CHARACTERIZATION OF INFLUENZA A VIRUSES AMONG HUMANS, PIGS AND POULTRY AND FACTORS ASSOCIATED WITH ACUTE RESPIRATORY ILLNESS AMONG PIG WORKERS AT THE HUMAN-ANIMAL INTERFACE IN SELECTED SITES IN KENYA

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2021

Characterization of Influenza A Viruses among humans, pigs and poultry and factors associated with acute respiratory illness among pig workers at the human-animal interface in selected sites in Kenya

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Epidemiology of the Jomo Kenyatta University of Agriculture and Technology

2021

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

I dedicate this work to my family who supported and encouraged me in various forms while undertaking work related to this thesis. To my wife, Millicent and our parents, Ephraim Muriuki, Beatrice Kerubo and Joyce Wanjiru.

ACKNOWLEDGEMENTS

This work has been made possible through the efforts of various persons and organizations. First I would like to thank my supervisors Prof. Zipporah Ng'ang'a and Prof. Kariuki Njenga, for their invaluable support and input throughout the process.

I thank the Ministry of Health, Ministry of Agriculture, and Fisheries, the County Government of Kiambu, and Kenya Medical Research Institute for their role in approving and allowing conduct of the study procedures in their institutions. I also acknowledge Penina Munyua and Clayton Onyango from the Centers for Disease Control and Prevention-Kenya for their technical assistance during the implementation of the study.

I wish to express my gratitude to the study participants for agreeing to participate in the study. My appreciation goes to the research assistants who had to work for extended hours during the study.

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ABBREVIATIONS AND ACRONYMS

ARI	Acute Respiratory Infections
BPS	Board of Post graduate Studies
CDC	Centers for Disease Control
CI	Confidence Interval
DNA	Deoxyribonucleic acid
ELISA	Enzyme linked immune-absorbent Assay
ERC	Ethical Review Committee
FAO	Food and Agriculture Organization of the United Nations
GISRS	Global Influenza Surveillance and Response System
GLMM	Generalized Linear Mixed Model
HA	Hemagglutinin
HAI	Hemagglutination inhibition assay
HH	Household
HPAI	Highly Pathogenic Avian Influenza
IAVs	Influenza A virus
IFA	Immunofluorence antibody staining
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KEMRI	Kenya Medical Research Institute
LPAI	Low Pathogenic Avian Influenza

M	Matrix protein
NA	Neuraminidase
NP	Nasopharyngeal
NS	Non-structural protein
OD	Optical Density
OP	Oropharyngeal
OR	Odds Ratio
PA	Polymerase A protein
PB1	Polymerase B1 protein
PB2	Polymerase B2 protein
PCR	Polymerase Chain Reaction
PPE	Personal Protective Equipment
RNA	Ribonucleic Acid
RR	Relative Risk
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SD	Standard Deviation
SIV	Swine Influenza Virus
USA	United States of America
VTM	Viral Transport Medium

WHO World Health Organization

OPERATIONAL DEFINITION OF TERMS

Pig exposure	Cleaning barns, feeding or slaughtering pigs as part of routine daily activities for the month (> 3 times a week) preceding the study interview.
Poultry exposure	Cleaning poultry houses, feeding or slaughtering poultry as part of routine daily activities for the month (> 3 times a week) preceding the study interview.
Pig workers	Participants with pig exposure
Poultry workers	Participants with poultry exposure
Acute respiratory Illness	Illness of less than 7 days duration presenting with cough with or without fever.
Influenza virus infection	Detection of influenza RNA in a respiratory swab sample by reverse transcription polymerase chain reaction

ABSTRACT

Transmission of Influenza A viruses between humans and pigs is associated with occupational and environmental exposures. The main objective of the study was to identify the influenza viruses circulating among humans, pigs and poultry and determine factors associated with acute respiratory illness among pig workers at household and slaughterhouse levels. The study was conducted in four repeated cross-sectional studies among humans, pigs and poultry with the household component conducted in Kiambu county while the slaughterhouse component was done in Kiambu, Siaya and Kisumu counties. Three participants were randomly selected in each selected household, while the pigs were sampled proportionate to herd size. Nasopharyngeal (NP) and Oropharyngeal (OP) swabs were collected from participants who reported acute respiratory illness (ARI) defined as cough with/without history of fever within the previous seven days. Nasal swabs and blood samples were collected from pigs and poultry. The human and animal swab samples were tested for viral nucleic acid by RT-PCR and animal sera tested by ELISA for antibodies. Data were collected using an interviewer administered questionnaire and a logistic generalized linear mixed effect model was implemented to assess the association between pig exposure and occurrence of ARI within 30 days of sampling. All study participants gave informed consent and the study obtained ethical approval. In the household component of the study, 1,267 including 384 (30.3%) pig workers and 883 (69.7%) non-pig workers were enrolled. Of 130 human NP/OP swabs tested, four (3.1%) were positive for Influenza A virus. Seroprevalence of animal sera was 6.2% (265/4273), including 11.6% (230/1990) in pigs and 1.5% (25/2283) in poultry. In the slaughterhouse component of the study, a total of 288 participants were sampled, 91.3% of them being male. Fifteen (5.2%) participants had ARI but the nine swabs collected from them were negative for influenza A virus by PCR. Of the 1,128 pigs sampled, five (0.4%) nasal swabs tested positive for influenza A/H1N1/pdm09 by PCR whereas seroprevalence was 19.8%. The adjusted odds ratio for the association between pig workers and reporting ARI was 1.12 (95%CI [0.77 – 1.63]) at household level and 0.48 (95%CI [0.24, 0.96]) at slaughterhouse level. Having a member of the household with an episode of ARI in the previous three months (3.6 [95%CI 2.28 – 5.68]) and chronic disease (1.96 [95%CI [1.26-3.06]) were associated with reporting ARI on multivariable regression. The study reports detection of influenza virus (A/H1N1/pdm09) among pigs, a virus associated with human seasonal influenza. There is need to conduct influenza surveillance among pig workers and pigs in slaughterhouses as an important early warning system for influenza related zoonotic events.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Influenza A viruses circulate widely in animals, including birds, humans, pigs, and other mammals and are the cause of epidemics and pandemics of influenza that have afflicted humans and animals for generations. Influenza infections have claimed millions of lives since they were first reported in the 15th century (Dawood *et al.*, 2012). The 1918-19 Spanish flu pandemic remains one of the most severe infectious disease pandemics in history, having claimed an estimated 50-100 million human lives globally. Other pandemics which occurred in the 20th century include the 1957-58 (H2N2, 70,000 US deaths) and 1968-69 (H3N2, 34,000 US deaths). The most recent influenza pandemic of 2009 was due to a new swine-origin influenza A virus resulting in up to 570,00 deaths globally (Jones *et al*., 2012; Neumann & Kawaoka, 2019).

Influenza is a significant important cause of acute respiratory infections (ARI), including pneumonia, and is associated with considerable morbidity, mortality and economic burden worldwide (de Francisco Shapovalova *et al.*, 2015; Iuliano *et al.*, 2018). According to the World Health Organization up to 650,000 deaths per year are associated with influenza (WHO, 2018). Children under five years, the elderly, pregnant women, and persons with chronic medical conditions have a higher risk of severe disease associated with influenza infections (CDC, 2009; Emukule *et al.*, 2015; Rudan *et al.*, 2008). In tropical sub-Saharan Africa, the impact of influenza is likely higher because of the prevalence of other infections and comorbidities such as malnutrition, Human Immuno-deficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) and Tuberculosis (TB) (Cohen *et al.*, 2015; Gessner *et al.*, 2011; Ortiz *et al.*, 2012).

While influenza studies and surveillance has been extensively done in developed countries, the data from developing countries is scarce. Studies from some countries in Africa suggest that influenza circulates and causes epidemics regularly. A study

among children in Gabon recorded levels of antibodies to influenza A of 40% by hemagglutination inhibition while another study reported that between 3 to 15% of outpatient ARI visits were due to influenza (Gessner *et al.,* 2011). Further, studies from several countries in Africa have reported levels of hospitalizations associated with influenza that are 2-fold or higher compared to estimates from developed countries (Emukule *et al.*, 2015; Ntiri *et al.*, 2016; Zhou *et al.*, 2012).

Influenza contributes to the burden of upper and lower respiratory tract infections which are the second most common cause of morbidity and mortality in Kenya (Institute for Health Metrics and Evaluation, 2017). In Kenya, it is estimated that from 2009 to 2011 influenza resulted in 57,000 to 81,000 cases of severe respiratory illnesses and between 960 to 1,420 deaths each year (Fuller *et al*., 2013). Studies in various parts of the country describing the relative frequency of influenza in the aetiology of acute respiratory infections, have reported influenza as the second or third most common virus detected when compared to other viruses such as respiratory syncytial virus, rhinovirus, adenoviruses and human metapneumovirus (Ndegwa *et al.*, 2014; O'Meara *et al.*, 2015).

Data on influenza in humans in Kenya from sentinel surveillance indicate that the incidence of disease is highest among those under 5 years, and especially under 2 years. Among patients attending health facilities with acute respiratory illness, influenza was detected in 5-27% of the patients and 5-10% among those admitted in studies in western Kenya and a refugee camp (Ahmed *et al.*, 2012; Dawa *et al.*, 2018; Feikin *et al.*, 2013; Waitumbi *et al.*, 2010).

In a review of surveillance data in Kenya, influenza was reported in 15% of respiratory specimens with majority of the influenza infections occurring in the months of March to August between 2008 and 2013 period (Emukule *et al.*, 2015; Magana *et al.*, 2013). In another study in Nairobi influenza was detected year round with only slight increases during the colder months (Gachara *et al.*, 2006).

Besides influenza epidemics which occur regularly, influenza causes pandemics which are difficult to predict and can result in significant morbidity and mortality and disruption of world trade. Pandemics occur when a novel influenza virus, to which

people have little or no immunity, is introduced to the human population and is able to transmit efficiently among people. With the wide host range, influenza viruses exchange genetic material through reassortment which can result in emergence of highly pathogenic viruses that cause epidemics and pandemics. The genetic changes can result in minor antigenic variants of the virus in a process called antigenic drift. When the genetic changes are major and result in a novel virus, the process is called antigenic shift (Ito *et al.*, 1998).

Interspecies transmission is one of the important mechanisms of establishment of a novel influenza virus through the acquisition of new antigenic material (Alexander & Brown, 2000; Gregory *et al.*, 2003). Pigs are thought to be important in the evolution of viruses of pandemic potential due to their inherent ability to allow replication of swine, avian and human influenza viruses and potential to have mixed infections (Ito et al., 1998; Kristen Van Reeth, 2007). For example, the 2009 Influenza A H1N1 pandemic virus was a result of re-assortment of circulating human influenza and avian influenza strains with pigs suspected as the mixing vessel (Dawood *et al.*, 2012).

Swine influenza virus (SIV) infection is a highly infectious respiratory disease that affects pigs. The virus is transmitted primarily through pig-to-pig contact, with the virus entering the body through the nasopharyngeal path, most likely through nose-to-nose contact or direct contact with mucus (Crisci et al., 2013; Torremorell et al., 2012). Studies in Kenya have reported influenza A virus prevalence of 16% among pigs in Kenya and other studies elsewhere in Africa and Asia have reported as high as 67% influenza virus prevalence among swine in live markets (Eugenie *et al.*, 2017; Munyua *et al.*, 2018; Snoeck *et al.*, 2015; Suriya *et al.*, 2008). In slaughterhouses, studies on influenza A virus seroprevalence reported findings ranging from 5% in Uganda to 49% in Vietnam (Baudon et al., 2015; Eugenie et al., 2018, 2017; Kirunda et al., 2014).

Avian influenza (AIV) refers to infection of birds with avian influenza type A viruses which is shed in saliva, nasal secretions, and feces. Some avian influenza viruses can be transmitted to other animals such as pigs and humans. Pigs are believed to be

more susceptible to AIV viruses compared to humans (K van Reeth, 2006). Human or swine infections with AIV viruses can occur through direct or indirect contact through hosts' eyes, nose or mouth, or through inhalation of infectious air droplets or dust.

1.2 Statement of the problem

Swine-to-human and human-to-swine influenza (reverse zoonosis) virus transmission events have been documented in all regions of the world such as North America, Europe, Asia and Africa (Gray *et al.*, 2007; Gregory *et al.*, 2003; Ma *et al.*, 2015; Myers *et al.*, 2007; Njabo *et al.*, 2012; Rith *et al.*, 2013). Severe morbidity and mortality can occur when infected with swine influenza virus (SIV), especially among humans with underlying medical conditions, although majority of infections are subclinical or cause mild respiratory symptoms (Embree, 2010; Gatherer, 2009). Reverse zoonosis of influenza virus is considered an important source of SIV diversity which reduce efficacy of vaccines to SIV in pigs (Nelson & Vincent, 2015).

Transmission of swine influenza viruses to humans is associated with occupational and environmental exposures and can result in spread to in-contact family members (Beaudoin *et al.*, 2010; Lopez-Robles *et al.*, 2012; Myers *et al.*, 2006). Studies have shown evidence of infection with newly emerging swine influenza viruses as well as higher prevalence of SIVs among persons whose occupations are associated with close contact with swine (Gray *et al.*, 2007; Lopez-Robles *et al.*, 2012). In the last decade, the Food and Agricultural Organization (FAO) has reported an increase of the number of influenza A virus subtypes and also genotypes circulating in farm animals worldwide (FAO, 2017b). The transmission of influenza virus between swine and humans is not only associated with occupational and environmental exposures, but also with influenza virus evolution and emergence of novel transmissible strains capable of infecting humans and spreading from person to person, which with efficient transmission can lead to a pandemic (Beaudoin *et al.*, 2010).

Results from a preliminary study of seroprevalence of influenza viruses in pigs from a local slaughterhouse carried out in Kenya in 2010 revealed influenza A

seroprevalence of 15% and a pdm2009 H1N1 seroprevalence of 12.3% by hemagglutination inhibition. Another survey from pigs sampled from four households in an informal settlement showed a seroprevalence of 8.9% and a pdm2009 H1N1 prevalence of 7.1% by hemagglutination inhibition (Munyua, 2014). These results suggest possibility of widespread transmission of influenza of human origin in pig herds in Kenya.

Pig slaughterhouses present an occupational environment for intense exposure between pigs and humans, which can facilitate inter-species transmission of influenza viruses. With increasing swine farming and commercialization in Kenya, and low uptake of biosecurity measures, the level of human-swine exposures will likely increase providing an opportunistic setting for inter-species transmission (FAO, 2012b). Pig workers can transmit zoonotic influenza virus amongst themselves and to their family and close contacts (Njabo *et al.*, 2012). In Kenya, studies on occupational exposure to influenza among persons working closely with pigs have not been documented.

1.3 Justification of the study

The occurrence of influenza pandemics cannot be predicted with the available tools and a focus area for pandemic preparedness is early detection through surveillance. At present, influenza surveillance in Kenya is based on medically reported respiratory illness and the system does not adequately identify or target those occupationally exposed to pigs or poultry (Katz *et al.*, 2014). Further, there is paucity of data on the utility of acute respiratory illness (ARI) among these occupationally exposed persons as a proxy for influenza infection and early identification of zoonotic influenza events. In the absence of routine surveillance targeting this population, studies to identify the influenza viruses circulating in humans, pigs and poultry, especially at the human-pig interface, remain a priority.

Understanding the occurrence and dynamics of influenza A virus at the pig-human interface will inform cost-effective surveillance strategies because the burden varies with factors such as farming practices and pig population. While several studies on influenza virus at the human-animal interface have been conducted across the world,

few such studies are from sub-Saharan Africa (Krumbholz *et al.*, 2010; Nelson *et al.*, 2014; Rith *et al.*, 2013) A study in a pig slaughter house in Kenya in 2010-2012, reported detection of influenza virus (A/H1N1/pdm09) among pigs, suggesting introduction from humans (Munyua *et al.*, 2013). Such viruses transmitted from humans to swine can undergo mutations and infect humans back, potentially causing epidemics or pandemics.

The growing demand for pork products in Kenya has resulted in rapidly increasing number of farmers engaged in intensive small-scale pig farming (FAO, 2012b). Although pig workers in such livestock production systems may be exposed to swine influenza viruses, studies on the risk of occupational exposure to influenza viruses have not been conducted in Kenya. Monitoring of acute respiratory illness (ARI) among pig workers can be a useful method to determine circulation of influenza viruses in this population and assess factors that could impact the spread of influenza A viruses at the pig–human interface.

The findings from this study will provide information for developing control programs for influenza, including zoonotic influenza, and form a basis of expanding the influenza surveillance system. Information collected will contribute to the estimation of the burden of influenza in both the animal and human population and possible mitigation and intervention measures for adoption.

This study was a series of cross-sectional studies among human and pigs at household and slaughterhouse levels conducted to identify the influenza A viruses in pigs and humans and assess the association between acute respiratory illness and pig exposure. The farming practices associated with the risk of influenza virus transmission among pig keepers were also assessed in the study area.

1.4 Research Questions

The research questions that the study sought to answer were:

1. Which influenza virus types are circulating among humans, pigs and poultry at household and slaughterhouse levels?

2. What is the seroprevalence of influenza A among pigs and poultry in households and among pigs in slaughterhouses?
3. What is the prevalence and factors associated with acute respiratory illness among pig-exposed persons?
4. What farming practices increase the potential for influenza virus transmission in Kiambu county?

1.5 General Objective

The general objective of the study was to characterize the influenza viruses circulating in humans, pigs and poultry and determine factors associated with acute respiratory illness among pig workers at the human-animal interface.

1.5.1 Specific Objectives

The specific objectives of the study were;

1. To identify the influenza viruses circulating among humans, pigs and poultry in households in Kiambu County and slaughterhouses in Kiambu, Siaya and Kisumu counties
2. To determine the seroprevalence of influenza A virus infection among pigs and poultry in households in Kiambu County and pigs presented in slaughterhouses in Kiambu, Siaya and Kisumu counties
3. To determine the prevalence and factors associated with acute respiratory illness among pig-exposed and non pig-exposed persons at household and slaughterhouse level in Kiambu, Siaya and Kisumu counties
4. To assess the potential risks of influenza virus transmission arising from farming practices among pig farmers in Kiambu county

1.6 Outcome Measures

The primary outcomes among human participants were the prevalence of acute respiratory illness (ARI) and the prevalence of influenza A virus. Among pigs and poultry, the primary outcome was the detection of influenza A virus.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

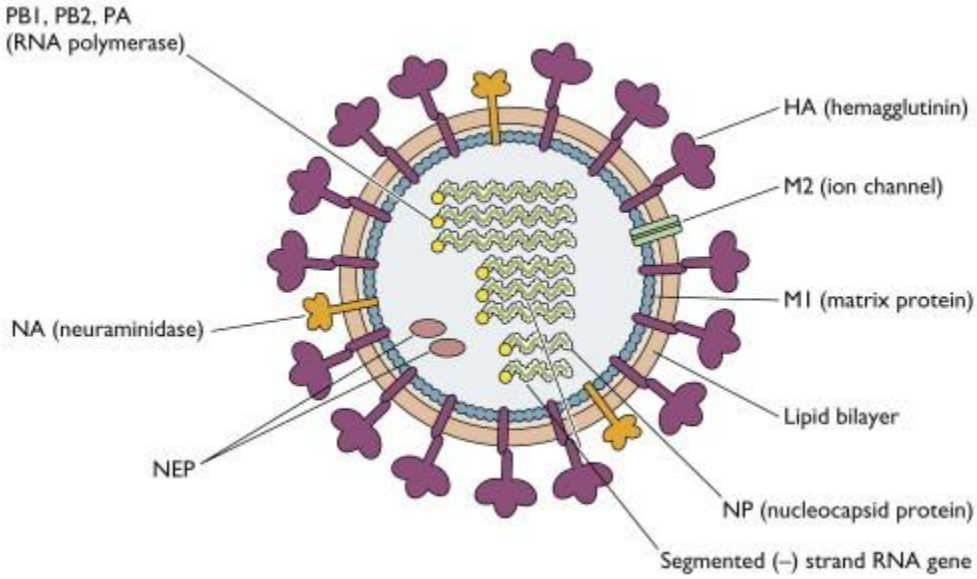
Influenza viruses belong to the Orthomyxoviridae family and are classified into four types – A, B, C and D – based on their antigenic differences (Dou *et al.*, 2018). Influenza A virus causes moderate to severe illness and infects humans and other animals and is perpetuated in nature by wild birds with most of them non-pathogenic to their natural hosts. Influenza B virus only infects humans and generally causes milder disease compared to Influenza A. It is more stable than Influenza A and has less antigenic drift and consequently more immunologic stability. Influenza C is rarely reported as a cause of human illness and has not been associated with any known epidemic while Influenza D viruses were recently described in cattle and are not known to cause disease in humans (Hause *et al.*, 2014). Influenza A virus is the type most associated with epidemics and pandemics because of its wide host range and is the focus of this study.

