MAKERERE  **UNIVERSITY**

EPIDEMIOLOGY OF SELECTED RESPIRATORY DISEASES AND THEIR IMPACTS ON SMALLHOLDER PIG PRODUCTION SYSTEMS IN LIRA DISTRICT, UGANDA

by

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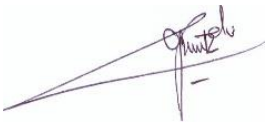
January, 2023

DECLARATION

I, **PETER OBA,** do hereby declare that this PhD thesis is my own original work and has not been published and/or submitted for any other degree award in any other university before.

Signed  **Date:** January 11th, 2023

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DEDICATION

I dedicate this thesis to my family: *Agnes Acom Oba* and children *Akengo Precious Diane, Amongin Leticia, Agadi Celina and Ebaat P. Peter.*

LIST OF ABBREVIATIONS

ADG – Average daily gain (gr/day)

App – *Actinobacillus pleuropneumoniae*

BCS – Body condition score

CDS – Clinical disease score

CoVAB – College of Veterinary Medicine, Animal Resources and Biosecurity

CPBP – Catarrhal purulent bronchopneumonia

CI – Confidence interval

ELISA – Enzyme-Linked Immunosorbent Assay

FAO – Food and Agriculture Organization of the UN

GIT – Gastro-intestinal parasites

IAV – Influenza A Viruses

ILRI – International Livestock Research Institute

MAAIF – Ministry of Agriculture, Animal Industry and Fisheries

M. hyo – *Mycoplasma hyopneumoniae*

NARO – National Agricultural Research Organisation

PRRSv – Porcine reproductive and respiratory syndrome virus

PCV2 – Porcine circovirus type 2

PLP – Pleuropneumonia

SBLS – School of Biosecurity, Biotechnology and Laboratory Sciences

RT-qPCR – Reverse transcriptase quantitative PCR

UBOS – Uganda Bureau of Statistics

UGX – Uganda shillings

USD – United States dollars

ZARDI – Zonal Agricultural Research and Development Institute

ABSTRACT

Respiratory diseases contribute significant economic losses to the swine industry globally. In Uganda, no detailed studies on pig respiratory pathogens have been undertaken previously. This doctoral thesis aimed to fill knowledge gaps on epidemiology of important respiratory pathogens, gastro-intestinal (GIT) parasites and their economic impacts on smallholder pig production systems in Uganda. The studies were conducted in Lira district from October 2018 to September 2019. Four respiratory pathogens of economic importance in pigs including porcine reproductive and respiratory syndrome virus (PPRSv), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae* (*M. hyo*) and *Actinobacillus pleuropneumoniae* (*App*) were studied. The first study was a desk systematic review on status and gaps of research on swine respiratory pathogens in Africa. This was followed by three cross-sectional studies: prevalence and risk factors for respiratory co-infections, a slaughter slab survey which correlated serology to 4 selected respiratory pathogens and pneumonia lesions and identify PRRSv genotypes. Tissue and blood samples were collected from pigs and used for genotypying PRRSv and to determine exposure to respiratory pathogens using ELISA assays, respectively. A mixed effects model was fitted to quantify economic losses due to exposure of pigs to respiratory pathogens and GIT parasites. Results highlighted major knowledge, information gaps on epidemiology, and economic impacts of the 4 studied pathogens reported in pigs in Africa. We found that there was dual circulation of both PRRSv PRRSv-1 and PRRSv-2 in Lira district with type 1 more predominant. A high prevalence and severity of pneumonia forms (17.4 – 74.2%) in slaughtered pigs was observed. The model showed that a grower pig in a given farm exposed to PRRSv and *Ascaris spp* infection had significantly lower average daily weight gain by 18.5 and 23.7 grams/pig/day respectively, compared to a similar unexposed pig. Monetary losses encountered by farmers due to PRRSv and *Ascaris spp.* infection amounted to USD 7.12 and USD 9.16 respectively, per pig during 200 days of fattening. In conclusion, these findings strengthened evidence of the role of housing, hygiene and biosecurity in reducing disease incidence in herds. The most important respiratory pathogens were PPRSv, *App, Ascaris spp* and risk factors were use of murram as floor type, poor hygiene, biosecurity practices, and concurrent GIT parasite infestations. Associations between serology and lung lesions suggests their potential role in lung disease precipitation. Farmers should pay close attention to proper housing, hygiene, biosecurity, wastes management and parasite control and limiting contacts with outside pigs. The findings from this study shall inform national policy in Uganda.

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CONTRIBUTION TO NEW SCIENTIFIC KNOWLEDGE

This study generated significant insights into the epidemiology and impacts of respiratory diseases/infections in pigs in Uganda. It revealed the identity and epidemiologic factors for occurrence and impacts of economically important respiratory pathogens in pigs in Uganda.

This thesis generated herd level management factors that could be used to reduce impacts of diseases in herds. Pigs that had GIT parasite infestations were more likely to have respiratory co-infections. Farms with poor hygiene and drainage level showed a higher likelihood of respiratory co-infections. Floor type played an important role in influencing the risk of infections with respiratory pathogens. Pigs raised in elevated platforms had a lower risk of infection, compared to those raised on concrete or mud floors. It strengthened previous evidence that improving hygiene and biosecurity is critical in reducing pathogen incidence in herds and their associated impacts.

To the best of our knowledge, this is the first study in Uganda to reveal the molecular identity of porcine reproductive and respiratory syndrome virus (PRRSv) circulating in domestic pigs. Two PRRSv types 1 and 2 were found to concurrently circulate in pigs in northern Uganda with type 1 more predominant. This study revealed a high prevalence and severity of pneumonic lesions in slaughter pigs. The risks of multiple pneumonia forms increased in pigs with multiple respiratory pathogens and with *Metastrongylus spp*. infestation, suggesting synergistic effects of their coinfections in lung pathology. Evidence of the magnitude of pneumonia associated with the studied pathogens was established. The findings that pigs infected with respiratory pathogens showed significantly decreased weight gains compared to uninfected pigs provides evidence of the contribution of these pathogens on pig growth rates and therefore justifies the need for interventions.

PUBLICATIONS

The key output of this doctoral thesis are the following peer reviewed publications:

i. **P. Oba*,** B. Wieland, F.N. Mwiine, J. Erume, E. Gertzell, M. Jacobson and M. M. Dione. Status and gaps of research on respiratory disease pathogens of swine in Africa. *Porc Health Manag* **6,** 5 (**2020**). https://doi.org/10.1186/s40813-020-0144-7

ii. **Oba, P*.**, Dione, M.M., Wieland, B., Mwiine, F.N. and Erume, J. Correlations between lung pneumonic lesions and serologic status for key respiratory pathogens in slaughtered pigs in northern Uganda. *Porc. Heal. Manag.* (**2021**) 7(1): 53. DOI: https://doi.org/10.1186/s40813-021-00233-y

iii. **Peter Oba*,** Michel M. Dione, Joseph Erume, Barbara Wieland, Christine Mutisya, Linnet Ochieng, Elizabeth A. J. Cook and Frank N. Mwiine. Molecular characterization of porcine reproductive and respiratory syndrome virus identified from slaughtered pigs in Lira district, Uganda. ***BMC Veterinary Research* (2022). 18(176)** https://doi.org/10.1186/s12917-022-03272-x

Submitted to a journal and currently under peer review:

i. "Co-infections of respiratory pathogens and gastro-intestinal helminths in smallholder pig production systems in Uganda", submitted to journal of ***Parasitology Research*, November 2022**

Upcoming papers ready to be submitted or under preparation:

i. Economic losses associated with respiratory and helminth infections in domestic pigs in Uganda **(*ready to be submitted to a journal*).**

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

In Uganda, agriculture remains the core sector employing 73% of the national population (NPA III, 2020). In the 2019/20 fiscal year, agricultural sector accounted for 21.5 percent of the country's GDP, up from 24.7 percent in 2017 (NPAIII, 2020). The value added to livestock grew by 3.1% in the fiscal year 2019/20 and contributed 16% of agricultural gross domestic (GDP) product (NPAIII, 2020). Livestock and their products make up a small portion of Uganda's exports and that per capita consumption of meat (10 kg per annum) is still low (ICPALD, 2020) compared to Kenya, whose per capita consumption is estimated at 14.9 kg (FAOSTAT, 2019). Fifty-eight percent of households depend on livestock for livelihoods (FAO, 2019).

Uganda's projected human population stands at 42.3 million as at 2020, with a growth rate of 3.0% per annum (UBOS, 2019). This rapid growth in human population requires a proportional increase in food production and distribution, to avert possible deficits, which could create instability in food prices. Reports indicate that pig population in Uganda has increased tremendously in the last decade to approximately 4.65 million pigs (UBOS, 2019). The proportion of households that own pigs in Uganda has also increased from 1,135 (10.13%) in 2008/09 to 1,345 (12.0%) in 2018 (UBOS, 2018). Recent trends show increase in the demand and consumption of pork in both urban and rural areas due to increase in human population and per capita purchasing power, as well as changes in consumption habits (Tatwangire, 2014). Per capita pork consumption in Uganda strands at 3.4 kg per annum, making Uganda the highest consumer in Africa, second to China in the world (FAO, 2018). However, available data reveals that the average supply of protein of animal origin has stagnated at 12 grams/capita/day between 2015-16 and 2016-17 (FAOSTAT, 2017). The proportion of children who were stunted stood at 33.7% in 2017, while 15.2 million people in Uganda are reportedly undernourished (FAOSTAT, 2017). There is therefore a need to increase food production, especially of animal origin to reduce the problem of malnutrition, which is prevalent in children in the country. This study is expected to generate important information to address critical constraints to pig production and productivity in Uganda.

The CGIAR research program on livestock and fish included the small holder pig value chains due to its potential and competitiveness in sub–Saharan Africa (Dione, Masembe, et al., 2016). Yet poverty indices show that women, youth and elderly are the most disproportionately affected and remain vulnerable to adversities of climate variability. Studies recommend increased involvement of women and youth in pig production and decision making as effective strategies for poverty reduction in the country. This is partly because pigs are highly prolific, are easy to manage and require less land compared to cattle or goats (ILRI, 2011).

1.2. Statement of the problem

Pig production provides a great potential for poverty alleviation and employment for many rural poor, as it meets livelihood needs (Perry and Grace, 2009). Despite the potential of the sector, its economic and productive performance (in terms of growth rates, reproductive indices, contribution to poverty reduction, etc.) is generally low or poor, which is a disincentive to making critical investments in the sector. Efforts to identify critical disease constraints to productivity are needed to overcome this challenge and enable producers benefit from the available market opportunities.

Livestock diseases disrupt both local and international trade, exacerbate poverty and pose significant public health risks, in case of zoonoses (VanderWaal & Deen, 2018). Pig respiratory diseases are known to suppress productivity in several ways. As a single or in many cases as mixed or co-infection, respiratory diseases contribute to economic losses due to their negative effect on growth, feed conversion, additional costs of treatment and loss of potential revenue (Rushton, 2008). Respiratory diseases also adversely affect reproductive performance, manifested in abortions, infertility and poor conception rates. Currently, limited information, if any, is available on the epidemiology of respiratory diseases in pigs in Uganda. Under these circumstances, design of prevention and control strategies is difficult. There is therefore a need to investigate the causes, transmission patterns, risk factors as well as cost benefits of available intervention strategies to guide small holder farmers.

Previous studies highlighted the need for follow up investigations to characterize the most important pathogenic serotypes and genotypes, population dynamics and their impact in the current small holder pig production systems in Uganda (Kungu et al., 2016; Ojok et al., 2013). Another study also recommended epidemiological investigations of infection patterns of respiratory

pathogens to establish population dynamics, risk factors and provide information for modeling studies (Fablet et al., 2011). Besides, the impact of a respiratory disease agent, acting singly or in combination with other infectious agents needs to be elucidated. While economic impacts of respiratory diseases have been documented elsewhere, there is lack of data and information on economically important respiratory pathogens in Ugandan pigs.

In Uganda, parasites and endemic diseases continue to constrain pig production (Muhanguzi et al., 2012). Among the diseases, diarrhea and respiratory infections are a common feature of Uganda's pig sector (Ikwap et al., 2014). The occurrence of several pig diseases constitutes a major setback to improving pig productivity in Uganda and limits the full potential of the sector (Muhanguzi et al., 2012). So far, the status of porcine circovirus 2 (PCV2) in pigs is unknown in many countries of sub-Saharan Africa (Jonsson, 2013). In Uganda, a recent multi-pathogen serological study demonstrated a high prevalence of *Mycoplasma hyopneumoniae* (20.9% in Lira district, 10.1% in Masaka district), *Actinobacillus pleuropneumoniae(*25.5% in Lira district, 20.5% in Masaka district, *Leptospira spp.*, porcine circovirus (50.8% in Lira district, 40.7% in Masaka district and *Streptococcus suis* (73% in Lira district, 68.2% in Masaka district in pigs (Dione et al., 2018); porcine circovirus type 2 (Jonsson, 2013; Ojok et al., 2013). Thus, identifying important circulating pathogens, risk factors for their occurrence and determining their genetic diversity in pig populations in Uganda provides a useful framework for the design of preventive and control interventions. This study therefore focused on establishing risk factors for important respiratory co-infections in pig herds, the molecular identity of PRRSv, impacts on growth and to quantify their economic losses to pig farmers in Uganda.

1.3. Study Objectives

1.3.1. Main Objective

To generate knowledge and information on epidemiology of important respiratory pathogens, gastro-intestinal parasites and their economic impacts on pig productivity in Lira district, Uganda

1.3.2. Specific Objectives

i. To determine the prevalence and herd level management risk factors for co-infections of key respiratory pathogens (PRRSv, PCV2, *M. hyo* and *App*) and gastro-intestinal helminths (*Ascaris spp* and *Strongyles spp*) in domestic pigs in Lira district, Uganda
ii. To determine molecular identity of porcine reproductive and respiratory syndrome virus (PRRSv) identified from slaughtered pigs in Lira district, Uganda
iii. To correlate serologic status and lung pneumonic lesions in slaughtered pigs in Lira district, Uganda
iv. To quantify economic losses (average daily weight gains) associated with exposure of pigs to respiratory infections in Lira district, Uganda

1.4. Research Questions

i. What is the prevalence and management risk factors for co-infections with key respiratory pathogens and GIT parasites in domestic pigs in Lira district, Uganda?
ii. What farm level biosecurity practices are associated with respiratory pathogen occurrence?
iii. What is the molecular identity of porcine reproductive and respiratory syndrome virus genotypes (PRRSv) isolated from slaughtered pigs in Uganda?
iv. Are there any associations between serologic responses to selected respiratory pathogens and lung lesion scores in slaughtered pigs?
v. What is the impact of exposure of pigs to respiratory pathogens on average daily weight gains, treatment costs and financial losses in farms in Lira district, Uganda?

1.5. Applicability of research findings – general outputs

This thesis contributed to improved knowledge and understanding of husbandry and management factors associated with pathogen occurrence in farms. This can be used to guide policy and the formulation of control interventions. The knowledge of important circulating respiratory pathogens and risk factors for respiratory disease in pigs in Uganda generated is useful to guide control interventions. The knowledge generated of the impacts of respiratory infections on weight gains and economic losses in pig herds in Uganda can be used to assess the benefits of control measures. It will provide a framework for formulation of advisory services to farmers for improving the productive and economic performance of the pig sector in the country. Results of this study are expected to guide Uganda's policy on pig imports (e.g., prescreening of breeder stock imports against PCV2, PRRSv), herd health management and support extension services delivery aimed to address disease constraints to pig production and productivity.

1.6. Specific outputs

i. The microbiological etiology, prevalence and herd level risk factors for occurrence of respiratory infections (PRRSv, PCV2, *M. hyo* and *App*) and gastro-intestinal helminths (*Ascaris spp* and *Strongyles spp*) in pig herds identified.
ii. Farm level biosecurity and husbandry practices associated with occurrence of respiratory pathogens in pig herds determined.
iii. The molecular identity of porcine reproductive and respiratory syndrome virus (PRRSv) genotypes circulating in pigs in Lira district determined.
iv. The economic losses (average daily weight gains and treatment costs) due to respiratory disease burden in pigs in Lira district Uganda quantified.
v. Herd health package for improving pig productivity in Uganda generated to support herd level interventions.

1.7. Justification for the Study

Whereas pig production offers substantial returns to investment due to their high fecundity and relatively short generation intervals, the development of the sector is hampered by limited investment (Waiswa, 2005). Economic costs associated with respiratory diseases in pigs can be substantial (Perry & Grace, 2009). The pig sector in Uganda is largely informal with poorly organized markets, limited access to technology, information and support services (Ouma et al.,

2013). In addition, losses associated with respiratory infections need to be quantified to guide decision making processes that aim to reduce the negative impacts of respiratory diseases. The proposed approaches used in this study were used in similar previous studies elsewhere (Sibila et al., 2008) and have proven useful in epidemiological surveys of pig respiratory pathogens.

Management systems and husbandry practices play a key role in determining efficiency and economic performance of any piggery enterprise. The profitability of a piggery enterprise is largely dictated by production factors such as housing, breeding, health, feeding and climate control. A lack of, or failure to provide sufficient environment in any of these factors often results in sub optimal performance, which manifests as slow growth and a failure to cope with environmental stressors (Bolhuis et al., 2006). Due to their relatively faster growth rates, pigs respond quickly to any changes in their environment.

Pig production is a sensitive venture that requires timely and effective decision making. However, most farmers in Uganda lack the necessary skills and knowledge of piggery production (Muhanguzi et al., 2012). Knowledge of market dynamics, biosecurity risks and product value chains, is equally critical to optimize management decisions, minimize production costs, and thus improve enterprise profitability. This lack of, or limited knowledge often translates into significant economic losses encountered by farmers. This study quantified the impact of respiratory diseases on pig productivity (average daily weight gains) in Lira district, Uganda.

This research project aligns with the third National Development Plan (III) (NDP III, 2020/21-2024/25) of the government of Uganda, which emphasizes development of livestock value chains and agro-industrialization through meat processing (Uganda National Planning Authority, 2020). To feed into this development agenda, increased production and productivity of pigs through reduction of diseases will be a prerequisite. Control of swine diseases improves the sector's profitability and facilitates greater access to local and regional markets, thereby contributing to foreign exchange earnings. It also contributes towards commercialization of agriculture, especially among smallholder farmers. This is expected to support attainment of Sustainable Development Goals (SDGs) 1): to end poverty in all its forms; and 2) to end hunger, achieve food and nutrition security. The results of this study will be used to guide stakeholders in the pig sector in Uganda on the design of interventions for improving pig productivity in the country. It constitutes an

important framework to guide appropriate policy formulation for enhancing the contribution of the sector to national development in Uganda.

A conceptual framework in the form of a directed acyclic graph (DAG) below (Figure 1.1) illustrates the relationships between management, environment contamination and pig level factors for infections with respiratory pathogens and their possible impacts on productivity.

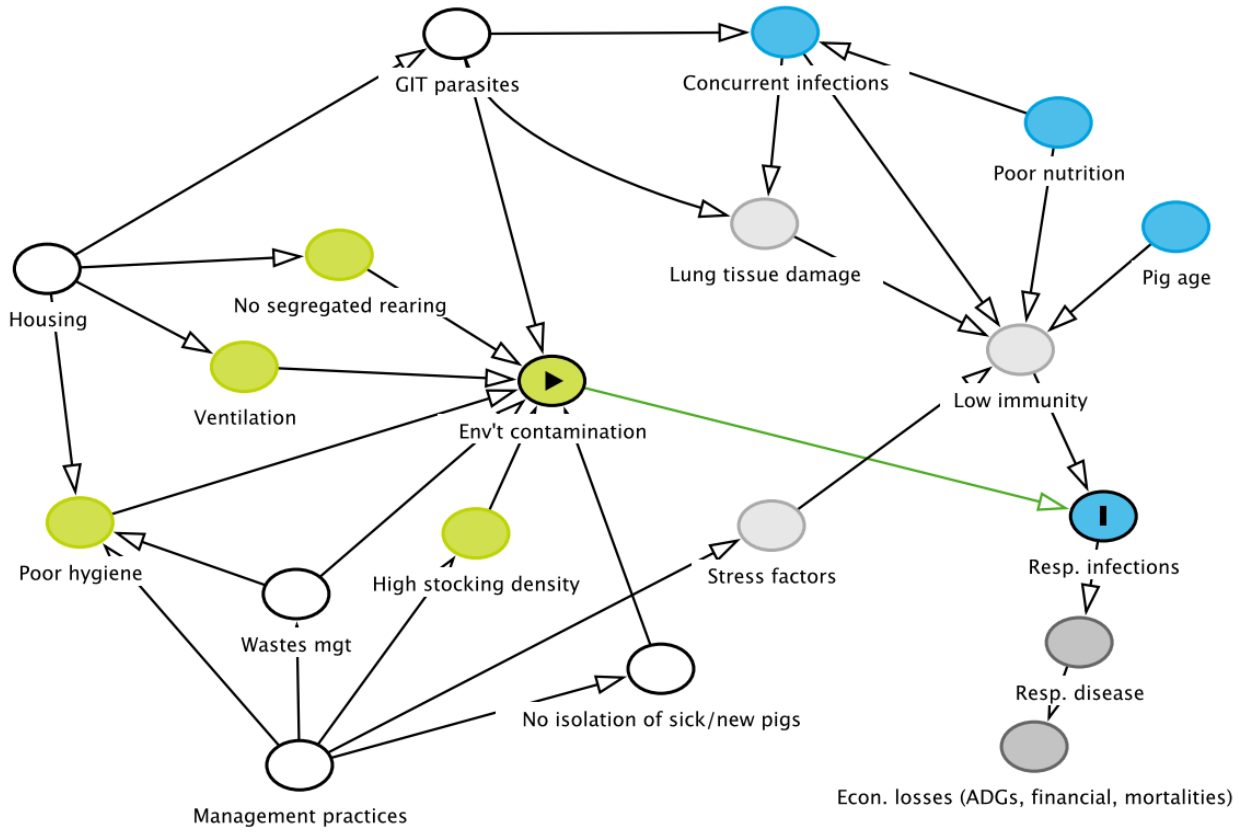

Figure 0.1: A conceptual framework of management factors, biosecurity practices and pig level factors for respiratory infections and their possible impacts on productivity

1.8. Presentation of the thesis

This thesis is arranged into eight (8) chapters. Chapter one (1) provides the general introduction and background to this study; Chapter two (2) highlights literature review which gives an overview of epidemiology of important respiratory pathogens in pigs, herd level risk factors for their occurrence and their economic impacts on swine productivity. Chapter three (3) gives a summary of the research methodology used. Chapter four (4) introduces a systematic review on the status and gaps of research on swine respiratory disease pathogens in Africa. Chapter five (5) focuses on objective one (1) of this study: establish prevalence and herd level risk factors for key respiratory co-infections in domestic pigs in Uganda. Chapter six (6) introduces objective 2: determine molecular identity of porcine reproductive and respiratory syndrome virus (PRRSv) in domestic pigs in Uganda. Chapter seven (7) describes objective three (3) which correlated lung pneumonic lesions and serologic profile in slaughtered pigs in Lira district Uganda.

Chapter eight (8) focused on quantifying direct economic losses (average daily weight gains, ADGs, financial and treatment costs) associated with exposure of pigs to key respiratory infections in Uganda. The last Chapter nine (9) gives the overall conclusions and recommendations from the study.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.1. The pig industry, health, and production systems in Uganda

In Uganda, pig production is largely dominated by small scale production systems, with limited focus on larger or growing markets. Three pig production systems are broadly identified in Uganda: intensive, semi-intensive and extensive systems (Tatwangire, 2014), while (Ikwap, et al., 2014) found four production systems in Soroti and Gulu districts, based on type of housing structures. Most households in Uganda (approx. 90%) are engaged in extensive production system (Tatwangire, 2014). Most pig farmers keep a small number of pigs, and usually allow them to roam around, increasing the risk of disease transmission between neighboring herds. These free-range or semi-intensive production systems have a range of limitations, among which is high incidence of epidemic diseases, notably African swine fever (ASF), and more recently swine influenza (Kirunda et al., 2014).

African swine fever (ASF) is a highly infectious disease of pigs endemic in Uganda, which may account for 80 - 100% herd mortality (Atuhaire et al., 2013). However, other respiratory diseases are known to occur, but information on their temporal and spatial distribution is unavailable. This is because limited studies have been conducted to-date. Much of the research work in pigs in Uganda has focused on African swine fever (ASF) due to its acute economic impact as compared to other diseases, whose mortality is comparatively lower, but cause reduced weight, market value and represent a public health threat in case of zoonoses.

The importance of the pig sector is evidenced by the rapid growth in pig population and the increasing number of farmers engaging in the enterprise (Tatwangire, 2014). In small scale production systems, pigs play a role in risk diversification, food security and as livelihood assets that can be sold to meet basic household needs such as school fees, clothing and medical care (Nantima et al., 2015). In Uganda, it is reported that pig production and consumption increased by 20% annually from 1980 to 1990 and by 3% annually from 1990 to 2000 (FAO, 2005). In 2011, estimates were that Uganda had one of the highest per capita consumption of pork in sub-Saharan Africa, reaching 3.4 kg/person/year (FAO, 2010). This represents a ten-fold increase in the last 30 years (Ouma et al., 2013). In this context, pigs are important for addressing the challenge of

malnutrition. The demand and market for pigs or their products is rapidly growing in many urban and rural areas across the country. However, several constraints exist, among which are diseases, which hamper the productivity of the sector.

The Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) is responsible for policy formulation, infrastructure development and regulatory enforcement, among other core functions. Veterinary services, which fall under MAAIF, are structured from the national to sub county level (MAAIF, 2012). The Directorate of Animal Resources is legally mandated, under the Animal Diseases (Amendment) Act of 2006, to enforce regulations for control and prevention of economically important livestock diseases. However, animal health systems, especially at local government level are ill equipped to enforce livestock disease control due to inadequate manpower and logistical infrastructure. Consequently, illegal and uncontrolled movements of animals or animal products across many parts of the country continue unabated. This partly accounts for persistent outbreaks of animal diseases (especially of epidemic nature).

2.2. Etiology of swine respiratory diseases

Several etiological agents have been identified as responsible for respiratory diseases in pigs (Høie et al., 1991). Respiratory diseases occur as a single pathogen (rarely) or as frequently, a mixed or co-infection, resulting into more severe disease (Choi et al., 2003). *Mycoplasma hyopneumoniae* (*M. hyo*) is known to be the primary agent of porcine respiratory disease complex (PRDC), with significant economic consequences in swine herds worldwide (Thacker et al., 1999). Pneumonia due to *M. hyo* is usually complicated by co-infections with porcine reproductive and respiratory syndrome virus (PRRSv) or with porcine circovirus type 2 (Thacker et al., 1999). Post weaning multisystemic wasting syndrome (PMWS) is an infectious disease of swine caused by porcine circovirus 2 (PCV2) infection in pigs (Allan & Ellis, 2000). However, combinations of other viral and bacterial agents are usually involved in the pathology of PMWS.

2.3. Epidemiology of swine respiratory diseases

Pig respiratory diseases are some of the most prevalent diseases affecting growing finishing pigs reared under intensive conditions (Fablet et al., 2011) and contribute significant economic losses (Perry and Grace, 2009). In the polymicrobial disease referred to as Porcine Respiratory Disease Complex (PRDC), several microbial agents are involved (Choi et al., 2003; Hernandez-Garcia et

al., 2017). *M. hyo* is the most prevalent and important respiratory pathogen associated with PRDC (Fano et al., 2005; Thacker et al., 1999). *M. hyo* often occurs in combination with other bacterial agents as a co-infection with *Haemophilus parasuis*, *Pasteurella multocida* or *Streptococcus suis.* It may also occur in combination with viral pathogens such as Swine Influenza Virus (SIV) and PRRSv. These agents constitute the most important causes of swine respiratory diseases (Sorensen et al., 2006).

The combined infection of several pathogens leading to PRDC results from synergistic effects through mechanisms that range from host immunosuppression, altered macrophage function and excessive cytokine responses, as well as inhibition of muco-ciliary clearance pathways in the respiratory tract, which allows bacterial colonization (Hernandez-Garcia et al., 2017). Disease severity thus results from a combination of the effects of the individual pathogens on the host, often leading to a clinical syndrome associated with the causative agent(s). Co-infections of highly pathogenic PRRSv and PCV2 were reported to induce more severe clinical disease, suggesting synergistic effects of both viruses (Fan et al., 2013).

Previous studies describe concomitant interactions of different infectious agents in the production of respiratory disease (Opriessnig & Halbur, 2012; Patterson et al., 2011). These infections are known to produce a more severe respiratory disease than those induced by a single pathogen (Thacker et al., 1999). A study showed that herds with a high percentage of seropositive pigs to Aujeszky's disease virus or Porcine Influenza serotype H3N2 showed a distinctly higher prevalence of pigs with lung abscesses at slaughter, compared to those with a lower extent of seropositivity (Elbers et al., 1992). This was associated with the effect of secondary bacterial infections with organisms such as *P. multocida* and *App,* which are known to cause lung abscesses. In addition, the degree of seropositivity in herds may reflect disease spread within herds (Morrison RB, 1989) . This is of epidemiological significance for control, but also highlights the challenges of possible disease persistence within herds. Concomitant infections that have been reported to result in respiratory disease are between PPRSv and *M. hyo* (Thacker et al., 1999) and between *App* and *M. hyo* (Marois et al., 2009). Concurrent infections of *P. multocida* and *M. hyo* are associated with respiratory disease in pigs (Ciprián et al., 1988).

Studies reveal disease outcome may be influenced by the infection pattern and infection pressure in growing-finishing pigs, especially for *M. hyo* (Sibila et al., 2004). Other authors reported that

the sow herd constitutes a reservoir for the continuous circulation of respiratory pathogens (Calsamiglia & Pijoan, 2000; Sorensen et al., 2006). Thus, the health status of sows during pregnancy and after farrowing largely impacts that of piglets, as several infectious agents can be easily vertically transmitted to their offspring.

Ascariasis, anteroventral pneumonia and atrophic rhinitis are common in all swine producing areas globally (Maes et al., 1996). The pathological effects of ascarid worm infestations include damage to the lung tissues during larval migration, which could induce losses (due to tissue condemnation at slaughter) and a possible increased susceptibility to pneumonic pathogens (Morrison et al., 1985). Indirect effects of *Ascaris suum* are due to enhanced susceptibility or pathogenicity to bacterial or viral infections, related to its migratory and immune-modulatory capacity (Nissen et al., 2011). A study showed that *A. suum* significantly compromised the effect of *M. hyopneumoniae* vaccination (Steenhard et al., 2009). Losses due to ascarid infestations in pigs can therefore be due to reduced growth, tissue condemnations, treatment costs and their ability to induce co-infections (Nissen et al., 2011). The impact of reduced vaccine efficacy caused by a common gastrointestinal helminth emphasizes the importance of parasite control.

