, OR = 0.11; 95% CI 0.02, 0.64), "intensive" method *versus* "semi-intensive" method ($p = 0.048$, OR = 0.12; 95% CI 0.01, 0.96)

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and cleaning feeders after every two days *versus* daily ($p = 0.017$, OR = 0.18; 95% CI 0.05, 0.72). This study has revealed a high prevalence of infection of piglets and weaners with diverse non-typhi *Salmonella* serovars and highlights the potential role of pork and pork products as sources of these organisms for humans. In addition, this study has identified protective factors that could be promoted to control *Salmonella* spp. and in antimicrobial resistance reduction programs in rural pigs from Uganda.

1. Introduction

The greatest risk of *Salmonella* infections is their zoonotic nature (Wegener and Baggesen, 1996; Bonalli et al., 2012). All non-typhi *Salmonella* spp. (over 2500 serovars) are considered as human pathogens (WHO, 2005). Non-typhoidal salmonellosis (NTS) is one of the most common food-borne zoonoses in the world (Gomez et al., 1997). The NTS is more common among children, the elderly and the immunocompromised persons (Shaw et al., 2008). In Uganda, with the advent of the Human Immune Deficiency Virus (HIV) infections, many people are now highly susceptible to clinical and life-threatening NTS with increased prevalence in persons with very low $CD4^+$ cell counts (Gilks, 1998).

In pigs, clinical salmonellosis is considered uncommon (Kranker et al., 2003) and only a few serovars namely *Salmonella enterica* subspecies *enterica* serovar Cholerasuis (*S.* Cholerasuis), *S.* Typhimurium, *S.* Enteritidis and *S.* Derby have been implicated in clinical disease (Fedorka-Cray et al., 2000). In piglets and growing pigs, *Salmonella* infections may cause enterocolitis, septicemia and death. However, subclinical infections are common (Lo Fo Wong et al., 2002; Aragaw et al., 2007; Vigo et al., 2009) and therefore, pork and pork products are considered to be among the major sources of NTS for humans world over (EFSA, 2008).

Invasive NTS in humans is treated by the use of antimicrobials. In Uganda, the most commonly used drugs are chloramphenicol, ciprofloxacin and nalidixic acid (Kalule et al., 2012). Lately, cases of drug-resistant non-typhi *Salmonella* spp. have been reported in a number of countries in Africa (Kariuki et al., 2006). With increasing and rampant use and misuse of antibiotics in developing countries (Sirinavin and Dowell, 2004; Byarugaba, 2004), this situation is bound to worsen. One of the possible ways to ameliorate this situation is to prevent contamination of pork and pork products through control of *Salmonella* infections right from the farm level to fork by identifying possible risk factors. However, no studies have been done to identify possible risk factors for *Salmonella* infections in Ugandan village pigs, which may be targeted in a control program to reduce the prevalence of infection and antimicrobial resistance. The aim of this study was to (1) determine the prevalence and identify serovars of *Salmonella* spp. in piglets and weaners from two districts in northern and eastern Uganda, (2) determine the prevalence of *Salmonella* spp. at herd level from two districts in northern and eastern Uganda, (3) characterize antimicrobial resistance of the isolated *Salmonella* spp. and (4) establish any epidemiological association between management practices and the herd *Salmonella* status.

2. Materials and methods

2.1. Study area and design

This study was carried out during 2011 and 2012 in Gulu and Soroti districts, located in northern and eastern Uganda, respectively. The location of Gulu district is between longitude 30°21′ east to longitude 32° east and latitude 2° north to latitude 4° north. The location of Soroti district is between longitude 30°01′ east and longitude 34°18′ east and latitude 1°33′ north and latitude 2°23′ north. These two districts were purposively selected because of their large pig populations as compared with the neighboring districts. The study households selected were keeping pigs including piglets and/or recently weaned pigs, *i.e.* at most 2 weeks after weaning. Data were collected from 6 sub-counties and Gulu municipality in Gulu district, and 4 sub-counties and Soroti municipality in Soroti district. The study involved collection of fecal samples for bacteriological analysis and administration of a questionnaire to collect data on the pig management practices and health to identify potential risk factors. The questionnaire also captured information on the demographics of the household heads.

