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# The importance of on-farm biosecurity: Sero-prevalence and risk factors of bacterial and viral pathogens in smallholder pig systems in Uganda

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ABSTRACT

*Background:* The productivity of pigs in smallholder systems is affected by high disease burden, most of which might not be obvious, with their epidemiology and impact being poorly understood. This study estimated the seroprevalence and identified the risk factors of a range of bacterial and viral pathogens of potential economic and public health importance in domestic pigs in Uganda. A total of 522 clinically healthy pigs were randomly selected from 276 pig farms in Masaka (142) and Lira (134) districts of Uganda in 2015.

*Results:* Overall the highest animal prevalence was found for *Streptococcus suis* 73.0% (CI95: 67.0–78.3) in Lira and 68.2% (CI95: 62.7–73.4) in Masaka; followed by *Porcine circovirus* type 2 with 50.8% (CI95: 44.5–57.2) in Lira and 40.7% (CI95: 35.2–46.5) in Masaka and *Actinobacillus pleuro-pneumoniae*, 25.6% (CI95: 20.4–31.6) in Lira and 20.5% (CI95: 16.2–25.6) in Masaka. Mycoplasma *hyopneumonia* prevalence was 20.9% (CI95: 16.2–26.6) in Lira and 10.1% (CI95: 7.1–14.1) in Masaka, while *Porcine parvovirus* was 6.2% (CI95: 4.0–9.7) in Masaka and 3.4% (CI95: 1.7–6.6) in Lira. Less common pathogens were *Influenza A*, 8.5% (CI95: 5.6–12.8) in Lira and 2.0% (CI95: 0.9–4.5) in Masaka and *Porcine Reproductive and Respiratory Syndrome Virus*, 1.7% (CI95: 0.7–4.3) in Lira and 1.3% (CI95: 0.5–3.5) in Masaka. Even less common was Rotavirus A with 0.8% (CI95: 0.2–3.0) in Lira and 0.7% (CI95: 0.2–2.5) in Masaka; the same was for Aujeszky virus with 0.4% (CI95: 0.7–2.4) in Lira and 0.0% (CI95: 0.0-0.1) in Masaka. Co-infection with two pathogens was common and there was a significant association of *M. hyo* and PCV2 co-occurrence ($p = 0.016$). Multivariate analysis showed that for *S. suis* the use of disinfectant reduced odds of sero-positivitey (OR = 0.15; $p = 0.017$) and pigs less than 6 months were more likely to be infected than older pigs (OR = 3.35; $p = 0.047$). For *M. hyo*, crossbred pigs had higher odd of infection compared to local breeds (OR = 1.59; $p < 0.001$).

*Conclusions:* The studied pathogens have high prevalences in smallholder pig production systems and might be silent killers, thus affecting productivity and there is a possibility that some pathogens could spread to humans. Given the limited knowledge of veterinary workers and the poor diagnostic capacities and capabilities in these systems, the diseases are potentially usually under-diagnosed. These findings constitute baseline data to measure the impact of future interventions aiming to reduce disease burden in the pig production systems in Uganda.

## 1. Introduction

Pig production in Uganda is stifled by several constraints. Besides limited feed availability and poor access to markets, high disease burden has been identified as a key challenge (Ouma et al., 2015; Dione et al., 2014). From a study carried out in 2013, where farmers' perceptions on occurrence of pig diseases was assessed, the most known and reported pig diseases by farmers and animal health workers were African swine fever (ASF) and parasite infections. Farmers also listed clinical signs that they often observed in their pigs; however those signs

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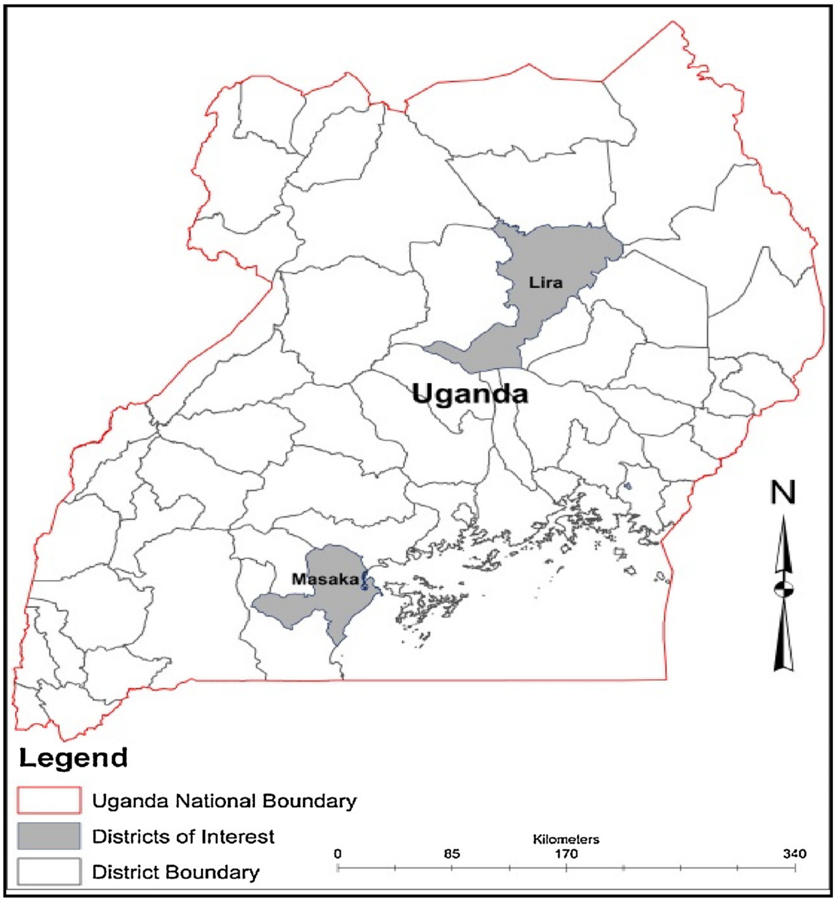