In Uganda, limited information exists on the current etiological agents of respiratory diseases in pigs, associated risk factors and their impact on pig productivity. Moreover, the identity of important circulating serotypes or strains, as well as their molecular epidemiology is unknown. Thus, design of cost-effective interventions may be difficult, if not impossible. This study therefore aims at generating information useful for designing control interventions against respiratory infections in swine herds in Uganda. This is expected to address some of the key constraints to pig production and productivity and hence improve the profitability of the sector in Uganda.

2.4. Risk factors for occurrence of respiratory diseases in pigs

Some risk factors are associated with occurrence of respiratory disease in pig herds in various countries. Management factors including increased herd size, increased stocking density and being farrow-to-finish herd have been associated with increased risk of lung lesions (Fraile et al., 2010a; Stärk, 1998), while implementing an all-in-all-out system has been demonstrated to be protective (Cleveland-Nielsen et al., 2002; Jäger et al., 2012). In Spain, risk factors associated with a higher prevalence of pneumonia in pig herds were presence of pleuritis and frequent purchases of pigs (Meyns et al., 2011). Management procedures such as poor ventilation and high stocking density

are associated with increased incidences of respiratory diseases in pigs (Choi et al., 2003; Martínez et al., 2009a). In addition, use of replacement stock from unknown sources increased occurrence of respiratory disease. In Uganda, the only risk factors for African Swine Fever (ASF) outbreaks have been elucidated and include purchase of pigs in the previous year and feeding of swill to pigs (Nantima et al., 2015). These studies show that management and husbandry factors are critical components for ensuring better herd health and welfare for good productivity of pig herds.

2.5. Economic costs associated with respiratory diseases and their impacts on pig productivity

For many of the pathogens involved in respiratory diseases both horizontal and vertical transmission is known to occur (Maes et al., 2008; Van Alstine, 2019; Zimmerman et al., 2006). These pathogens can be categorized as primary agents, which suppress the host defense mechanisms, or are secondary opportunistic pathogens, that take advantage of the weakened immune systems of the host, leading to more serious disease (Hernandez-Garcia et al., 2017). *M. hyo* is known to alter the immune response of the host and concurrent infection with PCV2 or PRRSV increases the severity and duration of mycoplasmal pneumonia (Opriessnig et al., 2004). However, the impact of these agents is largely determined by several factors, among which is the immune status of the herd, nutrition, management systems and biosecurity practices on the farm. Porcine Respiratory Disease Complex (PRDC) is recognized as an important respiratory disease of swine characterized by retarded growth, dyspnea, reduced feed efficiency, anorexia and cough (Van Alstine, 2019). Both porcine parvovirus and PRRSv infection is the common cause of reproductive failure in pregnant sows and respiratory disorders in growing pigs, leading to infertility (Thacker et al., 1999).

Respiratory diseases impact negatively on live weight gain, feed consumption and reproductive performance (Galdeano et al., 2019; Nathues et al., 2017a; Segalés et al., 2013). In addition, they cause increased mortalities, thus contributing to significant losses. Economic losses due to respiratory diseases in swine herds have been estimated to range between 2-25% reduction in average daily gain (ADG) (Hurnik et al., 1993). There are indications that the losses are proportional to lung lesion severity (Straw et al., 1990). Economic losses are largely a function of a combination of factors such as herd health immunological status, management systems, and age as well as environmental factors, all of which determine disease severity. In Uganda, economic

losses associated with pig respiratory diseases are unknown, as no such studies were conducted before. This thesis attempted to fill this critical knowledge and information gap, which could inform further research and health interventions.

2.6. Current status of swine respiratory diseases and research gaps in Uganda

In Uganda, a recent multi-pathogen study revealed a high prevalence of M. hyo, App, Leptospira spp., porcine parvovirus, Influenza A viruses, Streptococcus suis, and porcine circovirus type 2 in pigs (Dione et al., 2018). Other agents of low prevalence detected in the above study were PRRSv and Aujeszky's disease. Related studies in Uganda reported occurrence of PCV2 in pigs (Eneku et al., 2018; Jonsson, 2013). Most research studies on swine in Uganda have focused on African swine fever (ASF), enteric bacterial infections, such as Salmonella spp., E. coli and gastro-intestinal parasites (Atuhaire et al., 2013; Ikwap et al., 2014; Muhangi et al., 2014; Muhanguzi et al., 2012). However, information on swine respiratory disease pathogens in Uganda and elsewhere in Africa is largely absent or scanty. A recent review of the status of research and gaps on swine respiratory pathogens in Africa revealed scarcity of information, which warrants detailed investigations (Oba et al., 2020).

While these few studies revealed occurrence of respiratory infections in Ugandan pigs, information on epidemiological factors for their occurrence in swine herds, and their genetic diversity is unavailable. In Uganda, no previous study was found that documented the impacts of respiratory diseases on the growth of pigs. Consequently, it is difficult to propose any interventions for control and prevention. Thus, the potential contribution of the pig sector to Uganda's national economy cannot be fully harnessed as information and knowledge of important pathogens is generally lacking.

Based on available evidence of their economic importance in swine production in other regions, we identified four key respiratory pathogens which, in recent studies were reported in Ugandan pigs (Dione et al., 2018; Eneku et al., 2018; Jonsson, 2013). These are PRRSv, PCV2, *M. hyo* and *App*. A recent study reported a high prevalence and severity of pneumonia associated with key respiratory pathogens in slaughtered pigs in Lira district, Uganda (Oba et al., 2021).

Below is a short description of the clinical presentation, diagnosis, treatment, control and prevention of the four key pathogens detected in pigs in Uganda. However, this list may not be

fully exhaustive of all respiratory pathogens, as other respiratory pathogens are present and may contribute to economic losses to swine producers in the country.

2.7. Clinical signs, diagnosis, treatment, control and prevention of viral respiratory diseases in pigs

2.7.1. Clinical signs of porcine circovirus 2 (PCV2) infection

Porcine circovirus 2 infection in pigs is recognized as a principal cause of post weaning multisystemic wasting syndrome (PMWS) (Allan et al., 2004; Segalés et al., 2013). Many experimental studies show that full expression of the disease requires involvement of other agents, as PCV2 infection alone does not lead to overt clinical disease. PCV2 has been reported in South Africa (Drew et al., 2004) and recently in Uganda (Eneku et al., 2018; Jonsson, 2013a; Ojok et al., 2013), who recommended further studies to characterize circulating strains and the genetic diversity of PCV2 isolates in Uganda. PMWS manifests with a range of clinical signs, which include debility, dyspnea, palpable lymphadenopathy, diarrhea, icterus and hepatomegally (Allan & Ellis, 2000). However, studies reveal concurrent involvement of porcine parvovirus (PPV) enhances PCV2 replication and leads to induction of gross pathological lesions (Allan & Ellis, 2000).

2.7.2. Diagnosis of porcine circovirus 2 (PCV2)

ELISA is the recommended test for sequential or cross-sectional studies of swine populations (Opriessnig et al., 2007). A recent ELISA IgG (Ingezim PCV IgG and IgM PCV2 ELISA (Ingezim IgM) has been developed. To determine the timing of PCV2 infection, a comparison of IgG and IgM values is useful. When the IgM value is greater than or equal to IgG value, this implies early active infection (within the first 21 days); when IgM value is less than IgG value, this implies early infection (between 20-50 days) and when IgM value is lower than IgG value or when IgM value is negative, this indicates late or resolving infection (Segalés et al., 2005). Serum antibodies to PCV2 wane with time, and due to the problem of cross reactivity between PCV2a & PCV2b isolates (Ssemadaali et al., 2015), PCR-based methods, considered definitive are preferred.

2.7.3. Treatment of PCV2 infections in swine

No specific treatment is available for PCV2 infections. However, co-infections with other agents such as *S. suis, P. multocida, H. parasuis* should be treated with antimicrobials (Tanja Opriessnig et al., 2007). Use of chlortetracycline at 22 mg/kg of feed in pigs experimentally co-infected with *M. hyopneumoniae* has been found to reduce lesions due to PCV-associated disease (Opriessnig et al., 2006).

2.7.4. Control and prevention of PCV2 infection

Several commercial PCV vaccines (CIRCOVAC® and Ingelvac CIRCOFLEX™) are currently available and have been proven to reduce mortality in growing pigs and breeder herds (Opriessnig et al., 2007). PCV2 is resistant to inactivation by common disinfectants; and apparently good biosecurity measures does not assure freedom from disease. However, prompt diagnosis, reduction of stress, improvement of nutrition (www.thepigsite.com) and removal of diseased pigs appears the only method of controlling losses due to PCV infection (Allan & Ellis, 2000).

2.7.5. Clinical signs of Porcine Reproductive and Respiratory Syndrome Virus (PRRSv)

PRRS virus, first discovered in the US in 1987, is a multifactorial disease of swine with important economic implications worldwide (Nathues et al., 2017a). It is an infectious disease of swine characterized by reproductive failure in breeding sows and respiratory disorders in growing pigs. Infection causes late-term abortions, a high incidence of stillborn, mummified fetuses, early embryonic death and infertility (Brewer & Greve, 2011; T Opriessnig et al., 2004). Induction of clinical disease due to PRRSv infection requires co-infection with other viral or bacterial pathogens (Thacker et al., 1999). In neonatal and growing pigs, PRRSv induces inapparent disease mild respiratory disease or moderate to severe dyspnea and tachypnea (Halbur et al., 1996; Rossow et al., 1994).

2.7.6. Diagnosis of PRRSv

An ELISA test (IDDEXX HerdCheck PRRS 2XR ELISA, Maine, USA) is used to detect PRRSv from serum samples (Duinhof et al., 2011a). For a cross sectional study to detect exposure of pigs to PRRSv, pigs of 2.5 -15 months were sampled. For a longitudinal study, although previous studies showed that pigs of 9 -16 weeks were the most preferred age group to detect PRRSv virus

in herds without clinical signs of disease (Duinhof et al., 2011b), we sampled pigs of 2.5 – 8 months to increase the power of the study.

2.7.7. Treatment of PRRSv

No specific treatment is available for PRRSv infections. However, broad-spectrum antibiotics may be helpful in controlling secondary opportunistic infections. Anti-inflammatory agents are commonly administered during acute disease (https://vetmed.iastate.edu/vdpam/FSVD/swine/index-diseases/porcine-reproductive).

2.7.8. Control and prevention of PRRSv

The principal method used to control and prevent porcine reproductive and respiratory syndrome virus (PRRSV) outbreaks is by vaccination. Both inactivated and attenuated vaccines for the prevention of PRRSV in swine herds are available (Murtaugh & Genzow, 2011). However, modified live-attenuated (MLV) PRRSv vaccines do not confer complete protection against existing and genetically variant field isolates (Renukaradhya et al., 2015). Regular serological monitoring by ELISA testing and removal of persistent carriers in herds is recommended.

2.8. Clinical signs, treatment, control and prevention of swine bacterial respiratory diseases

2.8.1. Clinical signs of *Actinobacillus pleuropneumoniae (App)* infection in swine

App is a primary respiratory pathogen in pig populations worldwide. Gross pathological lesions of *App* are well described (Van Alstine, 2019a) and are characterized by demarcated lesions in the middle, cranial and caudal lobes of the lung (Marsteller & Fenwick, 1999). Pneumonic areas of the lung are dark and consolidated, while chronically affected pigs typically have pleural adhesions, fibrinous pleuritis with necrotic lesions in the lungs (Van Alstine, 2019).

2.8.2. Diagnosis of *Actinobacillus pleuropneumoniae* in pigs

The ELISA test has been used to test for antibodies against *App* in previous studies (Fablet et al., 2010, 2012). However, due to its higher test sensitivity and specificity (97.8% and 100% respectively), the *App*-ApxIV Ab ELISA (IDDEXX, Westbrook, Maine, USA) was used in the present study. A recent multi-pathogen sero-survey (Dione et al., 2018b; Dione, Masembe, et al., 2016) demonstrated a high prevalence of *App* (23%) in pigs in Uganda. To date, twelve distinct serotypes of *App* and serotype variants have been identified (Marsteller and Fenwick, 1999), with

closely related antigens. Cross reactions between serovars 1, 9 and 11, serovars 3, 6 and 8 have been reported (Marsteller & Fenwick, 1999). The App-ApxIV Ab ELISA test (IDDEXX) used in the present study detects all serotypes of *App.*

2.8.3. Treatment of *App* infections in pigs

Treatment of *App* is by use of appropriate antibiotics (Amoxycillin, tetracyclines or potentiated sulphonamides) in water or feed, which have been shown to be effective (Van Alstine, 2019a).

2.8.4. Control and prevention of *App* infections in pigs

Management practices play a big role in the prevention of *App* disease in pigs. Attention must focus on adequate stocking, ventilation, hygiene and prevention of opportunistic viral co-infections. In-feed administration of tilmicocin (Pulmotil, Elanco) is highly effective against *App* and can be used in the treatment of acute disease and for the control of chronic outbreaks (White, 2018).

2.8.5. Clinical signs of Mycoplasma hyopneumoniae (M. hyo)

M. hyo is the most common and important respiratory pathogen associated with porcine respiratory disease (PRDC) complex (Thacker et al., 1999). PRDC is characterized by pneumonia, slow growth, fever, cough and dyspnea. The primary clinical sign due to *M. hyo* infection is a sporadic, dry, nonproductive cough The gross lesions are characterized by dark red (acute) or tan-grey (chronic) areas of cranioventral consolidation (Thacker et al., 1999). Microscopically, *M. hyo* infection alone causes a pneumonia characterized by perivascular, peribronchial and peribronchiolar cuffings, pneumocyte hypertrophy and alveolar inflammation, predominated by macrophages, alveolar cells and neutrophils (Calsamiglia et al., 1999). However, concomitant infections of *M. hyo* with *P. multocida* induces high fever, severe cough and dyspnea with extensive exudative lung lesions (Ciprián et al., 1988).

2.8.6. Diagnosis of *M. hyo* infections in pigs

Of the serological methods routinely used to detect *M. hyo* in live animals, ELISA is the most sensitive (Bereiter et al., 1990). Sera were screened for presence or absence of *M. hyo* antibodies using an ELISA assay according to manufacturer's instructions (IDDEXX, Westbrook, Maine, USA).

2.8.7. Treatment of M. hyo infections in pigs

Tetracyclines and macrolides are used for the treatment of enzootic pneumonia (Van Alstine, 2019a). The use of Valnemulin (Econor) and chlortetracycline combination has been found effective for treatment of *M. hyo* infections in swine. Other medications are Tetramutin and lincomycin or with chlortetracycline (Stipkovits et al., 2001).

2.8.8. Control and prevention of *M. hyo* infections in pigs

Commercial vaccines for prevention of *M. hyo* are available (Mengeling *et al.,* 2000). Vaccination strategies vary depending on the type of herd, production system and the number of vaccine doses (Maes et al., 2008; Sibila et al., 2008).

2.9. Clinical signs due to gastro-intestinal nematode infestations in pigs

Pig nematode infections contribute acute illness, which may lead to death of pigs. Indirect losses can be due to weight loss, growth retardation and small litter size (Coles et al., 1992; Stewart & Hale, 1988). Helminth infestations in pigs are characterized by coughing, unthriftiness and diarrhea (Coles et al., 1992). The present study incorporates gastro-intestinal helminth infections due to their associations with respiratory pathogens. Larval migration through the liver and lungs may induce losses due to tissue condemnation at slaughter and possible enhanced susceptibility to pneumonic pathogens (Ferraz et al., 2020; Pagot et al., 2007).

2.9.1. Treatment and control of helminth infections in pigs

Several anthelmintic drugs are available for treatment and control of helminth infections in pigs. Benzimidazoles (e.g. thiabendazole, albendazole), macrolides (ivermectin) and imidazothiazoles (e.g. levamisole) are currently in commercial use (Van Alstine, 2019). However, their use needs to be adapted to local climate conditions, depending on production systems, age and distribution of nematode species.

2.10. Pathological evaluation: assessment of clinical disease and lung lesion scores in pigs

Lung lesion scoring provides useful information for assessing risk factors for occurrence of respiratory disease, such as pneumonia (Martínez et al., 2009b) and for monitoring pig health. It is a valuable tool to assess effectiveness of control interventions. The use of lung inspections enables an investigation of the quantitative association between occurrence of pneumonic lesions and production parameters, especially average daily gain (ADG) and feed conversion (FCR) ratio (Hurnik et al., 1993).

CHAPTER THREE

3.0. GENERAL METHODOLOGY

3.1. Study Area

These studies were conducted in Lira district, mid-northern Uganda, from March to September 2019. The coordinates of Lira City are 2°14'50.0"N 32°54'00.0"E (Latitude: 02.2472; Longitude: 32.9000) (https://en.wikipedia.org/wiki/Google_Maps). The city lies at an average elevation of 1,063 metres (3,488 ft), above sea level (http://www.floodmap.net/Elevation/ElevationMap/?gi=230166).

The studies used market access to select subcounties based on value chain domains into rural production for urban consumption (R-U) and urban production for urban (U-U) consumption (Ouma 2017). In all, studies were conducted in 4 selected subcounties as follows: peri-urban (Lira municipality and Railways) and rural-urban consumption (Ngetta and Adekokwok subcounties).

3.2. Study Design

Overall, five (5) studies were undertaken as summarized below:

1. Systematic literature review: In the first study, a systematic literature review was conducted on gaps and status of research on swine respiratory pathogens in Africa. This was a desk study done to identify research gaps to inform the current study. Online search tools from *GoogleScholar, PubMed and ScienceDirect* were used. The search was limited to published articles and official reports from veterinary authorities of governments in Africa. The *Preferred Reporting Items for Systematic Review* (PRISMA, 2009) guidelines were followed during the reporting (Moher, et al., 2009). The following search terms were used: *Africa, swine or porcine, respiratory pathogens, M. hyopneumoniae, App, PCV2, PRRSv, IAV, economic impacts, prevention and control*, in combination. Only original articles and official reports were considered and only original studies reporting random selection of study subjects were considered. Using *Harzing's Publish or Perish* software, this scoping review identified 133 published articles from which 74 articles were retained for the review.

2. Following the systematic literature review, three (3) cross-sectional studies were conducted as summarized below:

   i. **Study two (Objective one)** focused on establishing prevalence and herd management risk factors for co-infections of pigs with selected respiratory pathogens and gastro-intestinal parasites. This was done in ninety (90) randomly selected farms. For this study, blood samples were collected from pigs to establish exposure to selected pathogens using ELISA tests. Fecal samples were collected and screened to establish infections with 2 important gastro-intestinal parasites (*Strongyles spp* and *Ascaris spp*). A semi-structured questionnaire was designed, pretested, and administered to household heads. The questionnaire was used to collect farm husbandry practices associated with co-infections. For analysis, logistic regression was performed to identify risk factors for respiratory co-infections and cluster analysis was done to identify and characterize farms based on housing, biosecurity, husbandry practices and pathogens. **Study three (Objective two)** was undertaken in three (3) purposely selected slaughter slabs. At post-mortem, tissue samples were collected from grossly affected (with visible lung lesions) tissues (lungs, kidneys, liver and lymph nodes) and preserved in RNALater tissue stabilization solution. Lungs were extracted from the thoracic cavity and scored for possible pneumonia forms as previously described (Halbur et al. 1996). Upon securing export permit from the Directorate of Animal Resources Ministry of Agriculture Animal Industry and Fisheries, and import permit from the Directorate of Veterinary services, Republic of Kenya, samples were transported to ILRI campus Nairobi where RNA extraction was performed, followed by reverse transcriptase quantitative PCR (RT-qPCR) to identify circulating PRRSv genotypes. Descriptive statistics and Chi square tests were done to compare lung lesion scores and presence or absence of PRRSv genotype.

   ii. **Study four (Objective three)** correlated serologic responses to selected respiratory pathogens and pneumonic lesions in pigs brought for slaughter. In this study, a slaughter slab survey was undertaken in three (3) purposely selected slaughter slabs in Lira district. At ante mortem, pigs were identified, and live weight measurements taken. Blood samples were collected, sera prepared and later screened for exposure to select

respiratory pathogens using ELISA assays as per manufacturer's instructions. At post motem, lungs were extracted from the thoracic cavity and scored for possible pneumonic lesions as previously described (Halbur et al. 1996). Wilcoxon rank sum tests and regression analysis were done to compare total pneumonia scores and serologic status to each selected respiratory pathogen.

iii. **Study five (Objective four)** was longitudinal and conducted in a repeated measures design – in randomly selected farms and pigs. This study sought to quantify economic losses in form of average daily live weight gains (ADGs) and estimated financial losses encountered by farmers due to exposure of pigs to the studied pathogens. For this study, blood samples were collected from pigs and tested using ELISA, while fecal samples were collected to establish infection with two important gastro-intestinal parasites (*Ascaris spp* and *Strongyles spp*). Other pig level data were collected: live weight measurements (kg), body condition scores (BCS), pig age, sex and respiratory clinical disease scores. Farm level data – on feed types used, house characteristics (floor, wall and roof types), hygiene level, income from pig sales as well as treatment costs incurred. A mixed effects model was fitted with farm as a random effect to quantify economic (ADGs) and financial losses encountered by farmers due to exposure of pigs to selected pathogens.

3.3. Laboratory diagnostic assays used

i. Enzyme-linked immunosorbent assays (ELISA)

ELISA tests were prerformed on sera collected from pigs. Sera were screened against all the 4 respiratory pathogens (PRRSv, PCV2, *M. hyo* & *App*) as per manufacturer's instructions for each test kit.

ii. Detection of gastro-intestinal (GIT) helminth infestations in pigs

Fecal samples collected from pigs were analyzed using a Baermann method and a modified McMaster methods to identify helminths species and quantify nematode eggs in faecal samples, respectively (MAFF, 1986a).

iii. RNA extraction was done using the AllPrep DNA/RNA Mini Kit (cat. no. 80204) according to the manufacturer's protocol (Qiagen®, Denmark).

iv. ***A real-time (quantitative) reverse transcriptase PCR PCR (RT-qPCR)*** was performed in the same laboratoryusing the KiCqStart(R) OneStep Probe RT-qPCR ReadyMix™ Low ROX™ (SigmaAldrich®). Real-time RT-qPCR and complementary DNA synthesis were performed in a GeneAmp® PCR System 7500 Fast version 2.3 (Applied Biosystems®).

3.4. Ethical approvals

Following approval of this thesis, the following ethical approvals were secured from the relevant institutions:

i. Makerere University College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB) – IRB # SBLS/REC/18/008 (August 2018)

ii. Two (2) research ethical approvals were secured from International Livestock Research Institute (ILRI): Institutional Animal Care and Use Committee (IACUC 2018-22 – in September 2018) and Institutional Research Ethics Committee (IREC-2018-23 (November 2018)

iii. Uganda National Council of Science and Technology (Permit # A590) – (September 2018)

All the ethical approvals are enclosed in this thesis as appendices 1 – 2(i-iii)

To begin with, a systematic review of the status and gaps of research on swine respiratory pathogens was necessary to identify research gaps to inform (future) areas for research, to which this doctoral study aimed to address. The systematic review is presented in **Chapter four (4)** of this thesis.

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CHAPTER FOUR

4.0. SYSTEMATIC REVIEW: STATUS AND GAPS OF RESEARCH ON RESPIRATORY DISEASE PATHOGENS OF SWINE IN AFRICA

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Porcine Health Management

REVIEW Open Access

Status and gaps of research on respiratory disease pathogens of swine in Africa

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4.1. Abstract

Over the last two decades, the pig population in Africa has grown rapidly, reflecting the increased adoption of pig production as an important economic activity. Of all species, pigs are likely to constitute a greater share of the growth in the livestock subsector. However, constraints such as respiratory infectious diseases cause significant economic losses to the industry. The increasing intensification of pig production, fueled by rapid population growth, calls for improvements in management and biosecurity to minimize disease impacts. The extensive production systems predominant in Africa, result in high potential for disease transmission. However, reliable information on prevalence and incidence of economically important swine respiratory pathogens in pigs in Africa is lacking because of limited research in the area. Such knowledge is necessary to guide interventions for prevention and control. In this review, we highlight the occurrence and distribution of five economically important swine respiratory pathogens: porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae (M. hyopneumoniae), Actinobacillus pleuropneumoniae* (*App*) and swine influenza A viruses (IAV). Of these five key respiratory pathogens, PCV2, PRRSv and IAV have been more researched, while for *App* and *M. hyopneumoniae* only few reports were found in the literature. This review highlights knowledge and information gaps on epidemiologic aspects as

well as economic impacts of the various pathogens reported in swine in Africa, which calls for further studies.

Key words: Africa, pig production, epidemiology, PRRSv, PCV2, *M. hyopneumoniae, App*, IAV.

4.2. Introduction

Pig production accounts for a large share of growth in the livestock subsector worldwide (FAO, 2014). The growing global human population creates an increased demand for animal source foods. To meet this demand, pigs are one of the preferred species due to their efficient feed conversion and fast growth rates (FAO, 2014). Accordingly, there has been a substantial increase in the volume of pig meat produced (38% of the world livestock meat consumed) in the last 20 years (FAO, 2018), often associated with intensification of production and increased movement of pigs between countries.

In Africa, the top three countries in terms of pig population are Nigeria with 7.49 million (FAOSTAT, 2019), followed by Uganda, 4.23 million (UBOS, 2018a) and Malawi, 3.65 million (FAOSTAT, 2019). The trends show an increase in imports and exports of pigs and pork, with South Africa being the leading exporter of pigs to other African countries (FAOSTAT, 2019). While pig production offers immense opportunities for both commercial and smallholder producers, the industry faces several constraints (Afolabi, 2017a, Dione et al., 2014) Transboundary diseases such as African swine fever (ASF) pose a threat to international trade, livelihoods and food security due to its high economic impact. The growing trade with potentially sub clinically infected carrier animals or contaminated vehicles, constitutes a risk of disease spread between countries. Besides ASF, respiratory pathogens such as porcine reproductive and respiratory syndrome virus (PRRSV), porcine circovirus type 2 (PCV2), *Mycoplasma hyopneumoniae (M. hyopneumoniae), Actinobacillus pleuropneumoniae* (*App*) and swine influenza A viruses (IAV) are widespread and are known to account for economic losses (M. M. Dione et al., 2014; Drew, 2011; OIE, 2008) due to pig mortalities, reduced growth, poor feed conversion efficiency and reduced reproductive performance (Rushton et al., 1999).

PRRS is a multifactorial, viral infectious disease of swine with important economic implications described worldwide (Alarcon et al., 2013). Infection causes late-term abortions, a high incidence of stillborn, mummified fetuses, early embryonic death and infertility (Osorio et al., 2002; Yu et

al., 2015). Clinical disease due to PRRS infections in grower – finisher pigs manifests as fever, anorexia, tachypnea and/or dyspnea (Halbur et al., 1995). The economic effect of PRRS infections in pig herds is due to deaths, reduced daily weight gain and feed efficiency and reproductive losses (Nathues et al., 2017).

PCV2 infection in pigs is recognized as a principal cause of post weaning multisystemic wasting syndrome (PMWS) (Allan & Ellis, 2000; Segalés et al., 2004). PMWS is a multi-factorial syndrome (Gillespie, Opriessnig, Meng, & Pelzer, 2009) characterized by weight loss, labored respiration with coughing and dyspnea, and a dark-colored diarrhea (Kekarainen & Segale, 2013; Opriessnig et al., 2007). Clinical expression requires involvement of other agents, such as pathogens of the porcine respiratory disease complex (PRDC), or husbandry and environmental stressors, as PCV2 infection alone may not lead to overt clinical disease (Kekarainen & Segale, 2013; Opriessnig et al., 2007). Economic losses associated with PCV2 infections include post-weaning mortality (Baekbo et al., 2012), reproductive disorders and poor growth (Chae, 2005). Concurrent infection with PCV2 and other respiratory pathogens has been linked to increased severity and duration of pneumonia (Thacker et al., 1999). Another study showed that the greatest economic losses due to PCV2 infections occurs in swine herds suffering from subclinical infections (Alarcon et al., 2013).

Swine influenza outbreaks in pigs are characterized by a sudden onset of high fever (40.5-41.5 °C), anorexia, huddling, tachypnea and coughing (OIE, 2015). The disease is caused by swine influenza A viruses, subtyped based on hemagglutinin and neuraminidase proteins. The common subtypes identified in pigs include H1N1, H1N2 and H3N2 (OIE, 2019). The economic impact of the disease is associated with weight loss in affected pigs and reproductive failure (OIE, 2019).

M. hyopneumoniae is the etiological agent of swine enzootic pneumonia (EP), a chronic debilitating disease characterized by a mild, dry nonproductive cough (Thacker, 2004). *M. hyopneumoniae* contributes to the porcine respiratory disease complex (PRDC). A study showed that the average daily weight gain (ADG) of pigs experimentally inoculated simultaneously with *M. hyopneumoniae* and PCV2 was reduced by 90 grams between 63 to 133 days post inoculation and the mortality increased by 14% (Kim et al., 2011). *M. hyopneumoniae* often occurs as a co-infection with viral or other bacterial agents such as PRRSv or *P. multocida*, increasing the

likelihood of the development of severe disease and subsequent high economic losses (Brockmeier et al., 2001; Ciprián et al., 1988).

Actinobacillus pleuropneumoniae (App) is one of the most important causative agents of porcine pleuropneumonia, an economically important disease of global distribution. The economic consequences of porcine pleuropneumonia can be severe and are mainly due to death, reduced average daily weight gain and feed conversion ratios, and intervention costs (Gottschack, 2012). The main clinical features of acute *App* infection are depression, fever, anorexia, coughing and dyspnea (Gottschack, 2012), while the chronic form is characterized by fibrous adherences between the lungs and the pleural cavity, caused by pleuritis and lung abscesses (Gottschack, 2012). The daily weight gain of *App*-affected pigs may be reduced by up to 33.6% (Wallgren, et al., 1999). However, variations between studies produce incoherent results, largely due to differences in design, husbandry systems, and environmental factors, which influences disease severity and growth (Hill et al., 1993; Straw et al., 1989).

While swine respiratory pathogens have been sporadically reported in some African countries, knowledge and information on their distribution, genetic diversity and economic impact remains scanty. The purpose of this review is to compile existing knowledge on the occurrence and distribution of key respiratory pathogens of pigs in Africa, their economic impacts and to provide an update on the status of research and knowledge to target future research on pig health and production.

4.3. Materials and methods

4.3.1. Literature search strategy

Search tools from online biological databases in GoogleScholar, PubMed and ScienceDirect were used to search for published articles using the Preferred Reporting Items for Systematic Review (PRISMA 2009) guidelines (Moher, et al., 2009). We searched databases for published papers that reported descriptive and analytic studies, conference proceedings and other official reports. From published papers and reports, information on research status, spatial and temporal distribution of the five targeted respiratory pathogens of pigs in Africa were compiled. Full text articles and/or those with abstracts, all published in English were considered for this review.