2.2 Structure of influenza A viruses

Influenza A viruses (IAV) are enveloped single-stranded RNA viruses with glycoprotein projections of haemagglutinin and neuraminidase covering the surface of the particle ((Source: http://www.virology.ws).

Figure 0.1) (Cox & Subbarao, 1999). The IAV comprise eight separate segments which form the ribonucleoprotein (RNP). The eight segments code for the following proteins; Polymerase B2 protein (PB2), Polymerase B1 protein (PB1), Polymerase A protein (PA), Haemagglutinin (HA or H), Nucleocapsid protein, Neuraminidase (NA or N), Matrix protein (M): M1 constructs the matrix and M2 acts as an ion channel pump to lower or maintain the pH of the endosome and Non-structural protein (NS) ((Source: http://www.virology.ws).

Figure 0.1).



(Source: http://www.virology.ws).

Figure 0.1: Simplified influenza A virus structure

Three proteins -- PB2, PB1 and PA -- form the RNA polymerase which participates in replication and transcription. The NS1 and NS2 proteins support the formation of viral components in cells which are infected (Harper *et al.*, 2002). The virus envelope is a lipid bilayer originating from the infected cell and has prominent projections formed by HA and NA, and the M2 protein.

Influenza A viruses are divided into subtypes based on two glycoproteins on the surface of the virus: the hemagglutinin (H) and the neuraminidase (N). There are 18 different hemagglutinin subtypes and 11 different neuraminidase subtypes (H1 through H18 and N1 through N11 respectively) (Fouchier *et al.*, 2005). While many combinations of influenza A subtypes are possible, only a few are known to cause epidemics or pandemics in humans.

Influenza A viruses are named based on a nomenclature published by WHO in 1980. The approach applies the following components in the naming; antigenic type, host (if not human), geographical origin, strain number, year of isolation and hemagglutinin and neuraminidase antigen description for influenza A viruses (WHO,

1980). For example, a human origin influenza A virus isolated in Kansas in 2017 is designated as A/Kansas/14/2017 (H3N2).

2.3 Antigenic variability of influenza A viruses

Influenza A viruses undergo evolution which is most prominent in the surface glycoproteins and the genetic make-up. This evolution comes about following mutations, reassortment of generic material or both. Mutations are the more common cause of genetic diversity in influenza viruses due to the lack of proof-reading function during replication. With the segmented nature of the influenza genetic material, the chance of reassortment is increased with attendant changes in the virus antigenicity. Influenza virus antigenic variation is classified as either antigenic shift or antigenic drift depending on the extent of the changes (Kilbourne, 2006).

Antigenic drift involves minor changes to the haemagglutinin and neuraminidase glycoproteins in both Influenza A and influenza B virus. Antigenic drift results from a series of point mutations in the HA and NA, which progressively decrease the neutralizing capacity of existing antibodies and increasing the susceptibility of persons to infection. Compared to human and swine influenza A viruses, avian influenza viruses undergo antigenic drift at a lower rate (Cox & Subbarao, 1999).

Antigenic shift refers to a more fundamental change in which the HA subtype is replaced in progeny virus. Genetic reassortment involving whole segments or mixing from different viruses is described as the most likely mechanism for antigenic shift. If the new virus is able to transmit efficiently among humans and the population immunity is low, an epidemic or pandemic can follow (Kilbourne, 2006; Zimmer & Burke, 2009).

2.4 Burden of influenza

2.4.1 Global and regional burden of seasonal influenza

Influenza is a highly contagious respiratory disease transmitted from one person to another via droplet infection and contact with contaminated hands, surfaces and equipment. The influenza virus has an incubation period of between one and 14 days

with an average incubation period of two (2) days. Common signs and symptoms include fever, cough, headache, sore throat, muscle ache, and exhaustion. Infection may lead to secondary bacterial respiratory infection, death or still births in pregnancy, neonatal death, low birth weight and premature birth. In the majority of cases individuals will recover from two to seven days after symptoms appear (WHO, 2018).

Influenza A viruses circulate widely in animals, including birds, humans, pigs, and other mammals and are the cause of epidemics and pandemics of influenza that have afflicted humans and animals for generations. Influenza infections have claimed millions of lives since they were first reported in the 15th century (Dawood *et al.*, 2012). Influenza is an important contributor to acute respiratory infections (ARI), including pneumonia, and results in substantial morbidity, mortality and economic burden globally (de Francisco Shapovalova *et al.*, 2015; Iuliano *et al.*, 2018). According to the World Health Organization, influenza is estimated to be responsible for up to 1 billion infections, 3 to 5 million cases of severe illness and 300,000 to 500,000 deaths annually (WHO, 2019). Children aged less than five years, pregnant women, the elderly, and persons with underlying medical conditions have an increased risk of severe disease associated with influenza infections (CDC, 2009; Emukule *et al.*, 2015; Rudan *et al.*, 2008).

While influenza studies and surveillance have been extensively done in developed countries, the data from developing countries is scarce. Studies from some countries in Africa suggest that influenza circulates and causes epidemics regularly. A study among children in Gabon recorded high seropositivity (>40%) to both influenza A and B subtypes, a possible indication of the variability of the influenza strains, while another study reported that between 3-15% of outpatient Acute Respiratory Illness (ARI) visits were due to influenza (Gessner *et al.*, 2011). In tropical sub-Saharan Africa, the impact of influenza could be substantial due to the prevalence of other infections and comorbidities such as Human Immuno-deficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS), Tuberculosis (TB), and malnutrition. Studies conducted in several African countries have previously estimated rates of influenza-associated hospitalizations that are more than two-fold higher than

estimates from the US and other industrialized countries (Emukule *et al.*, 2015; Ntiri *et al.*, 2016; Zhou *et al.*, 2012)

A comprehensive modelling study on global influenza associated excess mortality rate reported higher estimates than previously reported. In the study, the highest proportion of excess mortality due to influenza were reported from Asia (25%), Western Pacific (25%) and Sub-Saharan Africa (17%) (Iuliano *et al.*, 2018).

2.4.2 Burden of influenza in Kenya

Data on influenza in Kenya is based on reports from sentinel surveillance or modelling studies. In a population-based surveillance study, influenza virus was detected as the second or third most common virus among patients with respiratory illness. The incidence of disease was highest among those under five years, and especially under two years. Among patients attending health facilities with acute respiratory illness, influenza was detected in 5-27% of the patients and 5-10% among those admitted in studies in western Kenya and a refugee camp (Ahmed *et al.*, 2012; Feikin *et al.*, 2013; Waitumbi *et al.*, 2010).

A health utilization adjusted study in 2013, reported that from 2009 to 2011 influenza resulted in 57,000 to 81,000 cases of severe respiratory illnesses and between 960 to 1,420 deaths each year (Fuller *et al.*, 2013). From a population-based study, the adjusted incidence rates among hospitalized children under five years with respiratory symptoms was 2.7–4.7 per 1,000, compared to that among persons above five years of 0.2–0.4 per 1,000 among persons. This findings reflect the likely higher incidence of severe disease among younger persons (Emukule *et al.*, 2015). Findings from sentinel surveillance and population based studies in Kenya indicated an incidence of medically attended influenza which is two to four times higher than rates reported in Europe and the United States (Fowlkes *et al.*, 2013).

In a study based on surveillance data, influenza was detected in 15% of all respiratory specimens with majority of the influenza infections occurring in the months of March to August of the study period (Magana *et al.*, 2013). In an earlier study in Nairobi, it was concluded that influenza was an infection of public health

importance and was present throughout the year with only slight increases during the colder months (Gachara *et al.,* 2006).

With the established high burden of influenza in sub-Saharan Africa and the zoonotic nature of influenza, studies at the human animal interface can provide important insights on some of the drivers of influenza transmission. Investigating acute respiratory illness among poultry and pig workers could help understand how it compares to the general population and if existing surveillance approaches need to target this population.

2.4.3 Pandemic influenza

Besides influenza epidemics which occur regularly, influenza causes pandemics which are difficult to predict but have the potential to cause significant morbidity and mortality and substantial disruption of world trade. Pandemics occur when a novel influenza virus, to which people have little or no immunity, is introduced to the human population and is able to transmit efficiently among people. With the wide host range, influenza viruses exchange genetic material through reassortment which can result in emergence of highly pathogenic viruses that cause epidemics and pandemics. The genetic changes can result in minor antigenic variants of the virus in a process called antigenic drift. When the genetic changes are major and result in a novel virus, the process is called antigenic shift (Ito *et al.,* 1998).

The 1918-19 Spanish flu pandemic remains one of the most severe infectious disease pandemics in history, having claimed an estimated 50-100 million human lives globally. Other pandemics which occurred in the 20th century include the 1957-58 (H2N2, 70,000 US deaths) and the 1968-69 (H3N2, 34,000 US deaths). The most recent pandemic influenza was in 2009 resulted in up to 570,000 deaths globally (Jones *et al.,* 2012).

While it is postulated that influenza pandemics occurred in the 19th century, the first confirmed pandemic was the 1918-19, which was likely caused by an avian origin H1N1 virus (Taubenberger *et al.,* 1997). The virus continued to circulate until 1957 when a novel reassortant H2N2 virus, also from an avian source, caused the next

pandemic with an estimated 1,000,000 deaths globally. In 1968, another new virus, H3N2 caused a pandemic whose impact was lower than the previous two likely because of some level of immunity in the population from circulating strains. The most recent pandemic of 2009 was caused by a novel H1N1 virus which had both swine and avian origins (Neumann & Kawaoka, 2019).

2.5 Transmission of influenza viruses between species

Avian influenza viruses occur naturally among wild aquatic birds. From these sources, they can infect varied animal species including domestic poultry and other wild birds, and also humans (Gaidet *et al.*, 2007; Olsen *et al.,* 2006).

The H glycoprotein binds to the host cell and fuses with the cell membrane to allow the viral contents to enter the cell. The H is responsible for the species specificity of influenza viruses although mutations can result in cross species transition. A primary determinant for influenza virus infectivity is the H receptor link in host cells. Avian viruses generally bind to NeuAcα2,3Gal sialic acid (SA) receptors while human viruses generally bind to NeuAcα2,6Gal SA receptors found in human respiratory epithelium. Both NeuAcα2,3Gal and NeuAcα2,6Gal SA receptors are present in the trachea of swine, which allows pigs to be infected by both avian and human viruses (Baigent & McCauley, 2003)

In domestic poultry, avian influenza can manifest as a mild disease form, termed the low pathogenic avian influenza (LPAI), or a severe and often lethal form, termed the highly pathogenic avian influenza (HPAI). The LPAI viruses can evolve into HPAI viruses when introduced into poultry populations, resulting in mortalities of up to 100%. The HPAI virus subtypes, H5N1, has been associated with both human and animal influenza outbreaks. The H5N1 virus is now endemic in parts of Asia and Africa with >800 human cases in sixteen countries confirmed between 2003-2017 and case fatality rate over 50% (WHO, 2017). This is in addition over 400 million birds, including poultry that have died or been culled because of H5N1 virus infection (FAO, 2012a).

In Africa, HPAI was first reported in Egypt between 1923 and 1945, and thereafter in South Africa in 1961. While the HPAI subtype associated with the infections in Egypt was not known, the outbreak in South Africa was caused by the H5N3 virus (Swayne & Suarez, 2000). More recently (2015-2017), HPAI outbreaks in poultry and wild bird populations were reported primarily in West Africa i.e. Burkina Faso, Cameroon, Côte d'Ivoire, Ghana, Niger, Nigeria and Togo (H5N1 subtype) and in Cameroon, Democratic Republic of the Congo, Niger, Nigeria (H5N8 subtype). Outside this region, the H5N8 subtype has been reported in the southern parts of Africa (South Africa and Zimbabwe) and more recently in East Africa (Uganda) (FAO, 2017b). The outbreak in Uganda was detected following the death of 1200 terns (in a population of 2000 terns) between mid-December 2016 and early January 2017. By late January, the virus had spilled over to domestic birds killing 7 out of 20 birds that showed clinical signs, in a population of 30,000 birds (FAO, 2017a). Of 213 samples collected during this outbreak, 10% were influenza A positive, with ducks being the most susceptible. The HPAI virus subtype associated with this outbreak was confirmed as the H5N8 HPAI clade 2.3.4.4 virus of group B (FAO, 2017b). No human cases were reported in any of these outbreaks.

Interspecies transmission is one of the important mechanisms of establishment of a novel influenza virus through the acquisition of new antigenic material (Alexander & Brown, 2000; Gregory *et al.*, 2003). Genetic mutations of influenza virus can result in interspecies transmission of the virus to humans or animals. The new host can be a dead-end host, in which the virus is not propagated or establish infection and transmission resulting in outbreaks. Pigs are believed to play a critical role in the evolution of viruses of pandemic potential due to their inherent ability to allow replication of swine, avian and human influenza viruses and potential to have mixed infections (Ito et al., 1998; Kristen Van Reeth, 2007). For example, the 2009 Influenza A H1N1 pandemic virus was the product of re-assortment of circulating human influenza and avian influenza strains with pigs suspected as the mixing vessel (Dawood *et al.*, 2012).

Pig-to-human and human-to-pig influenza (reverse zoonosis) virus transmission events have been documented in North America, Europe, Asia and Africa (Gray *et*

al., 2007; Gregory *et al.*, 2003; Ma *et al.*, 2015; Myers *et al.*, 2007; Njabo *et al.*, 2012; Rith *et al.*, 2013). Severe disease following these zoonotic events has been reported in persons with chronic medical conditions, although most such infections are mild or subclinical (Embree, 2010; Gatherer, 2009). Reverse zoonosis of influenza virus is considered an important source of swine influenza viruses (SIV) diversity which reduces efficacy of vaccines to SIV in pigs (Nelson & Vincent, 2015).

The transmission of influenza viruses between pigs and humans is not only associated with occupational and environmental exposures, but also with the virus evolution and emergence of novel transmissible strains capable of infecting humans and spreading from person to person that can lead to pandemics (Beaudoin *et al.*, 2010; Gray *et al.*, 2007; Myers *et al.*, 2007) ((Source: National Institute of Allergy and Infectious Diseases).

Figure 0.2).

Studies have shown evidence of infection with newly emerging SIVs as well as higher prevalence of SIVs among persons whose occupation involves close interaction with pigs (Gray *et al.*, 2007; Lopez-Robles *et al.*, 2012). Findings from a preliminary study in pigs from a Kenyan slaughterhouse revealed an overall influenza A seroprevalence of 15%, including >12% seroprevalence of the pandemic 2009 H1N1 influenza virus, suggesting transmission of influenza viruses from humans to pigs (Munyua, 2014).

2.6 Influenza seroprevalence in pigs and poultry

Swine influenza virus (SIV) infection is an acute and contagious respiratory disease of pigs and the primary route of virus transmission is pig-to-pig contact, with the virus entering the body via the nasopharyngeal route, most probably through nose-to-nose contact or following direct contact with mucus (Crisci et al., 2013; Torremorell et al., 2012)

Swine influenza virus (SIV) infection is a highly infectious respiratory disease that affects pigs. The virus is transmitted primarily through pig-to-pig contact, with the virus entering the body through the nasopharyngeal path, most likely through nose-to-nose contact or direct contact with mucus (Crisci et al., 2013; Torremorell et al., 2012). Studies in Kenya have reported influenza A virus prevalence of 16% among pigs in Kenya and other studies elsewhere in Africa and Asia have reported as high as 67% influenza virus prevalence among swine in live markets (Eugenie *et al.*, 2017; Munyua *et al.*, 2018; Snoeck *et al.*, 2015; Suriya *et al.*, 2008). In slaughterhouses, studies on influenza A virus seroprevalence reported findings ranging from 5% in Uganda to 49% in Vietnam (Baudon et al., 2015; Eugenie et al., 2017, 2018; Kirunda et al., 2014).

Avian influenza (AIV) refers to infection of birds with avian influenza type A viruses which is shed in saliva, nasal secretions, and faeces. Some avian influenza viruses can be transmitted to other animals such as pigs and humans. Pigs are believed to be more susceptible to AIV viruses compared to humans (K van Reeth, 2006). Human or swine infections with AIV viruses can occur through direct or indirect contact through hosts' eyes, nose or mouth, or through inhalation of infectious air droplets or dust.

Studies on avian influenza seroprevalence among backyard chicken in in several countries in Asia have reported varied estimates ranging from 20% in Bangladesh to 71% in Pakistan. (Biswas et al., 2009; Chaudhry et al., 2021). In Nigeria, a seroprevalence survey among poultry for several viruses including AIV did not detect any antibodies against AIV among sampled poultry (Owoade et al., 2006). The higher prevalence in Asia could reflect the higher incidence AIV likely associated with more intense poultry production. Determining the seroprevalence of AIV is a relatively quick method to understand the circulating strain and the intensity of transmission among poultry flocks. Suh information is useful in targeting AIV surveillance activities.

There have been few and sparse studies on influenza seroprevalence in pigs or poultry in Kenya. In the absence of systematic surveillance, such studies are

necessary to monitor transmission of influenza and could inform the utility or need to adopt specific farming practices. The influenza seroprevalence studies could be an indicator of the exposure workers among the pigs and poultry are exposed to.