**Fig. 1.** Map of Uganda with study sites highlighted.

could not be associated with any specific disease because of lack of accurate diagnosis. These clinical signs included abortion, pneumonia, cough, sudden death, nervous signs and stunted growth (Dione et al., 2014). From the diseases they knew, ASF was the most feared because of its high fatality rate which translates in severe lowering of the income of farmers relying on piggery. Gastrointestinal parasite infections have also been reported to occur in most pig herds. Recent studies reported that 60% of pig farms in major pig producing districts such as Masaka, Mukono and Kamuli tested positive for one or more gastrointestinal helminths, namely Strongyles, *Metastrongylus spp*., *Ascaris suum*, *Strongyloides ransomi*, and *Trichuris suis.* Coccidia oocysts were found in 40.7% of all pigs sampled. Gastrointestinal helminthes can cause high morbidity leading to pig body weight losses and poor growth, consequently lowering the market value of the pigs. However, farms that routinely removed manure and litter from their pens and used disinfectants had less gastro-intestinal helminthes burdens on their farms (Roesel et al., 2017).

Most research in pig diseases in Uganda has focused on ASF and parasites, thereby leaving the investigation of other diseases patchy. Farmers often cite "swine fever" to refer to ASF, but it is unknown how much of the "swine fever" may be due to ASF virus (ASFV), or some other diseases causing fever given that accurate diagnosis is a challenge to the animal health workers because of lack of both technical and logistical capacities (Dione et al., 2014). There are many other pathogens, both bacterial and viral causing diseases that affect pig productivity.

Among the bacterial causes is *Mycoplasma hyopneumonia* (*M. hyo.*), the primary agent of enzootic pneumonia, which causes a typical chronic disease in pig herds with low mortality but high morbidity (Thacker et al., 2001). This disease causes economic losses in swine production, through retarded growth, poor feed conversion and predisposition to bacterial pulmonary infections(Thacker et al., 2001). Indeed pigs that are infected with both *M. hyo*. and Swine *influenza* (SIV) agents usually exhibit more severe clinical signs (Thacker et al., 2001). *Actinobacillus Pleuropneumonia* (APP), is a primary respiratory pathogen and one of the leading causes of bacterial pneumonia in pigs globally leading to retarded growth rate, with the severity of the clinical signs caused depending on the serotype involved (Marsteller and Fenwick, 1999). APP can infect pigs of all ages, but clinical signs are mainly seen in 12–16weeks-old pigs (Chiers et al., 2002). *Strepococcus suis* (S. *suis*) is a significant pathogen of swine and one of the most important causes of bacterial mortality in piglets after weaning; it also has a high public health importance because human infections can result in septicemia, meningitis, permanent hearing loss, endocarditis, and arthritis (Dee, 2014).

Among the viruses, *Porcine parvovirus* (PPV) and *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV), are the most common cause of porcine reproductive failure (Keffaber, 1989; Mengeling, 1978; Mengeling et al., 2000a). Another important virus is *Porcine circovirus* type 2 (PCV2) which has been associated with enteric, respiratory and reproductive disorders (Segales, 2012), but most importantly it causes Post-weaning Multi-systemic Wasting Syndrome (PMWS). SIV, an acute, highly contagious respiratory disease caused by type A influenza virus is another viral condition that hinders pig productivity (Sreta et al., 2009).

The national pig diseases surveillance programs focus predominantly on ASF, leaving out other diseases. Therefore the true burden and impact of pig diseases in Uganda which is cited above needs to be urgently investigated. In the current situation, the diseases are likely underdiagnosed or mis-diagnosed despite them contributing significantly to productivity losses encountered by farmers and thus directly affecting livelihoods. A better understanding of the epidemiology of the pathogens that are commonly found in smallholder pig