4.3.2. Inclusion and exclusion criteria

Based on reported economic importance on swine productivity, five key swine respiratory disease pathogens were identified*: M. hyo*, *App*, PCV2, PRRSv and IAV. In the initial screening, the title and abstract of full text articles and/or abstracts displaying the following search terms were considered: Africa, swine or porcine, respiratory pathogens, *M. hyopneumoniae*, *App*, PCV2, PRRSV, IAV, economic impacts, prevention and control, in combination. Only papers that reported on the presence of swine respiratory pathogens in Africa and that were relevant for the review were retained. The quality criteria used for the selection of articles were based on study design, laboratory methods used and data analysis methods. Only articles that reported observational studies and undertook random selection of study animals were considered. Apart from those review papers that describe epidemiologic characteristics of selected pathogens which were retained, the rest were excluded as they could not present original data. The search was limited to papers published from January 1995 to December 2018. All selected articles were manually checked, and duplicates removed.

4.3.3. Results

Altogether, 133 articles and other information sources that reported swine respiratory pathogens were identified. Figure 4.1 provides a summary of the systematic review.

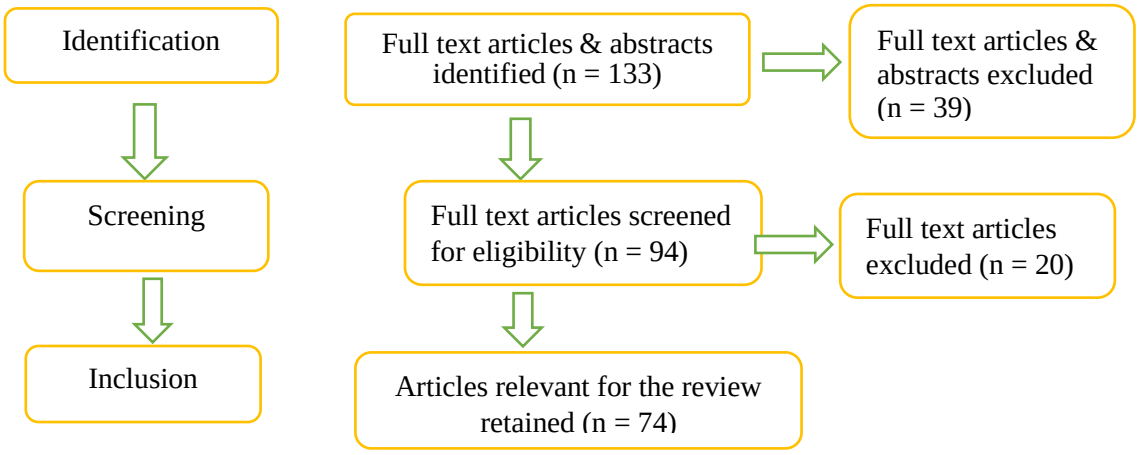

Figure 0.1: A PRISMA flow chart used for the systematic literature review

In total, 74 epidemiological studies were retained for this review. Of these, only 42 (56.7%) studies reported occurrence of selected respiratory pathogens in swine in Africa. Out of the 42 studies, only 17 (40.5%) demonstrated the actual presence of specific pathogens (based on immunohistochemistry or PCR), while most studies 25 (59.5%) were based on serologic assays, implying exposure of pigs to these pathogens or closely related strains. Figure 4.2 visualizes locations where the five pathogens of interest were reported.

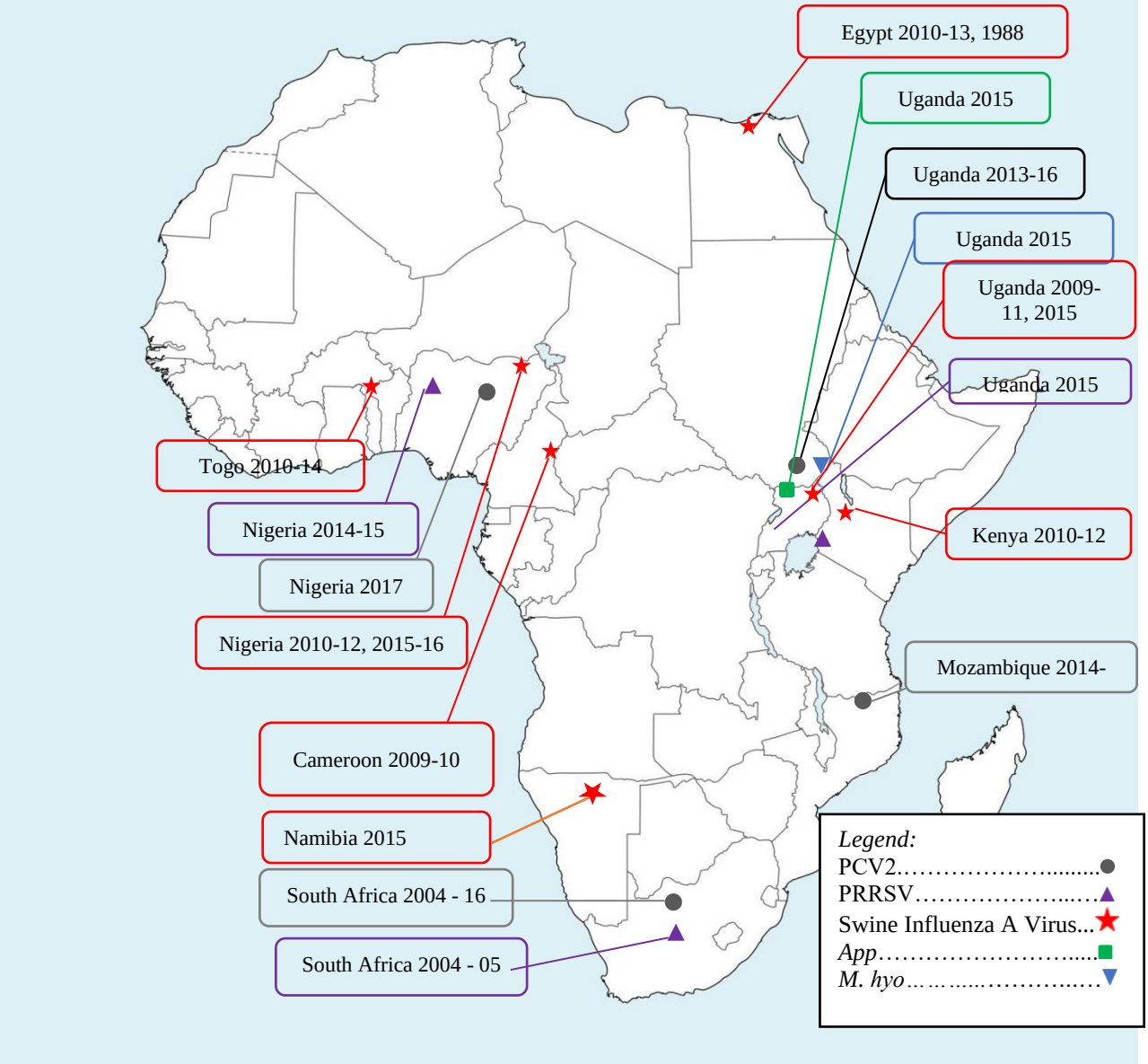

Figure 0.2: Map of Africa showing the reported occurrence of targeted respiratory pathogens of swine.

Of the 42 studies that reported occurrence of targeted respiratory pathogens in Africa, only 54.8% (n=23) were prevalence studies, as presented in Table 4.1 below, while the rest (45.2%, n=19) only reported the occurrence and molecular characteristics.

Table 0.1: Summary of the prevalence of swine respiratory pathogens in Africa

Pathogen reported	Country	Prevalence (%)	Sample size	Diagnostic method(s)	Year	References
PRRSv	Uganda	1.5	522	ELISA	2018	Dione et al. 2018 (36)
	Uganda	1.3	684	ELISA	2018	Dione et al. 2018 (36)
	South Africa	NA	NA	-	2004	OIE, 2004 (34)
	Nigeria	53.8	368	ELISA	2017	Aiki-Raji et al. 2017 (72)
	Nigeria	33.3	129	RT-qPCR	2018	Meseko et al. 2018 (39)
PCV2	South Africa	15.9	339	PCR, sequencing	2017	Afolabi et al. 2017b (46) OIE, 2004 (34)
	Nigeria	1.4	364	ELISA	2018	Aiki-Raji et al. 2018 (52)
	Uganda	12.0	25	IHC, PCR	2013	Ojok et al. 2013 (45)
	Uganda	77.0	91	RT-PCR	2013	Jonsson, 2013 (41)
	Mozambique	54.0	111	PCR, sequencing	2018	Laisse et al. 2018 (51)
	Uganda	25.0	25	IHC, PCR	2018	Eneku et al. 2018 (44)
	Uganda	4.2	522	ELISA	2018	Dione et al. 2018 (36)
	Nigeria	90.1	91	HI	2010	Adeola et al. 2009 (53)

Swine influenza A	Nigeria	8.0	75	ELISA	2015	Adeola et al. 2015 (54)
	Ghana	10.0	50	ELISA	2015	Adeola et al. 2015 (54)
	Togo	2.5-12.3	325	RT-qPCR	2012	Ducatez et al. 2015 (60)
	Ivory Coast	0	498	RT-PCR	2009-10	Couacy-hymann et al. 2012 (58)
	Benin	0	1112	RT-PCR	2009-10	Couacy-hymann et al. 2012 (58)
	Nigeria	31.0	227	RT-qPCR, HI	2014	Meseko et al, 2014 (56)
	Nigeria	14.0	50	HI	2009	Adeola et al. 2009 (53)
	Uganda	4.9	522	ELISA	2018	Dione et al. 2018 (36)
	Egypt	1.67- 4.6	240	HI, RT-PCR	2010	El-Sayed et al. 2010 (62)
	Egypt	2 - 4	93	HI, ELISA	2013	El-Sayed et al. 2013 (63)
	Uganda	1.4	511	RT-PCR	2014	Kirunda et al., 2014 (65)
	Kenya	16.9	759	ELISA	2015	Munyua, 2015 (66)
	Kenya	15.9	1084	ELISA	2018	Munyua et al. 2018 (67)
	Nigeria	33	129	RT-qPCR	2018	Meseko et al. 2018 (55)
	Cameroon	2.0	104	RT-PCR	2012	Njabo et al. 2012 (57)
	Nigeria	31.0	227	RT-qPCR, HI	2014	Meseko et al, 2014 (56)

App	Uganda	22.8	522	ELISA	2018	Dione et al. 2018 (36)
M. hyo	Uganda	9.9	522	ELISA	2018	Dione et al. 2018 (36)

Key: HI=Haemagglutination Inhibition; IHC=Immunohistochemistry; RT-PCR= Reverse Transcriptase Polymerase Chain Reaction; RT-qPCR = Reverse transcriptase real-time PCR.

4.3.4. Porcine reproductive and respiratory syndrome virus (PRRSv)

The first official report of PRRSV was from South Africa in June 2004 when 2,407 pigs from 32 farms were slaughtered in the Western Cape province (OIE, 2004). Two small outbreaks were reported in 2007 from the same area (Njeumi, et al., 2007). A recent report suggests Ugandan pigs were exposed to PRRSV, with an estimated seroprevalence of 1.3% (Dione et al., 2018). In West Africa, a serological study in Nigeria found 3 out of 221 (1.45%) samples testing positive for PRRSV antibodies by ELISA (Meseko & Oluwayelu, 2014), while another study in Southwest Nigeria reported a seroprevalence of 53.8% (Aiki-Raji, et al., 2018) and 33.3% (Meseko et al., 2018). Accordingly, most African countries, to date have never reported outbreaks of PRRSV, its economic impact or investigated the seroprevalence (OIE, 2018).

4.3.5. Porcine circovirus type 2 (PCV2)

The status of PCV2 is unknown in many countries of sub-Saharan Africa (Afolabi, Iweriebor, Okoh, et al., 2017; Jonsson, 2013b). In a study to unravel the transmission patterns of PCV2 at the wildlife-livestock interface in Murchison Falls National Park in Uganda, 91 pigs were sampled and screened for PCV2 antibodies (Jonsson, 2013b). This study revealed a prevalence of 77% of PCV2b, a genotype associated with PMWS (Ssemadaali et al., 2015). Other studies in Uganda reported a PCV2 overall seroprevalence of 45.2% (n=236) in Masaka and Lira districts (Dione et al., 2018) and 25% (n=5) of clinically sick pigs from four districts in central Uganda (Eneku et al., 2018). A study by Ojok et al. confirmed the presence of the PCV2 genotype as PCV2b by PCR and IHC (Ojok et al., 2013). Although limited by sample size (n=35), this study demonstrated the occurrence of PCV2 in Ugandan pigs, as has been shown by others (Jonsson, 2013b).

In the eastern Cape province of South Africa (Afolabi et al., 2017) reported a prevalence of 15.9% by PCR, with two distinct genogroups (PCV2b and PCV2d) identified by genome sequencing. In 2001, a study by Drew et al. (2004) confirmed the presence of PCV2 in pigs with clinical signs of

PWMS. They concluded that the PCV2 strain found in South African pigs is believed to originate from North America (Drew et al., 2004). PCV2d is reportedly a highly infectious genogroup associated with high virulence in pigs (Patterson et al., 2011). The occurrence of 2 genogroups (PCV2b and PCV2d) in South African pigs suggests a possibility for the emergence of new genotypes by natural recombination, as has been demonstrated to occur between PCV2a and PCV2b viruses (Cheung, 2009; Olvera et al., 2007). In Southern Mozambique, a recent study aiming to characterize PCV2 genotypes found that PCV2 DNA was detected in 62 out of 111 (54%) samples tested and 23 out of 31 (78%) farms (Laisse et al., 2018). This study revealed the presence of three PCV2 genotypes (PCV2b 1A/B & PCV2d) and suggested that different PCV2 genotypes circulate in Mozambican pigs. However, the number of pigs sampled in some districts was too low (average 12 pigs per district, range 2-26 pigs) to allow extrapolation to the whole pig population in Mozambique. A higher within-herd prevalence of PCV2 (78%) probably suggests the widespread occurrence of the virus in other swine-producing districts in Mozambique. In Nigeria, a recent serological study revealed a PCV2 prevalence of 1.4% in pigs (Aiki-Raji et al., 2018a). For most countries, the status of PCV2 remains unknown, confirming that overall, PCV2 is poorly studied in most of Africa (Ojok et al., 2013). In the published literature on Africa, no papers were found on the economic impact of PCV2 infection on pig production. Although studies have demonstrated economic losses to the swine industry in industrialized production systems, their findings are difficult to extrapolate to less intensive production systems predominant in Africa.

4.3.6. Swine influenza A viruses (IAV)

We found four studies from Nigeria investigating the occurrence and geographical distribution of IAV in pig populations, which confirmed the presence of H1 and H3 subtypes (Adeola et al., 2009; Adeola, Olugasa & Folitse, 2019; Meseko & Oluwayelu, 2014) and revealed the concurrent circulation of H1 and H3 in pigs (Meseko et al., 2014). One study reported from a sentinel surveillance program found a prevalence of 13.7% (n=31) of influenza A in pigs that presented with influenza-like illness, and of the isolates identified, 18% were of pandemic A/H1N1/2009 subtype (Meseko et al., 2014). In another study, Adeola et al. (2009) reported that H1N1 and H3N2 influenza A subtypes were isolated from 14% (n=7) of apparently healthy Landrace pigs in Oyo State, southwestern Nigeria (Adeola et al., 2009). A recent study showed that 44.4% (n=222) of

pigs in Nigeria were serologically positive (by ELISA) to virus nucleoprotein and that 8.4% (n=42) reacted positive by HI (Meseko et al., 2018). Influenza A virus (pandemic A/H1N1/2009) was also confirmed in Cameroon with a prevalence of 28% by a competitive ELISA assay (Njabo et al., 2012) and 88.9% of the positive pigs had high titer values (>1280). In a surveillance activity to determine the extent of circulation of influenza viruses in animals in Cote d'Ivoire, Benin and Togo, 2009-2010, Couacy-Hyman et al. (2012) found a low sero-prevalence of influenza viruses in domestic birds and pigs (Couacy-hymann et al., 2012). These three countries lie along the wild bird migratory flyways of the Mediterranean coastline, suggesting wild birds to be responsible for the introduction of HPAI into West Africa (Ducatez et al., 2007). The pandemic influenza (H1N1) was also detected by RT-PCR in pigs in Togo, with a prevalence of between 2.5 and 12.3% in pooled samples (Ducatez et al., 2016).

In North Africa, six studies documented the occurrence of influenza A viruses in pigs, and in wild and domestic birds. In Egypt, the occurrence of Highly Pathogenic Avian Influenza (HPAI) viruses (H1N1 and H5N1) subtypes was confirmed in pigs (El-Sayed et al., 2013; El-Sayed et al., 2010; Gomaa et al., 2018) which, though rare, highlights the potential for generation of new reassortant viruses following co-infection (Drew, 2011). While different IAV subtypes (H1 and H3), have been reported in pigs, little is known about the actual incidence of swine IAV. Egypt is considered a hotspot of IAV transmission since it lies along crossroads of two major wild bird migration pathways (Kim, 2018).

In Uganda, a survey using RT-qPCR, 1.4% (7/511) of the pigs tested positive for swine influenza A virus and the H1 subtype was detected in swine sera (Kirunda et al., 2014). A recent sero-survey also suggested the presence of influenza A viruses in Ugandan pigs (Dione et al., 2018). In Kenya, a cross-sectional study on influenza A viruses in domestic animals (pigs, ducks, chicken, dogs and cats), reported a sero-prevalence of 17.1% (Munyua, 2015) and 71.5% by a haemagglutination inhibition assay (Munyua et al., 2018). The same study revealed a close relationship between hemagglutinins of human and pig isolates suggesting possible re-assortment of the existing subtypes. A more recent study revealed an influenza A seroprevalence of 15.9% (172/1084) in pigs as investigated by ELISA (Munyua et al., 2018). The virus isolated from pigs (influenza A (H1N1)pdm09) was genetically similar to that of humans, suggesting that the virus was transmitted to pigs from humans (Munyua et al., 2018). In other African countries, information on influenza

A viruses in pigs was not available. Due to lack of data or studies, no published reports have documented the economic impact of IAV on swine production in Africa.

4.3.7. *Mycoplasma hyopneumoniae* (*M. hyo*)

Studies on occurrence and distribution of *M. hyopneumoniae,* the causative agent of EP, in Africa are scanty. A recent serological study conducted in the Lira and Masaka districts, Uganda suggested the occurrence of *M. hyopneumoniae* in pigs, with a reported seroprevalence of 10.1% and 20.9% in the respective districts (Dione et al., 2018). However, to our knowledge, no information is available on the genetic diversity of *M. hyopneumoniae* strains, their pathogenicity or distribution in pig populations in Africa. We did not find any published literature on the presence of *M. hyopneumoniae* or its economic impact in other African countries.

4.3.8. *Actinobacillus pleuropneumoniae* (App)

Apart from a recent, cross-sectional serological study on *App* in Ugandan pigs (Dione et al., 2018), which revealed a prevalence of 20.5% and 25.6% in the Masaka and Lira districts respectively, no other study has documented the *App* occurrence and distribution anywhere in Africa. To the best of our knowledge, no vaccination is currently being practiced against *App* in Uganda, which suggests exposure of Ugandan pigs to this pathogen. We did not find any study in Africa that characterized *App* serotypes or reported on its economic impact.

4.4. Prevention and control options for respiratory diseases of pigs in Africa

Outside Africa, a lot of research has been conducted on the development of diagnostic tools and vaccines for prevention of swine respiratory diseases. For PRRSv and PCV2, inactivated and attenuated vaccines are available (Kimman et al., 2009; Murtaugh & Genzow, 2011; Opriessnig et al., 2007). Approval for commercial applications, however, is still limited to the US, Europe, China and some Asian countries. Vaccines and therapeutic drugs for the treatment of *M. hyopneumoniae* and *App* infections are available (Maes et al., 2008; Ramjeet et al., 2008), but based on this review, these products are not routinely in use in Africa. In large parts of Africa, the use of vaccination is constrained by problems of vaccine accessibility, costs, distribution channels as well as limited cold chain facilities. Despite being the major pig producers, information on vaccine types used against PCV2 or PRRSv in Nigeria, Uganda, Malawi and South Africa was not available. In

general, knowledge gaps exist on the identity of circulating genotypes/strains, for which specific vaccine types can be targeted.

4.5. Discussion

This review compiled research on the occurrence and distribution of swine respiratory pathogens in Africa. The review only included studies published in English and accessible scientific journals online and may thus have missed papers in French or other languages and did not include "grey" literature. The studies retrieved were mainly undertaken in Nigeria, Egypt, South Africa and Uganda. This is surprising given the importance of the pig sector in other countries such as Kenya and Mozambique and the significant number of pig populations in several West African countries. Of the targeted pathogens, most studies focused on IAV, followed by PCV2 and PRRSv. Serological evidence of *M. hyo* and *App* were only reported from Uganda. The focus on swine IAV highlights its importance for public health. However, the distribution, genetic diversity, as well as the economic impacts of these pathogens is largely unknown, emphasizing the paucity of data and information.

In addition, sample sizes used in most studies may be insufficient to extrapolate findings at a national level. And several studies focused on a small number of provinces or districts in Nigeria (Adeola et al., 2019; Aiki-Raji, Adebiyi, & Oluwayelu, 2018; Aiki-Raji et al., 2017) and Uganda (Dione et al., 2018). Another important issue is the lack of multi-pathogen surveys, with only one study addressing several diseases (Dione et al., 2018). As shown by Dione et al. (2018), co-infections are however common, and that up to 68.9% (n=162) of the pigs studied in the Lira and 51.9% (n=149) in the Masaka districts of Uganda, tested positive for at least two pathogens. Multi-pathogen surveys are also important in attempts to estimate the burden of disease or to assess the impact of disease complexes such as porcine respiratory disease complex (PRDC).

While studies have documented the occurrence of PRRSv and PCV2 in various African countries, knowledge on the epidemiology remains scanty. In addition, no published studies exist on the economic impacts of PRRSV and PCV2 on pig production and productivity in Africa. Other reports also reveal that PCV2 is grossly underreported (Afolabi et al., 2017; Ojok et al., 2013), which highlights knowledge gaps on the status of PCV2 and PRRSv, and their occurrence, distribution and circulating genotypes.

M. hyopneumoniae and *App* have hardly been studied in Africa, and thus it is likely that their role for swine health and productivity is underestimated. Due to limited studies, information on their distribution, identity of pathogenic strains or their economic impact on pig productivity is unavailable. Epidemiological databases on the distribution of *App* serovars, Apx toxins, as well as approved diagnostic protocols are thus urgently needed (Sassu et al., 2018).

With respect to swine influenza viruses, we found significant knowledge gaps on circulating viral subtypes, and their spatial and temporal distribution in Africa, which call for further epidemiologic studies (Meseko & Oluwayelu, 2014). Most of the IAV studies were conducted in response to the swine influenza pandemic in 2009 and most likely were largely driven by public health risk concerns. While the prevalence of swine IAV found for Africa were low compared to other regions, which may be due to the low population density of pigs, more research on the distribution of influenza A subtypes is needed. Studies on IAV suggest that close linkages at the human-swine-bird interface in West Africa, has implications for continuous virus circulation and possible reassortment of human, swine and avian IAV subtypes, which justifies enhanced surveillance efforts in the region (Adeola et al., 2009).

Beside vaccination, biosecurity measures remain the best methods for prevention of pathogen entry into a herd, including respiratory pathogens (Alarcon, Rushton, Nathues, et al., 2013). Importantly, the success of vaccination strategies requires evaluation of technical and socio-economic aspects in the context of local production systems, which calls for further studies. However, the predominant production systems in Africa are characterized by poor farm biosecurity and lack of vaccination programs against economically important diseases, resulting in a high disease burden. The lack of data limits any attempts to estimate the economic losses caused by these diseases. In general, swine diseases are not considered a priority for surveillance, which hampers the estimation of their contribution to losses at national level and in turn does not provide evidence for need of more investment in swine health. In contrast, a lot of research and surveillance efforts have focused on African swine fever (ASF) due to its high mortality and absence of a vaccine.

4.6. Conclusions and recommendations

This review highlights critical research gaps on potentially economically important respiratory pathogens of pigs in Africa, to an extent that makes it impossible to estimating their impact, let alone providing convincing evidence that warrants more attention to these disease problems through intervention. Moreover, surveillance systems in many African countries are poor, due to limited manpower and infrastructure capacity and if available, focus on single diseases, such as ASF, instead of undertaking a more holistic approach that would allow to gauge the breadth of pig diseases and their impact and thus providing better insights to target interventions. In view of the potential devastating effects of these diseases, the need for further epidemiological studies in African pigs cannot be overemphasized.

This review highlights apparent gaps in research which calls for future epidemiological investigations. In Uganda, other than a cross sectional serologic study by (Dione et al., 2018b), no other study was found that carried detailed investigations on swine respiratory pathogens. Given the above research gaps, it was necessary to conduct detailed epidemiologic investigations on farm management factors that could be associated with respiratory pathogens, as a basis for future interventions. This is the focus of **Chapter five (objective one),** which is to determine prevalence and farm level management risk factors for co-infections of respiratory pathogens and GIT parasites in smallholder pig production systems in Lira district, Uganda.

4.7. Declarations

4.7.1. Competing interests

The authors declare that they have no competing interests

4.7.2. Funding

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4.7.3. Authors contributions

PO and MD conceived the study; PO conducted the literature search and wrote the first draft of the manuscript; EG, BW, MJ, MMD, FNM and JE contributed to the interpretation of findings. and writing of the manuscript. All authors read and approved the final manuscript.

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CHAPTER FIVE

5.0. OBJECTIVE ONE

5.1. CO-INFECTIONS OF SELECTED RESPIRATORY PATHOGENS AND GASTRO-INTESTINAL PARASITES IN SMALLHOLDER PIG PRODUCTION SYSTEMS IN UGANDA: PREVALENCE AND RISK FACTORS

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5.2. Abstract

A cross-sectional study was conducted from October to December 2018 to assess the exposure of important pathogens such as porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome virus (PPRSv), *Mycoplasma hyopneumoniae* (*M. hyo*), *Actinobacillus pleuropneumoniae* (*App*) and gastro-intestinal (GIT) parasites in smallholder pig production systems in Lira district, northern Uganda. A structured questionnaire was used to collect information on management and biosecurity practices associated with these co-infections. A total of 90 farming households were included in the study and 259 pigs aged 2.5-15 months were sampled. Sera were screened for antibodies against four pathogens using commercial ELISA tests. The Baerman's method was used to identify parasite species in faecal samples. Logistic regression was done to identify risk factors for infection. A cumulative link mixed model was fitted to estimate the odds of husbandry practices on occurrence of respiratory co-infections, with farm as a random effect. At a threshold of $p<0.05$, nine variables were selected for cluster analysis, using a hierarchical K-means (hkmeans) partitioning algorithm. Individual animal seroprevalence of PCV2 was 6.9% (95% CI 3.7–11.1), PRRSv 13.8% (95% CI 8.8 – 19.6), *M. hyo* 6.4% (95% CI 3.5–10.5) and *App* 30.4% (95% CI 24.8–36.5). The prevalences of GIT parasites were: *Ascaris spp* 12.7% (95% CI 8.6-16.8), *Strongyles spp* 16.2% (95% CI 11.7-20.7) and *Eimeria spp* 56.4% (95% CI 50.3-62.4). Pigs infested with *Ascaris spp* were more likely to test positive to PCV2, with odds ratio (OR) of 1.86 (CI 1.31-2.60; p=0.0002). Routine use of preventive drugs (anthelmintics & antibiotics) reduced the risk of PRRSv infection (OR 0.12, p=0.001). For *M. hyo*, infection with *Strongyles spp* was a risk factor (OR 12.9, $p<0.001$). Pigs that had parasite infestations were more likely (*Strongyles spp.* and *Ascaris spp.* ORs 3.5 and 3.4, $p<0.001$, respectively) to have respiratory

co-infections. Overall, 3 farm clusters were identified based on housing, biosecurity practices and mixed pathogens. Farms with poor hygiene and drainage level showed a higher likelihood of respiratory co-infections. This study provides further evidence that improved housing, hygiene and biosecurity is critical in reducing pathogen incidence in herds and their associated impacts.

5.3. Introduction

In Uganda, pig production has grown rapidly in recent years from approx. 0.7 million pigs in 1990 to 4.2 million in 2017 (UBOS, 2018). This reflects a rise in the demand for pork (Ouma et al., 2014), which offers significant opportunities to pig producers for livelihoods improvement. In Uganda's current production systems, the lack of implementation of biosecurity measures constitute key factors for the spread of swine diseases such as African swine fever (Dione et al., 2016; Muhangi et al., 2014; Muhanguzi et al., 2012). Previous studies reveal that among diseases, respiratory and gastro-intestinal (GIT) helminth infections are common in Ugandan pigs contributing to the disease burden and thus affecting productivity in the sector (Ikwap et al., 2014; Roesel et al., 2017). In Lira district, Uganda, a recent multi-pathogen study revealed occurrence of *Mycoplasma hyopneumoniae (M. hyo)*, *Actinobacillus pleuropneumoniae (App)*, *Leptospira spp.*, porcine reproductive and respiratory syndrome virus (PPRSv) and porcine circovirus (PCV2) type 2 (Dione et al., 2018). Other studies confirmed presence of PCV2 in Ugandan pigs (Eneku et al., 2018; Jonsson, 2013; Ojok et al., 2013). Three main production systems are identified: farrow to finish, farrow to wean and wean to finish. In some farms, pigs are often not segregated by age groups and are fed together. Coupled with low biosecurity, this exposes pigs to infectious diseases. In Uganda, no pig vaccines were available, and no vaccination was carried out against any pig disease during this study.

However, there is no information on pathogen co-infections in pig herds and associated management factors. This hampers efforts for the design of effective interventions for improvement of biosecurity at farm level. Evidence from previous studies shows *Metastrongylus spp.* and *Ascaris spp.* compromise lung function due to the damage induced by their migratory larvae, thereby exacerbating the effect of other viral and bacterial agents (Adedeji et al., 1989a; Van Alstine, 2019). This interaction increases disease duration and/or severity, with associated negative effects on productivity (Thacker et al., 1999). This study was designed to (i) identify risk factors for co-infections with respiratory pathogens (ii) investigate associations between pathogens

occurrence, farm management and biosecurity practices, with a view to inform control and preventive measures at herd level.

5.4. Materials and methods

5.4.1. Study area

This study was conducted in Lira district, mid-northern Uganda, where the International Livestock Research Institute (ILRI) had previously implemented a smallholder pig value chain development project (SPVCD) since 2011. In this project, a value chains assessment was conducted to select study sites using pig density, poverty levels and market access (Ouma, 2017). This study used market access to select subcounties based on value chain domains into rural production for urban consumption (R-U) and urban production for urban (U-U) consumption (Ouma, 2017). The total pig population in Lira district was estimated to be 30,000 in 2020 (*DVO personal communication)*. Pigs are produced under housed, tethered and free-range systems (Kungu et al., 2019). Under these systems, pigs are housed in permanent or temporal structures made of cement, wood or papyrus. Tethering is when pigs are tied on a rope (on a pole) to graze around the homestead, while free-range is when pigs are allowed to freely roam in the neighborhood in search of their own feeds and water. Routine preventive measures such as anthelmintics are generally not practiced, until pigs show visible signs of illness.