2.2. Identification of households and administration of the questionnaire

There was a lack of information on the households keeping pigs with piglets or weaners and therefore, households were identified by the snowballing method to redundancy (Kagira et al., 2010; Pondja et al., 2010). Briefly, the first household was identified with the help of the district animal husbandry officers and the local area council chairpersons. The research team visited the first household to fill in the questionnaire that contained questions on the demographics of the household head and pig ownership, management, health and marketing. With the help of the previous pig farmers, the subsequent households were then identified and questionnaires were filled in. With permission from the household heads, the questionnaires were filled in by personal interviews to household members who commonly took care of the pigs. The questionnaire was written in English and the questions and answers were at each visit communicated between the research team and the persons from the local communities in the local languages. The local languages used in this study were Luo in northern Uganda and Ateso and Kumam

in eastern Uganda. Before data collection, the questionnaire was pretested by selected veterinary officials and pig farmers in the study area and thereafter reviewed by the research team.

2.3. Definition of methods of management

In this study, a pig herd was considered "roaming" when the pigs of all ages were let loose and allowed to move freely from place to place. The pigs were considered "tethered" when the adults and weaners were tied with ropes to pegs but the piglets were let loose. The pigs were considered to be under an "intensive" system of management when they were housed and therefore, prevented from escaping to the outside. Lastly, the pigs were considered to be under "semi-intensive" system when they were housed, but also allowed to move within an enclosed space without a roof. However, during the administration of the questionnaire, it was found that the two categories, "tethering" and "roaming" were not possible to conclusively separate and therefore, these two were merged into one category called "tethering and roaming" in the analysis.

2.4. Collection of fecal samples

Fecal samples were collected from all piglets or weaners when the litter size at the time of sampling was 1–5 piglets or weaners. If the litter size exceeded 5 piglets or weaners, fecal samples were collected from 5 piglets or weaners per sow selected at random. In a litter with diarrhea and having more than 5 piglets or weaners, fecal samples were collected from all diarrheic piglets or weaners and from 5 non-diarrheic piglets or weaners selected at random. Therefore, each sampled pig was scored as being diarrheic or not. Individual fecal samples were collected from the rectum using sterile swabs (Heinz Herenz, Hamburg, Germany) and immediately placed into 5 mL of sterile and chilled Stuart transport medium (Oxoid, Basingstoke, England) in bijour bottles. The fecal samples were then transported on ice in a cool box to the laboratory at the College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, within 24 h of sampling for bacteriological culture and isolation.

2.5. Bacteriological culture, isolation and confirmation

The bacteriological cultivation was performed in accordance with standard procedures (ISO, 2002). Briefly, each fecal swab was separately cultured in 9 mL of pre-enrichment medium (2% w/v buffered peptone water, Mast group Ltd, Merseyside, UK) at 37 °C for 18 h. Thereafter, 0.1 mL of the turbid pre-enrichment medium was transferred to 9.9 mL of Rapparport Vassiliadis (RV) enrichment broth (Oxoid, Basingstoke, England) for enrichment at 42 °C for 24 h. Subsequently 0.1 mL of the turbid RV broth were inoculated onto Xylose Lysine Desoxycholate (XLD) agar (Mast group Ltd, Merseyside, UK) and incubated at 37 °C for 24 h. Three black colonies/isolates with a red periphery typical for *Salmonella* spp. were subcultured and biotyped using triple sugar iron agar (Mast group Ltd, Merseyside, UK) for sugar fermentation and H_2S production, tryptophan broth (Sigma, USA) for indole production and urea agar (Mast group Ltd, Merseyside, UK) for urease production. Suspected *Salmonella* colonies (glucose fermenter, non-lactose fermenter, H_2S producer, indole and urease negative) were further biotyped using API® 20E kit (Biomerieux, France) following the manufacturer's instructions. The *Salmonella* spp. biochemically confirmed were then serotyped (one isolate per sample) at the Swedish *Salmonella* Reference Laboratory, National Veterinary Institute (NVI) according to the Kauffmann–White scheme (Grimont and Weill, 2007).