**Table 1**
Demographic characteristics of pig farmers (n = 313).

| Demographic characteristics | Masaka N (%) | Lira N (%) |
|---|---|---|
| Sex of the household head | | |
| Male | 121 (76.1) | 126 (81.8) |
| Female | 38 (23.9) | 28 (18.2) |
| Sex of the respondent | | |
| Male | 57 (35.9) | 78 (50.6) |
| Female | 102 (64.1) | 76 (49.4) |
| Age of respondent (years) | | |
| Mean (SD) | 44(14) | 40(13.5) |
| Min-Max | 18-80 | 15-75 |
| Marital status of respondent | | |
| Married (monogamous) | 107 (67.3) | 120 (77.9) |
| Married (polygamous) | 7 (4.4) | 6 (3.9) |
| Widow/widower | 17 (10.7) | 20 (13.0) |
| Divorced/separated | 5 (3.1) | 1 (0.6) |
| Single/unmarried | 18 (11.3) | 6 (3.9) |
| Other | 5 (3.2) | 1 (0.6) |
| Highest education level | | |
| Never went to school | 5 (3.1) | 25 (16.2) |
| Non-formal education | 1 (0.6) | 0 (0.0) |
| Primary education | 84 (52.8) | 79 (51.3) |
| Secondary education/high school | 46 (28.9) | 35 (22.7) |
| Tertiary/vocational training | 16 (10.1) | 9 (5.8) |
| University | 6 (3.8) | 6 (3.9) |
| Adult literacy | 1 (0.6) | 0 (0.0) |
| Main source of income | | |
| Crop farming | 79 (49.7) | 101 (65.6) |
| Pig keeping | 18 (11.3) | 23 (14.9) |
| Salaried employed | 12 (7.5) | 7 (4.5) |
| Self-employed on-farm | 29 (18.2) | 11 (7.1) |
| Others[a] | 21 (13.2) | 12 (7.8) |

[a] Casual labourer, charcoal burning, boar keeping, cattle keeping, motorcycle rider.

farms will help identify potential areas for further investigation and guide the prioritization of interventions. Consequently, the main objective of this study was to assess the level of occurrence of selected bacterial and viral pathogens which are most likely to affect productivity of pigs under the current production system, and describe the co-infections patterns and risk factors.

## 2. Material and methods

### 2.1. Site selection

The study was carried out in Masaka and Lira districts of Uganda representing two different pig production systems. Masaka is located in the central region and has the highest pig population density in the country (> 50 heads/km$^2$) (UBOS, M.A., 2009), while Lira is located in the northern part of the country with a lower pig population density than Masaka (Fig. 1). These districts are part of the "Smallholder Pig Value Chains Development Project (SPVCD) in Uganda". A detailed selection process of the districts is described elsewhere (Dione et al., 2016). In each district, villages with high pig population density were identified through a census. The top 16 villages were then selected and enrolled in the study, making 32 villages in total.

### 2.2. Sample size

The sample size was calculated considering an infinite population (no recent census data) using the following formula: $n = [Z^2P(1-P)]/d^2$ where: n is the required sample size; Z is the multiplier from a standard normal distribution (1.96) at a probability level of 0.05; P is the estimated prevalence which is most conservatively estimated to be 50% considering that there is no reliable prevalence of the diseases studied, and d is the desired precision for the estimate (+/-5%). A sample size of 384 pigs was required for the study in each district. After correction for intra-cluster correlation due to clustering at village level with a coefficient of 0.2, the adjusted sample size was estimated at 461 for both districts. However, whenever possible, more pigs were sampled to increase study power. In each village 10 pig keeping households were randomly selected for the study. However, 276 farms were reached during the sample collection exercise given their availability, the consent of the farmers and logistics.

### 2.3. Pathogen selection

Pathogen selection took into account farmers' perceptions on clinical signs observed (Dione et al., 2014), and support by a literature review on the most common diseases that are associated with the reported symptoms in pigs. For diseases associated with reproductive failure PPV (Mengeling, 1978; Mengeling et al., 2000b), PRRS (Keffaber, 1989; Mengeling et al., 2000b), and PCV2(Segales, 2012) were considered as important; for pneumonia, SIV (Dee, 2014; Sreta et al., 2009) and APP (Marsteller and Fenwick, 1999); for cough and retarded growth rate, *M. hyo* (Dee, 2014) and PCV2; for sudden death ADV (Wittmann, 1986); for diarrhoea especially in piglets, Rotavirus A (Bohl et al., 1978); and neurological signs, *S. suis* for (Dee, 2014).

### 2.4. Data collection

Eighty per cent of the households keeping pigs were typical smallholder type with 1 to 3 sows for the piglet production units or 1 to 4 growers for pig fattening units. In each household, an average of two pigs, which represents around 50% of the pigs of an average household, were randomly selected for whole blood and serum collection. Pigs less than three months old and pregnant sows were excluded from the study because young pigs may carry antibodies against these pathogens and for animal welfare reasons respectively.

Bio-data was collected from individual pigs in relation to age, sex and breed. In addition, a semi-structured questionnaire was administered to all pig keepers to collect information on pig management system, husbandry and biosecurity practices. The information included age, sex and level of education of the farmer, pig feeding and housing, biosecurity, disease management, and farmer's access to animal health services (Table 1).

### 2.5. Laboratory analysis

Serological analyses were carried out at the Molecular Biology Laboratory in the College of Natural Sciences, Department of Zoology, Entomology and Fisheries Sciences of Makerere University in Uganda, using commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kits. Each serum sample was tested for all pathogens following the kit manufacturer guidelines. The ELISA kits were sourced from IDEXX Laboratories, Maine, USA (for ADV, SIV, PRRS, APP and *M. hyo*), KRISHGEN BioSystems, Ashley Ct, Whittier, CA 90603, USA (for PPV, PCV2 and *S. suis*).

### 2.6. Data management and statistical analysis

Household questionnaires were completed on paper in the field and entered into CSPro5.0 software database for storage and subsequent analysis. All data was imported into Microsoft Excel and statistical analysis was performed using Stata 14 (StataCorp LP, College Station, TX, USA). Prevalence of each pathogen and 95% confidence intervals were calculated. For each positive pathogen, we evaluated the proportion of co-infection with another pathogen. In addition, we assessed the proportion of accumulative co-infection patterns among seven pathogens by each district.