5.4.2. Study design, sampling of subcounties, parishes and villages

A cross-sectional serologic study was conducted from October to December 2018. We used multistage sampling to select subcounties and villages. In the first stage, four subcounties were selected (from a total of 9): two (Central division and Railways) representing U-U consumption and two (Adekokwok and Ngetta) representing R-U consumption. In stage two, two (2) villages with the highest pig density were selected for the study.

5.4.3. Sample size determination

To determine the sample size, a formula for simple random sampling was used (Dohoo et al., 2003a). A previous study in Lira district found a seroprevalence of *M. hyo* in pigs of 20.9% (Dione et al., 2018b). *M. hyo* was selected as it is considered the most important bacterial pathogen.

Adjusting for test sensitivity and specificity, true prevalence was computed to be 24%. The required sample size of pigs was obtained from equation (1):

$$n = Z^2_{\alpha/2} pq/d^2 \text{---------- } Eq\ (1)$$

where n = is the required sample size; Z_α is the standard z-score from a normal distribution (1.96), p = estimated prevalence of disease (24%) and q = 1-p (76%); d = allowable error (6%). Using this formula, an unadjusted sample size of 195 pigs was computed. To adjust for within-farm clustering, we sampled 3 pigs per herd, thus the design effect (*Deff*) was obtained from equation 2 below:

$$\text{Deff} = 1 + icc\ (n_1 - 1) \text{---------- } Eq\ (2)$$

where icc is the intra-cluster corrrelation (0.2) for respiratory disease (Dohoo et al., 2003a), n_1 is the number of pigs sampled per herd (3), thus the *Deff* calculated is 1.4. The adjusted sample size was calculated from the equation: N = n_1 x number of pigs sampled per cluster/herd (3). From this, the adjusted sample size of 273 pigs was derived.

5.4.4. Sampling of farms and pigs

In each selected village, a list of pig keeping households/farms was obtained from the district veterinary office and the area local councils. Random sampling of farms was done, until the required sample size was obtained. We sampled farms regardless of health status or anthelmintic treatments to examine differences in farm husbandry practices and how these influence occurrences of specific pathogens in farms. Using a sampling frame of all pig farmers generated with field research assistants, one (1) to three (3) pigs per herd were sampled until the required sample size was reached. Only pigs ≥2.5 months old were selected for sampling, since pigs below that age are reported to retain maternal antibodies to PCV2 and PRRSv post weaning, which could interfere with serologic tests (Gillespie, Opriessnig, Meng, Pelzer, et al., 2009; Opriessnig et al., 2004b). *App* acquired colostral antibodies were reported to decay within 2 months postpartum (Vigre et al., 2003). We sampled pigs from 2.5 months and above regardless of health status, clinical signs or feed types given to pigs.

5.4.5. Data collection methods

A structured questionnaire with closed questions was designed, pre-tested by the first author in Mukono district and revised before use. Research assistants were trained in its use before it was administered to each household head or farm manager. To ensure consistency, all questionnaire

questions were translated to a local language spoken in the area (Langi). The questionnaire captured data on potential risk factors for infection with respiratory pathogens.

5.4.6. Blood sample collection and storage

Each pig was properly restrained as described in the ILRI Standard Operating Procedures (SOPs) manual, section 2, part (c) & (d) (ILRI, 2004). Smaller pigs (2-25 kg) were restrained by hand, while larger ones (>25 kg) were restrained with a metallic pig catcher (*Model BZ002, MG. Livestock, Shandong, China*) placed behind the upper incisor teeth and the snout raised upwards. Blood was then collected from the cranial vena cava or jugular vein, using a 21G, 1.5" needle into plain 5 mL BD® vacutainer tubes. The tubes were labeled with animal identification details (serialized) and placed in an ice box at 4-6 °C. After collection, samples were delivered (within 3 hours) to the district veterinary laboratory for temporary storage. Blood samples were left to stand at room temperature (20°C) overnight and serum harvested the following day into 2 mL cryotubes (Sarstedt®, Germany), labelled and stored in a fridge at -20°C until testing.

5.4.7. Serological analysis of sera

In the lab, sera were screened using ELISA assays according to manufacturers' instructions for each pathogen: *M. hyo* and *App*-ApxIV (IDDEXX, Westbrook, Maine, USA); for PRRSv and PCV2 assays (Krishgen Biosystems, India). Cut-off sample to positive ratios (S/P%) for *M. hyo* were >0.40 (positive) and <0.30 (negative), *App* were ≥ 50% (positive) and <40% (negative). PCV2 and PRRSv S/P cut-off ratios for positive and negative samples were ≥ 0.2 and < 0.2 respectively. Suspect samples were re-tested. Test sensitivity (*Se*) and specificity (*Sp*) for *M. hyo* ELISA (IDDEXX) were 85.6% and 99.67% respectively; *Se* and *Sp* for *App*-ApxIV Ab ELISA test (IDDEXX) were 97.8% and 100%, respectively. *Se* and *Sp* for PRRSv (Krishgen Biosystems, India) were 94% and 94%, while for PCV2 *Se* and *Sp* (Krishgen Biosystems, India) were 92% and 94% respectively. Test *Se* and *Sp* were used to calculate true prevalence of respiratory infections at α=0.05 significance level. The ELISA test procedure is described in the general methodology section (Chapter 3) of this thesis.

5.4.8. Faecal sample collection and analysis

Faecal samples (~ 3gr) were collected from the rectum of each pig using gloved hands into 10 mL plastic containers, labelled and placed in ice box at 4ºC. Samples were taken to the district veterinary lab for temporary storage at 4ºC. Samples were transferred to the central diagnostic

laboratory, College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University for analysis, 1-2 weeks after collection. Helminth species were identified using the Baermann method (MAFF, 1986).

5.5. Data analysis and presentation

Data was coded and entered into *Excel 16.0* and any errors in entry corrected by cross-checking with questionnaires. RStudio was used for data analysis and presentation (R Core Team, 2019a). True prevalence was computed by adjusting apparent prevalence using *prevalence 0.2.0* package in R, considering test sensitivities and specificities (R Core Team, 2019). Multivariable logistic regression analysis of risk factors for each pathogen was performed. The response variable was the ELISA test result, with predictors: pig age and husbandry practices (house type, parasites, drug use, pig mixing, hygiene score and drainage). The model below was fitted to predict a single respiratory infection, as a function of pig characteristics and husbandry practices (house type, parasites, drug use, pig age, no pig mixing, hygiene and drainage):

$$\ln \frac{\hat{p}}{(1-\hat{p})} = \beta_0 + \beta_1 x_{1i} + \beta_2 x_{2i} \ldots\ldots \beta_p x_{pi} \text{ ----------Eq (3)}$$

where $\ln \frac{\hat{p}}{(1-\hat{p})}$ is the expected log of the odds of infection, β_0 is the model intercept; $\beta_1, \beta_2,$ βi are coefficients for the respective explanatory variables. Interaction terms were tested for each model and confounding was checked by inclusion and exclusion of variables to observe a change in model coefficients. A cumulative link mixed effects (CLMM) model was fitted to estimate the odds of co-infection (2 or more pathogens) with farm as a random effect. The CLMM model from R package *'ordinal'* was used to select 9 variables for cluster analysis. Only significant variables (at $p<0.05$) were retained in the model. However, pig age and sex were dropped because they both run across all farms, which maximizes between-cluster homogeneity. A hybrid hierarchical K-means (hkmeans) partitioning algorithm was used to identify and characterize farm clusters. Chi square tests were used to examine associations between pathogens and GIT parasites. Residual plots and R-square statistics were used to assess the fitted models.

5.6. Results

In all four subcounties, a total of 259 pigs were sampled from 90 farms. Data of 12 pigs was incomplete (missing values) as such was excluded from the analysis. The median age category of

the respondents was 36-50 years, with a min-max age of 24-70 years. Of the 90 respondents, 41 (45.5%) were males while 49 (54.5%) were females. The median herd size per housed herds was 11 pigs (range 5 – 18 pigs), and for tethered herds was 4 pigs (range 1 – 7 pigs). Male pigs constituted 53.7% (n=139) of pigs in the sample, while females were 46.3% (n=120). The median age of sampled pigs was 5 months, and the age range was from 2.5 to 15 months. Table 5.1 below shows a summary of demographic characteristics.

Table 0.1: Demographic characteristics of respondents

Characteristic	Category	Males n (%)	Females n (%)	Total n (%)
Age (yrs)	18-35	16 (17.8)	21 (23.3)	37 (41.1)
	36-50	15 (16.7)	15 (16.7)	30 (33.3)
	51-75	10 (11.1)	13 (14.4)	23 (25.5)
Location / Subcounty	Adekokwok (peri-urban)	18 (20.0)	29 (32.2)	47 (52.2)
	Central division (urban)	5 (5.5)	5 (5.5)	10 (11.0)
	Ngetta (rural)	9 (10.0)	9 (10.0)	18 (20.0)
	Railways div. (peri-urban)	9 (10.0)	6 (6.7)	15 (16.7)
Education level	None	1 (1.1)	2 (2.2)	3 (3.3)
	Primary	10 (11.1)	31 (34.4)	41 (45.5)
	Secondary	15 (16.7)	7 (7.8)	22 (24.5)
	Tertiary	15 (16.7)	9 (10.0)	24 (24.7)
Occupation	Farmer	29 (32.2)	36 (40.0)	65 (72.2)
	Business	7 (7.8)	8 (8.9)	15 (16.7)
	Employee	3 (3.3)	4 (4.4)	7 (7.7)
	Others	2 (2.2)	1 (1.1)	3 (3.3)
Management system	Housed	26 (28.9)	28 (31.1)	54 (60.0)
	Tethered	15 (16.7)	21 (23.3)	36 (40.0)
Breeds kept	Local	4 (4.4)	4 (4.4)	8 (8.8)
	Exotic	7 (7.8)	5 (5.5)	12 (13.3)
	Cross-bred	28 (31.1)	36 (40.0)	64 (71.1)
	Mixed	2 (2.2)	4 (4.4)	6 (6.6)
Totals		41 (45.5)	49 (54.5)	90 (100.0)

5.6.1. Prevalence of respiratory pathogens and GIT parasites

Table 5.2 below shows true herd and individual level prevalence of selected respiratory pathogens. Results showed that *App* was the most prevalent pathogen, while PCV2 was the least prevalent at both pig and herd level. Of the GIT parasites, *Eimeria spp* was the most prevalent while *Trichuris*

spp was the least prevalent parasite found. Of the 259 pigs sampled, 54.8% (n=142) had single respiratory infections.

Table 0.2: True prevalence of tested respiratory pathogens and helminths in pigs

Pathogen	Individual pig level (n=259)		Herd level (n=90)	
	% Prevalence	95% conf. int.	% Prevalence	95% conf. int.
PCV2	3.5 (n=9)	0.4 – 7.7	23.9	15.6 – 33.1
PRRSv	14.0 (n=36)	9.1 – 19.5	32.6	23.4 – 42.6
M. hyo	6.9 (n=18)	3.8 – 10.6	16.2	9.5 – 24.6
App	30.5 (n=79)	25.0 – 36.2	45.7	35.6 – 55.9
Ascaris spp	12.7 (n=33)	9.2 – 17.3	28.9	20.5 – 38.9
Strongyles spp	16.2 (n=42)	12.2 – 21.2	34.4	25.4 – 44.7
Trichuris spp	1.5 (n=4)	0.6 – 3.9	4.4	1.7 – 10.7
Eimeria spp	56.4 (n=146)	50.3 – 62.3	81.1	71.8– 87.8

5.6.2. Prevalence of co-infections

Co-infections with two or more pathogens were observed in this study. Among respiratory pathogens, the highest prevalence of co-infections was between PRRSv and *App*, followed by between PCV2 and *App*. For co-infections between respiratory pathogens and parasites, highest co-infections occurred between *Eimeria spp*, followed by *Strongyles spp* and respiratory pathogens. Only 5 pigs were co-infected with 3 pathogens and that 42.5% of pigs sampled had at least 2 co-infections. Table 5.3 below shows a summary of co-infections.

Table 0.3: Prevalence of co-infections between respiratory pathogens and GI helminths

Pathogen	PCV2 % (n)	PRRSv % (n)	*M. hyo* % (n)	*App* % (n)	Total coinfections
PCV2	-	1.93 (n=5)	1.15 (n=3)	3.47 (n=9)	6.56 (n=17)
PRRSv	1.93 (n=5)	-	1.54 n=4)	5.80 (n=15)	9.26 (n=24)
M. hyo	1.15 (n=3)	1.54 (n=4)	-	1.54 (n=4)	4.24 (n=11)
App	3.47 (n=9)	5.80 (n=15)	1.54 (n=4)	-	10.81 (n=28)
Ascaris spp	3.08 (n=8)	3.47 (n=9)	2.31 (n=6)	5.40 (n=14)	14.28 (n=37)
Strongyles spp	2.31 (n=6)	3.08 (n=8)	3.86 (n=10)	6.17 (n=16)	15.44 (n=40)
Eimeria spp	5.40 (n=14)	11.96 (n=31)	3.86 (n=10)	21.23 (n=55)	42.50 (n=110)

Note: only five pigs (1.93%) were co-infected with 3 resp. pathogens (PCV2, PRRSv & M. hyo; PCV2, PRRSv & App; and PRRSv, M. hyo & App)

5.6.3. Multivariable logistic regression model of risk factors for individual respiratory infections

Results showed that parasite infections, pig confinement (only for *App*), drug use (anthelmintics and antibiotics) and pig age were significant predictors of respiratory infections (Table 5.4). Drugs used by farmers were antibiotics, anthelmintics and multivitamins.

Table 0.4: Multivariable logistic regression model of risk factors for individual respiratory infections

Pathogen	Variable	Coeff	Std error	OR	95% CI	z-value	p-value
PCV2	(Intercept)	-2.4358	0.3214	0.087	0.044 - 0.157	-7.578	3.52e-14***
	Ascaris spp. infection	1.5590	0.4949	4.753	1.738 - 2.382	3.150	0.00163**
	No pig mixing	-0.690	0.485	0.501	0.182- 1.260	-1.421	0.155
PRRSv	(Intercept)	-2.530	0.726	0.079	0.017 - 0.307	-3.485	0.000***
	Pig age	0.181	0.069	1.199	1.046 - 1.378	2.614	0.008**
	Herd size (>20 pigs)	0.016	0.005	1.016	1.005 - 1.028	2.935	0.003**
	Hygiene score_1	1.003	0.584	2.728	0.953 - 9.949	1.716	0.086.
	Hygiene score_2	0.030	0.775	1.031	0.216 - 4.938	0.039	0.968
	Drug use	-2.125	0.669	0.119	0.028 - 0.406	-3.176	0.001**
	Farmer sex (fem.)	-1.207	0.430	0.299	0.126 - 0.692	-2.803	0.005**
	Drug use*farmer sex (females)	2.021	0.937	7.550	1.116 - 47.929	2.157	0.031*
M. hyo	(Intercept)	-3.5154	0.4771	0.0297	0.010-0.068	-7.369	1.72e-13***
	Strongyles spp infection	2.5628	0.5824	12.971	4.300-44.171	4.401	1.08e-05***
	Drainage	-0.7957	0.6885	0.4512	0.096-1.570	-1.156	0.248
App	(Intercept)	-2.881	0.50961	0.056	0.019 - 0.145	-5.654	1.56e-08 ***
	Housed	1.138	0.358	3.122	1.591 - 6.541	3.178	0.00148 **
	Pig age > 6 months	0.12313	0.055	1.131	1.014 -1.263	2.205	0.02745 *
	Eimeria spp infection	0.793	0.303	2.212	1.232 - 4.061	2.620	0.00880 **

*Drugs used by farmers: antibiotics, anthelmintics and multivitamins. Hygiene score_0= poor, hygiene score_1= moderate; Hygiene score_2=best. Hygiene score was made by physical observation of the level of hygiene in pens during sampling. *=p<0.05, **=p<0.01, ***=p< 0.001.*

5.6.4. A cumulative link mixed model for mixed respiratory infections with farm as random effect

Table 5.5 below shows a cumulative link mixed model (from R packages *'ordinal, factoextra'*) of factors for respiratory co-infections with farm as a random effect. This model was fitted to predict co-infections (regardless of pathogens involved), as this has synergistic effects in the induction of respiratory disease. The model shows that farmer occupation, wall, floor type and no contacts with outside pigs were protective against co-infection, while pig age, management method (confined *vs* tethered pigs) and helminth infestations increased the risks of respiratory co-infections.

Table 0.5: A cumulative link model for respiratory co-infections in pigs

Independent variable		Estimate	Std. error	OR	95% CI	z-value	p-value
Thresholds	0|1	3.02	0.69	20.62	5.26 - 8.08e+01	4.34	-
	1|2	5.55	0.77	257.54	56.80 - 1.16e+03	7.19	-
	2|3	7.68	0.91	2185.51	366.93 - 1.30e+04	8.44	-
Occupation_2		-1.14	0.40	0.32	0.14 - 7.08e-01	-2.81	0.004**
Occupation_3		-1.82	0.69	0.16	0.04 - 6.27e-01	-2.63	0.008**
Occupation_4		-0.38	0.79	0.68	0.14 - 3.20e+00	-0.48	0.626
Pig age		0.20	0.05	1.23	1.10 - 1.38e+00	3.57	0.0003***
Herd size		0.01	0.00	1.01	1.00 - 1.02e+00	2.95	0.003**
Floor type cement		-1.14	0.45	0.31	0.13 - 7.79e-01	-2.50	0.012*
Floor type raised		-1.51	0.65	0.22	0.06 - 7.95e-01	-2.31	0.020*
Wall type 1 (mud)		2.88	0.81	17.91	3.66 - 8.76e+01	3.56	0.0003***
Wall type 2 (timber)		1.10	0.56	3.03	1.00 - 9.13e+00	1.97	0.048*
Wall type 3 (bricks)		0.35	0.48	1.42	0.55 - 3.66e+00	0.73	0.468
Hygiene score 1		0.83	0.46	2.31	0.93 - 5.73e+00	1.80	0.070.
Hygiene score 2		0.96	0.59	2.62	0.81 - 8.48e+00	1.61	0.106
Cleaning frequency 1		0.83	0.50	2.30	0.85 - 6.20e+00	1.65	0.099.
Cleaning frequency 2		1.88	0.48	6.58	2.54 - 1.70e+01	3.89	0.000***
Cleaning frequency 3		1.46	0.44	4.32	1.80 - 1.04e+01	3.27	0.001**
No contacts		-0.78	0.45	0.45	0.18 - 1.10e+00	-1.73	0.082.
Strongyles spp		1.25	0.37	3.50	1.68 - 7.29e+00	3.35	0.0008***
Ascaris spp		1.23	0.42	3.45	1.49 - 7.95e+00	2.91	0.003**

*For categorical independent variables, higher or better quality were coded with higher values; contacts with outside pigs was coded as (1=no, 0=yes); Occupation_2=business, Occupation_3=employee, Occupation_4=other; continuous variables (pig age, herd size) were entered as counts. Differences in practices between farms account for the random effect. OR=Odds ratio, *= $p < 0.05$, **= $p < 0.01$, ***= $p < 0.001$.*

5.7. Cluster analysis

In this method, the hierarchical K-means initially uses a K-means algorithm to determine the number of clusters, and then employs it to perform hierarchical clustering (Tung-Shou et al., 2005). The method generates a tree-like dendrogram and a cluster plot used to visualize the clusters. Results of the cluster analysis revealed three distinct clusters of farms (Fig 5.1).

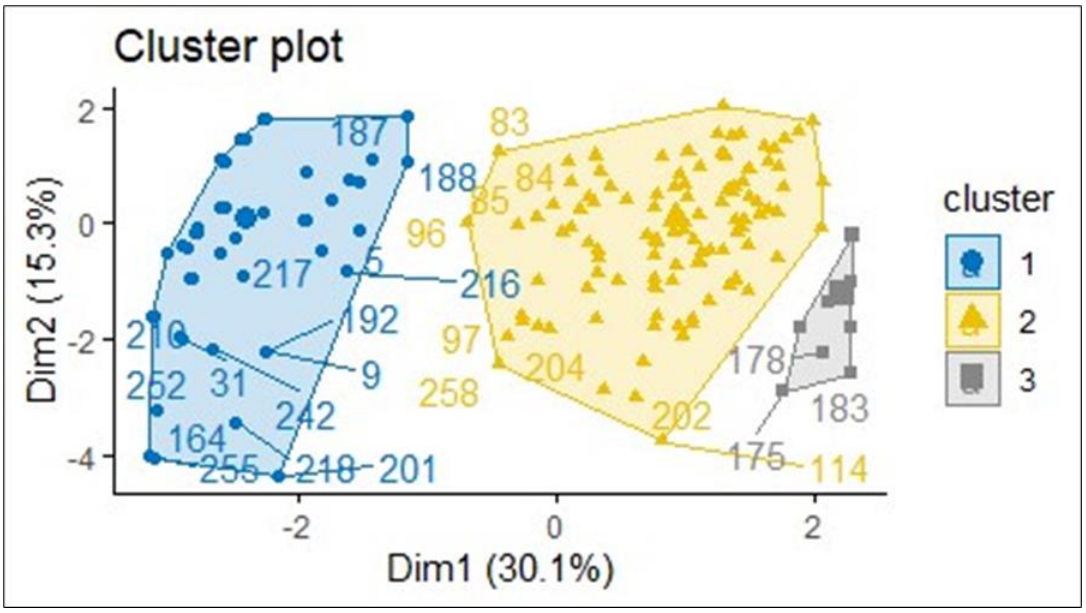

Figure 0.1: Three clusters of farms identified by husbandry practices, biosecurity level and respiratory pathogens. Management system, house characteristics, drainage systems, biosecurity level and pathogens were used to characterize farm clusters

Table 5.6 below shows a description of the 3 clusters revealed by the algorithm.

Table 0.6: Summary of description of farm clusters identified

Cluster #	Cluster category	No. of farms, n (%)	Farm cluster characteristics
One	Housed, cemented floors or raised wooden platforms; good housing, biosecurity level group	27 (30.0)	These had low herd sizes (mean ±SD = 7.5±4.0), had cemented floors or raised wooden platforms, good biosecurity, best floors, good drainage systems and hygiene scores. Compared to cluster 2, these were sparsely clustered together. Farms in cluster 1 were least affected by mixed parasites and respiratory pathogens.
Two	Tethered, poor "housing" and	56 (62.2)	These farms tether pigs, characterized by low herd sizes (mean ±SD, 5.0±3.3), poor biosecurity (poor hygiene,

	biosecurity level group		drainage). Farms in this cluster were more affected by *M. hyo,* had high mixed parasites *(Ascaris spp, Strongyles spp)* infestations. Farms in this cluster were most predominant and closely packed together
Three	Housed, cemented floors, poor to moderate housing, biosecurity level group	7 (7.7)	They had highest herd sizes (mean ±SD, 78±54), floor types were mostly made of concrete, had poor hygiene scores and drainage systems, and lower frequency of cleaning. These farms were fewer, kept younger pigs (compared to cluster 1 & 2 farms) and affected most by mixed respiratory infections. However, cluster 3 farms were least affected by parasite infections, but more affected by *App.* Compared to those in cluster 1 and 2, cluster 3 farm characteristics were unique and were sparsely clustered.

The following variables were used to define biosecurity level: fencing (1=present or 0=absent); use of disinfectant(s) on the farm (1=yes, 0=no), rearing of different age groups in the same pen (0=yes or 1=no), cleaning frequency (0=Never, 1=1-2times/week, 2=3-4 times/week, 3=daily), isolate sick pigs (1=yes or 0=no). The total biosecurity level/score was computed as the sum of individual variable scores with higher codes given to "best" or recommended practices.

5.8. Associations between respiratory pathogens and GIT parasites

There were significant associations between pathogen co-infections (PCV2, PRRSv, *M. hyo* and *App*) and parasites (*Ascaris spp, Strongyles spp*). At individual pathogen level, significant associations were observed between respiratory pathogens and GIT parasites. Table 5.7 below shows a summary of Chi squared tests.

Table 0.7: Chi squared tests of associations between respiratory pathogens and GI parasites

Response variable	Predictors	χ^2	df	p-value
Mixed pathogens	Mixed parasites	44.787	6	5.16e-08***
PCV2	Mixed parasites	-	2	0.007335**
PRRSv	Mixed parasites	-	2	0.4714
M. hyo	Mixed parasites	-	2	7.513e-06***
APP	Mixed parasites	5.078	2	0.07891
PCV2	*Ascaris spp.*	9.8567	1	0.001692**
	Strongyles spp.	1.3653	1	0.2426
PRRSv	*Ascaris spp.*	1.4748	1	0.2246
	Strongyles spp.	4.02e-31	1	1
M. hyo	*Ascaris spp.*	26.017	1	3.385e-07***
	Strongyles spp.	8.1978	1	0.004194**
App	*Ascaris spp.*	2.2624	1	0.1325
	Strongyles spp.	1.2353	1	0.2664

**= p < 0.05, **= p < 0.01, ***= p < 0.001*

5.9. Discussion

These results highlight widespread occurrence of selected respiratory pathogens in pigs in the study area. At both individual and herd level, *Actinobacillus pleuropneumoniae (App)* was found to be of highest prevalence, followed by porcine reproductive and respiratory syndrome virus (PRRSv), porcine circovirus type 2 (PCV2) and lastly, *Mycoplasma hyopneumoniae (M. hyo*. These findings are comparable with those from a recent study (Dione et al., 2018). However, compared to the findings of other studies (Eneku et al., 2018; Jonsson, 2013; Ojok et al., 2013), our study found a lower PCV2 seroprevalence. This may be due to differences in the sampling procedures, diagnostic methods used and the type of production system from which pigs were sampled. This study was conducted in peri-urban and rural smallholder production setting in which pigs were confined in temporary or permanent pig sheds or tethered around homesteads, while (Jonsson, 2013) sampled pigs from a wildlife-livestock interface (near Murchison Falls national park), which probably exposed them to a higher risk of infection from other roaming or wild pigs. Eneku et al., (2018) sampled pigs that presented with clinical signs of PCV2 and therefore had a higher probability of PCV2 detection while another research team (Ojok et al., 2013) sampled pig tissues from a local abattoir.

The PCV2 seroprevalence at individual pig and herd level found in this study was lower than (54% and 78%, respectively) reported in Mozambique (Laisse et al., 2018). Differences in the sampled population could account for variations in PCV2 prevalence, as the Mozambican study was done in slaughter places, which were likely to be older animals compared to the pigs sampled in this study. The results from this study also show that PCV2 seroprevalence was higher (6.9% vs 1.4%) than that found in Nigeria (Aiki-Raji et al., 2018b), lower than (15.9%) that was found in South Africa (Afolabi et al., 2017).

This study revealed a higher PRRSv seroprevalence compared to the previous findings (Dione et al., 2018). This could suggest either increased herd to herd transmission over the past few years, since PRRSv transmission can occur via several routes (Otake et al., 2010), and/or because the virus can remain in affected herds as a persistent infection (Murtaugh & Genzow, 2011). The finding that the odds of testing seropositive to PPRSv rises with increase in pig age is in consonance with findings from previous studies which showed that neutralizing and anti-PRRSv IgG antibodies can remain persistent for several months (Murtaugh et al., 2002; Nelson et al.,

1994). The increased odds of seropositivity to PRRSv due to lack of regular preventive treatments was demonstrated in this study. The role of PRRSv in inducing severe disease during co-infections with other pathogens has been previously reported (Halbur et al., 1996; Thacker et al., 1999). This suggests regular prophylactic treatments are important in reducing the risk of opportunistic co-infections. A similar observation was made for *App*, in which infection was dose-dependent, accounting for increased incidence in older pigs (Marsteller & Fenwick, 1999).

The effect of herd size was highlighted in this study. The observation that larger herds (> 20 pigs) increased the odds of PRRSv infection may be related with increased stocking density, as PRRSv is known to be highly infectious. The increase in the odds of PRRSv detection may also be due to its tendency to remain as a persistent infection after entry into a herd (Pileri & Mateu, 2016).

Co-infections in this study were lower than in other studies (Gillespie, Opriessnig, Meng, Pelzer, et al., 2009). Co-infections between PRRSv and *App* were the most prevalent, followed by PCV2 and *App*. The effect of PCV2 co-infection with other pathogens in increasing the severity and incidence of PCV2-associated disease has been reported in previous studies (Fablet et al., 2012; T Opriessnig et al., 2004; Segalés et al., 2013). Other studies reveal a diversity of pathogens involved in respiratory disease (Qin et al., 2018). The cumulative link model showed that better floor types (cement or raised platform) had protective effects on co-infections (Table 5). Similarly, changing from the use of mud to timber as material for the wall significantly reduced the odds of co-infections. Hygiene score and the frequency of cleaning of pens (while protective on single infections) did not appear to have any effect on the odds of co-infections. Probably some farmers did not provide honest answers to a question on how often they cleaned pig pens weekly.

This study revealed associations of particular respiratory pathogens and GIT parasite infections in pigs. The Chi square tests showed significant correlations between mixed parasites and pathogen co-infections and also between individual parasites and respiratory pathogens (Table 7). These results are comparable with findings from a study in southwest Uganda which reported a high prevalence of GIT helminth infections (Roesel et al., 2017b). The increased odds of *Ascaris spp* infection in tethered pigs illustrates the importance of biosecurity (e.g., confining pigs) in reducing the risk of infection. *Ascaris spp.* has been shown to compromise lung function through immunomodulatory mechanisms, thereby exacerbating the effect of other viral and bacterial agents, as well as increase disease severity (Adedeji et al., 1989b; Brewer & Greve, 2011).

While pigs infected with *Eimeria spp* may show no observable clinical signs, *Eimeria spp* have been reported to cause diarrhea in piglets as they damage intestinal mucosa increasing susceptibility to other pathogens.

In general, results showed that biosecurity variables had a major influence on pathogen occurrence in farms. The finding that *M. hyo* is correlated with PRRSv and mixed parasite infections confirms the significance of their associations (Table 5). Also, a positive correlation between PCV2 and mixed parasites infections probably explains why farms in cluster 3 with poor hygiene and low frequency of cleaning were more likely to have mixed pathogen infections (Tables 4 and 7). These findings are in consonance with previous studies which revealed that good housing, hygiene and reduced stress play a significant role in minimizing the effects of diseases such as PCVAD (Cargill, 2019; Gillespie, Opriessniget al., 2009). In contrast, farms in clusters 1 (Table 6, Figure 1), which had the best hygiene and biosecurity scores, were less affected by mixed pathogens. Farms in cluster 2 (free range) which had poor hygiene and biosecurity scores were mostly affected by GIT parasite infestations. Poor drainage and hygiene in cluster 1 and 2 farms may have raised the risk of re-infections with parasite eggs from contaminated feeds and water. In addition, the higher parasite infestations observed in cluster 1 and 2 farms may also have been due to lack of implementation of routine preventive measures such as deworming (compared to cluster 3 farms). These environmental stressors (poor hygiene, rearing of different age groups in the same pens, overcrowding and poor nutrition, etc.) are known to suppress immunological responses and therefore impede a pig's ability to fight off infection (Cargill, 2019). The role of good ventilation and proper cleaning practices in improving indoor air quality by reducing microbial contamination with respiratory pathogens has been documented (Banhazi et al., 2008; Cargill, 2019). Overall, the results of the cluster analysis agree with the logistic regression models which demonstrated the importance of improved hygiene and biosecurity in reducing mixed respiratory infections.