2.7 Influenza detection methods

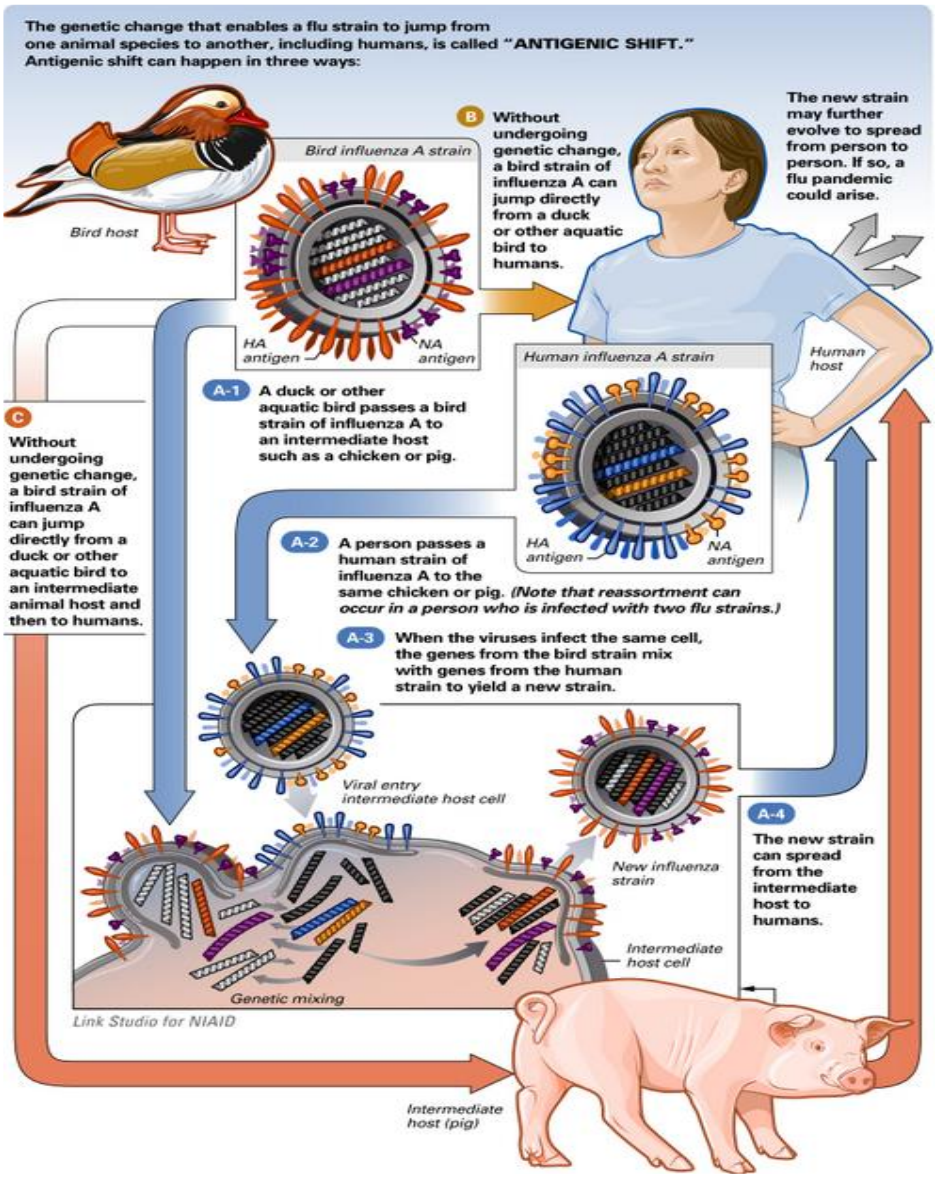
Influenza can be detected through the viral components or antibodies produced by the body against the virus. The methods include: Rapid antigen test, Immunofluorence antibody staining, Haemagglutination inhibition, Enzyme-linked immunosorbent assay, RT-PCR and virus isolation.

2.7.1 Rapid antigen tests

Rapid tests detect viral material (usually nucleoprotein) or enzyme activity. Rapid tests identify either influenza A only, influenza A or B without type specification. The sensitivity ranges between 40 and 80% and is greater earlier in the course of the disease and among children (Weinberg *et al.*, 2005). The predictive values of the antigen tests vary by prevalence of influenza and the tests are therefore not recommended for surveillance outside outbreak settings.

2.7.2 Immunofluorescence antibody staining

The principle of Immunofluorescence antibody staining (IFA) is that an antibody stains virus-infected cells, a phenomenon which is then observed under a fluorescent microscope. The IFA can be used on clinical specimens although it is best applied to virus isolates. The IFA can identify influenza virus species and H subtypes. The sensitivity of IFA is estimated at 60-80% and is influenced by the quality of the specimen, preparation of slides as well as the skills of the reader (Dziąbowska *et al.*, 2018).



(Source: National Institute of Allergy and Infectious Diseases).

Figure 0.2: Illustration of antigenic shift in Influenza viruses

2.7.3 Enzyme-linked Immunosorbent Assay

Enzyme-linked immunosorbent assay (ELISA) detects antibodies against the nucleoprotein common to all influenza A viruses, is not subtype specific and is designed to measure the relative level of antibody to influenza. Once the sample is incubated in the coated wells, an influenza specific antibody complex with the coated antigen is formed. Unbound material is washed away, and anti-AI monoclonal antibody enzyme conjugate is added to the wells. If the influenza antibodies are not in the sample, the conjugate will bind the influenza antigen on the plate. However, if there are anti-influenza antibodies in the sample, the anti-influenza conjugate does not bind to the antigen. Enzyme substrate is added after the unbound conjugate is washed. The color development has a negative relationship with the quantity of antibodies against influenza in the test sample (Shirley *et al.*, 2015). While ELISA techniques have been used for long, they have lower sensitivity and specificity compared to molecular methods (Leirs *et al.*, 2016).

2.7.4 Hemagglutination inhibition assay

The hemagglutinin glycoprotein on the influenza virus surface binds with red blood cells to cause agglutination when mixed. The principle of Hemagglutination inhibition assay (HAI) is therefore the prevention of this agglutination by anti-hemagglutinin antibodies in serum, following exposure, vaccination or infection, which bind to the hemagglutinin of the influenza virus making it unavailable to bind to the red blood cells. In the presence of influenza virus antibodies, agglutination is inhibited, but in the absence of the antibodies, agglutination occurs because of binding between the test virus and the red blood cells. The titers of HAI are determined in this test and a four-fold rise in titers is diagnostic of influenza infection. An advantage of HAI is that it is possible to determine the influenza A subtype by using specific antibodies (Li *et al.*, 2017).

2.7.5 Real time RT-PCR for influenza

Real time RT-PCR amplifies specific gene sequences for both detection and quantification. Quantitative reverse transcription PCR (RT-qPCR) is used because influenza in an RNA virus in which the RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase from total RNA. The cDNA is then applied as the template for the qPCR reaction. The procedure follows the general principle of PCR which is logarithmic amplification of a target fragment of a genome; its key feature is that the amplified DNA is detected as the reaction progresses in real time. This is different from conventional PCR, where the product of the reaction is detected at the end of the process after resolution of the PCR products by gel electrophoresis. Sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter permits detection after hybridization of the probe with its complementary DNA target (Dziąbowska *et al.*, 2018; Wang & Taubenberger, 2010).

2.7.6 Virus Isolation by Cell Culture

Virus isolation through cell culture is considered the "gold standard" of influenza testing. In cell culture, the clinical specimen is inoculated in embryonated eggs or mammalian tissue, incubated to allow growth for about a week and the cytopathic effect of the virus is observed. Isolates from the cultured virus can then be identified by various methods including staining and molecular techniques. Virus isolation therefore allows for strain characterization which a key component of global influenza surveillance and monitoring (Dziąbowska *et al.*, 2018).

2.8 Prevention and control of interspecies transmission

Prevention of interspecies transmission of influenza is important for a couple of reasons. First, viruses transmitted to humans could establish efficient transmission and result in an epidemic or pandemic. Viruses transmitted from humans to animals could cause big economic losses to the agricultural and food industries. Finally, viruses transmitted across species increase risk of further mutation and/or reassortment which could lead to pandemic strains (Alexander & Brown, 2000;

Rabinowitz *et al.*, 2013). Biosecurity measures are important in reducing interspecies transmission of influenza virus (McCune *et al.*, 2012). Basic biosecurity measures in farm operations include; separating pigs and isolating them from other herds and birds, management practices such as "all-in, all-out" and quarantine and isolation practices and controlling the number of people, vehicles, and equipment coming onto the farms; and ensuring that anyone or anything coming onto the farm is cleaned and disinfected. Other measures include preventing humans with respiratory symptoms from entering swine facilities and practicing personal hygiene including use of personal protective equipment

Vaccination may reduce the risk of co-infection and development of novel viruses. Influenza vaccinations are available for humans, swine, poultry, horses, and dogs. Vaccination does not eliminate viral replication or shedding of virus but may reduce clinical signs and the amount of virus shed (Yoo *et al.*, 2018).

2.9 Conceptual framework

The conceptual framework models the relationship and association between the independent and the dependent variables. The primary dependent variable in this study was the occurrence of acute respiratory illness and the secondary dependent variable was influenza A infection. Figure 0.3 is an illustration of the conceptual framework which is based on framework developed by Henry Mosley and Lincoln Chen, whose central tenet was that factors which were proximate to disease occurrence operated under a background and influence of sociocultural and economic factors (Mosley & Chen, 1984).

The independent variables were broadly divided into socio-demographic and economic and the pig husbandry characteristics. The sociodemographic factors which likely had a relationship with the dependent variable include age, sex, preexisting immunity to acute respiratory illness causing pathogen and household socio-economic status. Pig husbandry characteristics refer to those associated with work in pig farms and are associated with respiratory infection in humans. These include pig exposure, the level of circulation of influenza in the pig herd, the

biosecurity practices and use of personal protective equipment (PPE) by the pig workers.

The independent variables are modulated in their association with the dependent variables by seasonality of respiratory illness, household overcrowding and pollution (Figure 0.3).

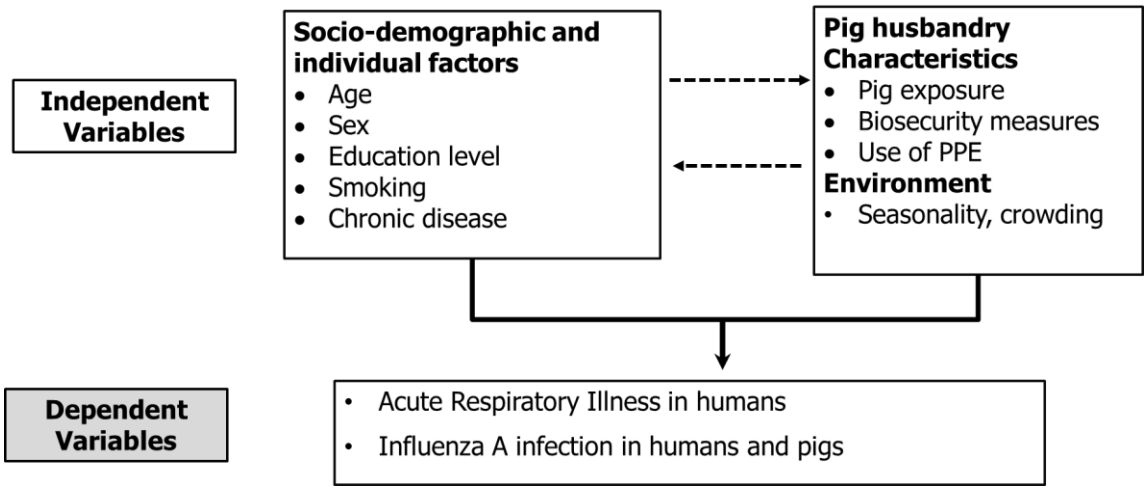


Figure 0.3: Conceptual framework of the association between the dependent and independent variables

CHAPTER THREE

MATERIALS AND METHODS

This section outlines the methods and procedures used in the study. The household and slaughter components of the study are described separately.

3.1 Study design and sampling

3.1.1 Household Component

3.1.1.1 Study Area

The study was conducted in Kiambu County, an administrative county in central Kenya with the headquarters in Kiambu town. The county is to the north of Nairobi and has a population of 2,417,735 (Kenya National Bureau of Statistics, 2019) and an area of 2,543 km² (County Government of Kiambu, 2018). With rich highland soils coupled with favourable climatic conditions, agriculture plays an important role in the county's economy. Intensive and extensive agricultural systems are practiced in the county including tea, coffee, dairy and pig farming.

Kiambu county that has the highest proportion of intensive small-scale pig farmers in Kenya (FAO, 2012b). Within Kiambu county, two sub-counties were selected that had high number of pig farms (Figure **0.1**). According to the 2019 population and housing census, there were 84, 991 pigs and 3,661,661 indigenous, layers and broiler chicken in Kiambu county (Kenya National Bureau of Statistics, 2019).

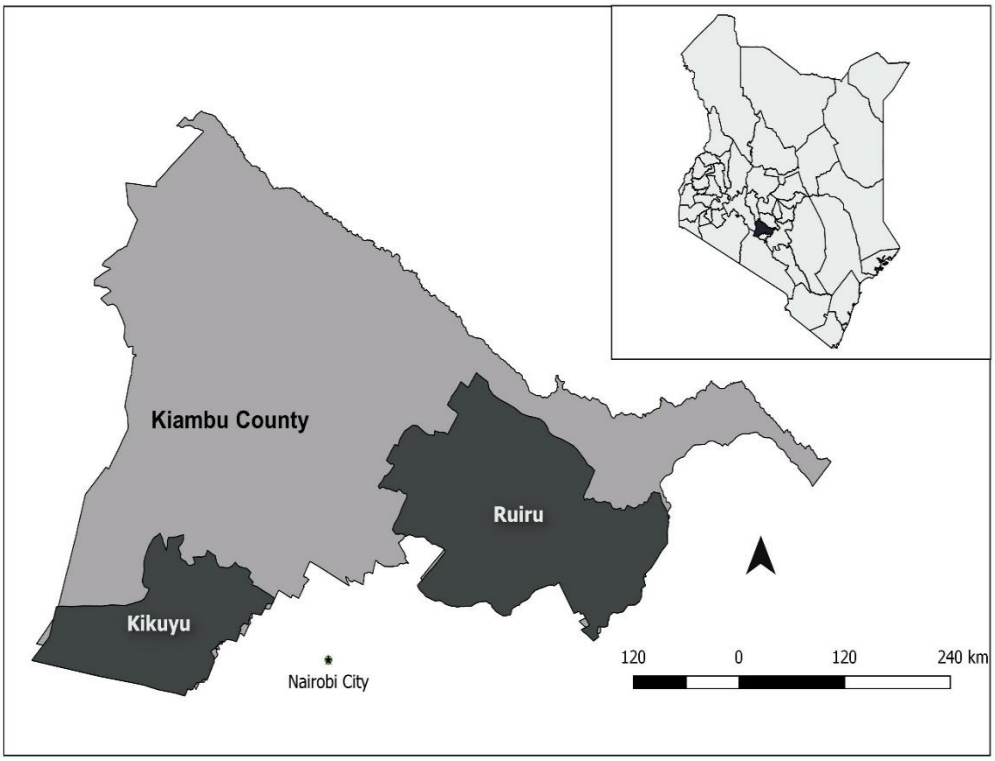


Figure 0.1: **Map of Kiambu county showing the selected administrative locations where households were sampled**

The households were sampled from within two sub-counties of Kiambu county – Kikuyu and Ruiru. Inset is a map of Kenya with Kiambu County highlighted in dark. Map created in QGIS

3.1.1.2 Study Design

We conducted four repeated cross-sectional studies among humans and animals (pigs and poultry) at household level. The cross-sectional studies were conducted in four waves over one year to account for potential seasonality differences in influenza transmission (Emukule *et al.*, 2016). Concurrent cross-sectional sampling of pigs and poultry in enrolled households was conducted at time of human sampling.

3.1.1.3 Study Population

The study population comprised of humans and livestock (pigs and poultry) in the enrolled households.

3.1.1.4 Inclusion and Exclusion Criteria for Human Participants

Persons in selected households aged 2 years and above were eligible for inclusion in the study. Children below 2 years were excluded because of the difficulty in collecting respiratory swabs from them in the field. Nasopharyngeal (NP) and Oropharyngeal (OP) swab collection was conducted in eligible persons in selected households that met the acute respiratory illness (ARI) case definition. The case definition for ARI was an illness of less than 7 days duration with history of cough with/without fever. The study excluded eligible persons who did not consent/assent. NP/OP swabs were not collected from persons that did not meet the ARI case definition

3.1.1.5 Inclusion and Exclusion Criteria for Pigs and Poultry

Pigs and poultry in selected households where humans were enrolled were eligible for inclusion. The study did not enroll pigs and poultry where the household head did not give consent for animal sampling.

3.1.1.6 Sample Size determination

3.1.1.6.1 Sample Size Determination for Human Sampling

The sample size was determined using Fleiss formula with continuity correction for cross sectional studies, comparing two proportions as described by Fleiss and based on the formula below (Fleiss et al., 2004).

$$n' = \frac{\left[Z_\alpha \sqrt{(1 + 1/m)\bar{p}(1 - \bar{p})} + Z_\beta \sqrt{p_0 (1 - p_0)m + p_1 (1 - p_1)}\right]^2}{(p_0 - p_1)^2}$$

$$\bar{p} = \frac{p_1 + m p_0}{m+1}$$

$$n = \frac{n'}{4}\left[1 + \sqrt{1\frac{2(m+1)}{nm|p_0 - p_1|}}\right]^2$$

n the estimated sample size per group

p_0 prevalence of acute respiratory illness among non pig workers (6%)

p_1 prevalence of acute respiratory illness among pig workers (18%)

n' sample size among exposed before continuity correction. The continuity correction brings normal curve probability in closer agreement with binomial probabilities

m number of unexposed individuals per exposed individual

$\bar{p}$ estimated average of p_0 and p_1

Z_α This is the Z value corresponding to the alpha error of 5%. The corresponding (two- tailed) Z values is 1.96

Z_β Z value corresponding to the beta error. The Z-value used was 0.80

The sample size was calculated for human participants assuming prevalence of acute respiratory illness of 18% among pig workers (exposed), and 6% among non-pig workers (unexposed) (Bigogo et al., 2013; Radon et al., 2001) translating to a sample size of 306 participants with 77 in the exposed and 229 in the unexposed group (exposed to unexposed ratio of 1:3). This sample size allowed for detection of odds ratio of 3.5 or higher with a power of 0.80 and a two-sided alpha of 0.05 (Dean et al., 2013). The sample size was inflated by a factor of 1.4 to account for clustering because of sampling multiple individuals in a household for a total of 429 participants (108 exposed and 321 unexposed) for each sampling wave.

A maximum of three persons were randomly selected in each household. Majority of the exposed (pig-workers) were drawn from pig-keeping households and therefore a minimum of 36 pig-keeping and 107 non pig-keeping households per sampling

wave. More households were enrolled to attain the estimated individual sample size because some of the households had less than 3 members.

3.1.1.6.2 Sample Size Determination for Pig Sampling

To determine the sample size for pigs and poultry the study applied the formula for cross sectional studies as described by Fleiss (Fleiss *et al.*, 2004).

$$n = \frac{{Z_{\alpha/2}}^2 * P * (1 - P) * D}{E^2}$$

Where,

N = sample size, Z= 1.96,

P is the estimated seroprevalence

E is the precision level

D is design effect

For pig sampling an assumption of the estimated seroprevalence of 15% (Munyua, 2014) was made with an absolute precision level of 5% and a design effect of two to account for clustering because of sampling of multiple pigs in a household. A minimum sample size of 392 pigs was therefore determined during each sampling wave.

For poultry sampling, an assumption of the estimated seroprevalence of 3% (Munyua, 2014) was made with an absolute precision level of 1.5% and a design effect of 1.5 to account for clustering at household level. A minimum sample size of 745 was determined based on these assumptions. Further assuming that 90% of the households kept poultry (Nyaga, 2007), we expected to sample an average of 6 poultry (expected average of 2 species) in each of these households.

3.1.2 Selection of Participants

3.1.2.1 Selection of Households

To enhance identification of persons with exposure to pigs, households were selected based on whether they kept pigs or not. Pig-keeping households were selected by systematic random sampling from a comprehensive list of pig farmers in the two sub-counties maintained by the sub county veterinary officers. To achieve systematic random selection, the total number of pig keeping households was divided by the total number of households required to obtain the sampling interval. A random number was selected to determine the starting point within the first sampling interval with subsequent household selections based on the sampling interval.