For the risk factor analysis, univariable logistic regression was applied to each pathogen with identified variables (month, sex, breed, deworming, spraying, vitamins, antibiotics and disinfectants). Variables that were significant in the univariable logistic regression analysis were included in multivariable logistic regression analysis with farm as a random effect. Variables with p-values < 0.05 were used for statistical significance in the model. Odds ratio (OR) and 95% confidence interval (CI) were calculated by exponentiation of the coefficients from the regression model. ArcGIS 10.3.1 ArcMap (ESRI, Redlands, CA, USA) was used to spatially visualize results on a map.

## 3. Results

### 3.1. Demographic characteristics of pig farmers

While in both districts there were more male headed than female headed households, 81.8% in Lira and 76.1% in Masaka, the majority of respondents were female in Masaka (64.1%) while Lira had equal proportions. The mean age of respondent was 44 and 40 years in Masaka and Lira respectively. The majority of farmers were married and monogamous (67.3% in Masaka and 77.9% in Lira), with the most common highest education level being primary school (52.8% in Masaka and 51.3% in Lira). The main income source for the households was crop farming. Pig rearing was the main income source for only 11.3% of farmers in Masaka and 14.9% of farmers in Lira (Table 1).

### 3.2. Pig husbandry practices and biosecurity

In Masaka, the majority of pigs (70%) were confined in pens throughout the year (Fig. 2); while in Lira, a high number of farmers practiced free-range pig rearing especially during the dry season (24.0% during dry season/day and 30.5% during dry season/night) (Fig. 3). The pig feeding patterns were the same in both districts, with crops residues being the main source of feed throughout the year. Feeding pigs on unprocessed swill was common in both districts. The use of disinfectant was low (Masaka, 8.8%; Lira, 21.4%), and the use of footbath on farms was very rare, with only 4 farmers in Masaka and 2 farmers in Lira reporting usage. The most common health management practice was routine deworming of the pigs. Lira farmers used more antibiotics (72.1%) than those of Masaka (24.5%); while administration of multivitamins was more common in Masaka (73.6%) than in Lira (27.7%). In both districts the main source of breeding was the communal village boar service. Also the majority of farmers did not use extension services (Masaka, 62.9%; Lira, 75.3%) (Table 2).

### 3.3. Animal level prevalence

A total of 287 samples were collected in Masaka District from 142 pig farms, and 235 samples were collected in Lira District from 134 pig farms. The pathogen with the highest prevalence in aninals was *S. suis* (73.0 in Lira and 68.2% in Masaka), followed by PCV2 (50.8% in Lira and 40.7% in Masaka); APP (25.6% in Lira and 20.5% in Masaka) and *M. hyo*. (20.9% in Lira and 10.1% in Masaka). Lower prevalence were recorded for PPV (6.2% in Masaka and 3.4% in Lira), SIV (8.5% in Lira and 2.0% in Masaka) and PRRSV (1.7% in Lira and 1.3% in Masaka). The lowest prevalences were recorded with Rotavirus A and ADV (Table 3).