A pig herd was considered *Salmonella*-positive when at least one fecal sample from the litter (s) tested positive for *Salmonella* spp.

2.6. Analysis for antimicrobial susceptibility

The *Salmonella* isolates serotyped were tested for antimicrobial susceptibility. This was done by determination of minimum inhibitory concentration (MIC) of 12 antimicrobials (ampicillin, ciprofloxacin, nalidixic acid, gentamicin, tetracycline, sulfamethoxazole, trimethoprim, chloramphenicol, kanamycin, streptomycin, cefotaxime and ceftazidime) by broth microdilution according to the protocol from Clinical and Laboratory Standards Institute (CLSI, 2008) using VetMIC™ GN-mo (version 4) test kits (NVI, Sweden). The MIC analysis was performed at Makerere University in accordance with the instructions from the manufacturer (NVI). *Escherichia coli* ATCC® 25922™ (USA) was used as quality control strain. The results were interpreted following the guidelines provided by CLSI (2012) and NARMS (2010).

2.7. Data analysis for risk factors

Data from the questionnaires and bacteriological analysis were first coded and entered into SPSS version 17 (SPSS Inc., Chicago, USA). The data were checked for any errors that may have occurred during entry. Errors were corrected by re-checking against the original questionnaires and laboratory result sheets. The data were imported into the SAS program 9.3 (SAS Institute, USA), described using summary statistics and analyzed using Chi-square, Fisher's exact test and logistic regression.

In the statistical analyses, the status of the herd was the dependent variable. All independent variables were cross-tabulated against the herd-level outcome (*Salmonella* spp. isolated or not) at univariable analysis using Chi-square or Fisher's exact test when the requirements for Chi-square test were not met. All variables with a p-value of ≤ 0.25 from univariable analyses and having ≥ 5 counts in each cell were offered as candidate variables to the multivariable analysis for model fitting. Collinearity among variables was evaluated by cross-tabulation of candidate variables using Fisher's exact test. Two variables were considered collinear when cross-tabulated, a p-value of ≤ 0.05 was obtained. Selection among the collinear variables for multivariable analysis was based on biological plausibility. Logistic regression was performed using SAS GLIMMIX procedure. From the selected variables, three models were fitted to the data using the logit function and the

parameters estimated by maximum likelihood. The fitness of the models was assessed using Akaike Information Criterion (AIC) and the ratio of Pearson Chi-square (deviance) to the degrees of freedom (DF). Only the best fitted model was reported and taken to be significant if the p-value was ≤ 0.05.

3. Results

3.1. Number of samples collected and prevalence of Salmonella infection

A total of 93 households were visited (49 from Gulu and 44 from Soroti districts), and overall, 465 fecal samples (271 and 194 from Gulu and Soroti districts, respectively) were collected and analyzed. Overall, the number of samples collected per household ranged from 1 to 12 with a mode and average of 5 and varied depending on the size of the litter, number of the litters and diarrhea in the litter. Of the 465 samples, 32 were from diarrheic piglets and weaners.

At individual pig level, the prevalence of *Salmonella* spp. was 12.2% ($n = 271$) in Gulu and 11.9% ($n = 194$) in Soroti with a total prevalence of 12% ($n = 465$). At the herd level, the prevalence of *Salmonella* spp. was 43% ($n = 49$) in Gulu and 34% ($n = 44$) in Soroti with a total herd prevalence of 39% ($n = 93$). Eighty-four percent (84%, $n = 56$) of the pigs that tested positive for *Salmonella* spp. were non-diarrheic. However using Fisher's exact test, there was a significant association between being *Salmonella* culture positive and having diarrhea ($p = 0.008$).