5.10. Conclusions and recommendations

This study highlights widespread occurrence of economically important respiratory pathogens in pigs in the study area. This may likely reflect the situation in swine herds in eastern and northern Uganda, where production systems are largely similar. The negative correlations between biosecurity variables and mixed pathogens signifies the role of improved biosecurity in reducing the risks of co-infections. In addition, the clustering of farms of poor biosecurity level with

pathogens provides further support to the above evidence. Further studies to identify PCV2 and PRRSv genotypes that circulate in pigs in this region, as well as quantify their economic impacts on swine productivity are warranted to guide the design of effective interventions.

Having understood herd level management risk factors for occurrence of selected respiratory pathogens and GI parasites, I investigated associations between pneumonia and serology to selected respiratory pathogens. This involved a slaughter slab survey. This study generated information on the potential role of respiratory pathogens in lung pathology in slaughtered pigs. This is the focus of the next **Chapter Six (Objective two)** of this thesis.

5.11. Strengths and limitations of the study

The principal investigator oversaw all aspects of data collection, laboratory analysis of samples, data entry and analysis, ensuring quality assurance. However, potential bias may have been introduced by inaccurate responses provided by some respondents to some questions in the questionnaire and misclassification errors of pigs due to imperfect assay sensitivities and specificities. Also, the time lapse between faecal sample collection and lab analysis reduces the sensitivity of the Baerman's test, as ability of larvae to hatch was reduced. The detection of antibodies may not reflect actual infection because antibody responses occur earlier than GIT helminth infections at the time of sampling. This may have led to overestimation of the observed relationships.

5.12. Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

5.13. Data Availability Statement

The original contributions presented in the study are publicly available. The dataset analyzed for this study can be found here**:** https://data.ilri.org/portal/dataset/multipathogen-survey-and-risk-factors.

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CHAPTER SIX

6.0. OBJECTIVE TWO

6.1. CORRELATIONS BETWEEN LUNG PNEUMONIC LESIONS AND SEROLOGIC STATUS FOR KEY RESPIRATORY PATHOGENS IN SLAUGHTERED PIGS IN NORTHERN UGANDA

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6.2. Abstract

Background: Several infectious agents are associated with pneumonia lesions in pigs. To establish the potential contribution of selected respiratory pathogens to pneumonia, it is important to assess lungs at slaughter. This information is useful to guide health management interventions.

Methods: A cross-sectional slaughter slab study was conducted in Lira district, Uganda, to (i) determine the prevalence and severity of pneumonia and (ii) establish relationships between pneumonia types and serological status for key respiratory pathogens. Using enzyme-linked immunosorbent assays (ELISAs), sera were screened for antibodies against *Mycoplasma hyopneumoniae* (*M. hyo*), *Actinobacillus pleuropneumoniae* (*App*), porcine reproductive and respiratory syndrome virus (PRRSv) and porcine circovirus type 2 (PCV2). At postmortem, lungs were grossly assessed for pneumonia types and pneumonic lesions. Pneumonia types were characterized as catarrhal purulent bronchopneumonia (CPBP), pleuropneumonia (PLP) and pleuritis. The percent of lung surface affected by pneumonia was determined by estimating the affected surface area of each lung lobe. Each lobe was assigned scores based on the approximate volume represented and the total percentage of lung surface affected obtained as a sum of individual lobe scores. *Metastrongylus spp.* helminth infection was determined by examining lungs for gross presence or absence. RStudio was used for data analysis and presentation. Wilcoxon rank sum tests were used to compare total median pneumonia scores and serostatus for each studied pathogen. An ordinal logistic regression model was fitted to evaluate the odds of multiple pneumonia forms, with pathogen serostatus and *Metastrongylus spp.* infection as predictors.

Results: One hundred sixty-seven (n=167) lungs were examined for pneumonia forms. The prevalences of CPBP, PLP and pleuritis were 29.9% (95% CI 22.9–36.9), 74.2% (95% CI 67.5–

80.9) and 17.3% (95% CI 22.4–36.3), respectively. The true prevalence of PCV2 was 9.7% (95% CI 4.5–16.8), that of PRRSv was 7.5% (95% CI 2.7–14.2), that of *M. hyo* was 11.5% (95% CI 7.2–18.0), that of *App* was 25.1% (95% CI 18.5–38.0), and that of *Metastrongylus spp.* was 29.3% (95% CI 22.9–36.6). The odds of multiple pneumonia forms increased in pigs with multiple pathogens (ORs 2.6, p=0.01) and *Metastrongylus spp.* infestation (OR 2.5, p=0.003), suggesting synergistic effects of coinfections in the induction of lesions.

Conclusions: This study revealed a high prevalence and severity of pneumonia in slaughtered pigs. It provides baseline information and evidence for the magnitude of pneumonia associated with the studied pathogens and justifies future studies on their potential economic impacts on Ugandan pigs.

Key words: Lira, lesion scores, lungs, respiratory, pigs, pneumonia, porcine, Uganda

6.3. Background

Respiratory diseases contribute significant economic losses to swine producers worldwide through increased mortality, retarded growth rates, and reduced feed conversion and reproductive performance (Holtkamp et al., 2013; Martínez et al., 2007; Straw et al., 1990). Other losses arise from additional costs of treatment (Baekbo et al., 2012), loss of potential revenue and vaccinations (Calderón Díaz et al., 2020; Nathues et al., 2017b). Various infectious agents are associated with lung lesions at slaughter (Holt et al., 2011a). Among these agents, *Mycoplasma hyopneumoniae* (*M. hyo*), *Actinobacillus pleuropneumoniae* (*App*), porcine reproductive and respiratory syndrome virus (PRRSv), porcine circovirus type 2 (PCV2) and swine influenza viruses are the most important agents associated with gross pulmonary lesions in pigs (Fablet et al., 2012; Fraile et al., 2010b). However, other bacterial or viral agents are known to contribute significant pneumonic lesions in pigs. For example, concurrent infections of *M. hyo* with other agents, such as PCV2 or PRRSv, have been found to increase the severity and duration of mycoplasma pneumonia (Thacker et al., 1999).

To establish the contribution of respiratory pathogens to lung lesions, it is necessary to assess lungs at slaughter. This enables monitoring of herd health (Holt et al., 2011b; Scollo et al., 2017) and provides baseline information for future epidemiologic studies. One of the cost-effective methods for this purpose is abattoir surveys, as they provide a valuable source of data and information supporting herd health management decisions. Rapid gross visual and detailed lung scores are used to accurately assess the extent of pathological lesions associated with enzootic pneumonia in pigs

due to the occurrence of distinct gross lesions (Hurnik et al., 1993; Martínez et al., 2009a). Serologic and clinical evidence provides useful information on the extent and severity of pulmonary lesions. In addition, it is important for monitoring growth, as pneumonic lesions (such as pleurisy) have been associated with growth retardation in pigs (Ferraz et al., 2020; Pagot et al., 2007).

In all types of production systems, pig growth is a key productivity indicator that is affected by respiratory disease in a herd, which in turn affects herd profitability. In Uganda, no information is available on the actual extent of pneumonia, its impact on growth and any associations of lung lesions with serologic or clinical profiles in pigs, as no studies have been previously conducted. Thus, the contribution of pneumonia to the overall economic performance of swine herds cannot be estimated, which hampers the design of effective interventions. In the selection of pathogens to be included, findings of previous prevalence and disease impact studies and DISCONTOOLS (O'Brien et al., 2017) were considered. Based on these considerations, PCV2, PRRSv, *M. hyo* and *App* were prioritized. The aims of this study were to (i) determine the prevalence and severity of pneumonia lesions in slaughtered pigs and (ii) establish the relationships between pneumonia lesions and serologic status for selected respiratory pathogens detected in slaughtered pigs in Lira district, mid-northern Uganda.

6.4. Materials and methods

6.4.1. Study area and design

We conducted a cross-sectional slaughter slab survey in Lira district, mid-northern Uganda, from March to September 2019. Lira district is located at latitude 2° 14' 59.64" north and longitude 32° 53' 59.46" east. Pigs sampled in this study were sourced from within Lira district (~70%) and from the neighboring districts of Dokolo, Agago, Alebtong and Kole (~30%). The study was conducted in three purposely selected slaughter slabs in the district based on high daily slaughter capacity (range 8–20 pigs). These slabs represented approximately 60% of all pigs slaughtered in the district (*DVO, pers. comm*). The three (3) slaughter slabs were Teso Bar, Adekokwok and Amach market. In each slab, pigs brought for slaughter from different sources were randomly sampled (approx. 40%) on a given day. Visits were made during early morning when slaughters were conducted and on days when the number of slaughters were known to be high.

6.4.2. Sampling of slaughter slabs and pigs

A list of all pigs brought for slaughter was made, and each was allocated a number (on a piece of paper) from which a simple random sample was drawn. Other characteristics of pigs (live weight using a measuring tape, body condition score (BCS) and sex) were recorded antemortem. The unit of measurement was the individual pig, and the outcome variable was the presence or absence of pneumonia.

6.4.3. Determination of sample size

A recent study reported a seroprevalence of 20.9% for *M. hyo* in pigs in Lira district (Dione et al., 2018a). A review of lung scoring methods by Garcia-Morante et al. showed that 80% of pigs infected with *M. hyo* had lung lesions (Garcia-Morante et al., 2016). Using these figures, we estimate that the prevalence of pneumonia in pigs in Lira district was at least 16%. We assumed no clustering effect within a slab since pigs were purchased from different farms. To determine the prevalence of gross pneumonia, the required sample size for a 5% level of significance was derived from the equation (Dohoo et al., 2003):

$$n = Z_{\alpha/2}^2\, pq/d^2 \text{---------- } Eq.\ (1)$$

where $Z_{\alpha/2}$ is the standard normal deviation for $\alpha = 1.960$; p = estimated proportion of pigs with gross pneumonia lesions = 0.16; q = the estimated proportion of pigs with no gross pneumonia = 1- p = 0.84; and d, the effect size, is estimated to be 6% (d = 0.06). Using the above equation, a sample size of 144 pigs was computed. During this study, we sampled 167 slaughtered pigs.

6.4.4. Blood sample collection

Antemortem blood samples were collected from pigs for serum preparation. Each pig was properly restrained as described in the ILRI Standard Operating Procedures (SOPs) manual, section 2, part (c) & (d) (ILRI, 2004). Blood was collected from the jugular vein using a 21G, 1.5" needle into plain 5 mL BD® vacutainer tubes. The tubes were labeled and then placed in an ice box containing ice packs at 4 °C. After collection, the samples were delivered to the district veterinary laboratory, where they were left to stand at room temperature (20 °C) overnight. After 24 hours, sera were harvested into 2 mL cryotubes (Sarstedt®, Germany), labeled and stored in a freezer at -20 °C until use.

6.4.5. Serologic analysis of sera

Serologic assays were performed at the College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University. Sera were screened using ELISA test kits for each of the four key pathogens using the protocols described by each manufacturer: *M. hyo* and *App*-ApxIV (IDDEXX, Westbrook, Maine, USA) and PRRSv and PCV2 (Krishgen Biosystems, India). The results were computed as a sample-to-positive ratio (S/P) using the equation:

$$S/P = \frac{(\text{Sample OD} - \text{Average of negative control})}{(\text{Average of positive control} - \text{Average of negative control})} \quad \text{---------- } Eq.\ (2)$$

Cutoff sample-to-positive ratios (S/P%) for *M. hyo* were >0.40 (positive) and <0.30 (negative) and for *App* were ≥ 50% (positive) and <40% (negative). PCV2 and PRRSv S/P cutoff ratios for positive and negative samples were ≥ 0.2 and < 0.2, respectively. Suspect samples were retested.

6.4.6. Lung lesion scoring procedures

For the animals from which sera were collected, detailed scoring of lung lesions was conducted postmortem. To ensure the accuracy of data collected, records for each pig were entered into a sheet of paper at antemortem. The first author performed the lesion scoring while being assisted by a research assistant to record observations on an Excel-designed sheet. Lungs were isolated from the thoracic cavity, placed on a flat clean surface, palpated and scored for visible pneumonic and pleuritic lesions. Palpation for hardened areas of hepatization (pneumonia or pneumonia-like) was performed, and the percent involvement per lung lobe was recorded. Incisions onto the lung parenchyma using a surgical blade were made to identify and characterize any deep-seated lesions. The gross lung lesion scoring procedures for CPBP, PLP and pleuritis were performed as previously described (Martínez et al., 2009a; Wallgren et al., 1994a).

Lesions were classified as catarrhal purulent bronchopneumonia (CPBP), pleuropneumonia (PLP) or pleuritis (Taylor, 1996). CPBP is characterized by cranioventral consolidation, reddish-to-pink areas, and mucous or purulent exudate on the cut surfaces of the lung. PLP includes lesions that are typical of one or more consolidated focus, mainly in the caudal lobes, with red-to-dark areas with fibrinous pleuritis and hemorrhages and necrosis on the cut surface (Martínez et al., 2009a). Pleuritis lesions were classified into 3 grades according to severity as described in previous studies (Pagot et al., 2007; Wallgren et al., 1994b). Using this method, grade 0 represents no pleuritis, grade 1 is where up to 5% of the lung surface is affected, and grade 2 is where >5% of the lung surface is affected (adhesions between lung lobes or between lobes and the thoracic cavity,

mediastinum or pericardium). The prevalence of pneumonia and the percent of lung tissue affected by pneumonia (proportion of lung surface visibly affected by pneumonia) were determined (Halbur et al., 1995). The percentage of pneumonia-affected lung area was based on the proportion of the lung surface that was abnormally firm and discolored (Bollo et al., 2008; Mousing & Christensen, 1993; Thacker et al., 1999). To estimate the surface area grossly affected by pneumonia, the method of Halbur et al. (Halbur et al., 1995) was used. Each lobe was assigned quantitative points based on the approximate volume represented by that lobe. A maximal score of 10 was possible for each: the right cranial, right middle, left cranial and left middle lobes. The accessory lobe was assigned 5 points. For the right and left caudal lobes, a score of 27.5 points (15 for dorsal and 12.5 for ventral parts) was possible, resulting in a maximum total of 100 points for the entire lung (Halbur et al., 1995). Score values for lung area affected by pneumonia ranged from 0 to 100%. For pleuritis scores, values ranged from 0 to 2. In brief, the following parameters were calculated:

i. Percent of lung surface affected by pneumonia $= \text{Total area affected}/_{100}$---------- *Eq. (3)*

ii. Percent prevalence of pneumonia $= \frac{\text{Number of lungs affected by pneumonia}}{\text{Total number of lungs examined}} \text{ x } 100$---------- *Eq. (4)*

6.4.7. Scoring for lung helminth infestations

Gross helminth infestations (*Metastrongylus spp.*) were detected by examining the diaphragmatic lung lobes (this is the predilection site for *Metastrongylus spp*) for wedge-shaped areas during the lung scoring procedures. Incisions were made on a grossly affected lung lobe with a surgical blade, or strips of one-centimeter tissue from the edge of diaphragmatic lung lobes were trimmed and squeezed to express adult worms (slender, 30–50 mm in length) from the bronchi (Van Alstine, 2019). Infestations were scored as present (coded=1) or absent (coded=0).

6.5. Data analysis

Data were coded and entered into *Excel 16.0* (*Excel Corp, TX*). Missing data were omitted from the analysis. RStudio (R Core Team, 2019) was used to analyze and present summary statistics (proportions, medians). Two response variables were defined: pneumonia type (CPBP, PLP or pleuritis), coded (yes=1, no=0) and total pneumonia scores (range 0–100%). The presence or absence of multiple pneumonia types in each lung (coded from 0–3) was used for ordinal logistic regression analysis. Multivariable logistic regression analysis was used to assess the odds of detecting pneumonia as an outcome variable with serostatus for respiratory pathogens as

predictors. An ordered logistic regression model was fitted to evaluate the odds of detecting multiple pneumonia types as a dependent variable, with serostatus for different pathogens as predictors. Adjustment for *Metastrongylus spp.* as a potential confounder was made by checking for a change in the model coefficient at a 10% cutoff when it was excluded from the model. Wilcoxon rank sum tests were used to compare the median pneumonia lesion scores and serologic status for each pathogen at a significance level of $\alpha = 0.05$.

6.6. Results

In total, 167 pigs were sampled and examined from three selected slaughter slabs. Overall, more female pigs (55.7%) were sampled, of which 17.2% (n=16) were pregnant. Live weights varied from 26 to 184 kg, while the age range was from 5 to 50 months, based on wear of teeth. Table 6.1 below shows the summary statistics of the pigs sampled.

Table 0.1: Summary statistics of pigs sampled

Slaughter slab	No. of pigs sampled, % (n)		Age (months)	Live weight (kg)
	Males	Females	Mean ± SD	Mean ± SD
Teso Bar	21.55 (36)	28.14 (47)	13.9±9.1	58.7±31.6
Adekokwok	2.4 (4)	5.4 (9)	13.6±3.8	70.3±16.8
Amach market	20.35 (34)	22.15 (37)	14.6±6.8	68.6±27.9
Totals	44.3 (74)	55.7 (93)	14.2±8.0	63.6±30.0

SD=standard deviation

6.6.1. Seroprevalence of studied respiratory pathogens

The true prevalence of PCV2 was 9.7% (95% CI 4.5–16.8), that of PRRSv was 7.5% (95% CI 2.7–14.2), that of *M. hyo* was 11.5% (95% CI 7.2–18.0), and that of *App* was 25.1% (95% CI 18.5–38.0). The prevalence of *Metastrongylus spp.* was 29.3% (95% CI 22.4–36.6).

6.6.2. Prevalence of pneumonia

Overall, the prevalence of pneumonia was generally high in all slaughter slabs. The prevalence of gross pneumonia in the three slaughter slabs was highest in the Amach market (80.28%, 95% CI 70.68–89.90), followed by Teso Bar (79.5%, 95% CI 77.16–92.60) and then Adekokwok (69.2%, 95% CI 61.93–76.47).

6.6.3. Prevalence of pneumonia forms (CPBP, PLP and pleuritis) and other lesions observed

The prevalences of CPBP, PLP, pleuritis and lung abscesses were 29.9% (95% CI 22.9–36.9), 74.2% (95% CI 67.5–80.9), 17.3% (95% CI 11.6–23.2) and 2.39% (95% CI 0.052–4.73), respectively. Overall, PLP was the most prevalent pneumonia type observed. Approximately 30% of sampled pigs also had *Metastrongylus spp.* nematodes in the lungs.

6.6.4. Relationships between total pneumonia scores and respiratory pathogen serologic status

Figure 6.1 shows that the total median lesion scores for pigs that tested seropositive to each of the 4 pathogens were higher than those for pigs that tested seronegative. *App*-positive pigs showed significantly higher median lesion scores than *App*-negative pigs. Additionally, PCV2-positive pigs were found to have marginally higher total median lesion scores than PCV2-negative pigs. Figure 6.1 below highlights the summary statistics of the total median pneumonia scores by pathogen type.

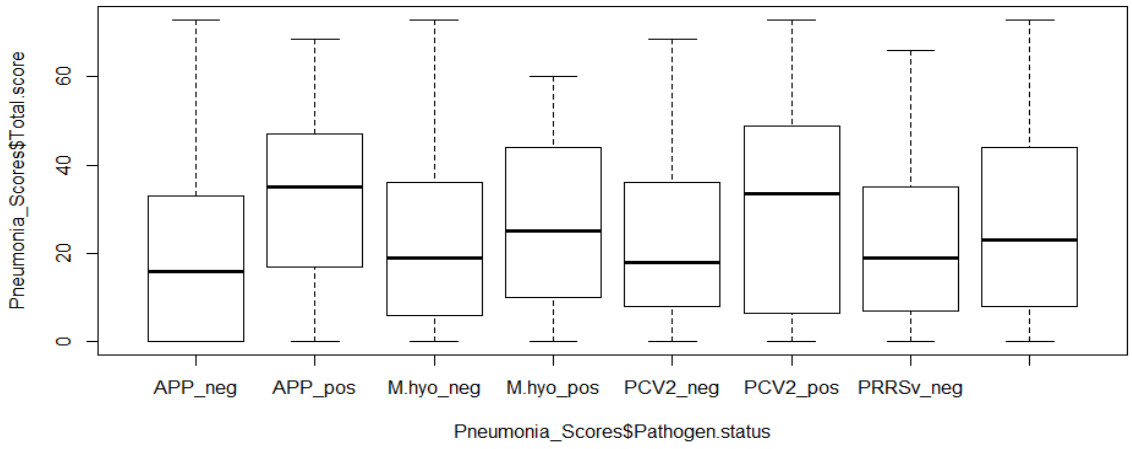

Figure 0.1: Boxplot of total lesion scores by pathogen serologic status.

6.6.5. Relationship between pneumonia types and pathogen serologic status

Table 6.2 below summarizes the relationships between serologic status and median lesion scores (MLSs) of different forms of pneumonia. The Wilcoxon rank sum tests showed significant differences in CPBP and PLP scores between pigs that tested positive and those that tested negative for PRRSv, PCV2 and *M. hyo*. For pleuritis, a significant difference in median scores was observed between pigs that tested positive and those that tested negative for *M. hyo*. Table 6.2 below shows a summary of the results.

Table 0.2: Results of the Wilcoxon rank sum tests of median lung lesion scores by serologic status

Pneumonia type	Pathogen	Wilcoxon rank sum statistic (W)	Diff. median scores	Diff. 95% CI	p value
CPBP	PCV2	11774	-7.992e-05	-7.81e-06 − -6.57e-05	0.0006268***
PLP	PCV2	5678	-0.999	-1.000 − -0.999	2.2e-16***
Pleuritis	PCV2	13527	-3.98e-05	-1.38e-05 − 5.50e-05	0.4552
CPBP	PRRSv	11523	-1.841e-05	-1.109e-05 − -2.014e-05	0.000108***
PLP	PRRSv	5427.5	-0.999	-1.000 − -0.999	2.2e-16***
Pleuritis	PRRSv	13276	-6.794e-05	-7.875e-05 − 7.91e-06	0.2209
CPBP	*M. hyo*	11189	-2.546e-05	-1.593e-05 − -2.73e-05	6.745e-06***
PLP	*M. hyo*	5093.5	-0.999	-1.000 − -0.999	2.2e-16***
Pleuritis	*M. hyo*	12942	-3.381e-05	-1.592e-05 − 5.80e-05	0.05723
CPBP	*App*	13193	-7.261e-05	-4.332e-05 − 5.17e-05	0.2697
PLP	*App*	7097.5	-0.999	-0.999 − -0.000	2.2e-16***
Pleuritis	*App*	14946	5.213e-05	-4.376e-05 − 2.098e-05	0.1074

p<0.05; **p< 0.01, p < 0.001**

Table 6.3 below presents results of the logistic regression models for pneumonia types and pathogen serology

Table 0.3: Results of the logistic regression models for pneumonia types and pathogen serology

Pneumonia type	Predictors	Estimate	Std Error	OR	95% CI	z-value	Pr (>|z|)
CPBP	Intercept	-1.262	0.241	0.282	0.172–0.446	-5.233	1.67e-07***
	App	0.540	0.384	1.717	0.799–3.638	1.405	0.1599
	Met. spp.	0.828	0.361	2.290	1.124–4.667	2.291	0.0219*
PLP	Intercept	0.645	0.202	1.906	1.291–2.859	3.194	0.00140**
	Met. spp.	1.392	0.515	4.023	1.583–2.398	2.704	0.00685**
	M. hyo	1.739	1.056	5.694	1.074–10.533	1.647	0.09956.
Pleuritis	Intercept	-1.8015	0.2647	0.165	0.095–0.269	-6.805	1.01e-11***
	PCV2	0.4978	0.5286	1.645	0.543–4.452	0.942	0.346
	App	0.5633	0.4436	1.756	0.714–4.130	1.270	0.204

*Met. spp.=Metastrongylus spp, ORs = Odds ratios, CI = Conf. intervals; ***p < 0.001, **p<0.01, *p<0.05*

6.6.6. Ordinal logistic regression model of the effect of coinfections on multiple pneumonia occurrence

Table 6.4 below shows that the odds of scoring positive for multiple pneumonia types significantly increased in pigs with concurrent *Metastrongylus spp.* and respiratory infections.

Table 0.4: Ordinal regression model for occurrence of multiple gross pneumonia types

Predictors		Coeff.	Std error	ORs	95% CI	t-value	p value
Single infection		0.3446	0.365	1.411	0.690–2.898	0.943	0.345
Coinfection (2 pathogens)		0.9876	0.416	2.684	1.192–6.125	2.373	0.0176*
Coinfection (3 pathogens)		0.2313	1.037	1.260	0.155–10.131	0.223	0.823
Metastrongylus spp.		0.9526	0.330	2.592	1.364–4.993	2.883	0.0039**
Intercepts	0|1	-1.2504	0.250	-	-	-4.989	0.0000***
	1|2	1.2995	0.248	-	-	5.234	0.0000***
	2|3	3.8065	0.444	-	-	8.563	0.0000***

**p<0.05, **p<0.01, ***p<0.001, CI = Conf. interval*

Table 6.4 above shows the effect of a single respiratory infection on the likelihood of detecting a given type of pneumonia. *Metastrongylus spp.* infestation of the lungs significantly increased the risks of occurrence of CPBP (OR=2.29) and PLP (OR=4.023). Infection with a single respiratory pathogen was not significantly associated with an increased risk of any form of pneumonia. However, coinfections with 2 respiratory pathogens and *Metastrongylus spp.* significantly increased the risks of occurrence of multiple pneumonia types (Table 6.4). Below are different

forms of pneumonia observed during the study. Figure 6.2 below shows gross pathologic forms of pneumonia detected during the study.

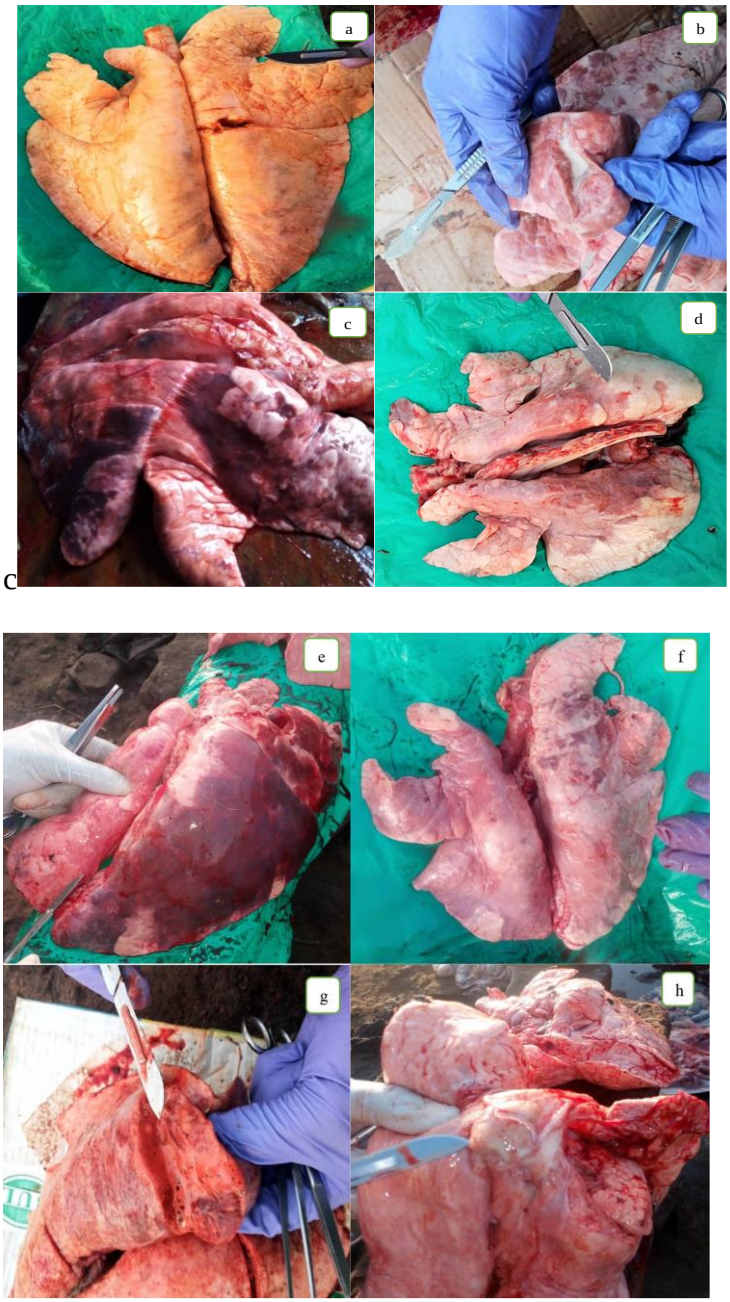

Figure 0.2: Pictures of normal lungs (a); lungs with purulent bronchopneumonia showing severe exudation (b) and cranioventral consolidation (c); lungs with diffuse interstitial pneumonia showing a rubbery texture (d); lungs with hemorrhagic bronchopneumonia showing failure to collapse (e) and pleuritis showing attachment of lung lobes (f); hemorrhagic pleuropneumonia, showing hemorrhages on cut surfaces of the lung (g) and a pulmonary abscess with thick caseous yellowish pus (h).

6.7. Discussion

This is the first study to document the magnitude of pneumonia prevalence and its relationship with respiratory pathogens in pigs in Uganda. This study revealed a high prevalence and severity of pneumonia in slaughtered pigs. The prevalence of pneumonic lesions (17.3–74.2%) in this study is comparable to that found in other studies, reported at 73.9% in Brazil (Galdeano et al., 2019). Our findings compare favorably with studies in other countries that reported pneumonia prevalence ranging from 6% to 81% (Alawneh et al., 2018; Galdeano et al., 2019; Leneveu et al., 2005; Ostanello et al., 2007; Pallarés et al., 2021; Scollo et al., 2017). Large variations in pneumonia and pleuritis (41–76% and 2–35%, respectively) have been reported in Brittany, France (Leneveu et al., 2005; Pagot et al., 2007). In Makurdi, Benue State, Nigeria, it has been reported that 36.4% of sampled pigs had lung lesions (Shima & Garba, 2014). In Ghana, it has been reported that only 5% of slaughtered pigs had pneumonia, although the form of pneumonia was not reported (Asenso et al., 2015). Differences in these studies likely reflect differences in scoring methods, production systems, hygiene and health status overall.