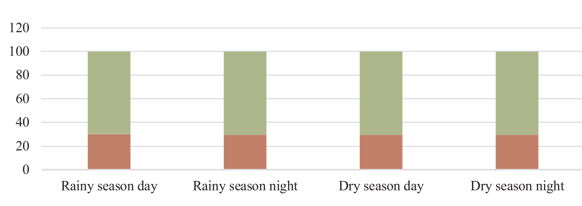


**Fig. 2.** Proportion (%) of pig keeping systems in Masaka District.

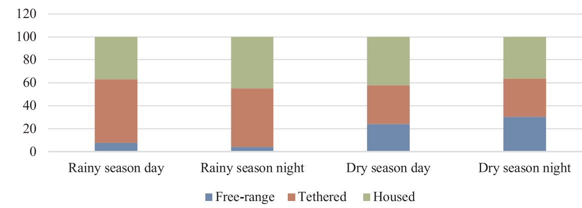


**Fig. 3.** Proportion (%) of pig keeping system in Lira District.

**Table 2**
Husbandry and biosecurity practices.

| | Masaka N (%) | Lira N (%) |
|---|---|---|
| Transportation of pigs | | |
| Truck | 29 (18.2) | 13 (8.4) |
| Motorcycle | 31 (19.5) | 48 (31.2) |
| Bicycle | 6 (3.8) | 56 (36.4) |
| Car | 3 (1.9) | 5 (3.2) |
| Walk | 85 (53.5) | 29 (18.5) |
| Other mean | 5 (3.1) | 3 (1.9) |
| Home slaughter of pigs | | |
| Yes | 25 (15.7) | 37 (24.0) |
| No | 134 (84.3) | 117 (76.0) |
| Isolation of new stock | | |
| Yes | 76 (47.8) | 70 (45.5) |
| No | 83 (52.2) | 84 (54.5) |
| Use of footbath on farm | | |
| Yes | 4 (2.5) | 2 (1.3) |
| No | 155 (97.5) | 152 (98.7) |
| Use of disinfectant | | |
| Yes | 14 (8.8) | 33 (21.4) |
| No | 145 (91.2) | 121 (78.6) |
| Swill feeding | | |
| Yes | 94 (59.1) | 90 (58.4) |
| No | 65 (40.9) | 64 (41.6) |
| Source of breeding | | |
| No breeding | 39 (24.5) | 45 (29.2) |
| Own boar on farm | 18 (11.9) | 46 (29.2) |
| Use of communal boar | 89 (56.0) | 47 (30.5) |
| Other | 13 (8.2) | 17 (11.0) |
| Deworming of pigs | | |
| Yes | 151 (95.0) | 121 (78.6) |
| No | 6 (3.8) | 21 (13.6) |
| No answer | 2 (1.3) | 12 (7.8) |
| Spraying of pigs | | |
| Yes | 52 (32.7) | 80 (51.9) |
| No | 95 (59.7) | 58 (37.7) |
| No answer | 12 (7.5) | 16 (10.4) |
| Administration of vitamins | | |
| Yes | 117 (73.6) | 38 (24.7) |
| No | 35 (22.0) | 97 (63.0) |
| No answer | 7 (4.4) | 19 (12.3) |
| Antibiotics use | | |
| Yes | 39 (24.5) | 111 (72.1) |
| No | 104 (65.4) | 31 (20.1) |
| No answer | 16 (10.1) | 12 (7.8) |
| Castration | | |
| Yes | 61 (38.4) | 46 (29.9) |
| No | 81 (50.9) | 89 (57.8) |
| No answer | 17 (10.7) | 19 (12.3) |
| Use of extension services | | |
| Yes | 55 (34.6) | 25 (16.2) |
| No | 100 (62.9) | 116 (75.3) |
| No answer | 4 (2.5) | 13 (8.4) |
| Hired labour for on-farm activities | | |
| Yes | 18 (11.3) | 12 (7.8) |
| No | 120 (75.5) | 119 (77.3) |
| No answer | 21 (13.2) | 23 (14.9) |

### 3.4. Co-infections

Table 4 and 5 describe co-infections with two pathogens. In Masaka District, the most common co-infection was *S. suis* together with PRRS, APP, PCV2, *M. hyo*., SIV and PPV, at 100%, 96.6%, 93.2% and 93.1%,

**Table 3**
Pathogen prevalence level per district.

| Pathogen | Masaka | | | Lira | | |
|---|---|---|---|---|---|---|
| | +VE | -VE | % (CI 95) | +VE | -VE | % (CI 95) |
| ADV | 0 | 287 | 0.0(0.0–0.1) | 1 | 234 | 0.4(0.7–2.4) |
| Rotavirus A | 2 | 285 | 0.7(0.2–2.5) | 2 | 233 | 0.8(0.2–3.0) |
| PRRS | 4 | 283 | 1.3(0.5–3.5) | 4 | 231 | 1.7(0.7–4.3) |
| PPV | 18 | 269 | 6.2 (4.0–9.7) | 8 | 227 | 3.4(1.7–6.6) |
| SIV | 6 | 281 | 2.0(0.9–4.5) | 20 | 215 | 8.5(5.6–12.8) |
| *M. hyo* | 29 | 258 | 10.1(7.1–14.1) | 49 | 186 | 20.9(16.2–26.6) |
| APP | 59 | 228 | 20.5(16.2–25.6) | 60 | 175 | 25.6(20.4–31.6) |
| PCV2 | 117 | 170 | 40.7(35.2–46.5) | 119 | 116 | 50.8(44.5–57.2) |
| *S. suis* | 196 | 91 | 68.2(62.7–73.4) | 171 | 64 | 73.0(67.0–78.3) |

**Table 4**
Proportion of co-infections by each positive pathogen in Masaka.

| Total sample: 287 | PRRS | SIV | *M. hyo* | APP | PCV2 | *S. suis* | PPV |
|---|---|---|---|---|---|---|---|
| PRRS (4) | 0.00% | 0.00% | 0.00% | 0.00% | 75.00% | 100.00% | 0.00% |
| SIV (20) | 0.00% | 0.00% | 0.00% | 33.33% | 66.67% | 83.33% | 0.00% |
| *M. hyo* (29) | 0.00% | 0.00% | 0.00% | 34.48% | 58.62% | 93.10% | 6.90% |
| APP (59) | 0.00% | 3.39% | 16.95% | 0.00% | 35.59% | 96.61% | 1.69% |
| PCV2 (117) | 2.56% | 3.42% | 14.53% | 17.95% | 0.00% | 93.16% | 5.98% |
| *S. suis* (264) | 1.52% | 1.89% | 10.23% | 21.59% | 41.29% | 0.00% | 5.68% |
| PPV (18) | 0.00% | 0.00% | 11.11% | 5.56% | 38.89% | 83.33% | 0.00% |

**Table 5**
Proportion of co-infections by each positive pathogen in Lira.

| Total sample:235 | PRRS | SIV | *M. hyo* | APP | PCV2 | *S. suis* | PPV |
|---|---|---|---|---|---|---|---|
| PRRS (4) | 0.00% | 25.00% | 25.00% | 0.00% | 50.00% | 50.00% | 0.00% |
| SIV (20) | 5.00% | 0.00% | 25.00% | 20.00% | 50.00% | 80.00% | 10.00% |
| *M. hyo* (49) | 2.04% | 10.20% | 0.00% | 22.45% | 57.14% | 71.43% | 4.08% |
| APP (60) | 0.00% | 6.67% | 18.33% | 0.00% | 51.67% | 60.00% | 1.67% |
| PCV2 (119) | 1.68% | 8.40% | 23.53% | 26.05% | 0.00% | 72.27% | 4.20% |
| *S. suis* (169) | 1.18% | 9.47% | 20.71% | 20.71% | 50.89% | 0.00% | 3.55% |
| PPV (7) | 0.00% | 28.57% | 28.57% | 14.29% | 71.43% | 85.71% | 0.00% |