3.2. Salmonella serovars isolated

In total 56 *Salmonella* spp. were isolated from 56 piglets and weaners and following analysis, 20 different serovars were identified. Of all the 36 herds that tested positive for *Salmonella* spp., multiple serovars were isolated from 4 (11%) of the herds. Table 1 shows the 7 predominant serovars identified in this study and their distribution by district. The other 13 serovars identified included *S.* Kenya, *S.* Virchow, *S.* Lodz, *S.* Leatherhead, *S.* Bolton, *S.* Bukavu, *S.* Loenga, *S.* Bofflens, *S.* Oslo, *S.* Loeben, *S.* Kingabwa, *S. enterica* subspecies enterica (I) Antigens = 4, 5:a:– and *S. enterica* subspecies enterica (I) Antigens = 4, 27:–:z6.

Table 1
Distribution of the 7 predominant *Salmonella* serovars, in the two major pig producing districts of Gulu and Soroti in northern and eastern Uganda, respectively.

Salmonella serovar isolated	No. of samples from Gulu	No. of samples from Soroti
S. Zanzibar	3	6
S. Heidelberg	3	4
S. Infantis	3	3
S. Typhimurium	5	0
S. Kampala	0	4
S. Stanleyville	5	0
S. Aberdeen	4	1

3.3. Antimicrobial susceptibility of Salmonella spp.

Only 53 of the 56 *Salmonella* isolates were available for susceptibility testing. The susceptibility of these isolates to 12 antimicrobials tested is shown in Table 2. Out of the 53 isolates, 19 (36%) were resistant to one of the drugs, 8 isolates (15%) were resistant to two of the drugs and 3 isolates (5.7%) were resistant to at least 3 and at most 5 of the drugs (Table 3). Of the 30 isolates resistant to at least one drug, the majority (60%) were from Gulu, northern Uganda. Overall, the majority (23/30) were resistant to sulfamethoxazole, 8 isolates to streptomycin, 7 isolates to trimethoprim, 3 isolates to chloramphenicol, 2 isolates to ampicillin, 2 isolates to tetracycline and 1 isolate to kanamycin. None of the isolates was resistant to cefotaxime, ceftazidime, gentamicin, ciprofloxacin or nalidixic acid (Tables 2 and 3).

Multidrug resistance (MDR), defined as resistance to or ability of the bacterium to grow in the presence of two or more antimicrobials that would normally kill it or limit its growth (Brichta-Harhay et al., 2011), was recorded in isolates from 7 serovars (Table 3). Seven of these MDR isolates were from "tethering and roaming" and 4 MDR isolates

Table 2
Antimicrobial susceptibility pattern of 53 *Salmonella* isolates categorized as "susceptible", "intermediate" and "resistant" to 12 antimicrobials used against a variety of infections in humans.

Drug	No. of susceptible isolates (MIC value)[c]	No. of intermediate isolates (MIC value)[c]	No. of resistant isolates (MIC value)[c]
Ampicillin	48 (≤8)	3 (16)	2 (≥32)
Cefotaxime	53 (≤1)	0 (2)	0 (≥4)
Ceftazidime	53 (≤4)	0 (8)	0 (≥16)
Gentamicin	53 (≤4)	0 (8)	0 (≥16)
Kanamycin	52 (≤16)	0 (32)	1 (≥64)
Tetracycline	51 (≤4)	0 (8)	2 (≥16)
Ciprofloxacin	53 (≤1)	0 (2)	0 (≥4)
Nalidixic acid	53 (≤16)	–[a]	0 (≥32)
Sulfamethoxazole	30 (≤256)	–[a]	23 (≥512)
Trimethoprim	46 (≤8)	–[a]	7 (≥16)
Chloramphenicol	50 (≤8)	0 (16)	3 (≥32)
Streptomycin	45 (≤32)[b]	–[a]	8 (≥64)[b]

[a] No MIC interpretive standard values (CLSI, 2012; NARMS, 2010).
[b] The MIC interpretive standard values (NARMS, 2010).
[c] In brackets- MIC interpretive standard values in μg/mL (CLSI, 2012; NARMS, 2010).