Of the studied respiratory pathogens, *Metastrongylus spp.* was found to be the most prevalent, followed by *App*. Our findings show that all the studied pathogens had low-to-moderate prevalence (from 7.5%-25.1%) in the study area. The present study demonstrates significant associations between pneumonia type and serologic status for the studied pathogens. Significant differences in CPBP and PLP scores were observed between pigs that tested positive and those that tested negative for 3 pathogens: PRRSv, PCV2 and *M. hyo*. Pigs that tested seropositive to *App* showed a significant difference in median PLP scores between seropositive and seronegative pigs. Figure 1 shows that *App*-seropositive pigs had significantly higher total median scores than seronegative pigs. These associations between pathogen seropositivity and lesion scores suggest their probable role in lung pathology. This finding could explain the high prevalence of pneumonia observed in this study. Our findings agree with previous studies that reported *M. hyo* as being strongly associated with lung lesions (Fano et al., 2007) and pulmonary consolidation (Maes et al., 2008). The finding that *App* was not significantly associated with pleuritis instead contrasts documented evidence that has shown *App* to be associated with fibrinous pleurisy (Marsteller & Fenwick, 1999). This discrepancy could be due to infection with *App* serotypes of low virulence since the test used detects all serotypes of *App*.

The results of the logistic regression model showed that the odds of detecting CPBP and PLP increased in pigs infested with *Metastrongylus spp*. The finding that approximately one-third of the pigs sampled had *Metastrongylus spp.* nematodes, frequently observed in the tips of diaphragmatic lobes, agrees with a previous study, which found a high prevalence of GIT nematodes in Ugandan pigs (Roesel et al., 2017b). GIT parasites such as *Ascaris suum* and *Metastrongylus spp.* are known to induce pulmonary tissue damage through their migratory larvae, increasing the susceptibility of pigs to various respiratory pathogens (Van Alstine, 2019).

This study showed that the odds of detecting multiple pneumonia forms increased with coinfections. This result strengthens previous evidence that showed that coinfections tend to produce more severe disease than single infections. This finding corroborates previous studies that have documented synergistic or potentiating effects of coinfections between PRRSv and other pathogens (Thacker et al., 1999) and PCV2 and other pathogens in the induction of respiratory disease (Fraile et al., 2010b; Opriessnig et al., 2007; Segalés et al., 2013). The ability of *M. hyo* infection to potentiate and prolong PRRSv-induced pneumonia clinically and macroscopically has been documented (Thacker et al., 1999). Notwithstanding differences in the study design by (Martínez et al., 2009a), which sampled only heavy pigs (100 kg), our study sampled pigs of varying ages and live weights from predominantly small-scale production systems. The disease progression from acute to chronic as pigs grow older may also explain the differences in the lesion scores observed. Apart from two studies in Nigeria and Ghana, there have been no other published studies in Africa (with comparable production systems) that have documented the magnitude of pneumonia prevalence in pigs. It is worth mentioning that in Uganda, no other published study or report on the magnitude of pneumonia prevalence in pigs exists. Thus, in the context of different pig production systems documented in Uganda (Ouma et al., 2013), our findings can be extrapolated only to the swine population in northern Uganda, with similar husbandry systems. This study showed that a high proportion of pigs brought for slaughter in the region presented with a high prevalence and severity of pneumonic lesions and that the association between lesions and serologic status suggests a significant contribution of the studied pathogens to lung pathology. Due to variations in the pathogenicity of *M. hyo* and *App* serotypes, further studies are required to elucidate the identity and their role in the induction of lung pathology in pigs.

6.8. Limitations of the study

The scoring methodology used in this study may have underestimated the actual magnitude of pneumonia since some pigs may have suffered early in life, and lesions could have resolved. In addition, due to the need to perform the scoring process quickly to match the slaughter speed, it is probable that some hidden lesions may have been missed. Since the ApxIV ELISA test detects all infections with *App* regardless of serotype, differences in virulence implies that not all lesions associated with *App* were correlated with positive serologic results.

6.9. Conclusions and recommendations

This is the first study to document associations of pneumonic lesions with serologic status for key swine respiratory pathogens in slaughtered pigs in Uganda. It revealed a high prevalence and severity of pneumonic lesions in slaughtered pigs in Lira district, and the association with respiratory pathogens suggests their potential contribution to lung pathology. The findings of this study establish critical baseline information for future studies on swine respiratory diseases. The high prevalence of pneumonic lesions justifies a need for future studies on the potential economic impacts of pneumonia on swine production and productivity in Uganda as a basis for designing future interventions.

Having known the potential contribution of exposure of slaughter-age pigs to respiratory pathogens to lung pathology, I sought to identify genotypes of porcine reproductive and respiratory syndrome virus (PRRSv) circulating in Lira and the neighboring districts. This is the focus of **Chapter Seven (Objective three)** of this thesis.

6.10. Declarations

6.10.1. Ethics approval and consent to participate

This study was approved by the Institutional Review Committee (IRB), College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University (IRB # SBLS/REC/18/008), Uganda National Council of Science and Technology (UNCST reg. no. A590); ILRI's Institutional Research Ethics Committee (IREC no. IREC2018-23) and ILRI's Institutional Animal Care and Use Committee (IACUC2018-22). Prior informed consent was obtained from district local authorities and owners of slaughter slabs before the study commenced.

6.10.2. Availability of data and materials

The datasets generated and analyzed during the current study are available at this link: https://data.ilri.org/portal/dataset/multipathogen-survey-and-risk-factors. We used the STROBE-VET checklist (https://strobevetstatement.files.wordpress.com/2016/09/strobe-vet-checklist.pdf) in the preparation of this manuscript.

6.10.3. Competing interests

The authors declare that they have no competing interests.

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CHAPTER SEVEN

7.0. OBJECTIVE THREE

7.1. MOLECULAR IDENTIFICATION OF PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME VIRUS GENOTYPES IDENTIFIED FROM SLAUGHTERED PIGS IN NORTHERN UGANDA

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7.2. Abstract

Background: Porcine reproductive and respiratory syndrome virus (PRRSv) contributes significant economic losses to pig producers worldwide. In Uganda, despite its reported occurrence, no information is available on the genotypes of PRRSv that circulate in the country.

Methods: A cross sectional study was conducted to detect and characterize PRRSv genotypes identified from slaughtered pigs in Lira district, northern Uganda. The study was conducted from March to September 2019 in three selected slaughter slabs. Pigs brought for slaughter were randomly sampled. At necropsy, lungs were extracted from the thoracic cavity and scored for pneumonic lesions. Seventy-three (73) pigs with gross lung lesions were sampled, from which one hundred and one (101) tissue samples were taken. A real-time reverse transcriptase PCR (RT-qPCR) was used to identify PRRSv genotypes.

Results: Out of the 73 pigs sampled, 27.39% (n=20) tested positive for PRRSv. The respective prevalence of PRRSv type 1 and PRRSv type 2 were 24.65% (n=18) and 2.73% (n=2). Of all the pigs sampled (n=73), only two pigs, 2.73% (n=2) tested positive to both genotypes. The likelihood of PRRSv detection decreased with pig age but increased with gross pneumonic pathology.

Conclusions: This study demonstrated dual circulation of both PRRSv type 1 and PRRSv type 2 genotypes in northern Uganda. The association between PRRSv and lung pathology suggests that it may be an important cause of lung disease in pigs in Uganda and hence loss of production. This calls for further investigations on potential economic impacts of PRRSv on pig productivity. These findings contribute to the need for surveillance and possible vaccination strategies against PRRSv in Uganda.

Key words: PRRSv, species, porcine, pigs, respiratory, Lira, Uganda

7.3. Background

In Uganda, pig production has increased over the last few years, from approximately 0.7 million in 1990 to 4.2 million pigs in 2018 due to a rising demand for pork (Ouma et al., 2017; UBOS, 2019). Pig production in Uganda is increasingly becoming an important economic activity for many households, providing a reliable source of livelihoods. However, disease constraints hinder pig production and productivity in the country (Muhanguzi et al., 2012). Recent multi-pathogen studies reveal occurrence of economically important respiratory pathogens such as porcine reproductive and respiratory syndrome virus (PPRSv), *Mycoplasma hyopneumoniae* (*M. hyo*), *Actinobacillus pleuropneumoniae* (*App*), and porcine circovirus (PCV2) type 2 (Dione et al., 2018a; Eneku et al., 2018; Jonsson, 2013a). Of the pathogens reported, PRRSv is known to be associated with high economic losses from mortalities, reproductive losses and increased costs of control (Holtkamp et al., 2013; Neumann et al., 2005; Zimmerman et al., 2006). In general, two genetically distinct genotypes of PRRSv have been described worldwide, with the European species (EU) designated as type 1 (PRRSv-1) and the north American species, designated as type 2 (PRRSv-2) (Walker et al., 2020). Furthermore, there is marked genetic diversity in PRRSv-2, leading to further classification into virus lineages (Kuhn et al., 2016). These two species are distinct in their virulence, antigenic characteristics and nucleotide sequences (Kapur et al., 1996; Meng, 2000). This has important implications for immunological responses and vaccine selection, as only incomplete protection can be achieved from heterologous field strains (Osorio et al., 2002). This diversity of the virus also compounds the challenges of disease control, due to differences in transmission rates, strain pathogenicity and its tendency to persist in infected herds.

In the US, PRRSv is reported to cost the swine industry up to $560 million annually, with up to 45% of these losses due to reduced growth and feed efficiency (Neumann et al., 2005). Overrall, losses due to PRRSv vary widely depending on epidemiological factors, production systems and farm characteristics. In a Dutch study, losses were found to range from €3 to €160 per sow per year (Nieuwenhuis et al., 2012). The economic impacts of PRRSv on swine productivity are justification for epidemiologic studies to generate knowledge to guide interventions.

In Uganda, no vaccines are currently in use for control or prevention of PRRSv. In particular, few studies on PRRSv in Uganda have mainly focused on serologic assays, providing evidence for past

exposure of pigs to the virus and possible virus circulation. Recent developments in the pig sector in Uganda show increased imports of breeder pigs from countries such as South Africa, where PRRSv has been reported (OIE, 2005). This poses a threat to the swine population, if no measures to contain virus spread are established. There is no information on the current epidemiological situation regarding PRRSv and its potential impacts on swine productivity in Uganda, due to lack of surveillance.

Despite the availability of several commercial vaccines in Europe, America and Asia to control PRRSv (Murtaugh & Genzow, 2011; Renukaradhya et al., 2015), the apparent lack of information on the identity of current PRRSv genotypes circulating in Ugandan pigs limits their use as effective tools for control and prevention. The aim of this study was to detect and characterize PRRSv genotypes identified from slaughtered pigs in northern Uganda.

7.4. Materials and Methods

7.4.1. Study design

A cross-sectional study was conducted from March to September 2019 in three purposely selected slaughter slabs in Lira district, northern Uganda. Slaughter slabs with the highest daily slaughter capacity (≥ 8 pigs) were selected for the study. Pigs slaughtered in Lira district were sourced from within the district (~60%), while the rest were sourced from neighboring districts of Apac, Kole, Amolatar and Pader. Figure 7.1 below shows a map of Lira district in Uganda where the study was conducted.

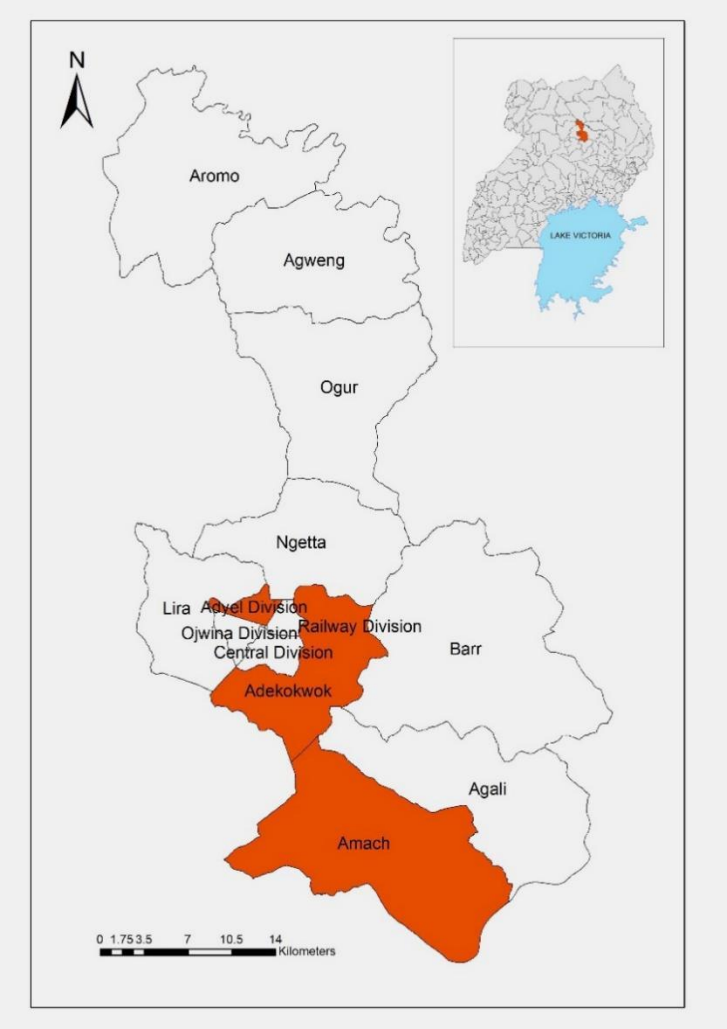

Figure 0.1: Map of Lira district showing study sites (subcounties)

7.4.2. Sampling of slaughter slabs and pigs

During this survey, three slaughter slabs: Teso Bar (Adyel division), Adekokwok (Adekokwok subcounty) and Amach market (Amach subcounty) were selected based on high daily slaughter capacity (≥ 8 pigs). In each slab, approximately 40% of pigs brought for slaughter were randomly selected on each day of sampling. At each slab on average, between 8 to 20 pigs were brought for slaughter per day, which represented approx. 8-12 farms. On each day, a list of all pigs brought for slaughter was made and each allocated a number, which were written on a piece of paper and folded. From this list, random sampling was done. Pig biodata was recorded at ante mortem (sex), while gross pathology (postmortem) was recorded as described in a related study (Oba et al., 2021). Traders were asked about the source(s) of the pigs, which were recorded.

7.4.3. Sample size determination

The number of pigs sampled represented approximately 40% of pigs slaughtered in the district *(DVO, pers comm*). The rest (60%) were slaughtered in other smaller slabs distributed throughout the district (~30 slabs), and whose daily slaughter capacity varied between 1-7 pigs. In a recent study, the seroprevalence of PRRSv in Lira district in pigs was found to be 1.7% (Dione et al., 2018a). To detect presence of PRRSv from an unknown population size (slaughter-age pigs), the equation below was used (Dohoo et al., 2003).

$$n = \ln(\alpha) / \ln(q)$$----------*Eq(1)*

where n = is the required sample size, α = 0.05, p = estimated prevalence of PRRSv (1.7%) and q = 1-p (98.3%). Using this equation, the computed sample size was 175 pigs. Assume 30% of slaughter age pigs show gross pneumonic lesions (Oba et al., 2021; Pallarés et al., 2021), a minimum sample size of fifty-three (53) pigs was required to detect PRRSv. During this study, we sampled a total of 73 pigs, from which 101 tissue samples (lungs, lymph nodes, spleen or kidneys) were taken. Only lungs with gross pathologic lesions were sampled, normal lungs or other organs were not sampled. In case a pig had > 1 organ with gross lesions, tissue samples were taken from all grossly affected organs. Out of 73 pigs sampled, 28 pigs had two (2) samples taken from 2 organs.

7.4.4. Examination of lungs and other tissues for gross pneumonic lesions and sample collection

At necropsy, the carcass was placed on a clean table, opened with knives to expose lungs and the pleura. The lungs were carefully extracted from the thoracic cavity and placed on a flat, clean surface. Examination of lungs for gross pneumonia forms scoring is described in a previous related study (Oba et al., 2021). Lesion samples were taken and cut into ~0.5-gram pieces, placed in a 2 mL cryovial (*Sarstaedt®, Germany*) containing RNA*later®* (*Thermo Scientific®, USA*) tissue stabilization solution. Other observed gross lesions were also recorded. The cryovial was labelled and then placed in an ice box containing ice packs at 4°C. To prevent cross contamination, a new sterile surgical blade was used for each pig lung, with disinfection of gloved hands and collection tools using 70% ethanol between samplings. Hand gloves were frequently changed to minimize the risk of cross contamination.

7.4.5. Tissue sample transport and storage

After collection, tissue samples were immediately (within 2 hours) transported to the district (Lira) veterinary laboratory for temporary storage in a fridge at 4°C. Later, samples were transported (in an icebox at 4°C) to Makerere College of Veterinary Medicine (CoVAB), Department of Biosecurity, Ecosystems and Veterinary public health laboratory and stored in a -20°C fridge. An export permit was secured from the Commissioner Animal Health, Uganda and an import permit from the Directorate of Veterinary Services of the Republic of Kenya to transfer samples to International Livestock Research Institute (ILRI) Kenya for molecular analysis. Upon receipt of an authorization to export samples, tissue samples were shipped by air in October 2019 to ILRI Nairobi, Kenya. The samples were packaged in an ice box containing ice packs at 4°C, where upon arrival they were placed in a -80°C fridge for subsequent RNA extraction and complementary DNA synthesis.

7.4.6. PRRSv RNA extraction and real-time reverse transcriptase PCR (RT-qPCR)

RNA extraction was done using the AllPrep DNA/RNA Mini Kit (cat. no. 80204) according to the manufacturer's protocol (Qiagen®, Denmark). A real-time (quantitative) reverse transcriptase PCR was performed in the same laboratory, in March 2020 using the KiCqStart(R) One-Step Probe RT-qPCR ReadyMix™ Low ROX™ (*Sigma-Aldrich®*). Real-time RT-qPCR and complementary DNA synthesis were performed in a GeneAmp® PCR System 7500 Fast *version 2.3* (*Applied Biosystems®).* The sequences of primers (Macrogen Europe, cat. no. OG200117-237) for full length cDNA synthesis and the dual-labeled Taq-Man probes are as shown in Table 7.1 below (Kleiboeker et al., 2005).

Table 0.1: Sequences of primers and dual-labeled probes used in the RT-qPCR assay

Genotype	Name	Orientation	Sequence
PRRSv-1	Primer 1	Forward	5'-CGA CCA CCT CAC CCA GAC-3'
	Primer 1	Reverse	5'-CAG TTC CTG CGC CTT GAT-3'
	Probe	Genomic	5'-6-FAM-CCT CTG CTT GCA ATC GAT CCA GAC-BHQ1-3'
PRRSv-2	Primer 2	Forward 1	5'-ATG ATG RGC TGG CAT TCT-3'
	Primer 2	Reverse	5'-ACA CGG TCG CCC TAA TTG-3'
	Probe	Genomic	5'-HEX-TGT GGT GAA TGG CAC TGA TTG ACA-BHQ2-3'

A qPCR master mix was made up of 4 µl molecular biology grade water, 1 µl of 10 µM Forward, 1 µl of 10 µM reverse primers, 1 µl of 10 µM probe and 10 µl of KICqStart Master mix (Sigma Aldrich, UK). The master mix was completely mixed by tapping the tube and a quick short spin. This master mix cocktail was adequate for one reaction. The components of the master mix were adjusted to suit the number of samples. The contents of the master mix tube were mixed thoroughly and dispensed 17 µl to each labelled sample and control tubes. An RNA template of 3 µl was then dispensed to each tube with a master mix. The tubes were placed in a 7500 Fast Thermocycler and the program which includes a Reverse Transcriptase (RT) at 50 °C for 10 min, pre-heating at 95 °C for 10 min, denaturation at 95 °C for 30 seconds and annealing at 60 °C for 1 minute was started. This was repeated for 45 cycles with the RT and preheating occurring just once.

7.5. Data analysis

Genotype identification was determined by plotting amplification curves of fluorescence signal detected versus cycle threshold values (Ct). Cycle threshold values of ≤ 42 were considered positive and Ct value > 42 were taken as negative. Summary statistics (prevalence, odds ratios) were derived in the R environment for statistical computing, *version 4.0.4* (http://cran.r-project.org/). The relationships between PRRSv positivity age, sex, location, and gross pathology were measured using Chi-squared analysis in the *epiDisplay* package in R. An individual pig was the unit of analysis; a pig was considered positive if any of the organs were found positive by RT-qPCR. Odds ratio (OR) values were calculated based on positivity of PRRSv-1.

7.6. Results

Seventy-three (73) pigs were sampled, from which 101 tissue samples were taken. Of the pigs sampled (n=73), the prevalence of PRRSv type 1 and type 2 were 24.65% (n=18) and 2.73% (n=2) respectively. Only two pigs, 2.73% (n=2) tested positive to both PRRSv type 1 and type 2, implying that the 2 pigs that had PRRSv-2 were also co-infected with PRRSv-1. There was a significant relationship between PRRSv positivity and the degree of lung pathology, Odds Ratio 3.74 (95% CI 1.14-15.05).

7.6.1. Reverse transcriptase quantitative (RT-qPCR) results

The figures 7.2 and 7.3 below show amplification plots of fluorescence detected versus cycle threshold for PRRSv type 1 and type 2 strains.

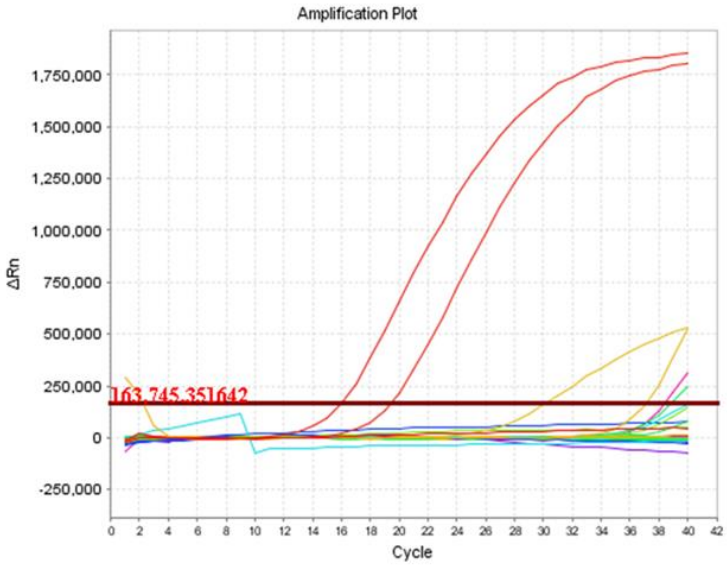

Figure 0.2: Amplification plot of fluorescence versus cycle number for PRRSv type 1, detected using the FAM probe. A horizontal thick dark brown line shows cycle threshold cutoff value.

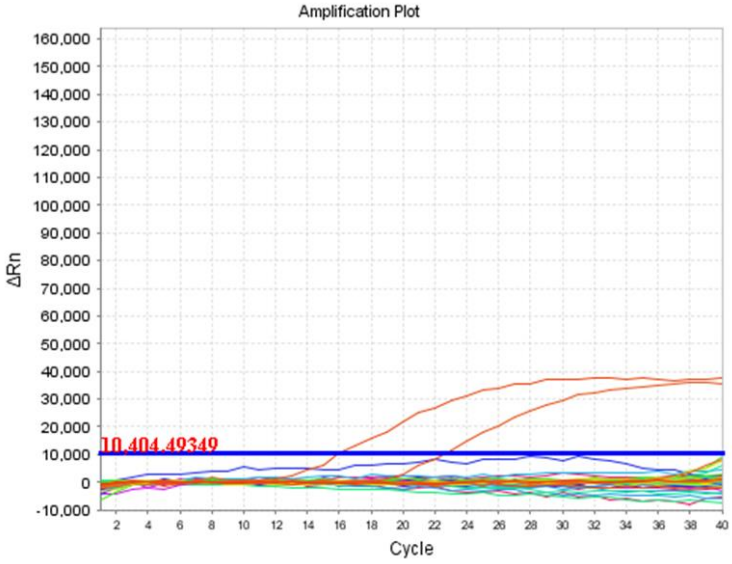

Figure 0.3: Figure 7.2 Amplification plot of fluorescence versus cycle number for PRRSv type 2, detected using the HEX probe. A horizontal thick blue line shows cycle threshold cutoff value.

Table 7.2 below shows a summary of Chi^2 results.

Table 0.2: Summary of the Chi^2 analysis for PRRSv-1 and PRRSv-2 positive samples collected from pigs in Lira district, Uganda

Variable	Category (N=73)	PRRSv-1 prev. % (n)	PRRSv-2 prev. % (n)	Odds Ratio (95% CI)	Chi^2 test, df, p-value
Pig sex	Males (n=35)	25.71 (n=9)	0 (n=0)	1.12 (0.38-3.32)	0.05, 1, 0.841
	Females (n=38)	23.47 (n=9)	5.26 (n=2)	1	
Pig age	≤12 months (n=48)	29.17 (n=14)	4.17 (n=2)	1	-
	> 12 months (n=25)	16.66 (n=4)	0 (n=0)	0.46 (0.13-1.59)	1.53, 1, 0.216
Gross pathology	0-24 % (n=33)	12.12 (n=4)	0 (n=0)	1	-
	25-72 % (n=40)	35.00 (n=14)	5.00 (n=2)	3.74 (1.15-15.05)	3.93, 1, 0.023*
Slaughter slab	Teso bar (n=39)	28.20 (n=11)	2.56 (n=1)	1.30 (0.37-5.05)	-
	Adekokwok (n=26)	23.07 (n=6)	3.84 (n=1)	1	
	Amach mrket (n=8)	12.56 (n=1)	0 (n=0)	0.49 (0.01-5.27)	0.936, 2, 0.626
Origin of pig	Lira (n=43)	20.93 (n=9)	4.65 (n=2)	1	
	Neighboring districts (n=30)	30.00 (n=9)	0 (n=0)	1.60 (0.53-4.83)	0.37, 1, 0.37

Note: *gross pathology represents percent estimate of lung surface area grossly affected by pneumonia; neighboring districts are Alebtong, Pader, Dokolo, Kole and Apac.*

7.7. Discussion

This study revealed circulation of both type 1 and 2 PRRSv genotypes in northern Uganda. However, PRRSv type 1 was found to be the more predominant genotype detected. Given the high animal movements for slaughter, restocking and breeding between regions (Atherstone et al., 2019) and the weak surveillance systems, the potential for spread of PRRSv may be substantial. This implies that PRRSv-1 may likely be prevalent elsewhere in Uganda, where its occurrence has not yet been investigated properly. This situation could have adverse implications for swine productivity in the country, herd economic performance and consequently livelihoods, if the virus becomes established in commercial breeding herds. Information about the predominant virus is important for implementing successful interventions for controlling the spread of the virus given its potential economic impacts on swine productivity. However, clinical manifestations and potentially economic impact might be very different between PRRSv-1 and PRRSv-2 infections.

These results showed the likelihood of PRRSv-1 detection decreased with pig age (range 5-50 months). While this was not statistically significant, it suggested a trend that needs further exploration with a larger sample size. This finding is consistent with the observation that the immune system of swine is able to completely eliminate PRRSv infection over prolonged periods of time (Murtaugh & Genzow, 2011). Pigs exposed to PRRSv become resistant to reinfection with a homologous strain, although the level of protection was incomplete (Shibata et al., 2000). This was also corroborated by a study which found age-dependent resistance to infection, shown by reduced viremia and viral load in the blood of adult pigs compared to younger pigs (Klinge et al., 2009). In contrast, other studies revealed that PRRSv tends to persist in infected herds (Nathues et al., 2017; Wills, et al., 2003), suggesting increased likelihood of detection in older pigs. However, this finding was specific for larger herds and where there were increased re-introductions of infected gilts (Evans et al., 2010). As part of a major longitudinal study (Oba et al. *unpublished*), most farms in the district were generally small in size (1-5 sows) and the replacements were infrequent. The estimated prevalence was obtained from randomly selected clinically healthy growing/adult pigs from households in the region. This implies that there exist age differences between the population in which the expected prevalence is drawn and the population from this study.

In a related study (Oba et al., 2021), the prevalence of gross pneumonic lesions ranged from 17.3% for pleuritis, 29.9% for catarrhal purulent bronchopneumonia (CPBP), to 74.2% for pleuropneumonia (PLP). The high prevalence of pneumonia forms in the above study is consistent with findings in the current study, which showed the likelihood of detection of PRRSv-1 increased with gross pathology. The increase in PRRSv detection rates associated with gross pathologic lesions conforms to previous studies. The ability of PRRSv to induce clinical and macroscopic pneumonia, often as a co-infection with other pathogens such as *M. hyo* has been documented (Thacker et al., 1999). No differences in detection rates between male and female pigs were observed in this study. While PRRSv type 1 was detected in both Lira and the neighboring districts, type 2 was detected only from Lira district. This suggests type 1 may be widespread compared to type 2. Apart from Lira district, pigs were also sourced from Apac, Kole, Amolatar and Pader districts and no localization was observed in any of the districts.

Our results are comparable to other studies which reported simultaneous circulation of both PRRSv type 1 and type 2 species in various regions and show increased circulation of PRRSv type 1. In Europe, both species circulate but there is a predominance of type 1, with marked genetic variation among species (Stadejek et al., 2017). In Asia, studies report the predominance of PRRSv type 1 in China, although the American type 2 has also been documented (Xingchen, et al., 2016). In the Republic of Korea, it was found that both type 1 and type 2 species circulated in pig farms during the period between 2013-2016. However, type 1 PRRSv was reportedly predominant (Kang et al., 2018).

The information on PRRSv in African countries is limited but there are official reports submitted to OIE by a few countries in Africa (DR Congo, Benin, Burkina Faso, Egypt, Ivory Coast, Nigeria) that document occurrence of PRRSv, although none of these studies reported its genetic diversity or molecular identity (OIE, 2005). The current situation regarding the PRRSv species circulating on the continent is largely unknown, as the few studies undertaken were based on serologic assays. In southwest Nigeria, a study reported a high seroprevalence of PRRSv of 53.8%, suggesting widespread exposure of pigs to the virus (Aiki-Raji et al., 2017a). However, the species of the virus was not determined.

In South Africa, the PRRSv strain responsible for the 2004 outbreaks was identified by RT-PCR as type 2 (Oosthuizen, 2010). Our results are contrary to expected since a large number of pigs are imported from South Africa and suggest a different source of the virus in Uganda, since PRRSv type 1 has not been reported in South Africa. The lack of reliable data on pig imports into Uganda limits our understanding of the likely sources of PRRSv introduction into the country. Further studies to understand the introduction and maintenance of PRRSv into Uganda are required. Knowledge gaps remain on the potential distribution of PRRSv species in other regions of Uganda especially in high pig dense areas, which justify further studies.

The method used to detect PRRSv in this study utilized primers that were designed to simultaneously detect both PRRSv-1 and PRRSv-2. This approach is reported to have high specificity and sensitivity, at differentiating PRRSv-1 from PRRSv-2 isolates. This method is reportedly efficient and rapid for large scale detection and differentiation of PRRSv species. However, this study was limited by the small sample size used and by the fact that the study was undertaken in only one region, implying that results cannot be extrapolated to other regions of the

country. Because we sampled only pigs that presented with gross lung lesions, the true prevalence of PRRSv and the distribution of species in all slaughtered pigs and in the general pig population still remains unknown and possibly is higher to what has been reported here. The future option of sequencing at least a portion of the genome of the PRRSv strains identified could be included with the aim to aid future epidemiology studies.