83.3% and 83.3% respectively. Co-infection was also common between PRRS and PCV2, SIV and *M. hyo*. at 75%, 66.7% and 58.7% respectively. In Lira District, the most common co-infections occurred between *S. suis* and PPV (85.7%), SIV (80.0%), PCV2 (72.3%), *M. hyo*. (71.4%), APP (60.0%) and PRRS (50.0%) respectively; and PRRS, SIV, *M. hyo*, APP, *S. suis* and PPV with PCV2 at more than 50% each. There was a significant association *M. hyo* and PCV2 co-occurrence ($p = 0.016$).

Co-infection with three pathogens was common in both districts with *M. hyo* (Masaka 12%; Lira 28%), APP (17%; 25%), PCV2 (32%; 43%) and *S. suis* (34%; 49%) (Fig. 4 and 5). Co-infection with four and more pathogens was less common.

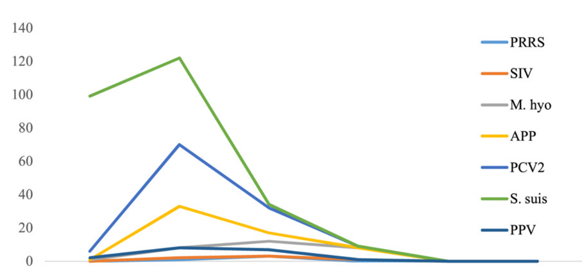


**Fig. 4.** Accumulative co-infection patterns of seven diseases in pigs in Masaka district.

### 3.5. Risk factors

Although univariate analysis generated a number of significant risk factors to pathogen exposure, the final multivariable logistic regression models resulted in limited significant risk factors. For *S. suis* the use of disinfectant reduced odds of infection (OR = 0.15; $p = 0.017$) and pigs less than six months old were more likely to be infected than older pigs (OR = 3.35; $p = 0.047$). For *M. hyo*, crossbred had higher odds of infection compared to local breed (OR = 1.77; $p = 0.03$). Use of disinfectants was associated with higher odds of SIV (OR = 8.39; $p < 0.002$); and crossed breeds were less likely to be infected with SIV compared to local breeds (OR = 0.17; $p = 0.003$) (Table 6).

## 4. Discussion

Successful swine production depends in part on preventing infectious diseases that affect reproduction performance and/or growth. To our knowledge, this is the first study in Uganda, exploring the epidemiology of a large number of viral and bacterial pig pathogens of production and public health importance. All these pathogens have been described in pig production systems worldwide and their presence in the Ugandan pig production system emphasizes their global spread and distribution. From a global perspective, PPV and PRRS virus are the most common viral causes of porcine reproductive failure. Major epizootics of PRRS have previously caused devastating losses to the swine sector of Vietnam in 2007–2010 (Zhang and Kono, 2012). There are vaccines available for prevention of both PPV and PRRS diseases (Keffaber, 1989; Mengeling, 1978; Mengeling et al., 2000a), but they are not used in Uganda because of lack of knowledge about the existence of these pathogens and the diseases in the country.

Over the past few years, the number of reported S. suis infections in humans has increased significantly, with most cases originating in Southeast Asia, where there is a high density of pigs (Wertheim et al., 2009a). Besides being the most important causes of bacterial mortality in piglets after weaning, *S. suis* is considered one of the most common causes of adult meningitis in Thailand, Vietnam and Hong Kong (Wertheim et al., 2009a, b). However, the disease is considered to be underdiagnosed and underreported in several countries including Uganda. Although statistical association between *S. suis* and PRRS was not found in our study, experimental studies have demonstrated that S. suis infection in pigs leads to increased severity of PRRS disease, and that PRRS virus infection increases susceptibility to S. suis (Galina et al., 1994; Xu et al., 2010). Some studies have even suggested the possibility of association between human S. suis and occurrence of PRRS outbreaks in pigs in northern Vietnam (Wertheim et al., 2009b). Hence, in addition to improved biosecurity on farms (Dee, 2005), a combined approach of surveillance for infection and disease diagnosis is needed to assist in effective control and/or elimination of PRRS from the pig population (Velasova et al., 2012).