Non-pig keeping households were randomly selected from the neighborhood of the selected pig keeping households. The study applied the spin the bottle method to randomly determine the direction in which the non-pig keeping households were selected. The spinning was done outside the entrance of the pig keeping household and the direction was determined by where the mouth of the bottle or pen tip pointed to. Every third household in the determined direction was selected for enrolment, for up to three households. If any of the selected household owned pigs they were replaced by an immediate neighbouring non-pig owning household.

3.1.2.2 Selection of of human participants and livestock

Once a household was identified for sampling, consent from the household head or eligible adult was sought for enrolment of the household. After the consenting process was completed, a list of all the residents of the household was written down on a study form. Each resident of the household above 2 years of age was allocated a number. The corresponding numbers were then written on pieces of paper and folded. Three numbers were drawn and the individuals corresponding to these numbers were approached for consenting if adults and assenting if children 7-17 years old, followed by individual interview and sample collection. If the selected person was not at home, one revisit was scheduled within three days of household

sampling. If a selected resident in a household declined consent, they were replaced by a person from the same household by drawing another number.

Sampling of pigs and poultry at the household during human sampling. For pigs, sampling was done from all age groups; piglet [birth to weaning], weaners [piglet after permanent separation with sow], growers [pig between weaning and sale], finishers [grower pigs over 70 kg live weight], sows [adult female pig], and boars [male adult pig]). Sampling of the pigs was proportional to size of the herd such that for small herds (< 10 pigs), all the pigs were sampled, for herds with sizes >10, a maximum of 15 pigs were sampled. For poultry three birds per species were sampled in households that kept poultry (**Figure** 0.2).

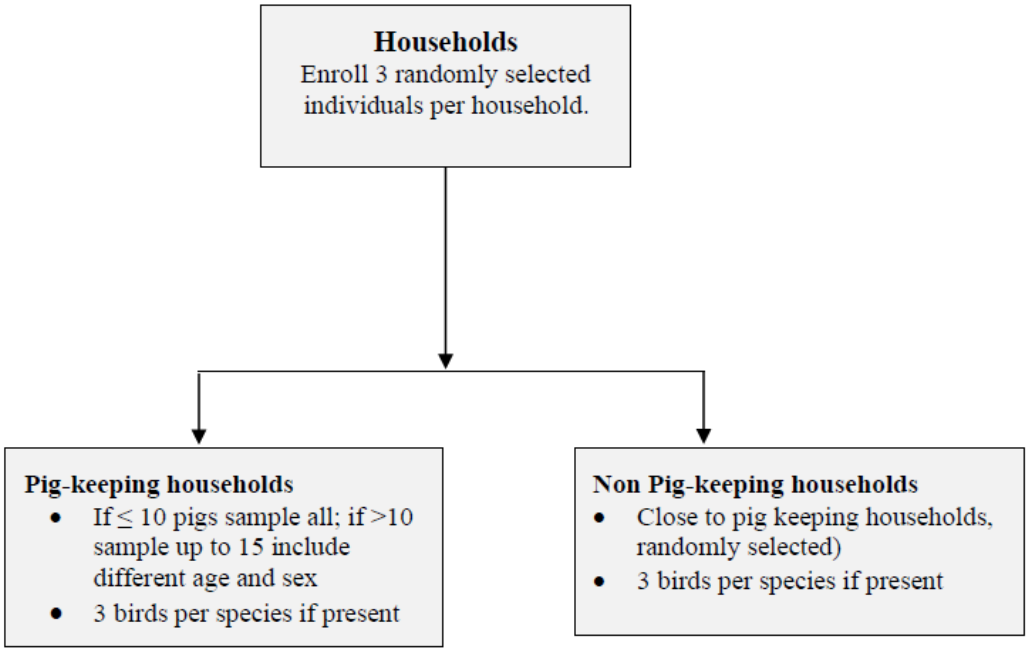


Figure 0.2: Sampling schema at household level

3.1.3 Slaughterhouse component

3.1.3.1 Study design and area

Four repeated cross-sectional studies were conducted over a period of one year among humans and pigs in three slaughterhouses in Kiambu (Uthiru slaughter

house), Kisumu and Siaya (Bondo slaughter house) counties (**Figure 0.3**). Uthiru slaughterhouse receives most pigs from the small scale farms in Kiambu that have intensive pig production system while the Kisumu and Siaya slaughterhouses receive pigs from more traditional, extensive pig production systems. These contrasting pig production systems represented varying degrees of contact between humans and pigs.

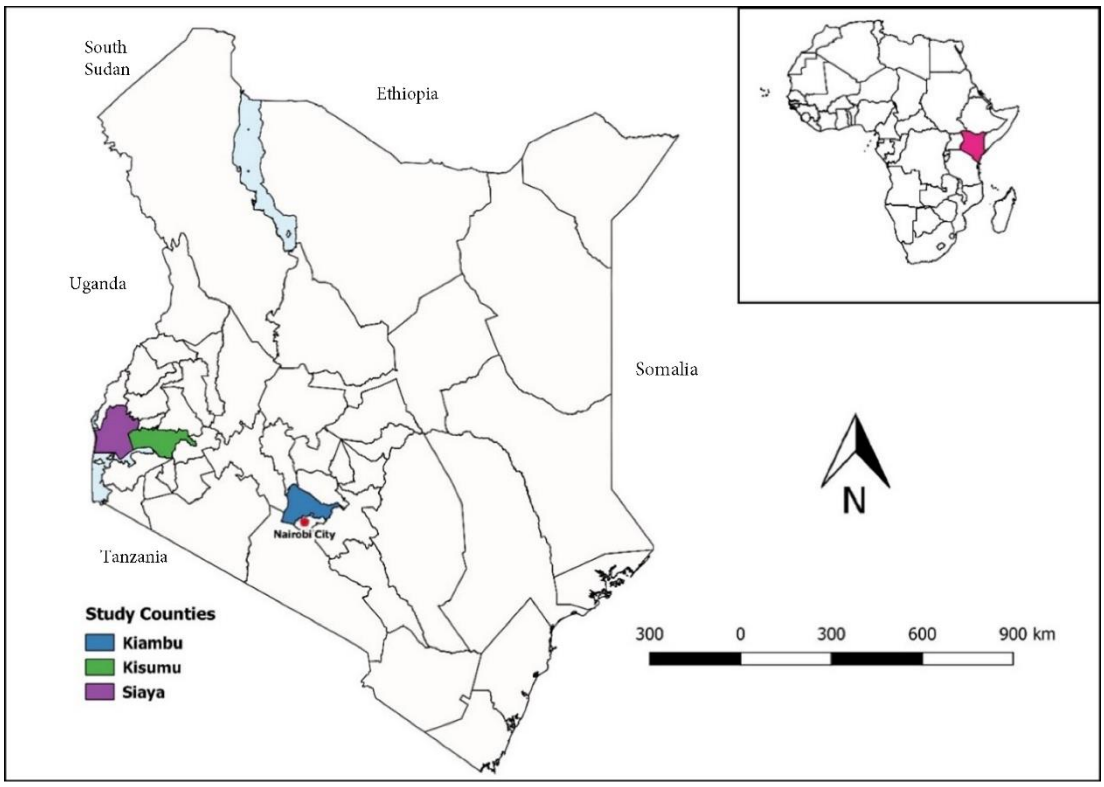


Figure 0.3: Map of Kenya highlighting the three counties where the slaughterhouse sampling was conducted.

Inset is a map of Africa with Kenya highlighted. (Map created in QGIS)

3.1.3.2 Study Population

The study population were the individuals within the precincts of the selected slaughterhouses and the pigs brought for slaughter.

3.1.3.3 Inclusion and Exclusion Criteria

All pig slaughterhouse workers, farmers and traders who visited the slaughterhouse to deliver pigs or to purchase meat, animal health personnel and others working in the slaughterhouse were eligible for inclusion. The study enrolled those that gave informed consent. NP/OP swabs were collected from enrolled participants that met the respiratory illness case definition

The study did not enroll eligible participants who declined consent/assent. All pigs brought for slaughter in the selected slaughterhouses during the study period and the owners consented for the sampling were included. We excluded pigs where the owners declined consent.

3.1.3.4 Sample size and sampling

All persons working or based in the slaughterhouse at the time of study were invited to participate. For pig sampling, an estimated seroprevalence of 20% was assumed, precision level of 5% and at 95% confidence level, design effect of 1.5. A population correction was applied because the estimated number of pigs presented at the slaughter houses during the sampling period was 1,000. The sample size was calculated using Fleiss formula and determined as 297 pigs per sampling period.

The slaughterhouses were visited each consecutive working day for 10 days to sample the pigs. For Uthiru (Kiambu) slaughterhouse, every other pig to a maximum of 25 per day was sampled per day. For Kisumu and Bondo (Siaya) slaughterhouse where volume of pigs slaughtered was low, all the pigs presented for slaughter on each day of sampling were targeted for sampling

3.2 Data and Sample collection

3.2.1 Data Collection

Standardized interviewer administered questionnaires (Appendix 6 & 7) in smart phones were administered to all participants. Data collected included demographics, clinical symptoms and exposure to risk factors including the specific activities they

were engaged in with reference to pig raising, transportation, slaughtering and dressing. For all animals sampled, data on demographic and risk factors (age, species present on the farm, herd size, species raised, and husbandry practices) were collected. By using a standardized questionnaire, the varying degree of contact for pig handlers and owners was assessed including pig husbandry activity, protective actions taken, influenza vaccination status, animal ownership at home and known illness. Additionally, information on knowledge, attitudes and practices of pig farmers/farm workers to influenza was collected.

For the slaughterhouse component of the study, a structured questionnaire was administered to participants to collect data on sociodemographic characteristics, frequency and level of contact with pigs, influenza vaccination history and history of respiratory illness. The questionnaire was administered electronically through smart phones using a windows-based application.

All participating households and participants were assigned a unique identifier (ID). Each participant at the slaughterhouse was also assigned a unique participant identifier that also included the slaughterhouse code. These unique identifiers were used to link all the specimens (human and animal) that were collected from the household.

The data were downloaded from the smart phones into a primary database and backed up daily on a secure server. Weekly frequency matches of the database were run to detect errors or inconsistencies that needed verification or correction. All data entered from the study results and serology were stored in a computer which was password protected and backed up daily to a central server. A central log that included location of study sample and participant unique identifier was kept in a secure location, along with the entry records in the smart phones and any paper field recordings of the study.

The study included research assistants (RAs) who worked under direct supervision of the principal investigator. The RAs were trained on the study rationale and objectives, methods, and the instruments. Field testing of the instruments was conducted with the RAs, with revisions made during the training period. The

research assistants who interacted with participants were trained on how to interview and answer questions from potential participants, procedures for obtaining informed consent, and how to interview enrolled participants. Additionally, training on sample collection, specimen transportation, processing and storage was provided with appropriate supervision throughout the study period. Training on informed consent procedures with both theoretical and practical sessions related to research ethics was also offered.

3.3 Sample collection

3.3.1 Human OP/NP swab collection

For the OP sampling, the participant was asked to open the mouth widely and the tongue held down by a tongue depressor. The posterior pharyngeal wall was then evenly swabbed, swad removed and the tip cut off after placing into a cryovial with viral transport medium (VTM). For the NP sampling, the polyester tipped swab was gently placed in either nostril towards the bottom of the nostril on the side by the septum. The swab was then gently moved parallel to the palate straight backwards until some resistance was met, turned three times, removed and the tip cut off into the same cryovial as the OP swab.

3.3.2 Sample collection in pigs and poultry

3.3.2.1 Pig blood sample collection

In households, the animal was restrained with the help of a pig snare and the puncture site (jugular vein or vena cava) swabbed with a cotton wool soaked in alcohol. In slaughterhouses the sample was collected after stunning. The vein was punctured and 9mls of blood (4mls from young pigs in household sampling) using a red-topped vacutainer. The needle was then removed, and pressure applied on the puncture site.

3.3.2.2 Nasal swab Collection from pigs

In the household, pigs were restrained by a snare before sample collection. The polyester-tipped swab was inserted into either nasal opening and then slowly withdrawn with a rotating motion. The swab was then placed into the cryovial containing viral transport media and the tip cut off using tips of a scissors. In slaughterhouses, the nasal swabs were collected post-mortem after the animal was stunned.

3.3.2.3 Poultry blood sample collection

Blood samples were collected form adult poultry. The puncture site (brachial vein) was swabbed with a cotton wool swab soaked in alcohol. The brachial vein was punctured with a needle and 2mls of blood drawn into a red-topped vacutainer, the needle withdrawn, and pressure applied on the puncture site using a dry swab.

3.3.2.4 Oropharyngeal swab collection from poultry

With the bird restrained, the beak was held open with one hand and the swab inserted with the other hand. The entrance of the trachea was gently swabbed with a rotating motion. The swab was then withdrawn and placed directly into the cryovial containing VTM.

3.3.3 Sample handling and shipping

All collected specimens were labelled using pre-printed barcode labels that had unique specimen ID. The specimen ID of each specimen was recorded in the study questionnaire and the sample tracking sheet that accompanied the sample to the laboratory. The unique participant ID was also recorded on the questionnaire and the sample tracking sheet. This specimen ID was used to link the laboratory results to the questionnaire data in both humans and animals. Personal protective equipment used during sample collection included latex gloves, coverall, gumboots and face masks. The gloves were changed between animals and hands sanitized.

The samples were placed into leak-proof secondary containers with absorbent material with extra sample labels (in a zip-lock bag) and sample collection tracking sheets accompanying the sample. Serum was separated from blood on the day of sample collection by centrifugation

Human NP/OP swabs were temporarily stored in a cool box at 2–8°C and transported to the Kenya Medical Research Institute (KEMRI) laboratory in Nairobi on the same day where they were stored at -80^{0}C until testing. Animal samples were temporarily stored in a cool box at 2–8^{0}C and transported to KEMRI laboratory in Kisumu where they were stored at -80^{0}C until testing. Animal samples collected in Nairobi were temporarily stored at the KEMRI laboratory in Nairobi before shipment to the KEMRI Kisumu laboratory.

3.4 Influenza testing

3.4.1 Serology for Influenza A virus

Animal sera were tested for antibodies against influenza A viruses using the IDEXX® ELISA (FlockChek AI MultiS-Screen Ab Test Kit®, Westbrook, Maine), following manufacturer instructions.

In brief, the test detected antibodies against the nucleoprotein common to all influenza A viruses and is not subtype specific and is designed to measure the relative level of antibody to influenza. The assay was performed in a 96 well plates that had been coated with influenza viral antigen. Upon incubation of the test sample in the coated wells, influenza specific antibody forms a complex with the coated antigen. After washing away unbound material, an anti-AI monoclonal antibody enzyme conjugate was added to the wells. In the absence of the influenza antibodies in the test sample, the conjugate was free to bind the influenza antigen on the plate. Conversely if there were antibodies to influenza present in the sample, the anti-influenza conjugate was blocked from binding to the antigen. Unbound conjugate was washed away, and enzyme substrate was added. Subsequent color development was negatively related to the level of anti-influenza antibodies in the sample (Shirley *et al.*, 2015).

For the specific steps, the test sample was diluted tenfold with sample diluent prior to being assayed. Wash concentrate was prepared and diluted in 1/10 with distilled water water. Thereafter, 100µl of the diluted test sample, negative and positive control were each dispensed into the corresponding wells of the plate. An antibody conjugate 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate solution as a color indicator and stop solution were serially added with incubation and washing between each step. The absorbance was then measured and read at 650 nanometers (nm) (Shirley *et al.*, 2015).

The ELISA data analyses were done using software from the manufacturer. Results were reported as the ratio of the sample optical density (OD) reading to the kit negative control OD reading (S/N). The manufacturer recommended cut-off of ≤ 0.5

for positive sera was applied for poultry. The test has been validated for use with pig sera with an adjusted cut-off of S/N ratio ≤ 0.673 applied since this was determined to increases test sensitivity to 72% and specificity to 99% in pigs sera (Ciacci-Zanella *et al.*, 2010; Munyua *et al.*, 2013).

3.4.2 Molecular detection of Influenza A Virus

3.4.2.1 Real time RT-PCR for influenza viruses

Human and animal swab samples were tested for influenza A virus RNA by real-time reverse transcriptase polymerase chain reaction (RT-PCR) using primers and probes that target the matrix gene of influenza A viruses and NS1 gene of influenza B viruses (Spackman *et al.*, 2002; Whiley *et al.*, 2009).

Briefly, all surfaces, pipettes, and centrifuges were wiped with RNase Zap to remove any potential contamination. The reagents were prepared by thawing aliquots of primers and probes and mixing RT-PCR buffers and probes by inversion and centrifuging them. The reaction mix was prepared using AgPath-ID One-step RT-PCR Kit. A master mix comprising the following was then made; Nuclease Free Water, RT-PCR buffer, Forward Primer, Reverse Primer, Probe and RT-PCR Enzyme Mix. The reaction mix was set up in the reaction plate and pipetted into each well/row. The whole plate was then covered with Alumaseal and moved to the next lab where 5µL of RNA template and positive controls were pipetted into the respective wells of the plate (Lilian *et al.*, 2015).

To set up equipment of the Real Time RT-PCR Quantification run; the Applied Biosystems 7500 SDS software was used, the relevant entries completed, and the plate loaded into the plate adapter. The instrument then conducted the PCR run, while displaying real-time status information in the instrument tab and recording the fluorescence resulting from cleavage of TaqMan probes in the presence of the target sequences. After the run was completed the real-time PCR results were viewed on the results tab using the amplification plot.

As part of the quality control, the no Template control reaction sets should not exhibit fluorescence growth curves that cross the threshold line, all clinical samples should exhibit reaction curves that cross the threshold line at or before 40 cycles, thus indicating the presence of sufficient RNA from human RNase P gene, an indicator that the specimen is of acceptable quality. Positive Template Control reactions should produce a positive result with the Flu A, Flu B and RNP reactions (Lilian *et al.*, 2015).

When all controls met stated requirements, the specimen was considered positive for influenza A or B virus if the Flu A or B reaction growth curves crossed the threshold line within 40 cycles respectively.

3.4.2.2 Subtyping of Animal Influenza A Virus Positive Samples

Subtyping was attempted for all PCR positive samples that had CT values <35 for seasonal human influenza, avian and swine influenza. Each sample RNA extract was tested by separate primer/probe sets: InfA, Swine H1, Swine H3, swine N1, swine N2, AH5a, AH5b, pdm InfA, pdm H1. Each run included a no template control (NTC) and a positive template control (PTC) (Lilian *et al.*, 2015).

Briefly on the steps, 5 µl of the first sample were pipetted into all the wells labeled for that sample on the plate. The column to which the samples were added was capped using Micro Amp Optical 8-Cap Strip to prevent sample cross-contamination and enable tracking of sample loading. This was repeated until all the samples are loaded.

The Applied Biosystems 7500 SDS software was then set up for the test run and analysis. When all controls had met the stated requirements, a specimen was considered positive for influenza A virus if the InfA reaction growth curve crossed the threshold line within 40 cycles. If the reaction for Influenza A is positive, it could also be positive for one of the following subtypes: Swine H1, Swine H3, swine N1, swine N2, AH5a, AH5b, pdm InfA, pdm H1. A specimen was considered positive for swine influenza A/H1,A/N1,A/N2, A/H3 or Influenza AH/5 (Asian Lineage) virus if both the InfA and the respective subtype (swine H1,swine N1,

swine N2, swine H3, AH5a AND AH5b) reaction growth curves crossed the threshold line within 40 cycles. A specimen was considered presumptive positive for pandemic influenza AH1 if both the InfA and the respective sub type (pdm InfA or pdm H1) reaction growth curves crossed the threshold line within 40 cycles.