7.8. Conclusions

This is the first study to document dual circulation of PRRSv type 1 and 2 species in pigs in Uganda. The relation between PRRSv and severe lung pathology suggests it may be an important and increasing cause of lung disease in pigs in Uganda and hence loss of production. This study reveals PRRSv-1 is the predominant genotype in circulation among slaughter-age pigs in Lira district in northern Uganda. However, in view of its reported genetic diversity, further characterization of possible PRRSv-1 subtypes and evaluation of their pathogenicity in pigs is justified, as well as investigate possible circulation of PRRSv in other parts of the country with the aim to establish surveillance. In addition, studies to evaluate efficacy of different control measures, such as vaccination, considering dual circulation of the two species and to quantify their economic effects in Uganda are recommended.

The results of the above study highlight the need to investigate economic impacts in the form of average daily live weight gains (ADGs) and financial costs encountered by farmers due to exposure of pigs to respiratory and GIT parasites. In the next **Chapter eight** of this thesis **(Objective four)**, I sought to quantify direct economic losses (daily weight gains and treatment costs) associated with exposure of pigs to selected respiratory pathogens.

7.9. Declarations

7.9.1. Ethics approval and consent to participate

This study received ethical approvals from the following institutions: Institutional Review Board (IRB), College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University (IRB # SBLS/REC/18/008), Uganda National Council of Science and Technology (UNCST reg no. A590); ILRI's Institutional Research Ethics Committee (IREC no. IREC2018-23) and ILRI's Institutional Animal Care and Use Committee (IACUC2018-22). Prior informed consent was obtained from district local authorities and owners of slaughter slabs before the study commenced.

All experiments were performed in accordance with relevant guidelines and regulations and that all methods were reported according to ARRIVE guidelines (https://arriveguidelines.org). No anesthesia procedures were performed on pigs.

7.9.2. Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on request. Both tissue samples and PCR products for this paper are stored at ILRI laboratory (Lab 5), ILRI campus, Nairobi, Kenya and can be obtained upon request.

7.9.3. Acknowledgements

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CHAPTER EIGHT

8.0. OBJECTIVE FOUR

8.1. ECONOMIC LOSSES ASSOCIATED WITH RESPIRATORY AND HELMINTH INFECTIONS IN DOMESTIC PIGS IN LIRA DISTRICT, UGANDA

8.2. Abstract

Respiratory diseases contribute significant economic losses to pig producers globally. This study sought to quantify direct economic losses (weight gains and financial losses) due to respiratory and gastrointestinal (GI) helminth infections in domestic pigs in Lira district, Uganda. In a repeated measures design, farm visits were made at 2-month intervals from Oct 2018 to Sept 2019. A total of 288 weaner and grower pigs aged 2-6 months were sampled from 94 farms in Lira district. The pigs were identified, monitored for growth and screened for exposure to 4 important respiratory pathogens: porcine circovirus type 2 (PCV2), porcine reproductive and respiratory syndrome virus (PRRSv), *Mycoplasma hyopneumoniae* (*M. hyo*), *Actinobacillus pleuropneumoniae* (*App*) using ELISA tests. Farm management practices were recorded and used to generate management level scores. Treatment expenses incurred (in UGX) were recorded throughout the study. A mixed effects model was fitted to quantify effects of respiratory and helminth infections on average daily weight gains (ADGs), with farm and pig as random effects. Analysis of variance (ANOVA) was used to compare mean treatment costs by farm management level. Financial losses were estimated from average carcass dressing percentage, ADG reductions and assumed fattening period (200 days). Results showed that a grower pig in a given farm in Lira district exposed to PRRSv and *Ascaris spp* infection had significantly lower daily weight gains (ADG) by up to 18.47 and 23.68 gr/pig/day respectively, compared to a similar unexposed pig of the same age. The mean treatment costs per pig declined with improvement in management level scores (MLS). We show that possible monetary losses encountered by farmers due to PRRSv and *Ascaris spp.* infection amounted to USD 7.12 and USD 9.16 respectively per pig during 6.5 months of fattening. This study strengthens evidence that improving herd management mitigates economic losses due to disease/infections. To guide interventions, further studies are required to unravel the full extent of indirect losses due to respiratory infections in pigs.

Key words: Average daily weight gains (ADGs), economic losses, respiratory pathogens, pigs, *Ascaris spp.* monetary losses, Lira, Uganda

8.3. Introduction

Respiratory diseases contribute significant economic losses to swine producers globally from reduced productivity, increased production costs and loss of market (Calderón Díaz et al., 2020;

Ferraz et al., 2020; Nieuwenhuis et al., 2012). However, economic losses vary considerably between countries, due to differences in production systems, study methodologies and the types of pathogens involved, whose interactions produce varying levels of disease severity. In Uganda's smallholder production systems with a high disease burden, the extent of possible economic losses may be considerable.

Economic losses due to swine diseases result from mortalities, reduced weight gains (Alarcon et al., 2013), poor reproductive performance, negative effects on feed conversion and increased costs of treatments (Cornelison et al., 2018; Nathues et al., 2017; Opriessnig et al., 2004). Reduced market value of sick animals represents indirect disease effects, which producers often encounter. Other losses result from carcass condemnations due to lung lesions or reduced carcass quality (Brombilla et al., 2019; Scollo et al., 2017). Studies show infections with multiple pathogens increases the severity and duration of clinical disease (Opriessnig & Halbur, 2012; Thacker et al., 1999). To provide a framework for design of interventions, it is necessary to quantify possible production losses. This information is useful for producers and extension services to support investment decisions that aim to improve herd profitability.

In Uganda, pig production offers huge opportunities for better livelihoods due to rising demand for pork (Ouma et al., 2017; UBOS, 2014). However, the sector's performance is hampered by poor management and biosecurity practices, which partly explain increased disease incidence in herds (Chenais et al., 2017; Dione et al., 2014; Gertzell et al., 2021). Recent studies reported occurrence of important respiratory pathogens in pigs. Among key pathogens reported are PCV2, PRRSv, *M. hyo*, *App* and *Streptococcus suis* (Dione et al., 2018; Eneku et al., 2018; Jonsson, 2013; Ojok et al., 2013). These agents are known to cause substantial economic losses (Nathues et al., 2017; Zhang et al., 2014).

In Uganda, respiratory disease impacts on pig growth and financial costs are unknown, as no such studies were conducted before. During this study (2018-19), no pig vaccines against any respiratory disease(s) were available (due to logistical constraints e.g., cold chain), implying no vaccination was done. Thus, a serological test that turns out positive can be interpreted as a likely result of natural infection. In this study, we monitored pigs prospectively for growth and exposure to four key respiratory pathogens (PCV2, PRRSv, *M. hyo* & *App*) using ELISA tests. The study scope was limited to estimation of direct costs (ADGs & treatments) due to respiratory and parasite

infections. These findings could guide intervention measures against respiratory diseases in Uganda and elsewhere with similar production systems.

8.4. Materials and methods

8.4.1. Study area

This study was conducted in Lira district, mid-northern Uganda, characterized by predominantly smallholder production systems. In this area, pigs are raised by mainly tethering in rural areas or confinement as in urban or peri-urban settings (Ikwap et al., 2014; Kungu et al., 2019). In these systems, farmers keep few pigs, usually between 2 and 30, and are mainly fed on local feed ingredients and/or indigenous forages. Further description of the study area and selection of subcounties and villages for this study is detailed in a previous study (Ouma, 2017). Anthelmintics and/or antibiotic treatments were given to pigs as preventive or curative measures in some farms.

8.4.2. Study design

A longitudinal observational study was conducted from October 2018 to September 2019. Data was collected in a repeated measures design at 2-month intervals for the duration of the study. A rolling recruitment procedure was used to enroll pigs for the study, because not all 2 pigs in each farm were available in the beginning. For pigs which died, got lost or were sold, new pigs of approx. same weight and age from the same herd were enrolled in the study.

8.4.3. Sampling of farms and pigs

We monitored farms in the urban and peri-urban settings only because this was where we could find enough confined pigs easy to access. In each farm, we targeted to sample at least 2 weaner and/or grower pigs. From a sampling frame of a list of pig keeping households (with $\geq$ 2 weaner/grower pigs) obtained with help from the local district veterinary office, random sampling was done. Informed consent was obtained from pig owners to voluntarily take part in the study and find out their willingness to keep pigs for $\geq$ 6 months. In each farm, weaner and grower pigs (aged 2.5–6 months) were randomly sampled. Enrolled pigs were identified by ear tagging and data recorded at the onset and on every subsequent visit.

8.4.4. Sample size determination

The following assumptions were made: a normal uninfected pig grows from 8 to 30 kg in 5 months. So, average daily gain (ADG) is 147 gr/day. An infected pig grows from 8 kg to 20 kg in 5 months; so ADG is 80 gr/day. The difference in ADGs is 147 – 80 = 67 gr/day. We assumed a standard deviation of 60 gr/day ($s = 0.06$); so, estimated common variance for the two groups, $\sigma^2 = 0.25$. To estimate effects of pathogen exposure on ADGs, a sample size was obtained from equation (1) (Dohoo et al., 2003):

$$n = 2\left(Z_{\alpha/2} + Z_{\beta}\right)^2 (1 + (n-1)\rho) \,/ n[(\mu_1 - \mu_2)\sigma^2]$$

Where $Z_{\alpha/2}$ is the standard normal deviate for $\alpha = 1.96$; $Z_{\beta} = 0.84$; $\mu_1 - \mu_2$, d = 67 gr/day and number of time points, n=3; Assumed correlations of repeated measures, $\rho = 0.3$. Thus, the required sample size, n = 233 pigs.

8.5. Data collection

8.5.1. Measurement of live weights, body condition scores and clinical disease scores

Excel sheets were designed and used to record data at farm and pig level. Data was collected at 2-month intervals in a repeated measures design, with replacements of pigs if they were sold or died. Data on farm management variables likely to be associated with infection and growth in pigs were captured. Data collected included rearing method (housed or tethered), pig age, sex, breed (local vs improved), live weights (kg) measured using a HiWeigh® BSR5300 weighing scale (*Shanghai, China*), feed types/quality used (4 grades), body condition scores (min-max: 1-5) and clinical respiratory disease scores (CDS). CDS were scored as follows, from 0 to 6: 0 = normal; 1 = mild dyspnea and/or tachypnea when stressed; 2 = mild dyspnea and/or tachypnea when at rest; 3 = moderate dyspnea and/or tachypnea when stressed; 4 = moderate dyspnea and/or tachypnea when at rest; 5 = severe dyspnea and/or tachypnea when stressed; 6 = severe dyspnea and/or tachypnea when at rest (Halbur et al., 1996).

To estimate direct production costs, treatment expenditures throughout the study period were recorded. Drug treatments were recorded per herd and divided by herd size to estimate average costs per pig during the 60-day sampling interval. These included types and costs of drugs bought (dewormers, antibiotics) and veterinary fees. Herd size and perceived herd value (based on farm-

gate prices) were recorded at the start and at each visit. All expenses and value of pigs were recorded in Uganda (UGX) shillings. Fixed and other variable costs (e.g., feeds) were omitted because most farmers lacked reliable records.

8.5.2. Blood sample collection and analysis

Pigs were monitored for exposure to 4 respiratory pathogens by blood sampling at each visit. Larger pigs were restrained with a metallic pig catcher (*Model BZ002, MG® Livestock, Shandong, China*), while smaller pigs were restrained by hand. With a pig properly restrained, blood was collected from the cranial vena cava or jugular vein, using a 21G, 1.5" needle into 5 mL plain BD® vacutainer tubes. The tubes were labeled with pig details and placed in icebox at 4-6 °C. Shortly after collection, samples were delivered to Lira district veterinary laboratory for temporary storage. Blood samples were left to stand at room temperature (20 °C) overnight and serum harvested the next day into 2 mL cryotubes (*Sarstedt®, Germany*), labelled and stored in a fridge at -20 °C until testing.

8.5.3. Serological analysis of sera

Sera were screened using specific ELISA assays according to manufacturer's instructions for each pathogen. Suspect samples were re-tested. Table 8.1 shows a summary of ELISA test characteristics.

Table 0.1: Summary of ELISA test characteristics

Pathogen	ELISA test kit manufacturer	Test sensitivity (*Se*) and specificity (*Sp*)	Cut-off sample to positive ratios (S/P%)
PCV2	Krishgen Biosystems, India	*Se* 92.0%, *Sp* 94.0%	positive ≥ 0.2; negative < 0.2
PRRSv	Krishgen Biosystems, India	*Se* 94.0%, *Sp* 94.0%	positive ≥ 0.2; negative < 0.2
M. hyo	IDDEXX, Westbrook, Maine, USA	*Se* 85.6%, *Sp* 99.6%	positive > 0.4; negative < 0.3
App	IDDEXX, Westbrook, Maine, USA	*Se* 97.8%, *Sp* 100%	positive ≥ 0.5; negative < 0.4

8.5.4. Faecal sample collection and analysis

At each farm visit, faecal samples (~ 5 gr) were collected from the rectum of each pig using gloved hands into 5 mL plastic containers, labelled and placed in ice box at 4ºC. Samples were screened for presence or absence of *Strongyles spp.* and *Ascaris spp* helminths at the College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University, Kampala. Helminth species were identified using the Baermann's method (MAFF, 1986).

8.6. Data analysis

Data was cleaned, coded, and analyzed using RStudio (R Core Team, 2019). To quantify effects of respiratory infection(s) on ADGs, we fitted a mixed effects (MEM) model (with farm and pig as random effect terms) which considers the multilevel structure of the data (pigs nested within farms). An individual pig was the unit of analysis, in which serostatus was defined as a positive ELISA test to any one respiratory pathogen during the period of observation. The average weight gain (ADG gr/day) per pig was computed using equation 2 below (Mutua et al., 2011).

$$\text{ADG (gr/day)} = \text{(Live weight (kg) at end)} - \text{(Live weight (kg) at start)} / \text{Time interval (days)} \text{--Eq(2)}$$

The following variables were included in a mixed effects model: rearing method, pig age, clinical disease score (CDS), pig sex, feed quality grade (1-4), respiratory pathogen serostatus and body condition score (BCS). The R packages "*lme4", "lmerTest", "reghelper"* and *"jtools"*(Bates et al., 2015) were used to fit the model. The mean ADG, Y_{ij} for an individual pig *i*, in a given farm *j*, was estimated from equation 3:

$$ADG_{ij} = \beta_0 + \beta_1 X_1 + \ldots\ldots \beta_k X_{ki} + \mu_{farm(i)} + \varepsilon_{ij} \text{----------Eq.(3)}$$

where β_0, β_1 are the fixed effect coefficients for the intercept and slope respectively; X_i, X_{ki} are the fixed effects regressors (i =1, 2...), $\mu_{farm(j)}$ is a random effect of farm *j*, and the residual error term, $\varepsilon_{ij} \sim N(0, \tau)$ assumed normally distributed. Random effect terms (1|farm/pig) were included to account for expected differences in ADGs between farms and pigs, which include different intercepts and random slopes.

As farm management practices directly or indirectly affect pathogen exposure, variables known or suspected to influence exposure rates and weight gains were captured. A *housing index (hi)* for

pigs was derived from the method of (Njuki et al., 2011) used as a proxy for poverty, but with slight modification. The index represented aggregated individual scores of pig house components which included floor, wall, and roof type. The higher the housing index, the better the quality of housing (range 1-24). Together with other management practices: routine drug use, floor hygiene level, access to extension services and whether farmers isolated sick pigs, a housing index was used to generate a management level score (MLS). Analysis of variance (ANOVA) was used to establish differences in mean treatment costs between 3 farm management levels at $p < 0.05$. The model was fitted using Residual Maximum Likelihood (REML) estimation procedure and selection was done using fit statistics, Akaike Information Criterion (AIC). Model diagnostics were evaluated by plotting a graph of fitted values *vs* residuals. Table 8.2 below shows variables used in fitting the model.

Table 0.2: Summary description of variables used for the analyses

Variables	**Measurement level**	**Description**
Farmer education level	Farm – continuous	Years of schooling
Pig sex	Pig – binary (1 or 0)	Male or female
Pig age (months)	Pig – continuous	Months (starting age at 2 months)
Live weight (kg)	Pig – continuous	Live weight (kg)
Pig breed	Pig – binary (1 or 0)	Local vs improved
Feed types (quality grade)*	Farm – categorical (1- 4)	1=poor, 4=best, compound feed
Body condition scores (BCS)	Pig – categorical (1-5)	1=very poor, 5=best, very fat
Total and average treatment costs	Pig level - continuous	Antibiotics &/or dewormers only
Average daily gain (ADG)	Pig – continuous	ADG, gr/day
Resp. clinical disease scores (CDS)	Pig - categorical (0-6)	0=none, 6=severe distress, under rest
Pathogen serostatus (PCV2, PRRSv, *M. hyo, App*)	Pig - binary (1 or 0)	ELISA test result (any of 4 pathogens)
GI helminths (*Ascaris spp & Strongyles spp*)	Pig - binary (1 or 0)	Baermann test result (positive or negative)

**Feed grades were classified into 4 grades: grade 1=sole grazing only, grade 2=sole grazing and maize bran, grade 3= maize bran and swill, grade 4= compounded feed.*

8.7. Results

8.7.1. Demographic characteristics

Table 8.3 below shows summary statistics for number of farms, sex of household head and number of pigs sampled. Overall, 288 pigs from ninety-four (94) farms were sampled and monitored. Male household heads constituted 53.38% (n=53), while females accounted for 43.62% (n = 41).

Table 0.3: Descriptive summary statistics by farm location and sex of household head

Characteristics	Category	No. of farms sampled (n=94)		Totals (%)
		Males (%)	Females (%)	
Location (Subcounty)	Adekokwok	28 (30.10)	25 (26.80)	53 (56.38)
	Central division	6 (6.40)	2 (2.10)	8 (8.50)
	Ngetta	7 (7.40)	10 (10.70)	17 (18.10)
	Railways	12 (12.9)	4 (4.30)	16 (17.00)
Farmer education level	Never attended	1 (1.06)	3 (3.20)	4 (4.25)
	Primary	19 (20.21)	23 (24.46)	42 (44.68)
	Secondary	14 (14.90)	8 (8.51)	22 (23.40)
	Graduate	15 (15.95)	7 (8.51)	22 (23.40)
	Post-graduate	4 (4.25)	0 (0.00)	4 (4.50)
Pig house type	Housed	27 (28.72)	24 (25.53)	51 (54.25)
	Tethered	26 (27.90)	17 (18.08)	43 (45.75)
	Totals	53 (53.38)	41 (43.62)	94 (100.0)

The mean live weight of pigs in this study was found to be 35 kg (range 4.2−101.0 kg), while the median age was 7.5 months (range 2 − 17 months). This suggests pigs were generally underweight for their age, as reflected by general poor body condition scores (from the data). Table 8.4 below shows summary statistics of farms.

Table 0.4: Summary of farm characteristics: herd size, pigs sold, number and value of dead pigs

Subcounty	# Farms	No. pigs sampled	Herd size per farm (mean ± SD)	Value of sold pigs (median)¶	# Pigs sold/farm (median)	Price/ pig sold, (mean)¶	# Pig deaths /farm (mean ± SD)*	Value of dead pigs/farm (mean ± SD)¶
Adekokwok	53	160	5.2 ± 3.5	69.10	2	34.55	0.80 ± 1.25	27.64 ± 43.19
Central division	8	26	11.8 ± 10.6	256.90	5	51.38	2.38 ± 2.80	122.28 ± 143.86
Ngetta	17	66	15.9 ± 33.7	555.25	13	42.71	6.40 ± 7.61	273.34 ± 325.00
Railways	16	36	14.8 ± 20.9	421.27	8	52.66	2.66 ± 5.24	140.10 ± 275.93
Totals	94	288	9.3 ± 17.2	410.00	7	45.32	2.52 ± 4.91	114.21 ± 222.52

¶*Values in USD, average exchange rate during study = 3620 UGX (2018-19). *Total number of deaths, regardless of cause.*

In all, ninety-nine (99) pigs were reported to have died during the study, whose perceived total value at farm-gate price before death was UGX 10,550,000 (USD 2,875). However, total

mortalities reported were due to several suspected causes, which were indistinguishable from each other.

8.7.2. Farm management level scores

At farm level, key factors known to influence occurrence of respiratory pathogens in herds include housing (influences hygiene, ventilation), floor type, isolation of sick pigs, access to extension services and routine use of dewormers and antibiotics (Cargill, 2019; Stärk, 1998). Using above management variables, a score of farm management level was derived, with a higher score reflecting higher, while a lower score indicates low management level. Herd size varied from 4.2 pigs for tethered pigs to 13.6 pigs per farm for housed pigs. Table 8.5 below shows summary measures for treatment costs by farm types and management level scores.

Table 0.5: Summary of rearing method, herd size and treatment costs by management level scores

Variables	Management level score (MLS)*		
	Poor (score 1)	Moderate (score 2)	High (score 3)
No. of housed farms, n (%)	0 (0.00%)	28 (29.80%)	18 (19.15%)
No. of tethering farms, n (%)	42 (44.68%)	6 (6.40%)	0 (0.00%)
Herd size (Mean±SD)	4.5 ± 2.3^{a}	8.6 ± 6.8^{b}	27.7 ± 38.9^{c}
Treatment costs/pig (USD) (Mean±SD)¶	1.13 ± 1.29^{a}	0.92 ± 0.79^{b}	0.95 ± 0.46^{b}

**MLS was obtained as the sum of scores of house type, feed grade, isolation of sick pigs, access to extension services, routine use of drugs (antibiotics & dewormers) and pen hygiene.* ¶*Different superscripts in the last two rows (within row) show mean values are statistically different at $p < 0.05$.*

The effect of management level score on average treatment costs was demonstrated. Analysis of variance revealed significant differences in mean treatment costs per pig between MLS 3 and MLS 1 level farms (F value =4.384, p=0.0128). High farm management level farms showed significantly lower mean treatment costs per pig compared to poor management level farms (Table 8.5).

8.7.3. Mixed effects model of ADG predictors with farm as random effect

Based on AIC and BIC, the model (Table 8.6) provided the best fit to the data ($\chi^2 = 25.10$, df = 4, $p < 0.000$). The mixed effects model showed that infection with PPRSv and *Ascaris spp.* were significant predictors of ADG. Pig level contributed the greatest variance to ADGs (80.14%), followed by farm level (12.83%). Residual or unexplained variance accounted for only 7.01%.

Table 0.6: Summary of a mixed effects model of predictors of ADG, with farm as random effect

Variables	Estimate	Conf. intervals	Std Err	df	t value	Pr(>|t|)
(Intercept)	114.902	83.31 − 146.60	16.071	621.828	7.150	2.45e-12 ***
PRRSv	-18.474	-36.48 − -0.45	9.166	842.922	-2.016	0.044159 *
Age (months)	3.901	1.78 − 6.02	1.079	853.984	3.615	0.000318 ***
Ascaris spp.	-23.682	-43.72 − -3.65	10.207	804.672	-2.320	0.020577 *
Feed grade	5.290	-5.43 − 15.98	5.419	679.626	0.976	0.329360

Table 8.7 below shows a summary of estimated monetary losses due to exposure of pigs to infections.

Table 0.7: Summary of estimated monetary losses from reduction in mean ADGs due to infections

Pathogen	Mean ADG reduction (gr/pig/day)	Total weight loss (gr) during fattening (200 days)	Est. carcass dressing %[§]	Mean weight loss (kg)	Est. total monetary losses per pig (UGX)	Total monetary losses (USD)*
PRRSv	18.47	3680	70	2.57	25,760	7.12
Ascaris spp.	23.68	4736	70	3.32	33,152	9.16

**Average exchange rate during the study, *USD=3620 UGX (2018-2019); days of fattening (assumed 200 days, from 2-8.5 months); [§]Carcass dressing percentage was obtained from (Kugonza et al., 2017) for pigs on maize bran-based diet. Average price/kg of pork during study (UGX 10,000, equivalent to USD 2.76).*

8.8. Discussion

This study highlights the role of farm management and the impacts of respiratory infections on pig daily weight gains in Uganda. To support farm decision making, a clear understanding of farm management practices and their relationship with weight gains and production costs is required. Ultimately, the goal of any producer is to minimize production costs, reduce or eliminate economic losses, which translate into better profit margins. These findings compare favorably with other studies elsewhere, which reveal negative effects of respiratory diseases on daily weight gains (Agostini et al., 2014; Gray et al., 2021), increased financial expenditures (Calderón Díaz et al., 2020; Nathues et al., 2017) and reduced profit margins (Renken et al., 2021). A recent study which reported high prevalences of pneumonic lesions in slaughtered pigs highlights the potential contribution of respiratory infections on lung pathology (Oba et al., 2021).

Our findings agreed with other studies elsewhere, which reported a drop in mean ADGs with increase in respiratory disease prevalence (Gray et al., 2021). In this study, pigs from the same farm and age exposed to PRRSv and infested with *Ascaris spp* gained significantly lower ADGs compared to those unexposed/uninfected (Table 8.6). As expected, age was a significant predictor

of ADGs. However, at the 6th data wave (from data), age was negatively associated with ADGs, implying a tendency of losing weight or gain lower ADGs with age. While feed grade increased ADGs, it was not statistically significant.

These findings are consistent with other studies which reported a reduction in ADGs by between 8% and 14%, and increased mortality of 19.9% in farms with a high disease challenge (Cornelison et al., 2018). A study reported a reduction in ADGs of between 16 and 29% for respiratory and between 8.4 and 19.4% for parasitic infections (Pastorelli et al., 2012). The mean ADG of pigs reported in this study is comparable to that in other studies in East Africa in similar settings. In Uganda, a study reported that the ADG of nursery pigs fed on forage-based diet was 160 gr/day (NA. Carter et al., 2017), while another recent study in Lira district reported 101 gr/day (Gertzell et al., 2021). In Western Kenya, (Carter et al., 2013) reported ADG of 130 gr/pig/day, while in Tanzania, Lipendele and colleagues reported ADG of 136 gr/pig/day (Lipendele et al., 2015). However, these ADG values are generally much lower compared to those in other countries, which attain 600 gr/day or higher (Cornelison et al., 2018; Gray et al., 2021). While other parasite infections (*Eimeria & Strongyles spp*) reduced ADGs, the decreases were not statistically significant.

The low ADGs of pigs in this study (compared to that in developed economies) could be explained by endemic infections, limited access to quality feeds and inferior genetics as reported in recent studies in Uganda (Carter et al., 2017; Gertzell et al., 2021; Lukuyu et al., 2017; Ouma et al., 2015). Other models (not shown here) showed that co-infection with 2 or 3 pathogens were associated with weight loss. The adverse effects of mixed infections on ADGs confirm findings from previous studies which showed mixed infections reduced ADGs, led to more severe and prolonged duration of respiratory disease (Niederwerder et al., 2016; Opriessnig & Halbur, 2012). The effect of infective dose, pathogen type and strains involved, their interactions with environmental stressors and the subsequent response of the pig's immune system play a significant role in the induction of clinical disease. These interactions lead to subclinical, mild, or severe disease outcome, producing varying effects on weight gains and other productive indices as previously reported (Alarcon et al., 2011; Brockmeier et al., 2001).

This study revealed that mean treatment costs declined with improvement in management level score. Farms with a high level of management (*MLS 3*) spent significantly lower mean treatment

costs compared to those with poor (*MLS 1*) management level (Table 8.5). This is unsurprising, since farms with poor management were reported to have higher disease incidence in previous studies (Gray et al., 2021; Merialdi et al., 2012).

In this study, we show that monetary losses per pig associated with PRRSv and *Ascaris spp* infections were substantial for smallholder farmers. These estimates, however, likely represent a fraction of potential total losses encountered as other productivity indices (e.g., abortions, mortalities) were not captured. In this study, partly due to underfeeding, farmers often kept pigs for longer than 200 days (6.5 months), which adds to possible losses from extra feeds needed to raise pigs to market weight. In the US, (Cornelison et al., 2018) reported that financial costs under commercial conditions in high disease challenge farms varied between USD 8.5 and 29.8 per marketed pig, while (Dee & Joo, 1994) reported costs due to PRRSv infection between 2005 and 2010 ranged from 10.5 to 12.5 USD per marketed pig. However, these studies were done in intensive, high-efficiency settings, in contrast to smallholder production systems in our study.

Housing type and quality have a direct and indirect effect on pathogen transmission between pigs. Floor types (deep litter, raised timber platform, cement and rammed soil/murram) influences pig welfare and hygiene. Farmer attitude and behaviour determines the frequency with which wastes are removed from pens. These management factors directly influence the pathogen load that may accumulate and multiply in pens, particularly if the floor was poorly designed. Accumulation of pathogens in pens due to lack of cleaning may facilitate transfer of infection(s) between pigs. (Cargill, 2019) reported that pigs reared in an all-in-all-out (AIAO) system with cleaning grew by 15% faster than pigs reared with no cleaning. The same study showed that pigs on dry floors gained higher ADGs (5% higher) than pigs on wet floors. A similar finding was reported in a study by Pastorelli *et al.,* which found that pigs raised in poor sanitary conditions gained 11% significantly lower ADG compared to those under good sanitary conditions (Pastorelli et al., 2012). Evidence of the role of good hygiene in pig health, overall welfare, and efficiency of the value chain, generating better financial returns to the producer is documented (Calderón Díaz et al., 2020). A related study (Oba et al. *unpublished*) that described 3 farm clusters based on biosecurity practices reflects 3 farm management level scores (MLS) found in this study and previously described. Our study provides a framework to measure the quality of housing and management level, both of

which influence health, welfare and productivity. It can be adapted to a given context to include other management practices (e.g., beddings), which most farmers did not provide for.

The fact that only a third of farmers in this study had access to extension services justifies a necessity to strengthen these services to provide technical advice on herd health and biosecurity. While prophylactic use of antibiotics against bacterial pathogens is known to reduce the burden of opportunistic infections (Adedeji et al., 1989; Steenhard et al., 2009), their judicious use should be promoted to guard against possible misuse, which could escalate the problem of AMR. The practice of isolating sick pigs helps minimize the risk of further pathogen spread between pigs. These herd preventive practices are critical as they influence the level of contamination, risk and extent of pathogen spread between pigs. A study showed routine management factors (e.g., routine removal of manure) had a greater impact on *Ascaris suum* infection than regular deworming (Roesel et al., 2017). This underscores the importance of "*good*" management in reducing adverse effects of disease. Management is a combination of a farmer's socio-economic practices, attitude, and behaviour, which reflects their skills and knowledge of possible disease impacts on herd performance. However, farmers' adoption of biosecurity measures for disease control should be supported by incentives that increase their financial returns, as previously highlighted (Ouma et al., 2018). The observation that farmers' education level directly correlated with management level underscores the importance of education in reducing adverse disease impacts. It is therefore important to consider the social context when designing health management interventions.