Table 3
Distribution by district of the *Salmonella* spp. resistant to at least two of the 12 antimicrobials used against a variety of infections in humans.

Name of isolate	Drugs resistant to	No. of isolates	District
S. Stanleyville[b,c]	Su, Cm, Tc	1	Gulu
S. Heidelberga[b]	Su, Tm	1	Gulu
S. Infantis	Su, Tm	2	Gulu
S. Aberdeen	Su, Tm, Sm	1	Gulu
S. Zanzibar	Su, Tm	1	Gulu
S. Zanzibar	Su, Am	1	Soroti
S. Typhimurium[a]	Su, Tm	1	Gulu
S. Typhimurium[a]	Su, Tm, Am, Tc, Sm	1	Gulu
S. Heidelberg	Su, Sm	1	Soroti
S. Leatherhead[c]	Su, Sm	1	Soroti

Su – Sulfamethoxazole, Tm – Trimethoprim, Am – Ampicillin, Cm – Chloramphenicol, Tc – Tetracycline, Sm – Streptomycin.
[a] or [b] *Salmonella* isolates that originated from piglets in the same herd.
[c] *Salmonella* isolates that originated from diarrheic piglets or weaners.

were from "semi-intensive" system. Similarly, 15 non-MDR isolates were from "tethering and roaming", 3 non-MDR isolates were from "semi-intensive" and 1 isolate was from "intensive" system of management.

3.4. Herd-level risk factors for Salmonella infection in piglets and weaners

Data from the two districts were combined for the analysis of risk factors since the management of the pig herds was similar in the two districts. From the univariable analysis, 11 variables with $p \leq 0.25$ were identified as possible risk factors. However, many of the variables were collinear (Table 4) and only 4 of them were included in the best fitted model (multivariable analysis).

The best fitted multivariable model with the lowest AIC value (120.01), deviance/DF value = 1.02 and judged as significant ($p = 0.011$, Table 5) included the following variables: feeding the adults and the piglets together or not, management method, diarrhea seen or not seen in some of the piglets/weaners in a herd during sampling and the intensity of cleaning of the feeders. Apart from diarrhea seen or not, the other 3 variables had p-values ≤ 0.05, as assessed using the Type III sums of squares test and therefore, considered significantly associated with *Salmonella* status.

From the goodness of fit statistics, clustering due to over-dispersion was not considered as a problem even if more than one sample was tested in each herd, since the deviance/DF value was close to 1. Therefore, the model fitted well to the data. This model identified feeding the adults and the piglets separately *versus* feeding together ($p = 0.043$, Odds Ratio (OR) = 4.3; 95% Confidence Interval (CI) 1.1, 17.4) as a risk factor for increased prevalence of *Salmonella* spp. However, a number of protective factors were identified and included "intensive" method of rearing *versus* "tethering and roaming" ($p = 0.016$, OR = 0.11; 95% CI 0.02, 0.64), "intensive" method *versus* "semi-intensive" ($p = 0.048$, OR = 0.12; 95% CI 0.01, 0.96) and cleaning feeders after every two days *versus* daily ($p = 0.017$, OR = 0.18; 95% CI 0.05, 0.72).