3.5 Data analysis

Data were cleaned and analyzed using R Statistical Software, version 3.5.1 (R Core Team, 2017). Descriptive statistics were determined for socio-demographic and other characteristics comparing pig workers and non-pig workers. Categorical variables were compared using chi-square test and fisher's exact test where applicable while continuous variables were compared using the Student's t-test.

In households, pig exposure was defined as cleaning barns, feeding or slaughtering pigs as part of routine daily activities for the month (> 3 times a week) preceding the study interview. Poultry exposure was similarly defined for those working with poultry. Participants with pig or poultry exposure were classified as pig workers and poultry workers respectively. Acute respiratory illness was defined as illness of less than 7 days duration with cough with/without fever.

In slaughterhouses, pig exposure was defined as any person who routinely skinned or stunned pigs, sold pork or offals at the slaughter house. Acute respiratory illness (ARI) was defined as an illness of less than 7 days duration with cough with/without fever. Chronic disease was any reported illness which required regular follow up by a health professional for at least 3 months.

Influenza A prevalence in humans and animals was calculated as the proportion of samples positive by RT-PCR against all the samples tested. Influenza seroprevalence for animal samples was calculated as a proportion of the number of samples that were positive by ELISA against all the samples tested and by species. A seropositive herd was defined as any farm with at least one pig positive for influenza A IgG antibody by ELISA.

In the household component, the prevalence of ARI within 30 days of sampling was calculated as the number of episodes reported by participants divided by the total number of participants. Crude odds ratios were determined for the initial assessment of association between pig exposure and reports of ARI.

The household level study design provided for clustering at household and individual level and a generalized linear mixed model (GLMM) using the logistic distribution was therefore applied to adjust the odds ratio between pig exposure and reporting ARI for potential confounding.

The predictor variables (fixed effects) included in GLMM to predict the odds of occurrence of ARI were pig workers, age, sex, occupation, education level completed, reported chronic disease, sampling month and poultry exposure. Clustering was accounted for at household and individual level (repeat sampling) by including the variables as random effects in the mixed model. The GLMM was done using the lme4 package in R statistical software where the estimation is based on maximum likelihood (Bates *et al.*, 2015)

Model selection was conducted using stepwise selection using Akaike information criterion and Bayesian information criteria measures where lower values indicate better model fit (Bolker *et al.*, 2009). The adjusted odds ratio and the 95% confidence intervals were then computed and statistical significance determined at a p-value of <0.05.

For the slaughterhouse level data, prevalence was determined as the proportion of samples positive for influenza A virus against all samples tested. Bivariable logistic regression was conducted to determine the association between any acute respiratory illness within 30 days of sampling and pig exposure status, sociodemographic factors, reported chronic disease and sampling month. Multivariable logistic regression was then applied to identify independent factors associated with ARI and estimating the magnitude of the adjusted odds ratio (aOR) for the assessed factors. The 95% confidence intervals (CIs) were computed for the aOR. Model selection was based on likelihood ratio tests for nested models. Model goodness of fit was

assessed by the Hosmer-Lemeshow Test with p-value of >0.05 indicating good fit (Hosmer et al., 2013).

3.6 Ethical Considerations

The study was approved by the KEMRI Scientific and Ethics Review Committee (Protocol number 2557 and KEMRI Animal Care and Use Committee (Appendix 8). The study also received administrative approval form the Ministry of Health and the Directorate of Veterinary Services (Appendix 8)

All data and specimens collected were kept confidential to the extent allowable by law or regulation. Participant's names were not used in any of the survey forms or on the specimens. All biological specimens and data forms were assigned a unique number as identifiers to ensure confidentiality throughout the study. Any data not stripped of identifiers were stored in a locked file to which only study personnel had access. All databases used for data analysis used codes only, without participants' names.

In each of the identified household (HH) that was visited, the household head/ or any eligible adult was approached for consenting by the RAs. Willing HH heads were taken through the consent form and if consent was given, the form was signed by the HH head and RA, with a copy remaining with the participant (Appendix 1). The household head consent allowed for sampling of animals and access to household members to obtain individual consent. The consenting process and questionnaire administration was done in a private area as practically as was possible.

All persons who were eligible as determined by the inclusion criteria were taken through the individual consent form (Appendix 2) and documenting the consent before participating in the study. For children aged 2-12 years, the parent permission (Appendix 3) was sought before participation. For children aged 13-17 years, parental permission and child assent (Appendix 4) were sought before participation.

Consent for obtaining animal specimens was sought during HH head consenting. Care was taken to minimize this stress of handling and restraining animals for

sample collection. Pigs were restrained using a pig snare while birds were manually restrained.

CHAPTER FOUR

RESULTS

4.1 Characteristics at household level

4.1.1 Introduction

The household component of the study was conducted in four sampling waves in September 2013 (Wave 1), December 2013 (Wave 2), May 2014 (Wave 3) and August 2014 (Wave 4). A total of 1,127 households were enrolled during the four waves – 310 in September 2013, 255 in December 2013, 324 in May 2014 and 238 in September 2014 (Figure 0.1). Among the 1,127 households, there were 3,784 residents giving a mean household size of 3.4. From among the 1,127 households enlisted, there were 634 (56.2%) distinct households of which 493 (77.8%) had two visits during the sampling waves.

From the 634 distinct households, 170 were pig keeping while 464 were non-pig keeping. The household characteristics are presented in Table 0.1. There. was a statistical difference in the sex of household respondent between the pig keeping and non-pig keeping households ($p < 0.041$), with pig keeping household having significantly more males compared to non-pig keeping households (55.9% vs 46.3%). There were no significant differences on the household respondents' level of education between pig keeping and non-pig keeping households with over 60% of the respondents having either secondary education or higher (Table 0.1). The median household size was three in both groups, with a arrange of one to 15 members.

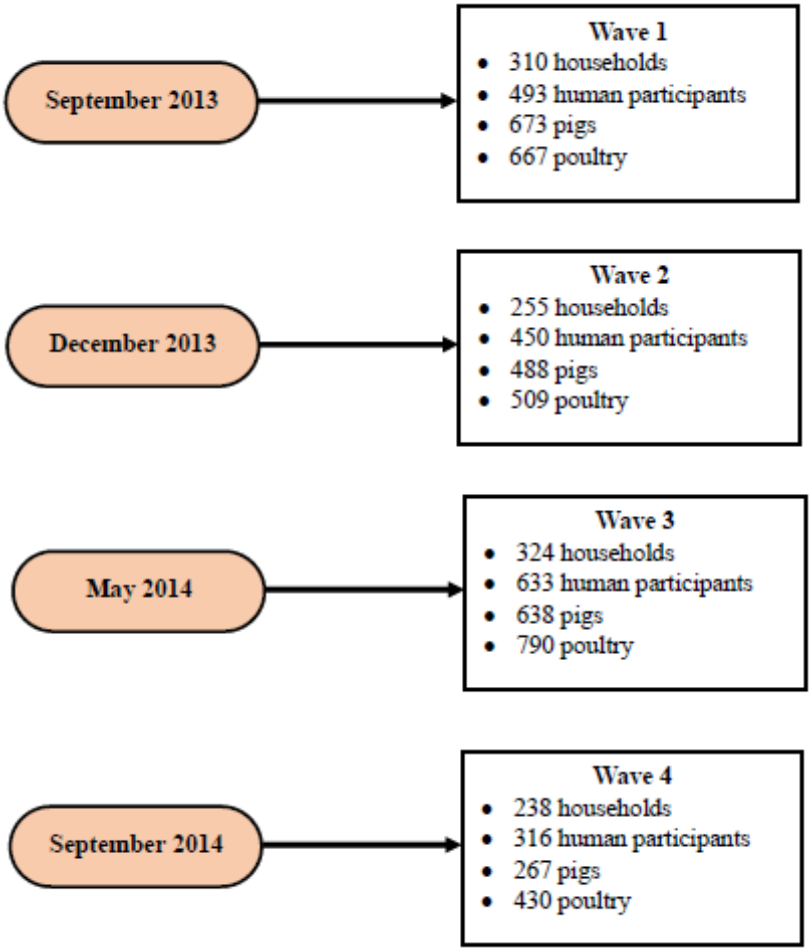


Figure 0.1: Schema of household and participant enrolment by sampling wave, Kiambu County, Kenya, 2013-2014

Nearly all pig-keeping households (97.6%) owned other livestock apart from pigs. About three quarters (72.4%) of the non-pig keeping households owned other livestock including cattle, goats, sheep and poultry. Chicken were the most commonly owned livestock among pig keeping households (80.6%) and non-pig keeping households (64.2%) followed by cattle, goats and sheep. Poultry such as geese and turkey were owned by less than 10% of the households. A higher proportion of pig keeping households owned cattle, chicken, ducks and turkeys compared to non-pig keeping households (Table 0.1).

Table 0.1: Characteristics of households and household respondents by pig keeping status, Kiambu County, Kenya, 2013-2014

Characteristic	Pig keeping household[α] N=170 % (95% CI)	Non-Pig keeping household[α] N=464 % (95% CI)
Sex		
Female	44.1 (36.5−51.9)	53.7 (49.0−58.3)
Male	55.9 (48.1−63.5)	46.3 (41.7−51.0)
Level of Education		
No formal Education	18.2 (12.7−24.9)	18.8 (15.3−22.6)
Primary	3.5 (1.31−7.52)	3.0 (1.66−5.01)
Secondary	38.8 (31.5−46.6)	35.8 (31.4−40.3)
Post-secondary	24.7 (18.4−31.9)	28.4 (24.4−32.8)
Other	14.7 (9.8−20.9)	12.9 (10.0−16.3)
Type of livestock owned[β]		
Cattle	45.3 (37.7−53.1)	31.7 (27.5−36.1)
Goats	18.2 (12.7−24.9)	12.5 (9.63−15.9)
Sheep	14.1 (9.26−20.3)	11.2 (8.48−14.4)
Chicken	80.6 (73.8−86.2)	64.2 (59.7−68.6)
Ducks	14.1 (9.3−20.3)	5.0 (3.2−7.4)
Geese	6.5 (3.27−11.3)	2.9 (1.5−4.7)
Turkey	5.3 (2.5−9.8)	1.3 (0.5−2.8)

[α]Only households with a follow up visit [a]Variable has some missing data [β]Categories not mutually exclusive

Among pig keeping households, the median number of pigs per household was 13 (range; 1 to 200) and over half (54.5%) of the households had reared pigs for at least 2 years. Besides pigs, majority of the farms reared chicken (80.6%). During the sampling waves, a total of 2,066 pigs were sampled, of which 1,118 (63.2%) were female and nearly half (58.9%) were either finishers or growers (Figure 0.2).

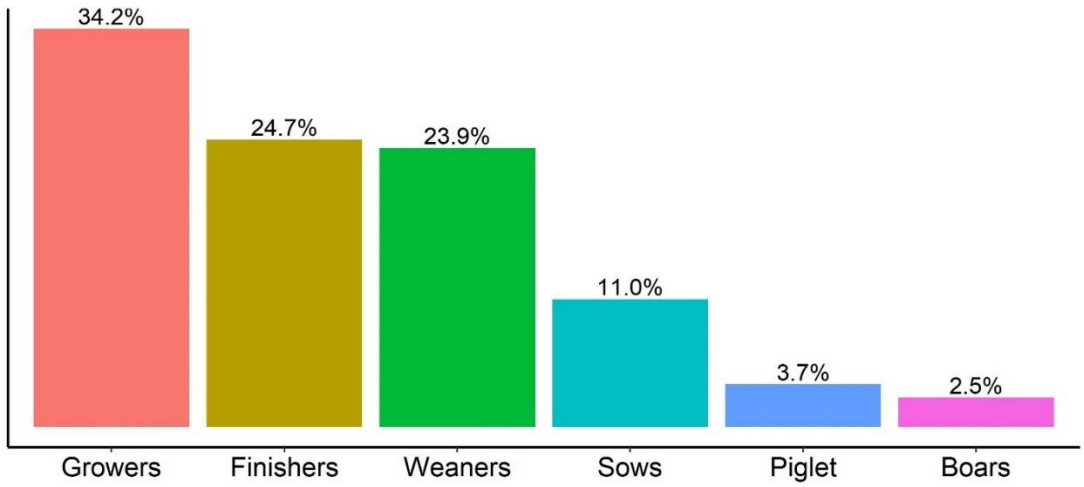


Figure 0.2: Proportion of pigs sampled in the households by age-group, Kiambu County, Kenya, 2013 – 2014

4.1.2 Human Participants at household level

A total of 1,892 respondents were enrolled in the four waves, representing about half of all the members in the enrolled households. Most of the participants were sampled in May 2014 (33.5%), followed by September 2013 (26.1%), December 2013 (23.8%) and September 2014 (16.4%) (Figure 0.1). Two thirds (1,267) of the respondents had one visit with one-third having two visits during the sampling waves.

The demographic characteristics of 1,267 participants who had at least one visit are presented in

Table 0.2 by pig worker status. Among the 384 pig workers, 81.5% were residents of pig keeping households, 58.1% were male, and the 21–40 years age-group accounted for 43.9% of the participants. Nearly four-fifths of the pig-exposed participants reported their occupation as farming, 76.8% had poultry exposure with 14 (3.7%) pig workers reporting no formal education. Non-pig workers were mostly female (56.2%), about one third (33.7%) were between 21 and 40 years old and 51.7% were farmers Among pig workers, 58.1% had secondary education or above compared to 54.4% among non-pig workers (

Table 0.2).

Table 0.2: Demographic and other characteristics of study participants by pig worker status, Kiambu County, Kenya, 2013-2014

Characteristic	Pig Workers N=384 % (95% CI)	Non-Pig Workers N=883 % (95% CI)	P-value[a]
Pig keeping household			
Yes	81.5 (77.3−85.3)	10.4 (8.5−12.6)	χ^2 = 619, df=1, p <0.001
No	18.5 (14.7−22.7)	89.6 (87.4−91.5)	
Sex			
Female	41.9 (36.9−47.0)	56.2 (52.8−59.5)	χ^2 = 21, df=1, p =<0.001
Male	58.1 (53.0−63.1)	43.8 (40.5−47.2)	
Age Category, years			
Below 10	0.0 (0.0−1.0)	4.9 (3.5−6.5)	
10 to 20	14.3 (11.0−18.2)	20.8 (18.2−23.7)	χ^2 = 33, df=4, p <0.001
21 to 40	43.8 (38.7−48.9)	33.6 (30.5−36.9)	
41 to 60	30.5 (25.9−35.3)	28.7 (25.7−31.8)	
Above 60	11.2 (8.2−14.8)	11.8 (9.7−14.1)	
Missing	0.3 (<0.1−1.4)	0.2 (<0.1−0.8)	
Level of Education[a]			
No formal Education	3.6 (2.0−6.0)	2.5 (1.6−3.7)	
Primary	37.8 (32.9−42.8)	39.0 (35.7−42.3)	χ^2 = 3.4, df=3, p = 0.334
Secondary	40.1 (35.2−45.2)	34.8 (31.6−38.0)	
Post secondary	18.0 (14.3−22.2)	19.6 (17.0−22.4)	
Missing	0.5 (0.1−1.9)	4.2 (3.0−5.7)	
Occupation[a]			
Unemployed	10.4 (7.5−13.9)	17.3 (14.9−20.0)	
Farmer	69.0 (64.1−73.6)	38.1 (34.8−41.3)	χ^2 = 106, df=4, p <0.001
Business	4.9 (3.0−7.6)	11.7 (9.6−14.0)	
Office Worker	4.7 (2.8−7.3)	6.6 (5.0−8.4)	
Missing	10.9 (8.0−14.5)	26.3 (23.4−29.3)	
Poultry Worker	76.6 (72.0−80.7)	55.7 (52.4−59.0)	χ^2 = 49, df=1, p <0.001
Use Tobacco	12.0 (8.9−15.7)	6.5 (4.9−8.3)	χ^2 = 10.3, df=1, p = 0.001
Reported Chronic Disease	13.3 (10.1−17.1)	15.4 (13.1−18.0)	χ^2 = 0.8, df=1, p =0.378

[a] Chi-square test used to test significance by pig worker status

There were statistically significant differences in residence in pig keeping household, sex, age category, occupation, use of tobacco and working with poultry between pig workers and non-pig workers ($p < 0.05$). There were no statistically significant differences in the level of education, reported chronic disease and pig worker status (

Table 0.2). Human influenza vaccination in the previous 12 months was reported by two pig workers and one non-pig worker.

4.2 Characteristics at slaughterhouse level

4.2.1 Slaughter houses

All three slaughterhouses (Kiambu, Siaya, Kisumu) operated for five days a week (Monday to Friday), receiving pigs mostly from farms within the respective and neighboring counties. The Uthiru slaughterhouse received an average of 50 pigs per day while the Bondo and Kisumu slaughterhouses received three to five pigs per day. All pigs slaughtered in the three slaughterhouses were adults.

4.2.2 Human participant characteristics

A total of 288 participants were sampled over the four sampling periods, 91 (31.6%) in September 2013, 43 (14.9%) in December 2013, 101 (35.1%) in May 2014, and 53 (18.4%) in September 2014. More than half (51.7%) of the participants were from Uthiru slaughterhouse.

Majority of participants were male (91.3%), and 35.4% (n = 102) were classified as pig workers. The mean age for the participants was 35.5 years with a significant difference between the mean age of pig-workers and non-pig workers (32.5yrs, vs 37.2 years, p = 0.001). Further, 5.4% of the pig workers were above 60 years of age compared to 2.9% of the non-pig workers. Although 55.9% of all participants had completed at least secondary education, 5% of non-pig exposed had no formal education whereas all pig exposed participants had some formal education (Table 0.3). None of the participants had received vaccination against influenza in the previous two years.

Table 0.3: Sociodemographic characteristics of participants by pig worker status in three slaughterhouses, 2018

Characteristic	Pig Worker	P-value[a]

	Yes (N=102) % (95% CI)	No (N=186) % (95% CI)	
Sex			$\chi^2 = 1.1$, df=1, p = 302
Female	5.9 (2.19−12.4)	10.2 (6.26−15.5)	
Male	94.1 (87.6−97.8)	89.8 (84.5−93.7)	
Education Level Completed			$\chi^2 = 11.8$, df=3, p = 0.008
No Formal Education	0.0 (0.0−3.6)	4.84 (2.2−9.0)	
Primary	5.9 (2.2−12.4)	15.6 (10.7−21.6)	
Secondary	44.1 (34.3−54.3)	39.2 (32.2−46.7)	
Post Secondary	50.0 (39.9−60.1)	40.3 (33.2−47.7)	
Occupation			$\chi^2 = 51.3$, df=3, p = <0.001
Slaughter House worker	82.4 (73.6−89.2)	38.7 (31.7−46.1)	
Pig farmer	6.9 (2.8−13.6)	15.6 (10.7−21.6)	
Pig trader	6.9 (2.8−13.6)	26.3 (20.2−33.3)	
Other	3.92 (1.1−9.7)	19.4 (13.9−25.8)	
Sampling Period			$\chi^2 = 10.9$, df=3, p = 0.012
Sep-2013	31.4 (22.5−41.3)	31.7 (25.1−38.9)	
Dec-2013	7.84 (3.45−14.9)	18.8 (13.5−25.2)	
May-2014	34.3 (25.2−44.4)	35.5 (28.6−42.8)	
Sep-2014	26.5 (18.2−36.1)	14.0 (9.34−19.8)	
Slaughter House			$\chi^2 = 3.1$, df=2, p = 210
Bondo	28.4 (19.9−38.2)	25.3 (19.2−32.1)	
Kisumu	26.5 (18.2−36.1)	19.4 (13.9−25.8)	
Uthiru	45.1 (35.2−55.3)	55.4 (47.9−62.7)	

[a] Chi-square test used to test significance by pig worker status

4.3 Influenza A virus PCR findings in humans and animals

4.3.1 Household samples

A total of 144 episodes of ARI were reported by participants at the time of sampling representing a prevalence of 7.6%. Most of these participants were female (58.3%). The most common symptoms reported by these participants were runny nose (79%)

and cough (68%) and sore throat (40%). Nearly one third (31.3%) of the participants with ARI episodes had one symptom while 25% had three or four symptoms.

