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

Kokas Ikwap a,*, Joseph Erume a, David Okello Owiny a, George William Nasinyama a, Lennart Melin c, Björn Bengtsson c, Nils Lundeheim b, Claes Fellström b, Magdalena Jacobson b

a College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, P.O. Box 7062, Kampala, Uganda
b Faculty of Veterinary Medicine and Animal Science, Swedish University of Agricultural Sciences, P.O. Box 7070, SE-750 07 Uppsala, Sweden
c National Veterinary Institute, 751 89 Uppsala, Sweden

A R T I C L E   I N F O
Article history:
Received 16 December 2013
Received in revised form 1 March 2014
Accepted 10 March 2014

Keywords:
Diversity
Non-typhi
Protective factors
Drug susceptibility

A B S T R A C T
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. Kampaign. 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)
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 (Kranzer 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 question-
naire 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
be under an “intensive” system of management when
they were housed and therefore, prevented from escap-
ting 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 with
out a roof. However, during the administration of the question-
naire, 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 wean-
ers 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 wean-
ers 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, Bas-
ingstoke, 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 accord-
ance 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. There-
after, 0.1 mL of the turbid pre-enrichment medium was
transferred to 9.9 mL of Rappaport Vassiliadis (RV) enrich-
ment 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 incu-
bated at 37°C for 24 h. Three black colonies/isolates with
a red periphery typical for Salmonella spp. were sub-
cultured and biotyped using triple sugar iron agar (Mast
group Ltd, Merseyside, UK) for sugar fermentation and H2S
production, tryptophan broth (Sigma, USA) for indole pro-
duction and urea agar (Mast group Ltd, Merseyside, UK) for
urease production. Suspected Salmonella colonies (glucose
fermenter, non-lactose fermenter, H2S 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 Vet-
eryinary 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 determi-
nation of minimum inhibitory concentration (MIC) of
12 antimicrobials (ampicillin, ciprofloxacin, nalidixic acid,
genamycin, 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 Mak-
erere 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 analy-
sis 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 lab-
oratory 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 mul-
tivariable 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. Boeffens, S. Oslo, S. Loeben, S. Kingabwa, S. enterica subspecies enterica (I) Antigens = 4, 5; a:– and S. enterica subspecies enterica (I) Antigens = 4, 27;:z:z6.

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, cefazidime, 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 (Brichita-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 1**

<table>
<thead>
<tr>
<th>Salmonella serovar isolated</th>
<th>No. of samples from Gulu</th>
<th>No. of samples from Soroti</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. Zanzibar</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>S. Heidelberg</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>S. Infantis</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>S. Typhimurium</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>S. Kampala</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>S. Stanleyville</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>S. Aberdeen</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

**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.

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of susceptible isolates (MIC value)</th>
<th>No. of intermediate isolates (MIC value)</th>
<th>No. of resistant isolates (MIC value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>48 (≤8)</td>
<td>3 (16)</td>
<td>2 (≥32)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>53 (≤1)</td>
<td>0 (2)</td>
<td>0 (≥4)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>53 (≤4)</td>
<td>0 (8)</td>
<td>0 (≥16)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>53 (≤4)</td>
<td>0 (8)</td>
<td>0 (≥16)</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>52 (≤16)</td>
<td>0 (32)</td>
<td>1 (≥64)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>51 (≤4)</td>
<td>0 (8)</td>
<td>2 (≥16)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>53 (≤1)</td>
<td>0 (2)</td>
<td>0 (≥4)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>53 (≤16)</td>
<td>–</td>
<td>0 (≥32)</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>30 (≤≥256)</td>
<td>–</td>
<td>23 (≥512)</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>46 (≤8)</td>
<td>–</td>
<td>7 (≥16)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>50 (≤8)</td>
<td>0 (16)</td>
<td>3 (≥32)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>45 (≤≥32)</td>
<td>–</td>
<td>8 (≥64)</td>
</tr>
</tbody>
</table>

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).
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 \( (p = 0.011, \text{ Table 5}) \) 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 \((p = 0.043, \text{ Odds Ratio (OR)} = 4.3; 95\% \text{ Confidence Interval (CI)} 
1.1, 17.4)\) 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” \((p = 0.016, \text{ OR} = 0.11; 95\% \text{ CI} 0.02, 0.64)\), “intensive” method versus “semi-intensive” \((p = 0.048, \text{ OR} = 0.12; 95\% \text{ CI} 0.01, 0.96)\) and cleaning feeders after every two days versus daily \((p = 0.017, \text{ OR} = 0.18; 95\% \text{ CI} 0.05, 0.72)\).

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. Choleraeus), 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.

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

*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.*

*The variable collapsed before univariable analysis due to very low response to one level or not possible to conclusively separate the levels.*

*Two households occasionally housed pigs only at night but tethered most of the time and were categorized under “tethering”.*

*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.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Estimate</th>
<th>S.E.</th>
<th><em>p</em></th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group feeding</td>
<td>Yes</td>
<td>1.46</td>
<td>0.71</td>
<td>0.043</td>
<td>4.31</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>No</td>
<td>−1.12</td>
<td>0.59</td>
<td>0.062</td>
<td>0.33</td>
</tr>
<tr>
<td>Management method</td>
<td>Yes</td>
<td>−2.19</td>
<td>0.88</td>
<td>0.016</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td>Intensive</td>
<td>Semi-intensive</td>
<td>−2.17</td>
<td>1.08</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td>Tethering and roaming</td>
<td>Semi-intensive</td>
<td>0.03</td>
<td>0.76</td>
<td>0.971</td>
</tr>
<tr>
<td>Cleaning feeders</td>
<td>≤2× week After every two days</td>
<td>1.4</td>
<td>0.72</td>
<td>0.053</td>
<td>4.07</td>
</tr>
<tr>
<td></td>
<td>≤2× week Daily</td>
<td>−0.3</td>
<td>0.54</td>
<td>0.575</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>After every two days Daily</td>
<td>−1.71</td>
<td>0.7</td>
<td>0.017</td>
<td>0.18</td>
</tr>
</tbody>
</table>

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 (Kikweto 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 viruses 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 (Cardinael 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.

Acknowledgements

This study was funded in part by the Swedish International Development Cooperation Agency (Sida) (75007369), Carnegie Corporation of New York (BR741.R01) and Makerere University (2010/HD17/18118U). We acknowledge the assistance provided by the District Veterinary Officers, the field staff, the farmers in Gulu and Soroti districts and Pauline Kibui for processing of samples and isolation of bacteria.

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.

References