4. Discussion

This study has revealed valuable information on the occurrence of *Salmonella* infections in village pigs in Uganda. The *Salmonella* fecal prevalence of 12% found in this study is comparable to the 8.6% prevalence reported from slaughter pigs in Kenya (Kikuvi et al., 2010), but lower than the 21.8% prevalence in slaughter pigs from Ethiopia (Molla et al., 2006). It is possible, that these results could have been affected by the methods employed in the selection of the herds, but we believe that by sampling to redundancy, it did not have a large impact. Interestingly, unlike in the previous studies in Kenya and Ethiopia (Molla et al., 2006; Kikuvi et al., 2010), the current study has shown a high diversity of *Salmonella* serovars (20 serovars, from 56 isolates). The reasons for this may be difficult to analyze, given the limited information on *Salmonella* spp. in animals from Uganda. However, this high diversity may suggest a complex flow of *Salmonella* spp. in the study area. This may include the transmission between domestic and wild animals, and humans. The average sample size per litter in this study was considered high taken into consideration the average litter size of 8 piglets or 5 weaners recorded in the households visited. In addition, the average sample size was also higher than the number of samples (3 piglets) used in another study (Funk et al., 2001). This was to increase the sensitivity for isolating *Salmonella* spp. in a herd since fecal swab samples used in this study have previously been reported to have low relative sensitivity (Funk et al., 2000).

Among the predominant serovars, *S.* Typhimurium and *S.* Stanleyville were only isolated from northern Uganda and *S.* Kampala from eastern Uganda. Although there is no previous information on the distribution of *Salmonella* serovars in these regions, this result may suggest a difference in predominant serovars in the different regions. Also, the predominant serovars in this study were different from all or some of those reported predominant in pigs from Kenya (*S.* Saintpaul and *S.* Heidelberg), Ethiopia (*S.* Hadar, *S.* Kentucky, *S.* Anatum and *S.* Blockley), South Africa (*S.* Typhimurium, *S.* Muenchen, *S.* Derby and *S.* Choleraesuis), in Europe (*S.* Typhimurium and *S.* Rissen), in the USA (*S.* Agona, *S.* Derby, *S.* Schwarzengrund, *S.* Typhimurium and *S.* Senftenberg) and Thailand (*S.* Rissen, *S.* Typhimurium, *S.* Stanley, and *S.* Weltevreden) (Bahnson et al., 2006; Molla et al., 2006; Dorn-In et al., 2009; Kikuvi et al., 2010; Kidanemariam et al., 2010; Vico et al., 2011). Geographical differences in the distribution of the predominant *Salmonella* serovars have also been reported in other countries (Davison et al., 2003). These differences strengthen the argument that there may be regional differences in common reservoirs and/or risk factors of infection. Most of the *Salmonella* spp. were from non-diarrheic piglets and weaners, an indication that these infections may have been subclinical at the time of sampling.

A majority of the MDR *Salmonella* spp. were from northern Uganda. Since antimicrobial use is a risk factor for increased drug resistance (McGarock, 2002; Byarugaba, 2004), this result may suggest a high antimicrobial use or misuse in northern as compared to eastern Uganda. The six MDR patterns found in this study were; sulfamethoxazole-trimethoprim or sulfamethoxazole-ampicillin or

Table 4
The percentage of the herds that tested positive for *Salmonella* spp., in relation to the 11 factors having a *p*-value of ≤0.25 at univariable analysis using Chi-square test.

Factor	Level	No. of herds	Percent *Salmonella* positive
Level of education of house hold head[a,c]	≤Primary	54	40.7
	Secondary	21	47.6
	Tertiary	18	22.2
Management method[c]	Tethering and roaming	67	43.3
	Semi-intensive	15	45.5
	Intensive	11	13.3
Cleaning feeders[c]	Daily	41	43.9
	After every 2 days	21	19
	≤2 times a week	31	45.2
Feeding the adults and the piglets together	Yes	78	35.9
	No	15	53.3
Cleaning the pig house[a,c]	Daily	11	27.3
	≤3 times a week	17[d]	23.5
	Do not use a house	65	44.6
Housing neonates/piglets[a,b]	Yes	21	19
	No	59	42.4
Sow emaciated[b]	Yes	35	28.6
	No	45	42.2
Receive professional vet care[a]	Yes	36	30.6
	No	57	43.9
Treat whenever pigs are sick[a,e]	Yes	37	32.4
	No	54	44.4
Type of boar used[a,c]	Own not shared	21	23.8
	Own, shared	23	60.9
	Others	49	34.7
Diarrhea observed in some of the piglets or weaners in the herd	Yes	20	50
	No	73	35.6