This study was limited to estimation of direct costs due to respiratory and worm infections. However, it was impossible to estimate other indirect economic costs (specific to respiratory diseases) attributable to deaths and salvage prices of sick pigs due to lack of reliable farm data. That these indirect costs were not captured suggests economic losses encountered by farmers in this study may have been underestimated. Besides, because not all farmers actually treated their pigs despite showing clinical disease due to lack of cash or no access to extension worker, it's probable that errors in estimates of treatment costs may have been introduced. Because the ELISA tests used reflect prior exposure of pigs to respiratory pathogens, knowledge gaps still remain on the duration of infection(s), and the time lapse to induce clinical disease during individual or mixed infections, both of which ultimately influence growth rates.

8.9. Conclusions and recommendations

To the best of our knowledge, this is the first study in Uganda to document evidence of adverse effects of respiratory and helminth infections on weight gains in pigs. We showed that a grower pig in a given farm exposed to PRRSv and *Ascaris spp* infection had significantly lower daily weight gain (ADG) by up to 18.47 gr/day and 23.68 grams/day respectively, compared to a similar unexposed pig of the same age. The mean treatment costs per pig declined with improvement in management level scores (MLS). We show that monetary losses encountered by farmers due to PRRSv and *Ascaris spp.* infection amounted to USD 7.12 and USD 9.16 respectively, per pig during the fattening period (200 days).

This study highlights the role good management plays in mitigating against adverse effects of respiratory infections in pigs. Good herd management using welfare concept and principles of proper housing, *"adequate*" nutrition, biosecurity are prerequisites for optimal growth and health of pigs, which enhances herd profitability. Further studies are required to establish the full extent of other potential indirect losses (e.g., reproductive disorders) considering pathogen interactions and variations in disease severity.

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8.11. Data availability statement

The original analyzed datasets presented in this study can be obtained from the authors upon request

8.12. Conflicts of interest

The authors declare no conflict of interest

8.13. References

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CHAPTER NINE

9.0. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

9.1. General discussion

This thesis provides critical insights on epidemiology of important respiratory pathogens of pigs in Africa and in Uganda in particular. Despite occurrence of these pathogens, information on spatial and temporal distribution on the African continent is generally scanty, as shown by the systematic review study. In East Africa, most reports on swine respiratory pathogens were from Uganda, mainly in the last decade. In West Africa, reports on PCV2 and PRRSv occurrence were only made in a few countries (Cameroon, Nigeria, Ivory Coast and Benin). In addition, no published studies exist on the economic impacts of PRRSv and PCV2 on pig production and productivity in Africa. With respect to swine influenza viruses, significant knowledge gaps existed on circulating viral subtypes, and their spatial and temporal distribution on the continent, which call for further epidemiologic studies. Due to its zoonotic nature, swine IAV were reported in a few North African countries (Tunisia, Egypt), West Africa (Cameroon, Nigeria, Togo) and in East Africa (Uganda, DRC and Kenya). The rest of the pathogens, *M. hyopneumoniae* and *App* have hardly been studied in Africa, and thus it is likely that their role in swine health and productivity is underestimated.

Based on the systematic literature review, we showed scarcity of information on swine respiratory pathogens on the African continent, despite growing demand for pork and the contribution of the pig sector to national economies. The molecular identity of important respiratory pathogens in Africa still remains unknown as only few studies conducted to date were based on serology, suggesting prior exposure of pigs to these pathogens. Likewise, the spatial and temporal distribution of important respiratory pathogens in pigs remain unknown in most of Africa. In all, no published study was found that described economic impacts of any important swine respiratory pathogen(s) on pig productivity in Africa. This highlights apparent gaps in research, and therefore justifies future epidemiological studies on swine respiratory pathogens with a view to inform future interventions. The use of vaccines as disease control tools particularly in the context of smallholder production settings need to be evaluated for efficacy and cost effectiveness.

The first objective aimed to identify key management risk factors for occurrence of important respiratory pathogens and GI parasites in pigs. To achieve this, a cross sectional study was undertaken in randomly selected farms. Serologic screening of pigs (using ELISA) is one of the methods employed to establish their exposure to selected respiratory pathogens. Serological screening of pigs to determine exposure to respiratory pathogens is a fast and cheaper way to generate information to guide farm management decisions. This method has been widely used in several previous studies elsewhere (Duinhof et al., 2011b; Fablet et al., 2011; Haimi-hakala et al., 2017). Farm level management and husbandry practices associated with pathogen exposure were collected using a pre-tested questionnaire (Annex 4(i)). This method is cheap, flexible and is a quick way to generate valuable information and knowledge of key risk factors for occurrence of important respiratory pathogens in pig herds. The knowledge generated is critical to inform farm-level interventions. The major limitation of the ELISA test used is that because it's not 100% sensitive and specific, there may have been false positives and false negatives. The lack of prior vaccination of pigs before the study reflects likely natural exposure to the studied pathogens. This study highlights the critical role of management, and in particular biosecurity play in influencing pathogen/disease occurrence in farms. It showed pigs that had parasite infestations were more likely (*Strongyles spp.* and *Ascaris spp.* to have respiratory co-infections. The negative correlations between biosecurity variables and mixed pathogens signifies the role of improved biosecurity in reducing the risks of co-infections. In addition, the clustering of farms of poor biosecurity level with pathogens provides further support to the above evidence. Such scientific evidence needs to be promoted to improve extension services for the benefit of farmers. These findings provide a good framework to guide herd level interventions. Overall, 3 farm clusters were identified and characterized based on housing, biosecurity practices and mixed pathogens. Farms with poor hygiene and drainage showed a higher likelihood of respiratory co-infections. This study provides further evidence that improving hygiene and biosecurity is critical in reducing pathogen incidence in herds and their associated impacts.

The second objective aimed to identify and characterize both species of PRRSv identified in slaughtered pigs. The diagnostic method (reverse transcriptase quantitative PCR) used was established to have a high diagnostic sensitivity of 100% and a specificity of 97.5% and thus recommended for differentiation of PRRSv genotypes (Kleiboeker et al., 2005; Lurchachaiwong et al., 2008). The RT-qPCR test used is able to distinguish PRRSv type 1 from type 2 since both

NA and EU PRRSv oligonucleotide primer sets and dual-labeled probes were included in each reaction mixture in a multiplex assay format, as previously described (Kleiboeker et al., 2005). The detection of both PRRSv genotypes in Lira district may have implications on swine productivity in the future, as the virus is prone to genetic mutations and recombinations. This necessitates establishment of a surveillance system to enable monitoring of virus characteristics and hence inform management interventions.

The third objective aimed to determine the relationships between serologic profile (exposure to respiratory pathogens) and lung pneumonic lesions in slaughtered pigs. The method used has been widely employed in several studies though with different modifications (Ferraz et al., 2020; Holt et al., 2011; Martínez et al., 2009; Scollo et al., 2017). It is a quick and cheap method suited for field situations to generate information on the status of herd health, endemic disease situation and importantly, the magnitude of pneumonia in pigs. Abattoir surveys provide a valuable source of information for this purpose. This study revealed that pneumonia in pigs brought for slaughter in Lira district was highly prevalent. This justifies why attention to control of respiratory disease and the interactions between pathogens is necessary as it is likely to be a key health and production constraint in smallholder production systems.

The fourth objective aimed to quantify economic losses due to exposure to respiratory pathogens. A longitudinal observational study was conducted in a repeated measures design. This method has been widely employed in several previous studies elsewhere (Cornelison et al., 2018, Gray et al., 2021). However, the approach needs to be contextualized depending on the prevailing production system, intensity of production, rate of pig flows in a farm and volume of data required for modelling purposes. The negative effects of exposure of pigs to respiratory pathogens was demonstrated in this study. While the mixed effects model only showed the negative effect of infection with PRRSv and *Ascaris spp*, other models (not shown) revealed that co-infections had negative effects on average daily weight gains (ADGs) in growing pigs. This suggests that the interactions between other pathogens (beyond the scope of this thesis) may play an important role in respiratory disease in pigs.

9.2. General conclusions

Based on the research methods used in this doctoral study, the findings and their interpretations, the following conclusions were arrived at:

i. There are significant knowledge and information gaps on swine respiratory pathogens in Africa. Information on spatial and temporal distribution of important respiratory pathogens in pigs remain unknown in most of Africa. Likewise, the economic impacts of important swine respiratory pathogen(s) remain unknown.

ii. This study highlighted widespread occurrence of economically important respiratory pathogens in pigs in northern Uganda. This may likely reflect the situation in swine herds in other regions of Uganda, where production systems are largely similar. Management practices largely influence types of pathogens that occur in herds: knowledge of associations between farm management practices, biosecurity and pathogens informs intervention measures.

iii. Key risk factors for single respiratory infections included higher herd size, prophylactic treatments (protective), and parasite *(Ascaris spp* and *Strongyles spp)* infestations. For respiratory co-infections, key risk factors identified included use of cement and elevated floor (protective), while use of earth/murram floor, timber wall and poor biosecurity practices (poor hygiene) and concurrent parasite infections increased risk.

iv. This study revealed dual circulation of both PRRSv type 1 and type 2 species in northern Uganda, and that PRRSv type 1 was the predominat type in circulation.

v. To the best of my knowledge, this was the first study in Uganda to document associations between pneumonia forms and serology to key swine respiratory pathogens in slaughtered pigs. It revealed a high prevalence and severity of pneumonia forms in slaughtered pigs and the association with respiratory pathogens suggests their potential contribution to lung pathology.

vi. Results showed that a grower pig in a given farm in northern Uganda exposed to PRRSv and Ascaris spp infection had significantly lower daily weight gains (ADG) by up to 18.47 and 23.68 gr/pig/day respectively, compared to a similar unexposed pig of the same age. The mean treatment costs per pig declined with improvement in management level scores (MLS). We show that possible monetary losses encountered by farmers

due to PRRSv and *Ascaris spp.* infection amounted to USD 7.12 and USD 9.16 respectively per pig during fattening (200 days). This study strengthens evidence that improving herd management mitigates economic losses due to disease/infections.

9.3. Recommendations

i. Farmers should pay close attention to proper *housing, hygiene, biosecurity, wastes management and parasite control*

ii. Limiting contacts with outside pigs reduces the risks of disease spread between herds

iii. For farmers and extension workers, the concept of *welfare* & *herd health management* are key to maximize productivity. Farmers should endeavour to minimize stress factors in pigs - such as poor hygiene, mixed-age rearing, improve the floor and space allowance – all these improve the general welfare and health of pigs.

iv. Given that PRRSv type 1 and PRRSv type 2 co-circulate in Lira district, there is need to establish a national surveillance system to monitor virus genotypes and disease impacts on swine productivity to guide future interventions

v. While these findings can be generalized in northern Uganda where production systems are homogeneous, similar studies should be conducted in other areas of the country where pig production is rapidly growing. This study established critical baseline information to guide future studies on swine respiratory diseases. In effect, it justifies why veterinary services need to prioritize control interventions against respiratory pathogens and GI helminths. To this end, the herd health management approach, focusing on animal welfare principles will be critical for extension services if improvements of pig health are to be made. I believe these findings shall inform national policy on swine health and disease control in Uganda.

9.4. Future direction of research

i. Further studies to identify PCV2 and PRRSv species that circulate in pigs in other regions of the country, as well as quantify their economic impacts on swine productivity are warranted to guide the design of effective interventions. It is necessary to do sequencing of PRRSv subtypes and determine their pathogenicity.

ii. In future, the clinical outcome(s) of infection with PRRSv genotypes need to be evaluated for their impacts on pig productivity, which could be used to guide future interventions.

Given the high rate of pig movements in the country for trade and breeding (Atherstone et al., 2019), there could be a likelihood that PRRSv is slowly spreading to other areas, which calls for establishment of a national surveillance program.

iii. The high prevalence of pneumonic lesions justifies a need for future studies on potential economic impacts of pneumonia on swine production and productivity in Uganda, as a basis for designing future interventions.

iv. To guide interventions, further studies are required to unravel the full extent of indirect economic losses due to respiratory infections in pigs. Further studies to quantify economic impacts of respiratory pathogen infections on reproductive indices are recommended.

v. Studies to understand interactions between nutrition and disease outcome and their effects on key productivity indices are justified.

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{Bibliography

LIST OF APPENDICES

The appendices below are included as attachments in this doctoral thesis:

1. Research participant consent form
2. Institutional ethical approvals and research permits obtained:
   i. Institutional Review Board ethical approval letter (IRB no. SBLS/REC/18/008), College of Veterinary Medicine, Animal Resources and Biosecurity (CoVAB), Makerere University
   ii. Research permit – from Uganda National Council of Science and Technology (UNCST reg no. A590).
   iii. ILRI's Institutional Research Ethics Committee approval (IREC no. IREC2018-23)

3. Raw data and collection tools

   i. Questionnaire used for a cross sectional study (for objective one)
   ii. Lung lesion score sheets (2) – used during a slaughter slab survey (for objective three)
   iii. Data sheet – excel for pig house type, live weight measurements, clinical disease scores (CDS), body condition scores (BCS) & hygiene scores – (used for objective four - quantify economic losses associated with respiratory infections in pigs).
   iv. Raw data can be found here: ..\COMPLETE_DATA\ECON_data_ALL_Jan22_for model.xlsx

4. ***Pictures taken*** *while collecting data in the field*

Appendix 1: Research participant consent form

FARMER CONSENT FORM ***Form F1 Nr……..***

Study: Epidemiology of Respiratory Diseases: Impacts on Smallholder Pig Production Systems in Uganda

Preamble

Dear Respondent,

I, Peter Oba, is a PhD student of Makerere University, undertaking research on pig respiratory diseases in Uganda. This purpose of this study is to identify important respiratory diseases and to determine their economic impact on pig growth in Uganda. This will be used to guide you and other stakeholders on the most effective strategies for prevention and control of these diseases, which could improve the productivity of your pig herds.

As a key stakeholder in this sector, we request you to take part in this study, which will last for about one year. The information collected here remains confidential and will be purely used for research purposes only. Please feel free to ask any questions regarding your pigs from the research team. We will share the results of this study with you in the end. This study is funded by the German Academic Exchange Service (DAAD) through the International Livestock Research Institute (ILRI), Kampala.

Thanking you for your participation.

…….

PETER OBA (PhD Student, ILRI/Makerere University)
Position: Principal Investigator/Researcher
Mobile: 0772 694099/ 0700 474633

I…….of…….Subcounty,

……village, herein referred to as *the farmer or animal owner*, hereby do consent and accept to enter into this memorandum of understanding to participate in this research study, led by **Peter Oba and Michel Dione** *(herein referred to as the principal investigator or researcher)* under the following terms and conditions:

1. The farmer shall allow the researcher(s) to collect information from him/her and samples (blood) from his/her pigs during the study period
2. The researcher(s) shall ensure the safety and welfare of pigs during all sample collection procedures
3. The farmer shall have the freedom, without hindrance to sell, slaughter or give away all or some of his/her pigs at any time during the study period
4. The farmer is free to decline to take part in this study or withdraw from the study at any time, and that no punishment whatsoever will arise if he/she chose not to participate or withdraw from the study.
5. The farmer is aware that taking part in this study is ***fully voluntary*** and that no monetary benefits or otherwise are attached to participating in this study.
6. The farmer shall have the freedom to seek for technical advice or ask any questions from the researcher(s) during the study period
7. The researcher(s) is obliged give feedback of research findings to the farmer(s) at the end of the study period
8. The study period will run from……. to ……..

This memorandum of understanding is below signed in the presence of the following witnesses:

Name of farmer…… Tel no:…….

Signature & date…….

Witness 1 name:…….. Tel no:……..

Title……

Signature & date…….

Name of Researcher: PETER OBA Signature & date……..……

Witness 2 name:…….. Tel no:……..

Title……

Signature & date…….

Appendix 2(i): ***IRB approval***

MAKERERE UNIVERSITY

COLLEGE OF VETERINARY MEDICINE, ANIMAL RESOURCES AND BIOSECURITY

OFFICE OF THE DEAN

SCHOOL OF BIOSECURITY, BIOTECHNICAL AND LABORATORY SCIENCES (SBLS)

P. O. Box 7062 Kampala, Uganda
Tel : +256414-554685
Fax : +256-414-554685

Cables : "MARUNIKA"
Email : sbls@covab.mak.ac.ug
Website : vvww.covab.mak.ac.ug

Your Ref : Our Ref SBLS.PO.2018 Date: 15th /08/2018

RESEARCH AND ETHICS COMMITTEE REVIEW AND ENDORSEMENT

Statement from the Institutional Ethical Review Board:

The REC only accepts for review and approval, research proposals that have been found both scientifically and ethically acceptable in accordance with guidelines on Institutional Ethical Review Boards.

We the Institutional Ethical Review Committee established by

COLLEGE OF VETERINARY MEDICINE, ANIMAL RESOURCES AND BIOSECURITY

do certify that we have reviewed the research proposal (SBLS/REC/18/008) entitled

Epidemiology of respiratory diseases: Impact on smallholder pig production systems in Uganda

submitted by

Dr. Peter Oba. ILRI, Kampala Uganda

We attest to scientific and ethical merit of this study and competency of the investigator(s) to conduct the research and hereby recommend the proposal to the Uganda National Council for Science and Technology (UNCST) for approval

SIGNATURES

	Name	Signature	Date
Ethics Committee Representative	Assoc. Prof. Clovice Kankya		15 /08/2018
Head of Ethics Committee	Assoc. Prof. Frank N. Mwiine	7	15 /08/2018

CONTACTS

Tel: +256 787 405 220
E-mail: mwiine@covab.mak.ac.ug
fmwiine@gmail.com

OFFICIAL STAMP OF INSTITUTION

DEAN
SCHOOL OF BIOSECURITY, BIOTECHNICAL & LABORATORY SCIENCES (SBLS)
15 AUG 2018
COVAB 1922
MAKERERE UNIVERSITY
P.O.BOX 7062, KAMPALA, UGANDA

Appendix 2(ii)

Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

UNCST

Our Ref: A 590 **10th September 2018**

Dr. Peter Oba
Principal Investigator
C/o International Livestock Research Institute (ILRI)
Kampala

Dear Dr. Oba,

Re: Research Approval: Epidemiology of Respiratory Diseases: Impact on Smallholder Pig Production Systems in Uganda

I am pleased to inform you that on **28/08/2018**, the Uganda National Council for Science and Technology (UNCST) approved the above referenced research project. The Approval of the research project is for the period of **28/08/2018** to **28/08/2021**.

Your research registration number with the UNCST is **A 590.** Please, cite this number in all your future correspondences with UNCST in respect of the above research project.

As Principal Investigator of the research project, you are responsible for fulfilling the following requirements of approval:

1. All co-investigators must be kept informed of the status of the research.
2. Changes, amendments, and addenda to the research protocol or the consent form (where applicable) must be submitted to the designated Research Ethics Committee (REC) or Lead Agency for re-review and approval **prior** to the activation of the changes. UNCST must be notified of the approved changes within five working days.
3. For clinical trials, all serious adverse events must be reported promptly to the designated local IRC for review with copies to the National Drug Authority.
4. Unanticipated problems involving risks to research subjects/participants or other must be reported promptly to the UNCST. New information that becomes available which could change the risk/benefit ratio must be submitted promptly for UNCST review.
5. Only approved study procedures are to be implemented. The UNCST may conduct impromptu audits of all study records.
6. An annual progress report and approval letter of continuation from the REC must be submitted electronically to UNCST. Failure to do so may result in termination of the research project.

LOCATION/CORRESPONDENCE

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P. O. Box 6884
KAMPALA, UGANDA

COMMUNICATION

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Uganda National Council for Science and Technology

(Established by Act of Parliament of the Republic of Uganda)

Below is a list of documents approved with this application:

	Document Title	Language	Version	Version Date
1.	Research proposal	English	N/A	August 2018
2.	Participant consent form	English	N/A	N/A
3.	Longitudinal survey: Effect of Resp. disease scores on weight gain	English	N/A	N/A
4.	HERD Inventory	English	N/A	N/A
5.	Variable costs of production	English	N/A	N/A
6.	Revenue/Income from pigs	English	N/A	N/A

Yours sincerely,

Isaac Makhuwa
For: Executive Secretary
UGANDA NATIONAL COUNCIL FOR SCIENCE AND TECHNOLOGY

Copied to: Dean, Makerere University School of Biosecurity, Biotechnology and Laboratory Sciences (SBL)

LOCATION/CORRESPONDENCE

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Appendix 2(iii)

12th November 2018

Our Ref: ILRI-IREC2018-23

International Livestock Research Institute

P.O. Box 30709 00100

Nairobi, Kenya.

Dear Michel Dione & Peter Oba,

REF: EPIDEMIOLOGY OF RESPIRATORY DISEASES: IMPACT ON SMALLHOLDER PIG PRODUCTION SYSTEMS IN UGANDA

Thank you for submitting your request for ethical approval to the International Livestock Research Institute (ILRI) Institutional Research Ethics Committee (IREC).

ILRI IREC is registered and accredited by the National Commission for Science, Technology and Innovation (NACOSTI) in Kenya, and approved by the Federalwide Assurance (FWA) for the Protection of Human Subjects in the United States of America.

I am pleased to inform you that ILRI IREC has reviewed and approved your study titled ***'Epidemiology of Respiratory Diseases: Impact on Smallholder Pig Production Systems in Uganda'***. The approval period is 12th November 2018 to 11th November 2019 and is subject to compliance to the following requirements:

• Only approved documents will be used;

Patron: Professor Peter C Doherty AC, FAA, FRS

Animal scientist, Nobel Prize Laureate for Physiology or Medicine–1996

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ILRI has offices in East Africa • South Asia • Southeast and East Asia • Southern Africa • West Africa

- All changes must be submitted for review and approval before implementation;
- Adverse events must be reported to ILRI IREC immediately;
- Access and Benefits Sharing (ABS) requirements, where applicable;
- Submission of a request for renewal of approval at least 30 days prior to expiry of approval period; and
- Submission of an executive summary report within 90 days upon completion of the study.

Please call on ILRI IREC on ILRIResearchcompliance@cgiar.org for any further clarification or information you may require.

Yours Sincerely,

Silvia Alonso, PhD
Chair, ILRI Institutional Research Ethics Committee

Documents received & reviewed:

- IREC Form
- Project Proposal
- Consent & Questionnaire

- UNCST approval

Patron: Professor Peter C. Doherty AC, FAA, FRS
animal scientist, Nobel Prize Laureate for Physiology or Medicine–
1996

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ILRI has offices in • East Africa • South Asia • Southeast and East Asia • Southern Africa • West Africa

Appendix 3(i): Household questionnaire

HH no……..

Prevalence and risk factors of respiratory infections in Ugandan pigs

Dear Respondent,

This study aims at identifying important respiratory pathogens and risk factors for their occurrence in pigs in Uganda. This will be used to advise you and other farmers on prevention and control measures.

As a key actor in this sector, you are requested to take part in this study. The information collected here remains confidential and will be used purely for research purposes only. We shall share the findings with you at the end of the study, which would help you improve the productivity and profitability of your piggery enterprise.

Thanks for your participation.

…….

Peter Oba *(PhD Student, ILRI/Makerere University)*
Mobile: 0772 694099/ 0700 474633

Part a: General information

Date of interview:……. (i) District ……. (ii) Sub county: …….

(iii) Parish:…… (iv) Village:……. (v) GPS reading:……

(vi) Name of respondent:…… (vii) Sex: 1= Male 2= Female

(viii) Age category: 1= 18-35 years
2= 36-50 years
3= 51-75 years
4= 76 and above

(viii) Education level: 1= Primary 2= Secondary 3= Tertiary 4= graduate 5=post-graduate

(ix) Religion: 1= Catholic 2= Protestant 3= Muslim 4= Pentecostal 5= Others

(x) Main occupation: 1 = Farmer 2 = Business 3 = Formal employee 4=other…

Part b: Herd specific information

1.1 Production system/type
1 = Intensive
2 = Semi-intensive

1.2 Herd structure

1.2.1 Breed of pigs kept: 1= Local 2 = Exotic 3 = Crossbreed 4 = Mixed (exotic and local)

1.2.2 If cross breed, specify breed:……

Age group	Breed	Number	Age (months)

Sows			
Gilts			
Growers/fatteners			
Weaners			
Piglets			
Boars			
Total			

2.0 Herd environment

2.1 **Housing system**

2.1.1 Housing structures 1 = housed
0 = not housed (tethered)

2.1.2 If housed:

Specify floor type: 2 = raised (with wood / timber above the ground)
1 = not raised (concrete floor)
0 =not raised, soil or murram

2.1.2 If not raised, specify type of floor

Type of floor	Est. size (m^2)	Comments
Concrete floor		
No concrete floor (rammed earth or murram)		
Timber boards		

2.1.3 Wall material: 4 = Bricks
3 = Mud and wattle
2 = Timber boards
1 = Wire mesh

2.1.4 Roofing material 2 = iron sheets
1 = grass thatched / papyrus
0 = other (specify)……

2.2 **Stocking density** (0 = >1 pig per sq. metre, 1 = 1 or less pig per sq. metre)

Pig age category	Number of pigs	Approx. area of pen (m^2)	Density (pigs/m^2)
Sows			
Gilts			
Growers/fatteners			
Weaners			
Piglets			
Boars			

2.4 Feeding: Feed types given to pigs (you can choose more than one)

Feed type used	Est. qty per day (kg)	No. of times pigs are fed per day
Household leftovers		
Local mixed feed		
Green forages		

Maize bran		
Commercial feeds		

Part c: Herd health performance

3.1 What disease control and preventive measures do you use ?
1= dewormers/antibiotics
0= do nothing)

Frequency of practice: 1 = 1-2 times/month
0 = no routine or less frequent)

3.2 **Preventive drugs used (in the last one month)**

3.4 In what circumstances do you use the drug(s) named above?

Frequency: 1= routine (1-2 times per month); 2= occasional (once every 3-4 months)
3 = rarely (once every 5-6 months)

Type of drug	Frequency	Reason for use	Comments

Reasons for use: 1= to keep pigs healthy
2 = only when pigs show signs of illness
3 = other (specify)….

3.5 Important signs of respiratory disease(s) observed in the last one month (you may tick more than one):

Yes = 1 No = 0

Code	**Sign**	**Yes**	**No**
1	Sneezing		
2	Coughing		
3	Difficult / labored breathing		
4	Fever (high temperature)		
5	Loss of appetite		
6	Sudden death		
7	Diarrhea		
8	Other (specify)……		

3.6 Age group most affected by respiratory signs (enter appropriate code)

Age group	Not affected	Slightly affected	Moderately affected	Highly affected
	0	1	2	3
Piglets				
Weaners				

Growers / fatteners				
Adults				

3.7 Period when most signs are observed

1= rainy season

2= dry season

3.8 When you see signs of respiratory disease, what do you do?

1= call a vet / extension worker
2 = buy drugs from local shop and administer myself
3= get local herbs and administer
4 = sell the pig(s)
5= do nothing
6 = other……..

3.9 Do you isolate sick pigs from healthy ones when you notice them?

1= yes

2 = no

Part e: herd management: *this section aims at understanding the general herd management practices that shall be used to identify possible risk factors for occurrence of respiratory disease*

4.1 Biosecurity on the farm:

1= Fenced

0= No fence

4.2 Disinfectant use: 1= Disinfectant used 0=No disinfectant used

4.6 Labour: Who takes care of or feeds the pigs?

1= Husband 2= Wife 3= Household member/child 4= Casual laborer (hired)

If own or hired labour, estimated cost per person per month (UShs)…….

4.7 Do you mix pigs of different age groups in the same pen/unit (apart from a lactating sow)?

1= no

0= yes

4.9 **Hygiene and sanitation:**

4.9.0 Hygiene score

2= clean

1=moderate

0=dirty/filthy

4.9.1 Who does the cleaning of pig house(s)? (you can choose more than one)

1= husband

2= wife

3= household member/child

4= hired laborer

4.9.2 Cleaning frequency per week?

0= Never,
1= 1-2 times/week
2= 3-4 times/week
3= daily

4.9.3 What is the type of drainage used in the pig houses?

0= None
1= sloping or pipe
2= sloping & pipe

4.9.5 Where do you dump pig wastes?

1 = > 10 m away from the pens
0 = < 10 m away from pens

4.9.6 How do you dispose of pig fecal material?

0=None
1=Thrown away
2=Buried

5.0 Visitors to the farm

How often do visitors or other people enter your farm (piggery unit)?

0= 'daily' 4-5 times/week,
1= 'occasionally' (1-2 times/week),
2= rarely/never)

5.1 How far or near are other pig farms from yours?

1 = < 20 m
2 = between 20 - 50 m
3 = more than 50 m

5.2 Do pigs from neighboring farms often mix with your pigs?

1 = no
0 = yes

5.3 How do you market or sell your pigs?

1 = take to the local market
2 = bring a trader to buy them from home
3 = slaughter in a nearby slaughter slab
4 = other (specify)…….

Part f: Herd breeding management

6.1 What breeding system do you use at your farm? 1 = natural service 2 = artificial insemination (AI)

6.2 If by natural service, which boar do you use?

0= borrowed from neighbor,
1= owned

6.3 If borrowed from a neighbor, how is the boar moved?

1= brought up to home 2 = a sow is taken where the boar is.

6.4 Do you buy replacement stock from outside your farm? 1= yes 2 = no

6.5 If yes, how often (in six months' time)? ……..

6.6 If yes, which age of pigs do you buy or obtain as replacement stock? (you may tick more than one)

1= adults
2 = gilts
3= weaners/growers
4 = piglets
5 = mixed age groups

6.7 Do you isolate new pigs (before mixing with your pigs) brought to your farm?

1= yes
0 = no

Any other comments you wish to state…….

……

End of questionnaire and thank you for your participation

Appendix 3(ii): Lung lesions score sheets

Appendix 3 (ii)

A) Lung lesions pneumonia form score sheet

Subcounty……..Village…….

Name of slab	Name of pig owner	Pig ID	Sample ID	Source of pig(s)	Breed	Sex	Live wt (kg)	BCS	CPBP score	PLP score	Pleuritis score	Remarks

B) Lung lobes pneumonia surface area score sheet

S/N	Name of owner	Pig ID	Dorsal view						Ventral view							Total score
			Right ant =5	Right mid =5	Right caudal =15	Left ant = 5	Left mid =5	Left caudal = 15	Right ant =5	Right mid =5	Right caudal =12.5	Left ant = 5	Left mid =5	Left caudal = 12.5	Access =5	

Appendix 3(iii) ***Longitudinal study: effects of respiratory infections on weight gains data sheet***

Subcounty	Village	Name of farmer	HH code	Confinement method	House type	Longitudes	Latitudes	Pig ID	Sample ID	Sex	Age	LW (kg)	CDS	BCS

Appendix : Pictures taken in the field (October 2018 – September 2019)

Principal investigator administering a questionnaire to one of the research participants in Lira, October 2018

Peter Oba after collecting data from a farmer's pigs in Lira district, December 2018

One of the field research assistants taking live weight measurements (A); and (B) Peter Oba and one of the assistants conducting a post-mortem to collect lung tissue samples from a weaner pig in Lira, April 2019

Peter Oba at Amach market, Lira district undertaking a slaughter slab survey of pigs, June 2019.

Some of the weaner/grower pigs tethered under a tree in Lira district Uganda, Feb 2019 (Photo Credit, Peter Oba, ILRI/Mak)