When compared by pig worker status, a higher proportion of the participants with ARI were non-pig workers. The differences in reported symptoms of cough, fever, missed work days and sore throat were not statistically significant (Figure 0.3).

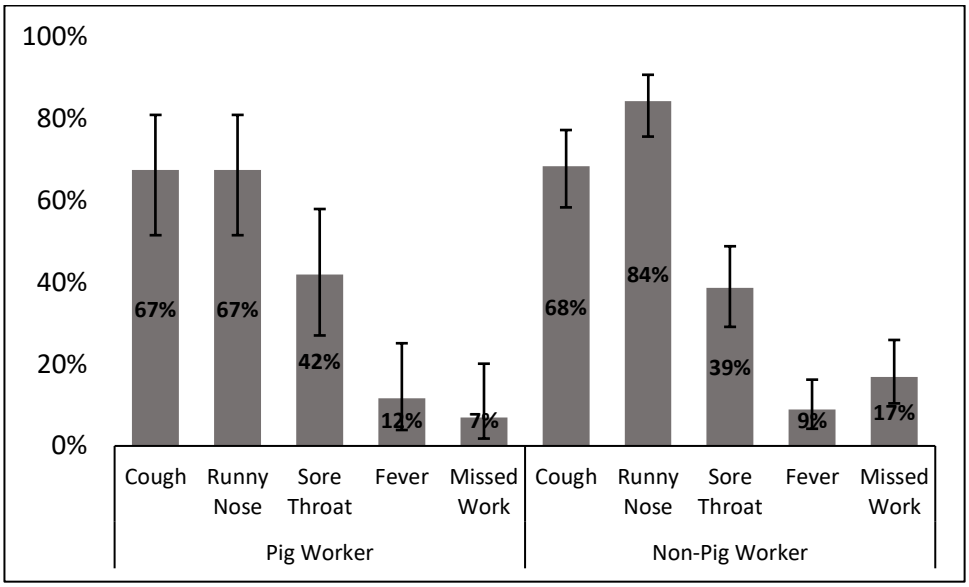


Figure 0.3: Symptoms of acute respiratory illness reported by participants at time of sampling by pig worker status, Kiambu County, Kenya, 2013-2014

Table 0.4 illustrates the distribution of symptoms of participants reporting ARI by sampling period. Over half (51.4%) of the reported ARI were in May 2014 with another 22% reported in September 2013. About 14% (20) of the participants reported that they had missed work for at least one day because of ARI. Among those reporting ARI during the sampling waves, pig workers constituted between 20% to 40% of the cases.

From among participants reporting ARI, 130 NP/OP swab samples were collected with five participants having samples collected during two different waves. Four human swabs (3%) tested positive for Influenza A virus by RT-PCR; one from a pig worker and three from non-pig workers. The positive samples had cycle threshold (C_T) values ranging from 37.4 to 39.7 and attempts to subtype the influenza virus were unsuccessful.

Table 0.4: Symptoms of among participants reporting ARI by sampling wave, Kiambu County, Kenya, 2013-2014.

Characteristic	Sampling wave			
	Sep-2013	Dec-2013	May-2014	Sep-2014
	N=31	N=24	N=74	N=15
	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
Fever	9.7 (2.0−25.8)	8.3 (1.0−27.0)	12.2 (5.7−21.8)	0.0 (0.0−21.8)
Cough	71.0 (52.0−85.8)	66.7 (44.7−84.4)	71.6 (59.9−81.5)	46.7 (21.3−73.4)
Sore Throat	41.9 (24.5−60.9)	37.5 (18.8−59.4)	41.9 (30.5−53.9)	26.7 (7.8−55.1)
Runny Nose	80.6 (62.5−92.5)	83.3 (62.6−95.3)	74.3 (62.8−83.8)	93.3 (68.1−99.8)
Missed Work	22.6 (10.3−41.5)	20.8 (7.9−42.7)	8.1 (3.3−17.4)	13.3 (2.3−41.6)

A total of 4,462 nasal and oropharyngeal swabs from animals were collected; 2,173 (48.7%) from chicken, 2,066 (46.3%) from pigs, 126 (2.8%) from ducks, 56 (1.3%) from geese and 41 (0.9%) from turkey. None of the swabs was positive for influenza A virus by RT-PCR.

4.3.2 Slaughterhouse samples

A total of 15 (5.2%) of the participants from slaughterhouses had acute respiratory illness during the four sampling periods. Nine OP and NP were collected for influenza A virus by RT-PCR testing and none of the samples were positive for influenza A virus.

Among pigs, 1,128 swab samples were collected for RT-PCR where 73% were from Uthiru slaughter house. Five (0.4%) swab samples were positive for Influenza A virus by RT-PCR. On subtyping using PCR, all the five samples were identified as Influenza A (H1N1) pdm09.

4.4 Seroprevalence of influenza A virus among pigs and poultry

4.4.1 Household samples

A total of 4,273 serum samples were collected from the animals, including 2,283 (53.4%) from poultry and 1,990 (46.6%) from pigs. Overall, 265 (6.2%) of the animal sera were positive for influenza A virus by ELISA, including 230 of 1990 (11.6%) pig sera and 35 of 2,283 (1.5%) poultry sera. Among poultry, the seropositivity for influenza A was 3.3% for geese, 2.9% for ducks, 1.4% for chicken and 0% for turkeys.

Fifty-eight (34.1%) of the pig keeping households had at least one seropositive pig during the sampling points. The median number of seropositive pigs among these households was two (range: 1 to 10). There was no noticeable clustering of seropositive herds compared to seronegative herds (Figure 0.4 and Figure 0.5).

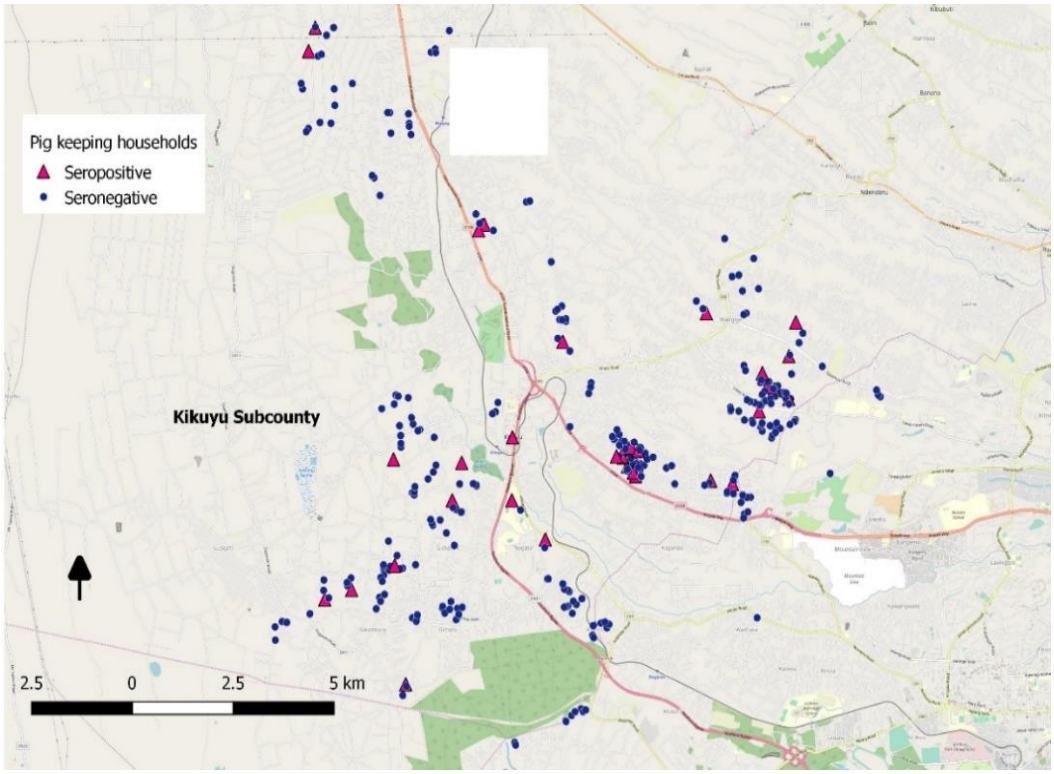


Figure 0.4: Spatial distribution of pig keeping households by herd serostatus, Kikuyu subcounty

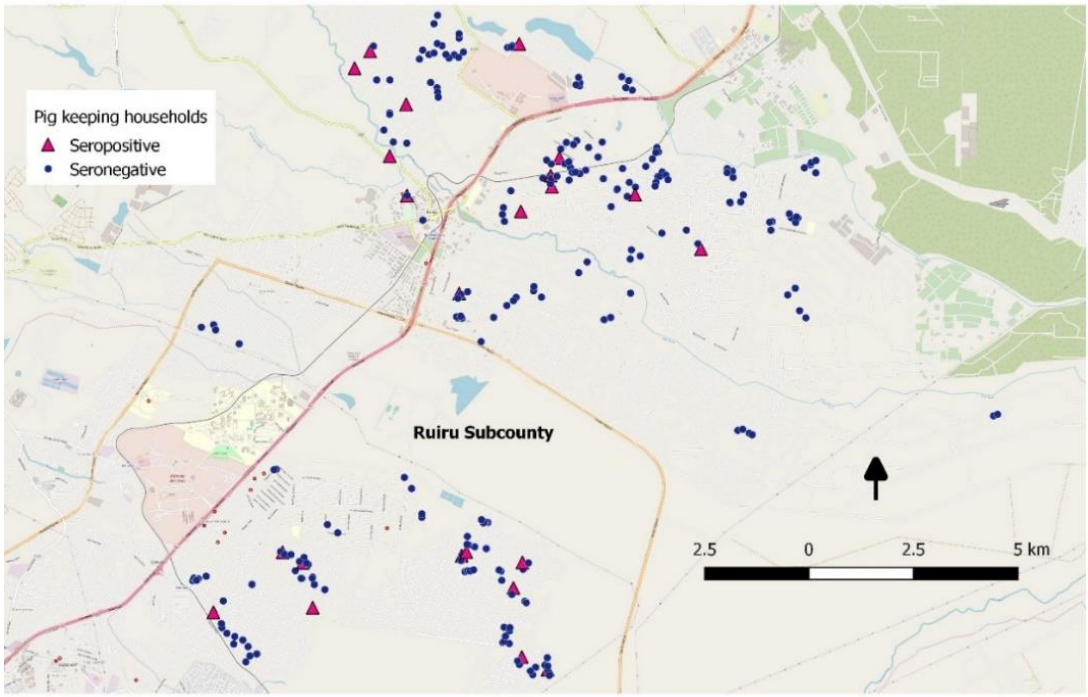


Figure 0.5: Spatial distribution of pig keeping households by herd serostatus, Ruiru subcounty

4.4.2 Slaughterhouse samples

A total of 1,082 serum samples were collected and tested by ELISA. Three quarters of the samples were from Uthiru slaughter house and between 21-28% of the samples were collected in each of the four phases. Nearly 20% (214) of the samples were positive for influenza virus by ELISA. Samples collected in September 2014 had the highest seroprevalence at 37.1%, followed by those collected in September 2013 at 19.8% (Table 0.5). Among the slaughter houses, about one-third of the samples from Bondo were seropositive for influenza A virus (Table 0.5). The farmers reported that they do not vaccinate their pigs against influenza.

Table 0.5: Seroprevalence of influenza A among pigs by sampling month and slaughter house, 2013-2014

		Samples Tested	Positive	Seroprevalence (%)	95% CI
All samples		1,082	214	19.8	17.5, 22.3
Sampling month	Sep-2013	237	47	19.8	15.3, 25.4
	Dec-2013	293	28	9.6	6.7, 13.5
	May -2014	301	46	15.3	11.7, 19.8
	Sep -2014	251	93	37.1	31.3, 43.2
Slaughter House	Bondo	87	30	34.5	25.3, 44.9
	Kisumu	93	21	22.6	15.3, 32.1
	Uthiru	902	163	18.1	15.7, 20.7

4.5 Prevalence and factors associated with acute respiratory illness among pig-exposed and non-pig exposed persons

4.5.1 Household participants

4.5.1.1 Prevalence of acute respiratory illness

Overall, 363 participants reported an episodes of ARI within 30 days of sampling. The prevalence of ARI within 30 days of sampling was therefore 19.2%. The prevalence on ARI among pig and non-pig workers was similar (18.8% vs 19.4%, p =0.764). While there were no significant differences on sex, participants reporting ARI had a significantly lower mean age compared to those without ARI (34.1 vs 38.7, respectively) (Table 0:6)

4.5.1.2 Factors associated with acute respiratory illness among pig-exposed and non pig-exposed persons

On univariable regression analysis, the odds of reporting ARI were lower across all age categories compared to the participants below 10 years. These odds ratios were statistically significant except for the 10 to 20 years age group. For participants aged 21 years and above the odds of reporting ARI were about 60% lower compared to those below 10 years. The mean age for participants with ARI had a significantly lower mean age compared to participants who did not report ARI. (34.1 years vs 38.7 years, p-value <0.001). Respondents enrolled in September 2014 had 63% lower odds of reporting ARI compared to those sampled in September 2013. Having a chronic disease and a household member who had ARI in the previous three months and missing work were associated with a higher odds of reporting ARI (Table 0:6.).

Table 0:6: Univariable model for association between acute respiratory illness and pig worker status at household level, Kiambu County, Kenya, 2013-2014

Characteristic	Acute Respiratory Illness		Unadjusted	P-value for odds ratio
	Yes (N=363) n (%)	No (N=1529) n (%)	OR (95% CI)	
Sex				
Male	172 (47.4)	737 (48.2)	0.97(0.77−1.22)	0.78
Female	191 (52.6)	792 (51.8)	Ref.	Ref.
Age category				
Below 10	19 (5.26)	41 (2.69)	Ref.	Ref.
10 to 20	95 (26.3)	227 (14.9)	0.90 (0.50−1.66)	0.731

21 to 40	119 (33.0)	581 (38.1)	0.44(0.25−0.80)	0.008
41 to 60	90 (24.9)	475 (31.2)	0.41(0.23−0.75)	0.005
Above 60	38 (10.5)	200 (13.1)	0.41(0.22−0.80)	0.009
Level of Education				
No formal Education	9 (2.62)	47 (3.13)	Ref.	Ref.
Primary	150 (43.6)	577 (38.4)	1.34(0.67−3.00)	0.426
Secondary	125 (36.3)	586 (39.0)	1.10(0.55−2.47)	0.802
Post secondary	60 (17.4)	292 (19.4)	1.06(0.51−2.43)	0.882
Occupation				
Unemployed	47 (18.9)	210 (16.7)	Ref.	Ref.
Farmer	155 (62.2)	812 (64.8)	0.85(0.60−1.23)	0.386
Business	28 (11.2)	141 (11.2)	0.89(0.53−1.48)	0.655
Office Worker	19 (7.63)	91 (7.26)	0.94(0.51−1.67)	0.828
Sampling Month				
Sep-2013	99 (27.3)	394 (25.8)	Ref.	Ref.
Dec-2013	88 (24.2)	362 (23.7)	0.97(0.70−1.33)	0.841
May-2014	149 (41.0)	484 (31.7)	1.22(0.92−1.63)	0.165
Sep-2014	27 (7.44)	289 (18.9)	0.37(0.23−0.58)	<0.001
Pig Worker				
Yes	102 (28.1)	442 (28.9)	0.96(0.74−1.24)	0.764
No	261 (71.9)	1087 (71.1)		
Poultry Worker				
Yes	208 (57.6)	957 (63.0)	0.80(0.63−1.01)	0.062
No	153 (42.4)	563 (37.0)	Ref.	Ref.
Reported Chronic Disease				
Yes	69 (19.1)	189 (12.4)	1.67(1.23−2.25)	0.001
No	293 (80.9)	1338 (87.6)	Ref.	Ref.
Use Tobacco				
Yes	23 (6.35)	126 (8.24)	0.76(0.47−1.18)	0.23
No	339 (93.6)	1403 (91.8)	Ref.	Ref.
Household member with ARI in previous 3 months				
Yes	109 (30.5)	161 (10.6)	3.71(2.80−4.90)	<0.001
No	248 (69.5)	1360 (89.4)	Ref.	Ref.
Missed Work because of illness				
Yes	24 (92.3)	29 (70.7)	4.61(1.09−34.77)	0.036
No	2 (7.69)	12 (29.3)	Ref.	Ref.

Multivariable logistic generalized linear mixed model was used to adjust the odds ratio (OR) of pig exposure for potential confounding against age, sex, poultry exposure, education, month of sampling, occupation and reported chronic disease. Household and individual identifiers were included in the model as random effects to account for clustering. A fixed effect model was applied and compared with a mixed model using the AIC and BIC values. The mixed effect model had lower values indicating better model fit.

The adjusted OR for pig workers was 1.12 (95%CI [0.77,1.63]), indicating pig workers had 12% higher odds of having ARI compared to non-pig workers although the finding was not statistically significant. Participants from households where members had reported acute respiratory illness in the previous three months had >3 times higher odds of reporting ARI. Those with chronic illness have 1.96 times higher odds of reporting ARI after adjusting for other predictors (**Table** 0.7).

Participants who were sampled in September 2014 had nearly 75% lower odds of reporting ARI compared to those sampled in September 2013. Variables such as age, sex, education levels and use of tobacco did not have significant associations with occurrence of ARI (**Table** 0.7, Figure 0.6).