[a] The variable dropped from the best fitted multivariable model due to collinearity with management method, feeding the young and adults together and/or cleaning feeders.
[b] The variable dropped from the best fitted multivariable model due to missing values from herds with only weaners.
[c] The variable collapsed before univariable analysis due to very low response to one level or not possible to conclusively separate the levels.
[d] Two households occasionally housed pigs only at night but tethered most of the time and were categorized under "tethering".
[e] None-response from two households.

sulfamethoxazole-streptomycin or sulfamethoxazole-trimethoprim-ampicillin-tetracycline-streptomycin or sulfamethoxazole-chloramphenicol-tetracycline or sulfamethoxazole-trimethoprim-streptomycin. According to the National Drug Authority (NDA) Uganda (2013), apart from chloramphenicol and sulfamethoxazole, the other drugs (trimethoprim, ampicillin, streptomycin and tetracycline) are also veterinary-licensed drugs and are

Table 5
The best fitted model for the multivariable analysis of *Salmonella* spp. in piglets and weaners included 4 variables *i.e.* feeding the adults and the piglets together or not, management method, diarrhea seen or not seen during sampling and the intensity of cleaning the feeders.

Variable		Estimate	S.E.	*p*	OR	95% CI
Group feeding						
No	Yes	1.46	0.71	0.043	4.31	1.1, 17.38
Diarrhea						
No	Yes	−1.12	0.59	0.062	0.33	0.1, 1.04
Management method						
Intensive	Tethering and roaming	−2.19	0.88	0.016	0.11	0.02, 0.64
Intensive	Semi-intensive	−2.17	1.08	0.048	0.12	0.01, 0.96
Tethering and roaming	Semi-intensive	0.03	0.76	0.971	1.03	0.23, 4.55
Cleaning feeders						
≤2× week	After every two days	1.4	0.72	0.053	4.07	1.00, 16.54
≤2× week	Daily	−0.3	0.54	0.575	0.74	0.26, 2.13
After every two days	Daily	−1.71	0.7	0.017	0.18	0.05, 0.72

Fit statistics: deviance/DF = 1.02; model: $p = 0.011$.

commonly used. This may explain the occurrence of multidrug resistance against these drugs. The diversity of the *Salmonella* spp., the MDR patterns found and the management system where drug resistance was mostly detected, suggest that the *Salmonella* spp. are circulating not only from pigs to humans, but also from humans to pigs possibly through contact with human feces, because of the poor sanitation and unhygienic conditions that may be common in these areas (UDHS, 2011). We therefore hypothesize that humans are one of the main reservoirs of *Salmonella* spp. in these areas and further studies are warranted to unravel the routes of transmission. Resistance to chloramphenicol and sulfamethoxazole may also be an indicator of illegal use/misuse of the drugs in animals.

Most of the drug-resistant *Salmonella* spp. (77%) were resistant to sulfamethoxazole and 23% to trimethoprim. The level of resistance to these drugs is worrisome since cotrimoxazole (trimethoprim-sulfamethoxazole combination) is the drug commonly used to control opportunistic infections in HIV positive persons in Uganda (Campbell et al., 2012). We recommend for a wider study that includes bacterial isolates from different regions and species including humans in order to assess the overall effectiveness of these two drugs in the country. All the *Salmonella* isolates in this study were susceptible to gentamicin, ciprofloxacin, nalidixic acid, cefotaxime and ceftazidime. These results are similar to the results from previous studies in Uganda (Kalule et al., 2012) and Kenya (Kikuvi et al., 2010). Apart from gentamicin, the other four antimicrobials (ciprofloxacin, nalidixic acid, cefotaxime and ceftazidime) are only licensed for the use in humans in Uganda (NDA, 2013). In addition, some of these drugs are very expensive and therefore not commonly used. This may be a possible reason for the high susceptibility of the pig-derived isolates of *Salmonella* to these drugs in the present study. However, resistance to fluoroquinolones and third generation cephalosporins in *Salmonella* spp. from pigs and other food animals is reported in monitoring programs in North America and Europe (USDA, 2010; CIPARS, 2011; EFSA and ECDC, 2013), and also from South-East Asia (Van et al., 2012). Although still mostly uncommon, occurrence of these types of resistance seems to be increasing in some *Salmonella* serovars in some animal species in these regions. There is therefore need for continuous monitoring, restrictions and judicious use of these critically important antimicrobials to ensure the future availability of effective antimicrobial drugs for use in human medicine.