From our study, pigs less than six months old were more likely to be infected with *S. suis* than older pigs. Newborn piglets may become colonized with S. suis via vertical transmission during farrowing and suckling. Previous studies have found that S. suis is frequently isolated from the tonsillar area of piglets before and after weaning (Baele et al., 2001). Infectious S. suis can spread to other animals during the production stage, or cause disease due to stress at weaning (Staats et al., 1997). In our study, it was reported that the use of disinfectant by farmers was associated with reduction of infection of *S. suis.* The pathogen is quickly killed by phenolic, chlorine and iodine based disinfectants (Band, 1990). Given the importance of these pathogens in the pig industry, the relationship between PRRS and *S. suis* and its implication for pig production and public health needs further investigation in Uganda

PCV2, the cause of porcine multisystemic wasting syndrome (PMWS) causes great economic losses in pigs and there are several studies on disease manifestations and lesions associated with this

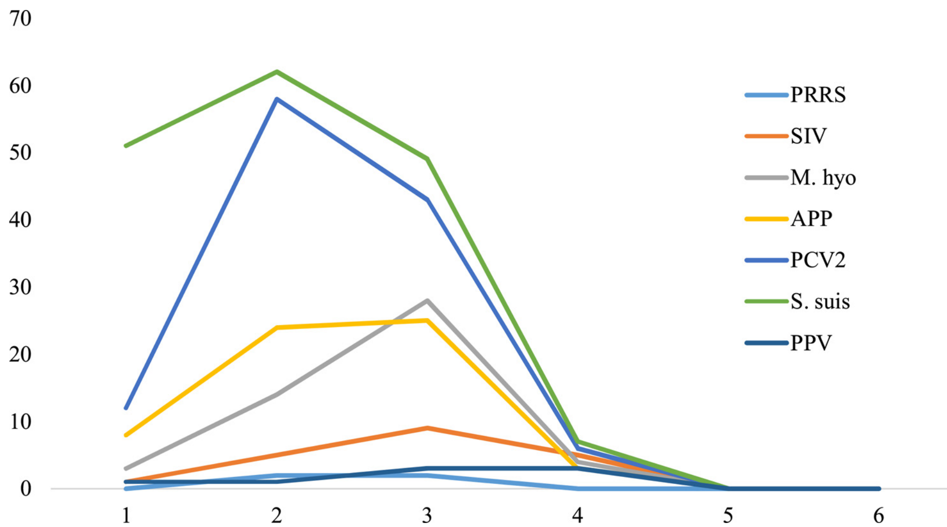


**Fig. 5.** Accumulative co-infection patterns of seven diseases in pigs in Lira district.

**Table 6**
Significant variables at p ≤ 0.05 in multivariate logistic regression analysis.

| Pathogen | Variable | Category | Adjusted odds ratio | 95% CI | p-value |
|---|---|---|---|---|---|
| *S. suis* | Disinfectants | | | | |
| | | Present | 0.15 | 0.32–0.71 | 0.017 |
| | Pig age in months | | | | |
| | | 12 < | Reference | N/A | N/A |
| | | ≤6 | 3.35 | 1.01–11.07 | 0.047 |
| | | 7-12 | 2.30 | 0.63–8.33 | 0.201 |
| *M. hyo* | Pig breed type | | | | |
| | | Local | Reference | N/A | N/A |
| | | Crossed | 1.59 | 0.87–2.91 | < 0.001 |
| SIV | Disinfectants | | | | |
| | | Present | 8.39 | 2.41–29.19 | 0.002 |
| | Pig breed type | | | | |
| | | Local | Reference | N/A | N/A |
| | | Crossed | 0.17 | 0.05–0.53 | 0.003 |

pathogen in pigs. Besides wasting, manifestation can include reproductive failure, predominately associated with increased numbers of mummies and non-viable piglets at parturition. However, the typical clinical signs with wasting and pathological features are still the cornerstones for suspecting and diagnosing disease associated with PCV2 infection (Segales, 2012). Co-infections especially with PPV, PRRS, and M. hyo., immune stimulation, immune suppression, environmental factors, including management practices, nutrition and housing, have been linked to PCV2 associated diseases (Gillespie et al., 2009; Madec et al., 2008; Opriessnig et al., 2007; Ramamoorthy and Meng, 2009; Alarcon et al., 2011). The significant association between *M. hyo* and PCV2 observed in our study is well documented in the literature. Coinfection with PCV2 and *M. hyo* which plays a primary role in the porcine respiratory disease complex, has been linked to increased PMWS severity and continues to have a major economic impact on the global swine industry (Kim et al., 2003). Past studies in Uganda reported molecular detection and characterization of PCV2 from pigs (Ojok et al., 2013). The three PCV2 sequences in the study were observed to cluster with PCV2b genotype which was originally referred to as the European cluster. Authors recommended that further studies be conducted so as to fully understand the true prevalence of the PCV2 in pig population of Uganda as well as their genetic diversity.

*M. hyo.* infection causes economic losses in swine production, through retarded growth, poor feed conversion and predisposition to bacterial pulmonary infections. Its occurrence has been associated with *Pasteurella multocida* and, in some cases, with *Haemophilus parasuis* or APP (Kobisch and Friis, 1996). A study found that pigs infected with both *M. hyo*. and SIV agents exhibited more severe clinical disease (Thacker et al., 2001). In this study, the most common pathogens found to occur with *M. hyo.* were APP, PCV2 and *S. suis.* The high occurrence of *M. hyo.* could be an indication of high incidence of related enzootic pneumonia diseases in the pig population given that pneumonia was among the most common undiagnosed syndromes reported by pig farmers in Uganda (Dione et al., 2014).