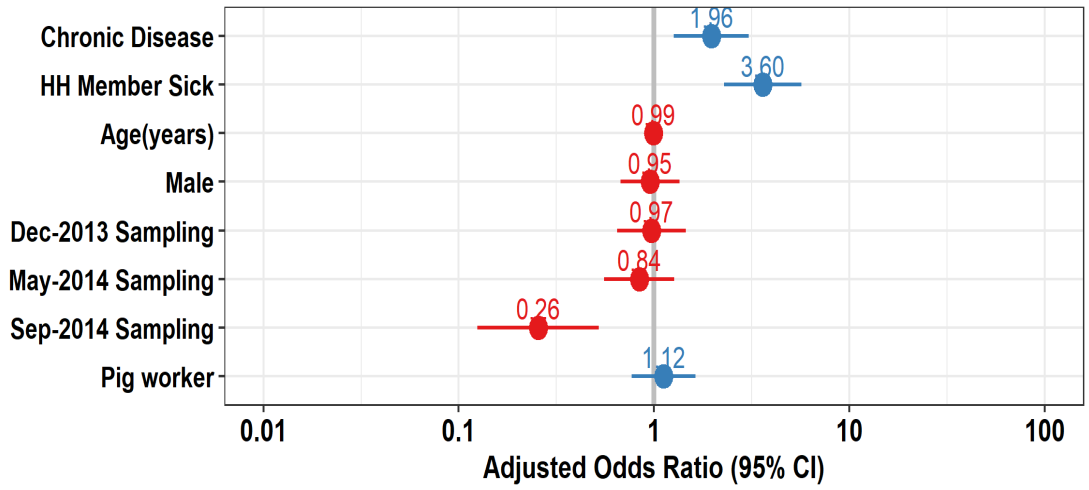


Figure 0.6: Mixed effects logistic regression plot for the association between acute respiratory illness and pig exposure and other factors at household level, Kiambu County, Kenya, 2013-2014

Table 0.7: Multivariable logistic generalized linear mixed effect model for the association between occurrence of acute respiratory infections and pig worker status at household level, Kiambu

Variables	Fixed Effect Model			Mixed Effect Model		
	Odds Ratios	**95% CI**	**p-value**	**Odds Ratios**	**95% CI**	**p-value**
Pig Worker						
Yes	1.07	0.78 – 1.46	0.67	1.12	0.77 – 1.63	0.551
No						
Household member with ARI in previous 3 months						
Yes	3.18	2.28 – 4.44	**<0.001**	3.6	2.28 – 5.68	**<0.001**
No						
Reported Chronic Disease						
Yes	1.75	1.22 – 2.51	**0.002**	1.96	1.26 – 3.06	**0.003**
No						
Sex						
Male	0.95	0.69 – 1.30	0.744	0.95	0.67 – 1.35	0.789
Female						
Age in years	0.99	0.98 – 1.00	0.219	0.99	0.98 – 1.01	0.382
Sampling month						
Sept 2013	Ref	Ref	Ref			
Dec 2013	0.97	0.67 – 1.41	0.887	0.97	0.65 – 1.46	0.892
May 2014	0.84	0.59 – 1.20	0.346	0.84	0.55 – 1.27	0.407
Sep 2013	0.28	0.14 – 0.53	**<0.001**	0.26	0.12 – 0.52	**<0.001**
Use Tobacco*						
Yes	1.01	0.59 – 1.73	0.977	--	--	--
No	Ref	Ref	Ref			
Level of Education*						
No formal Education	Ref	Ref	Ref	Ref	--	--
Primary	1.1	0.49 – 2.45	0.822	--	--	--
Secondary	0.8	0.35 – 1.83	0.602	--	--	--
Post secondary	0.92	0.39 – 2.16	0.852	--	--	--

*Not included in the mixed effect model

4.5.2 Slaughterhouse participants

On univariable analysis pig workers had a significant almost 50% lower odds of having ARI compared to non-pig worker. Other variables which had significant lower odds of having AR included sampling in Dec 2013 or in Sept 2014. Participants from Uthiru and those reporting chronic disease had >2 times higher odds of reporting ARI. Pig traders had a significant 3.5 times higher odds of reporting ARI compared to other occupations. Level of education completed or sex were not significantly associated with ARI (

Table 0.8)

On multivariable logistic regression, the adjusted odds ratio of ARI among pig workers was 0.48 (95%CI 0.24, 0.96), indicating that pig workers had about half the odds of getting ARI compared to non-pig workers. Participants who were sampled in Dec 2013 had lower odds of ARI compared to other sampling periods. Having chronic disease and male sex had >2 the odds of ARI compared to no chronic disease and female sex, respectively (Table 0.9, Figure 0.6). The model goodness of fit test p-value was >0.05.

Table 0.8: Univariable logistic regression model for the association between acute respiratory illness and pig worker status and other factors at slaughterhouse level, 2013-2014

Variable	Acute Respiratory Illness		Unadjusted OR (95% CI)	P-value
	Yes (N=56) n (%)	No (N=232) n (%)		
Pig Worker				
Yes	13 (23.2)	89 (38.4)	0.49 (0.24−0.94)	0.032
No	43 (76.8)	143 (61.6)	Ref.	
Sampling Month				
Sep-2013	20 (35.7)	71 (30.6)	Ref.	
Dec-2013	2 (3.57)	41 (17.7)	0.19 (0.03−0.69)	0.009
May -2014	30 (53.6)	71 (30.6)	1.49 (0.78−2.92)	0.229
Sep -2014	4 (7.14)	49 (21.1)	0.30 (0.08−0.86)	0.024
Slaughterhouse				
Bondo	7 (12.5)	69 (29.7)	Ref.	
Kisumu	11 (19.6)	52 (22.4)	2.06 (0.75−6.04)	0.162
Uthiru	38 (67.9)	111 (47.8)	3.30 (1.47−8.54)	0.003
Sex				
Female	2 (3.57)	23 (9.91)	Ref.	
Male	54 (96.4)	209 (90.1)	2.78 (0.78−19.2)	0.126
Chronic Disease				
Yes	18 (32.1)	41 (17.7)	2.21 (1.13−4.23)	0.022
No	38 (67.9)	191 (82.3)	Ref.	
Age in years, mean (SD)	37.0 (10.1)	35.1 (12.4)	1.01 (0.99−1.04)	0.290
Education Level Completed				
No Formal Education	1 (1.8)	8 (3.45)	Ref.	
Primary	23 (41.1)	95 (40.9)	1.73 (0.29−45.1)	0.607
Secondary	25 (44.6)	101 (43.5)	1.77 (0.30−46.0)	0.605
Post-Secondary	7 (12.5)	28 (12.1)	1.79 (0.25−50.8)	0.589
Occupation				
Other	4 (7.1)	36 (15.5)	Ref.	
Slaughterhouse worker	28 (50.0)	128 (55.2)	1.91 (0.69−6.93)	0.232
Pig farmer	8 (14.3)	28 (12.1)	2.50 (0.69−10.6)	0.164
Pig trader	16 (28.6)	40 (17.2)	3.47 (1.13−13.4)	0.028

Table 0.9: Multivariable logistic regression model for the association between acute respiratory illness and pig worker status and other factors at slaughterhouse level, 2013-2014

Variable	Categories	Adjusted OR (95% CI)	P-value
Pig Worker	Yes	0.48 (0.24− 0.96)	0.038
	No	Ref.	
Sampling Month	Sep-2013	Ref.	
	Dec-2013	0.16 (0.04− 0.77)	0.022
	May -2014	1.39 (0.69− 2.77)	0.356
	Sep -2014	0.38 (0.11− 1.34)	0.132
Slaughterhouse	Bondo	Ref.	
	Kisumu	0.94 (0.3− 2.94)	0.92
	Uthiru	2.17 (0.82− 5.74)	0.117
Sex	Female	Ref.	
	Male	4.31 (1.15− 16.22)	0.031
Chronic Disease	Yes	2.34 (1.06− 5.18)	0.036
	No	Ref.	

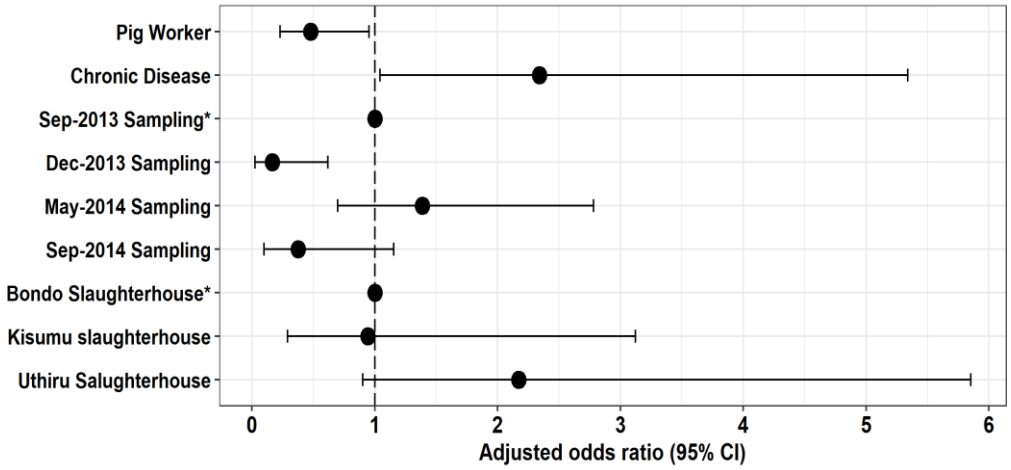


Figure 0.6: Multivariate logistics plot of the adjusted odds ratio for acute respiratory illness and pig worker status and other variables at slaughterhouse level

Variables in asterisk were used as reference categories.

4.6 Assessment of pig farming practices promoting influenza virus transmission

For assessment of the potential risks of transmission at the human-animal interface, the analyses were restricted to the sampled pig keeping households and was based on self- reports from the farmers. A total of 170 pig keeping households were enrolled in the study. The interviewed respondents from the households were predominantly male (55.9%). Nearly two thirds of the respondents (63.5%) had completed at least secondary school education and 18.2% of the respondents had no formal education. Median number of members per household was three with a range of one to 15.

The median number of pigs per household was 13, with a range of one to 200. Majority of the households (54.5%%) had kept pigs for at least 2 years. Besides pig raising, majority of the farms reared other animals (Table 0.10).

Table 0.10: Type of animals raised in the pig keeping households

Type of animals[α]	No. of households (%)	Median No. of animals	Range
Pigs	170 (100.0)	13	1 – 200

Chickens	137 (80.6)	14	1– 3000
Duck	24 (14.1)	3	1 – 40
Geese	11 (6.5)	3	1 – 16
Turkey	9 (5.3)	1	1 – 7
Goat	31 (18.2)	2	1 – 9
Sheep	23 (13.5)	3	1 – 25
Cattle	77 (45.3)	3	1 – 37

[α] Categories not mutually exclusive

Majority (88%) of the households had pens with concrete floors, with 30% of them using saw dust for beddings. While majority of the households fed the pigs with commercial feeds, about 60% of the households also used scraps/wastes from the household or the market.

Households regularly added new pigs to their herds with 11.1% of the households reporting buying new pigs within one month preceding the interview. Quarantine was practiced in 46% of the households on acquiring new pigs before mixing with the farm herd. Among those who quarantined, about half (52.5%) quarantined for three days the rest quarantined for less than 3 days. .Three quarters(77.7%) of those who quarantine reported that they do it all the time they receive new pigs.

About one quarter (26%) of the households vaccinated their pigs for various diseases and nearly all households dewormed the pigs.. Loss of appetite (59.9%) and diarrhea (21.6%) were the most common signs among sick pigs as reported by the respondents. Selling pigs to traders was practiced by 82% of the farms, with 30% of the respondents reporting that the traders buy the pigs at farm level. Although none of the households had slaughterhouse facilities, 9% of the respondents reported that they slaughtered the pigs in the farm and sold them dressed. A quarter of the households reported that the pigs mixed with other farm animals frequently.

Nearly half (46%) of the respondents working in pig farms reported that they used coveralls or dust coats at least at least 4 times each week when working in the farms. Majority (70%) did not use gloves but reported that they washed hands regularly after attending to the farm animals mostly because their hands got soiled.

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Introduction

The study was a linked human-animal (humans, pigs and poultry) study at household and slaughterhouse levels which provided a platform for concurrent investigation of influenza A virus occurrence and circulation at the inter-species level. Studies at the human-animal interface provide evidence on dynamics of cross-species transmission of epidemic prone pathogens and data which can inform decisions in addressing zoonotic diseases using the one health approach (Lebov *et al.*, 2017).

5.1.2 Household and participant characteristics

In the household study, 60% of the household heads in pig keeping and non-pig keeping households had secondary school education level or higher. This finding is consistent with the general high literacy level of the study area (County Government of Kiambu, 2018). Majority of households kept livestock consistent with findings from household studies in the same county (Osoro *et al.*, 2015), with nearly all pig keeping households having other livestock. The higher proportion of pig keeping households having other livestock could be due to greater social value and preference placed on cattle and chicken compared to pigs.

Almost half the pig keeping households had reared the pigs for less than two years, consistent with reports that pig farming is expanding in the county (FAO, 2012b). However, most of the farms are fairly small with a median of 13 pigs per household.

Among household participants, there were significantly more males than females among pig workers, likely due to the male preference of the mostly manual and strenuous work of pig farming in the study area. There were also differences in younger age categories (<20 years) between pig workers and non-pig workers likely because pig farming is mostly done by adults and the younger age groups include

children in school who would not be engaged in pig farming. However, the older age categories (>40 years) were similar in the two groups perhaps accounting for similar levels of reported chronic disease. Among the slaughterhouses, Uthiru was on average slaughtered 10 times more pigs than Bondo and Siaya in a day. Only one third of the participants from the slaughterhouses were classified as pig exposed likely because most of the participants were traders or farmers delivering the pigs and who were not handling the pigs.

Similar to the household participants, most slaughterhouse participants had at least secondary school education and with a significant majority (90%) being male. Fewer participants were recruited during Dec-2013 and Sep-2014 (33% vs 67%) recruitment periods. While December is considered peak season for pig slaughter (Alarcon *et al.*, 2017) and hence more personnel expected in the slaughter house, this study's sampling was done in early December before the peak of the season and could account for the lower numbers recruited.

5.1.3 Influenza viruses circulating among humans and pigs

In the household component of the study, 3% of human samples were positive for Influenza A virus. Subtyping of the samples was not successful, likely due to low viral load as evidenced by the high C_T values on PCR. This relatively low yield of positive samples for influenza A virus could be due to sampling a healthy population and who may not have had active influenza virus infection at the time of sampling or had the respiratory illness was due to a non-influenza cause. In a community cohort study in England, majority of influenza infections were found to be subclinical and only about 25% resulted in clinical illness (Hayward *et al.*, 2014).

Other studies on influenza infection at community level have found either higher or lower prevalence compared to this study. A community based study in Romania detected influenza A virus in 13% of participants with ARI while a study among pig exposed persons in slaughter houses in Nigeria did not detect influenza virus in any sample by PCR (Awosanya *et al.*, 2013; Coman *et al.*, 2014). Although a study with intense follow up could likely have a higher yield, such studies are costly and have a disadvantage of significant loss to follow up.

Among the slaughterhouse participants, none of the human samples tested were positive for influenza A virus suggesting low levels of influenza A circulation associated with clinical illness, especially among the participants in the study who were mostly middle-aged adults. Similar to the findings of this study, a study in Nigeria among pig workers in a slaughter house did not report any positive influenza A virus by PCR (Awosanya *et al.*, 2013). It is likely that serologic testing would have reported a higher prevalence of influenza A virus among human participants. However, attempts to carry out this testing were unsuccessful.

Among the slaughter house pig samples, five samples were found to have Influenza A (H1N1) pdm09, which is a human influenza virus introduced in Kenya in June 2009 and currently associated with seasonal influenza among humans (Emukule *et al.*, 2016; States & June, 2009). This finding adds to evidence of continued circulation of pandemic H1N1 virus among swine populations in the East Africa region. This finding is also consistent with a similar study in Kenya where 0.5% of sampled pigs were found to have Influenza A (H1N1) pdm09 (Munyua *et al.*, 2018).

The detection of human pandemic H1N1 influenza virus among pigs adds to the evidence from the few studies in the East Africa region of possible interspecies transmission of the virus. The increasing commercialization of pig farming in Kenya, provides a suitable environment for exposure and occurrence zoonotic events related to influenza A virus (FAO, 2012b). The evolution of reassortant influenza viruses and their transmission to humans is unpredictable making routine monitoring at the animal-human interface a priority. Such virological surveillance offers a mechanism to detect early any changes in the antigenic composition or zoonotic transmission events.

The occurrence of pandemic influenza in pigs has been documented in all regions of the world (Nelson & Vincent, 2015). In Africa, the occurrence has been reported in several countries including Kenya, Nigeria and Ghana and Cameroon (Adeola *et al.*, 2015; Munyua *et al.*, 2018; Njabo *et al.*, 2012). The study in Kenya reported that 72% of seropositive pigs were confirmed to be pandemic H1N1 influenza using hemagglutination inhibition test. This suggests that the predominant virus circulating

among swine populations in Kenya is Influenza A (H1N1) pdm09. Studies have shown that when pandemic H1N1 circulates in local swine populations, it undergoes antigenic changes over time which could result in reassortant virus (Vincent *et al.*, 2014). Our failure to detect Influenza A virus (IAV) from household animal samples by PCR probably reflects the generally very low detection levels in household studies. A review in South Asia on IAV among pigs reported low detection levels in households (0.8% from 12,400 samples) and slightly higher in slaughterhouse samples (1.7% from 5,316 samples). Other studies failed to detect influenza A by PCR among samples collected in Nigeria, Malaysia, Vietnam (Awosanya *et al.*, 2013; Suriya *et al.*, 2008; Trevennec *et al.*, 2011). These findings imply that studies on influenza virus at the human-pig interface are likely to be most efficiently when conducted at points where animal congregate such as slaughterhouses and live markets.

5.1.4 Seroprevalence of influenza A virus among pigs and poultry

In the household study, about 11% of the pigs were seropositive for influenza A virus by ELISA. This finding is lower than an earlier study that reported 16% influenza A virus prevalence among pigs in Kenya and other studies elsewhere in Africa and Asia reporting as high as 67% influenza virus prevalence among swine in live markets (Eugenie *et al.*, 2017; Munyua *et al.*, 2018; Snoeck *et al.*, 2015; Suriya *et al.*, 2008). Most of the studies with higher seroprevalence were conducted in live markets. Since swine influenza vaccination was not practiced by the farmers in this study, the level of seropositivity suggests exposure to circulating influenza A virus. The 1.4 – 3.3% prevalence of influenza virus among the poultry species that are commonly reared by farmers in Kenya (chicken, ducks and geese) reported in this study support the finding that Kiambu County is an environment of substantial animal influenza virus circulation. Various studies have shown that pig exposed persons are at increased odds of swine influenza infection compared to non-pig exposed individuals (Gerloff *et al.*, 2011; Kitikoon *et al.*, 2011; Ma *et al.*, 2015). The risk of interspecies transmission therefore exists among swine workers and is likely to increase with enhanced pig production in the country.

At the slaughterhouse level, the seroprevalence in pigs was higher (20%) than at the household level. The higher seroprevalence was likely because only adult pigs, which are more likely to have been exposed to influenza virus compared to younger pigs in the farms are presented for slaughter. This study provides evidence of intense circulation of swine influenza virus among pig populations in two distinct geographical regions of Kenya, located >350 kilometres apart. The higher prevalence reported in Bondo (34.5%) and Kisumu (22.6%) slaughterhouses located in Western Kenya may be due to the free-range nature of pig production there, when compared with Uthiru slaughterhouse in the central region of the country that mostly receives pigs from nearly counties where confined production system is practiced. This study's findings also point to higher influenza transmission during the colder months (July – September) as supported by the finding that almost two thirds of the pigs sampled during this period were seropositive. In addition, all the PCR positive samples were collected during the cold season. Trends in human seasonal influenza

in Kenya have also shown higher transmission during the colder months of June to August (Emukule *et al.,* 2016). Studies from other countries show varied findings on seroprevalence ranging from 5% in Uganda to 49% in Vietnam (Baudon *et al.,* 2015; Eugenie *et al.,* 2018, 2017; Kirunda *et al.,* 2014). The differences in seroprevalence with this study could be due to differences in sampling methodologies (farm level vs live market vs slaughterhouses) and some regions such as Asia may have large pig industries with higher transmission levels.