In this study, one factor associated with increased prevalence of *Salmonella* spp. was feeding the piglets and the adults separately. This finding may be difficult to explain, although it may suggest that *Salmonella* infections in rural pigs from Uganda are acquired through feeds. Although the importance of contaminated feeds in the epidemiology of *Salmonella* in pigs is contentious (Funk and Gebreyes, 2004), the Quantitative Microbiological Risk Assessment by EFSA reported that by feeding only *Salmonella*-free feeds, slaughter pig prevalence reductions of 10%–20% and 60%–70% in high and low prevalence EU member states respectively, could be expected (EFSA, 2010). In addition, studies in Europe have reported that feed physical and chemical composition and structure are associated with pig *Salmonella* prevalence (Funk and Gebreyes, 2004). This indicates the possible importance of contaminated feeds or different feed types given to pigs in the epidemiology of *Salmonella*. However in this study, the questionnaire did not include necessary information that could have been used to explain this finding.

In addition, "intensive" keeping of pigs as compared to "tethering and roaming" or "semi-intensive", and washing the feeders after every second day instead of daily were significantly associated with low *Salmonella* prevalence. Compared to "tethering and roaming" pigs, "intensively" kept pigs do not directly interact with the open environment and other animals that can be a source of infection and this might be an explanation to the lower infection rate in the "intensive" units. This argument is supported by previous findings (Cardinale et al., 2010; Gotter et al., 2012) that reported contact with other animals as a risk factor for *Salmonella* infections in pigs. From our observations during data collection, all "intensive" units had concrete floors whereas "semi-intensive" units had a larger part or the whole of the floor being mud. This probably meant that the "intensive" units were easy to clean which could have reduced the level of contamination. Moreover farmers who washed feeders after every two days possibly did it adequately, hence reducing the prevalence of *Salmonella* spp. in such units.

In conclusion, piglets and weaners in the study area were highly infected with non-typhi *Salmonella* spp. suggesting that pork and pork products could be a source of these bacteria for humans and the high diversity of *Salmonella* serovars suggests the presence of many reservoirs. Therefore we recommend a comprehensive study to include other possible carriers/reservoirs of these *Salmonella* serovars, including the possible re-cycling of the infection from humans to pigs. Although pork is generally consumed roasted or cooked in Uganda, there is possible spread of *Salmonella* during slaughter in rural areas due to poor hygiene and lack of sensitization on possible contamination of pork, persons and other materials. It is therefore, important to carry out studies on possible transmission of *Salmonella* in slaughter places. Antimicrobial resistance in these *Salmonella* spp. against some of the common drugs used in humans suggests drug misuse or circulation of the bacteria between humans, the environment and pigs. There is therefore a need for more elaborate studies of antimicrobial susceptibility of *Salmonella* spp. along the pig value chain so as to come up with policies to combat this problem. Lastly, this study has revealed possible protective factors such as "intensive" piggery that could be promoted in *Salmonella* and antimicrobial resistance reduction programs in rural pigs in Uganda.

Conflict of interest

There is no conflict of interest from any of the authors of this article.

Role of funders

The funders did not play any other role in the study, including in the writing of the manuscript or in its submission for publication.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.prevetmed.2014.03.009.

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