The association of use of disinfectants and SIV may indicate unsuccessful attempts of farmers to solve a disease problem. While the most used disinfectant reported by pig farmers in Uganda is sodium hypochlorite (NaOCL) commonly known as 'Jik', because it is cheap and available (Dione et al., 2018), many other disinfectants are in use. However, data on the exact disinfectant used by farmers in our study and their modes of application was not available. Sodium hypochlorite fulfills many requirements as the ideal disinfectant and furthermore it has an excellent cleaning action. However, its effectiveness in the cleaning and disinfection processes depends on the concentration of available chlorine and the pH of the solution (Fukuzaki, 2006). Insufficient knowledge of husbandry practices including type of disinfectant and mode of disinfection of pig pens and farm material was among the priority constraints faced by pig farmers in Uganda (Dione et al., 2014). The use of inappropriate disinfectant or wrong use of appropriate disinfectant could lead to inefficacy of the disinfection process. The lack of complementary information has made the interpretation of these findings difficult. Follow up studies are therefore needed to investigate the effect of available disinfectants for viruses affecting pigs in Uganda.

The classic type A infection with isolates of mild virulence may favor replication of ADV, *Haemophilus parasuis*, APP and *M. hyo.*, any of which may complicate outbreaks (Dee, 2005). However, with the test used in this study, it was not possible to distinguish between different strains of SIV, but the high prevalence indicates that more research on SIV is warranted. APP was highly prevalent in this study with animal level prevalence of 25.6% in Lira and 20.5% in Masaka. The severity of the clinical signs caused by APP may depend on the type of serotype involved (Marsteller and Fenwick, 1999). Therefore, further serotyping and linking presence of APP with clinical disease is necessary to have a clear picture of the situation.

The current poor biosecurity observed in most pig farms and reported in previous studies (Dione et al., 2014, 2016) could contribute to the high prevalence of these pathogens. In addition, environmental stress factors emanating from the poor housing observed could trigger onset of severe clinical signs and accelerate the occurrence of the diseases. It must be stressed that respiratory disease problems in pigs are frequently the result of multiple agents and rarely due to the effects of a single pathogen. A precise diagnostic of disease should take into consideration clinical history, symptomatic as well as serological data together with multiple factors associated with co-infection. Given the lack of pig disease diagnostic capacities in Uganda, there is need for building capacity in this area in order to improve disease control.

This study did not test for cross-reactivity across pathogens, which could have occurred. However, given the high specificity and sensitivity of the tests used, these results served the more explorative purpose of this study. Without doubts, there are plenty of pathogens that contribute to increasing the disease burden in the pig systems in Uganda, other than parasites which were not considered here since their level of endemicity is already known. Other bacterial pathogens that might have been worthwhile to consider are Haemophilus *parasuis* and Pasteurella. A more in-depth study on the respiratory disease complex would help to improve understanding of the different pathogens and role of co-infections with other reproductive and digestive infections in different management systems. Even though vaccines for most of the diseases reported in this study are available, no vaccination for pig diseases by neither pig farmers or the government is reported in Uganda. There was no history of vaccination for all the diseases we investigated. Since most of the diseases studied are typical production diseases, costs of vaccination would have to be covered by farmers. However little is known about pig farmers' willingness to pay for these vaccines but it is likely to be low given that their knowledge of these diseases is very limited. Before implementing any control programs for any of these diseases, a lot of disease awareness work would be required.

The results of this study also have implications for herd health in general. Serological surveys provide invaluable insight into the natural history and epidemiology of infection and can be used to estimate the burden of disaeses particularly in developping countries where diagnostic capacities are limited. However, serologic positivity is a *proxy* for infection, and may not accurately reflect actual infection rates, making it difficult to assess the exact time at which an animal acquired an infection. Despite some of the well-known limitations of serological surveys, the presence of antibodies against these pathogens in the serum collected in his study clearly indicates that pigs of the surveyed regions have already been exposed to the diseases and the pathogens in question have been circulating in the herd. Therefore, the reported diseases should be given adequate attention during herd health investigation. Also this study has pointed out keys areas for improvement in biosecurity and disease control in pig farms in Uganda.

## 5. Conclusions

Most pig health research in Uganda has previously focused on the obvious killer disease, ASF. This study however highlights the importance and widespread occurrence of other pathogens which also contribute to mortality and most importantly affect productivity and may result in zoonotic risks. The observed patterns of multiple infections indicate that biosecurity perceptions and practices of farmers provide important entry points to improving the current production systems and thus contributing towards reducing the disease burden and the related socio-economic impact of commonly occurring pig pathogens. These pathogens, which might be silent killers, are under-diagnosed by veterinarians; and farmers have little knowledge about how to prevent and control them. Any measures to combat ASF, for example improved biosecurity or strengthened veterinary services, will have an inherent positive impact on the other pig diseases. The findings of this study can thus be considered as a baseline to measure impact of future interventions aiming to reduce disease burden in the pig sector. However, follow up investigations should aim at determining specific pathogen's serovars and genotypes that are most virulent to pigs and humans, and study the dynamics and impact of these pathogens in current smallholder pig production systems in order to prioritize disease control programs.

## 6. Ethics approval and consent to participate

A consent form and an information sheet describing the aim of the study were handed over to literate farmers before the interview. Translation into local languages namely Luganda for Masaka District and Luo for Lira District was done for the farmers that were not conversant with English. Farmers who were willing to take part in the study signed the consent forms. The study was approved by the Research Ethics Committee of the College of Veterinary Medicine, Animal Resources and Biosecurity (COVAB) of Makerere University, Kampala, Uganda, with reference number SBLS.MD.2015; and the Ugandan National Council for Science and Technology (UNCST), with reference number A507.

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## Competing interests

Authors declare no conflict of interest.

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