5.1.5 Prevalence and factors associated with acute respiratory illness

The prevalence of ARI at household level was >3 times higher than a household survey in Siaya, Kenya in 2011 which reported a prevalence of acute respiratory illness of 6.1% in the two weeks before the study (Burton *et al.*, 2011). The study in Siaya sampled only households with children under 5 years of age and the current study included ARI cases in the previous 30 days compared to 14 days in the Saya study. These differences in target population and duration of ARI definition could account for the differences observed in the two studies.

Despite the reported level of influenza virus seroprevalence, pig workers in the household study had 12% higher odds, which were not statistically significant, of reporting ARI within 3 months of sampling compared to non-pig workers. This could be due to healthy worker effect which is a progressive selection bias where persons who work closely with pigs could have systematic differences associated with lower occurrence of ARI compared to the non-pig exposed persons (Chowdhury *et al.,* 2017). For example, there were fewer pig workers among participants in the <20 years-old age group, that includes young children with a higher risk of ARI, compared to non-pig workers (14.4% vs 25.8%, respectively). However, this study finding is inconsistent with other studies which reported that pig workers have significantly higher odds of respiratory illness compared to non-pig workers (Driesen, 2003; Radon *et al.*, 2001). However, most of these studies were conducted in high income countries to assess chronic respiratory health among farmers who were older. A higher proportion of non-pig workers (17%) missed work compared to pig-workers (7%) suggesting than non-pig workers had more severe ARI.

At slaughterhouse level, pig workers had about half the odds of reporting ARI compared to non-pig workers which is inconsistent with findings from other studies (Driesen, 2003; Radon *et al.*, 2001). Similar to this study's findings among household participants, this reported difference in odds could be related to the differences in mean age, with pig-workers having a higher mean age, that may also be associated with higher risk of ARI.

Our findings of lower odds of ARI among pig workers in slaughterhouse, and no difference in odds and probably less severe ARI among pig workers at household level suggests that monitoring ARI in the general population would likely miss potential zoonotic events. Zoonotic influenza events are likely to first appear among those working or exposed to swine or poultry, mostly young adults. With the reported levels of exposure to swine influenza among pigs in this study, including the pig workers in the influenza surveillance could enhance efforts to detect early zoonotic influenza events. Focused surveillance in an occupationally exposed group offers a potentially cost-effective mechanism to monitor trends of influenza, including influenza zoonotic events. A number of innovative and affordable approaches such as mobile based surveillance, could be applied to offer the needed early warning mechanism to identify increases in acute or severe respiratory episodes in this group (Lee & Wong, 2014).

In the multivariate logistic model at household level, having a household member with an episode of ARI in the previous three months was associated with >3.6 times higher odds of reporting ARI compared to participants not reporting ARI among household members. This findings is consistent with the known transmission of pathogens associated with ARI through close contact (Koskela *et al.*, 2005). At the slaughter house level, chronic disease was associated with higher odds of reporting ARI possibly reflecting the higher risk of ARI among those with chronic illness, as has been reported in other studies (Britto *et al.*, 2017; Haroon *et al.*, 2013).

5.1.6 Potential risks of influenza virus transmission in pig farms

This study assessed the known farming practices with potential to increase zoonotic influenza transmission such as low biosecurity measures and mixing of animals of difference species (Eugenie *et al.*, 2017).

Majority of pig keeping households kept poultry and reported frequent mixing of farm animals, such as within the same holding areas, of different species. With increasing intensive pig farming in Kenya, the mixing of farm animal species could serve as a bridge for zoonotic influenza transmission.

Studies on zoonotic influenza have documented that lack of quarantine and uncontrolled movement between farms are risk factors for transmission (Simon-Grifé *et al.*, 2011; Suriya *et al.*, 2008). Biosecurity practices such as quarantine and limiting movement of people into the farm were not practiced consistently in this study. Traders bought pigs at farm level and would often move between farms with purchased pigs. These transmission-promoting practices could be because of lack of knowledge and facilities for biosecurity measures such as restricting access to the farms and appropriate quarantine

The use of protective clothing such as coveralls, gloves and masks has been demonstrated to lower the risk of interspecies influenza transmission (Kelly *et al.*, 2008; Ramirez *et al.*, 2006). While hand washing was widely practiced by swine workers, most did not use gloves, protective clothing or any eye protection while working on the pig pens. Failure to use these personal protective measures can enhance viral transmission between pigs and humans. Similar findings on use of personal protective equipment have been documented in studies in Peru, Romania and Nigeria (Awosanya *et al.*, 2013; McCune *et al.*, 2012; Rabinowitz *et al.*, 2013)

5.1.7 Study limitations

The study had a number of limitations. Although the study was conducted over a period of one year to account for seasonality of influenza, the study was cross-sectional in design. A longitudinal design in which the participants were followed up over a period of time would provide a more accurate assessment of influenza circulation and reduce recall bias on the episodes of acute respiratory illness. In this

study, the assessment of ARI was limited to 30 days before sampling to reduce recall bias.

Another limitation is that hemagglutination inhibition tests to identify the specific strains among pigs was not conducted because the required reagents were not available. The findings could therefore be overstated due to cross reactivity and the specific strains responsible for infection could not be determined. However, another study in Kenya (Munyua *et al.*, 2018) reported 72% of seropositive pigs had influenza virus (A/H1N1/pdm09) by HI, findings which might reflect the diversity of influenza virus among pigs in this study.

5.2 Conclusions

Based on the results from the study, the following conclusions are made:

i. Three percent of the human samples from households were positive for Influenza A virus. Subtyping of the samples was not successful, probably due to low viral load. Among the slaughter house participants, none of the human samples tested were positive for influenza A virus suggesting low levels of influenza A circulation associated with clinical illness, especially among the participants in the study who were mostly middle-aged adults.
ii. The study detected Influenza A (H1N1) pdm09 among the slaughter house pig samples, which is a human influenza virus introduced in Kenya in June 2009 and currently associated with seasonal influenza among humans. The detection of human pandemic H1N1 influenza virus among pigs adds to the evidence from the few studies in the East Africa region of possible interspecies transmission of the virus
iii. There was low to moderate levels of exposure to influenza A in pig and poultry at household level. At the slaughterhouse level, the seroprevalence in pigs was higher. This suggests the study area, especially at the slaughterhouse level is an environment of significant pig influenza virus circulation
iv. Despite the reported level of influenza virus seroprevalence in pigs, this study found that pig workers had similar or lower odds of reporting ARI compared to non-pig workers at household and slaughterhouse level respectively.

v. Majority of pig keeping households kept poultry and reported frequent mixing of farm animals of different species. Biosecurity practices such as quarantine and limiting movement of people into the farm were not practiced by majority of the farmers. While hand washing was widely practiced by swine workers, most did not use gloves or protective clothing while working on the pig pens.

5.3 Recommendations

There is need for :

i. Sentinel surveillance for influenza A viruses among pigs in pig slaughterhouses by the Directorate of Veterinary Services to monitor the diversity of influenza viruses in pigs as well as detect changes to the virus which could results in epidemics if zoonotic events occur. This is informed by the detection of Influenza A (H1N1) pdm09 in slaughterhouses in pigs, which is the subtype currently causing seasonal influenza in humans. The surveillance would best be done in slaughterhouses because they are congregating areas from the feeder farms and would reflect the circulation in these farms.
ii. Sentinel surveillance for influenza among pigs by the Directorate of Veterinary Services should include hemagglutination inhibition assay to identify and monitor the types and levels of circulating influenza A virus among pigs. With the reported seroprevalence of influenza among pigs such a measure would be important to inform future needs for swine influenza vaccination.
iii. The Ministry of Health to expand the existing human influenza sentinel surveillance which is currently domiciled in hospitals to include pig workers in slaughterhouses. The study shows that the pig workers have lower risk of acute respiratory illness and are likely underrepresented among those seeking care in health facilities. Pig workers are most likely to experience zoonotic influenza events and would need to be specifically targeted to increase the chances of early detection of such events.

iv. The Directorate of Veterinary Services to educate pig farmers on the need to use appropriate personal protective equipment regularly and enhance biosecurity measures such as reducing mixing of farmed animals and appropriate quarantine of new pigs to reduce chances of cross-species transmission

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APPENDICES

Appendix I: Consent form for the household head -- English

Prevalence of Zoonotic Swine Influenza Viruses at Household and Slaughterhouse Level in Kenya

Introduction:

We are visiting your household as part of a research project to assess the relationship between human and animal health. This study is administered by researchers from KEMRI, CDC, Ministry of Public health and Sanitation, the Ministry of Livestock Development and University of Minnesota. You can talk about your participation with anyone you choose. Do not participate if the research team has responded to your questions to your satisfaction.

The goal of this project is to look at the diseases that can be transmitted from animals to humans and to design new ways of carrying out surveillance and control of infectious diseases in this part of Africa. A total of 1200 persons and close to 3500 animals will participate in the study.

Purpose:

Influenza (flu) is a common viral infection among humans. Animals also get influenza infection. Some human illnesses may be caused by germs that are carried by animals, including domestic livestock. We are doing a research study to see if the animals in this area are carrying these germs, and if they are passing them to people. To do this, we would like to collect samples from part or all the animals (pigs, chicken, ducks, geese, turkeys) that are kept by your household as well as from three people within your household, and test them for some of the germs that may possibly cause illness among humans and animals. The samples that we would like to collect include some blood, a swab from the nose and back of the mouth (throat) each. We would also like to ask you some questions about how the animals are managed. If you agree, we will visit you again ten weeks after this date and collect similar

samples and ask some questions. During each of these two visits, this process will take from one to a few hours, depending on the number of animals.

Alternatives

You are free to choose whether or not you will be in this study. The alternative to choosing to be in the study is to decline to be in the study.

Confidentiality: The facts about you and your family from this study will be kept private as much as allowed by the law. No names will be used on any of the study reports. To enhance confidentiality, special codes on the information will be used and will be stored in secure study offices. However, we will record the three names of the persons who we take samples from in your household in a separate book to allow us use unique numbers to identify them when we come for the second visit. Only staff involved in the study implementation will be allowed access to the research data. We will use computers with password protection to store the data electronically. For this study, each sample will be labeled only with a barcode and a unique tracking number to protect the confidentiality of the participant. Personnel at the storage facility and testing laboratories will not know your identity, or the participant ID code assigned to you for the study.

Handling of specimens:

We will test the samples collected from your animals and members of your household at the KEMRI/CDC laboratory in Nairobi, Kenya and at other laboratories abroad including CDC Atlanta and University of Minnesota, as not all tests can be carried out in Kenya. We would also like to ask if we can store these samples to do more tests at a later time. However, additional ethical approval will be sort from KEMRI prior to any tests in future on these specimens.

Benefits from being in the study:

Participants in this study will get free advice on management and animal health, including for those illnesses which are diagnosed in this study. Any information obtained from these tests that might be important for your family's health, or for your

animals' health and welfare, will be communicated to you through project staff. Identification of diseases affecting your animals will help improve their health and welfare, as well as that of your family.

We will offer you deworming medicines for your poultry and pigs during the first visit only.

Risks from being in the study:

Risks to humans: Taking swab samples from the nose cause temporary discomfort and might rarely cause temporary bleeding from irritation of the lining of the nose. Taking swab samples from the back of the mouth can cause a gagging sensation. Drawing blood can cause brief discomfort. Rarely, it might cause bleeding and bruising. Serious injury from taking swabs and blood are very rare. It is possible that other people will find out that you participated in this study. Additionally, if you are comfortable with it, we may ask you or members of your household to help with restraining the animals. This may expose you or your family members to risk of injury from the animals.

Risks to animals: Handling and restraining animals for sample collection can be slightly stressful for the animals and for people from the household who are participating. Every care will be taken to minimize this stress. Drawing blood can cause brief pain to the animals and may result in brief bleeding. Sampling the animals may take some time, as will answering the questions about the animals.

There may be unknown or unforeseen risks associated with study participation.

Voluntary participation:

Deciding whether or not to be in the study today is your choice. You can choose not to join, or to drop out at any stage. This will not adversely affect you in any way. Should any more questions arise or if you feel like you, your family or your animals might have been harmed by being in the study, please contact Dr Stellah Kiambi on

0724283920 or Dr Eric Osoro on 0722216391. For queries regarding your rights as a participant, reach out to the secretary, KEMRI/NERC (tel. 0202722541 or 0722205901 or 0733400003). We will give you a signed consent form to take away with you

The consent form has been explained to me and I agree for my household members and animals to take part in the study. I have been told that I am free to choose not to take part in this study at any time and that saying "NO" will have no effect on the members of my household or me.			
Head of family	Name:	Signature/Thumb print:	date☐☐/☐☐/☐☐
Witness	Name:	Signature:	date☐☐/☐☐/☐☐
Interviewer	Name:	Signature:	date☐☐/☐☐/☐☐

I agree to allow samples from my animals to be stored at KEMRI and CVL for possible future testing in Kenya and abroad. This testing will not include genetic testing.

Head of family	Name:	Signature/thumb print:	date☐☐/☐☐/☐☐
Witness	Name:	Signature:	date☐☐/☐☐/☐☐
Study Staff	Name:	Signature:	date☐☐/☐☐/☐☐

Appendix II: Adult Consent form -- English

Prevalence of Zoonotic Swine Influenza Viruses at Household and Slaughterhouse Level in Kenya

Introduction:

We are visiting your household as part of a research project to assess the relationship between human and animal health. This study is administered by researchers from KEMRI, CDC, Ministry of Public health and Sanitation, the Ministry of Livestock Development and University of Minnesota. Please take time to read the following information carefully before you decide whether you want to take part in this study or not.

The goal of this project is to look at the diseases that can be transmitted from animals to humans and to design new ways of carrying out surveillance and control of infectious diseases in this part of Africa. A total of 1200 persons and close to 3500 animals will participate in the study.

Purpose:

Influenza (flu) is a common viral infection among humans. Animals also get influenza infection. The virus that causes the human and animal infection is usually different but in some cases, it can be the same. People who work closely with different animals may get the infection from their animals. In Kenya, it is not known if this happens and to what extent this occurs. However, there are ways that humans can minimize getting influenza from their animals if indeed this is happening. Researchers from the Kenya medical research institute (KEMRI), the centres for disease control and prevention in Kenya, Kenya's Ministry of Health, Ministry of Livestock development and University of Minnesota would like to determine how much of influenza infection we have occurring between humans and animals by doing a study to find factors that contribute to infections in humans as well as animals.

Voluntary Participation. You are free to join the study or not to join. At any time, you can leave the study, for any reason. If you decide not to join or to drop out, you will not lose any health care services you are entitled to at the Hospital; neither will this affect your employment at the home or slaughterhouse at all. You will not get any direct benefit or payment for being in this study, but you will help us know more about this disease. Study staff will update you in a timely way about new information that might affect your decision to stay in the study.

Alternatives

The alternative to choosing to be in the study is to decline to be in the study.

Why You Have Been Chosen: We are testing persons including children from households selected because they keep pigs and some that do not keep pigs but have/or do not have other animals in Kiambu and Kisumu county. We are also testing persons working in pig slaughterhouses at any level.

Procedure: If you choose to be in this study we will draw 5 ml of blood (a teaspoon) from the vein in your, his or her arm. We will also collect a swab sample from the nose and the back of the mouth (throat) if you have a cough or running nose. The blood and swab sample will be tested for germs of the influenza virus, or other disease-causing germs at the KEMRI/CDC lab in Nairobi. Tests may show us that you may have been sick with influenza before or is sick with it now. A small number of blood and swab samples will be sent to CDC in Atlanta, Georgia U.S.A and/or other laboratories abroad. Researchers at these laboratories will do the test again to see if they get the same test results. The remaining amount of the sample will be stored in the freezer for possible testing for other germs in future. No human genetic testing, HIV and/or tuberculosis testing will be done on the sample**.** We will also ask you questions for 30 minutes. Neither of you have to answer the questions if you do not want to.

Handling of specimens:

We will test the samples collected from you at the KEMRI/CDC laboratory in Nairobi, Kenya and at other laboratories abroad, as not all tests can be carried out in Kenya. We would also like to ask if we can store these samples to do more tests at a later time. However, additional ethical approval will be sort from KEMRI prior to any tests in future on these specimens.

Confidentiality: Only staff involved in the conduct, oversight, or auditing of this study will be allowed work with your samples and to see your information. All samples will be coded at collection and will bear no information that can identify you. To enhance confidentiality, special codes on the information will be used and will be stored in secure study offices. Electronic data will be stored in password protected computers. For this study, each blood sample and naso/oropharyngeal swab will be labeled only with a barcode and a unique tracking number to protect your confidentiality. Personnel at the storage facility and testing laboratories will not know your identity, or the volunteer ID code assigned to you for the study. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

Risks. Taking swab samples from the nose cause temporary discomfort and might rarely cause temporary bleeding from irritation of the lining of the nose. Taking swab samples from the back of the mouth can cause a gagging sensation. Drawing blood can cause brief discomfort. Rarely, it might cause bleeding and bruising. Serious injury from taking swabs and blood are very rare. In addition, it is possible that other people will find out that you participated in this study. There may be unknown or unforeseen risks associated with study participation.

Benefits. There will be no benefit for you from this study. In addition, information obtained from this study may help the Ministry of Health decide when and how much influenza disease occurs. Any information obtained from these tests that might be important for your health, will be communicated to you through project staff.

Contact Persons: If you have concerns regarding, injuries please contact Dr Eric Osoro on 0722216391. If you have concerns regarding your rights in the study, reach out to the Ethics Review Committee, Kenya Medical Research Institute

(KEMRI), P.O. Box 54840-00202, GPO, Nairobi. Telephone 0202722541 or 0722205901 or 0733400003.

You will get your signed consent form to take away with you

Consent

This study has been explained to me. I have asked the questions I had. I have been informed that it is my free choice to be in this study and if I join the study, I can drop out at any time without any penalty.

If you agree to participate in the study, please sign/thumb print here _____
_____Date: _____/_____/_____

Name of Participant _____

Witness signature (when needed, e.g. if participant cannot read)

Name of Witness _____ Date: _____/_____/_____

Study Staff member who obtained this consent _____Date: _____/_____/_____

Appendix III: Parental Permission Form for Children 2-17 -- English

Prevalence of Zoonotic Swine Influenza Viruses at Household and Slaughterhouse Level in Kenya

Introduction:

We are visiting your household as part of a research project to assess the relationship between human and animal health. This study is administered by researchers from KEMRI, CDC, Ministry of Public health and Sanitation, the Ministry of Livestock Development and University of Minnesota. Please take time to read the following information carefully before you decide whether you want to take part in this study or not.

The goal of this project is to look at the diseases that can be transmitted from animals to humans and to design new ways of carrying out surveillance and control of infectious diseases in this part of Africa. A total of 1200 persons and close to 3500 animals will participate in the study.

Purpose:

Influenza (flu) is a common viral infection among humans. Animals also get influenza infection. The virus that causes the human and animal infection is usually different but, in some cases,, it can be the same. People who work closely with different animals may get the infection from their animals. In Kenya, it is not known if this happens and to what extent this occurs. However, there are ways that humans can minimize getting influenza from their animals if indeed this is happening. Researchers from the Kenya medical research institute (KEMRI), the centers for disease control and prevention in Kenya, Kenya's Ministry of Health, Ministry of Livestock development and University of Minnesota would like to determine how much of influenza infection we have occurring between humans and animals by doing a study to find factors that contribute to infections in humans as well as animals.

Voluntary Participation. You are free to allow for your child to join the study or not to join. Your child may leave the study at any time, for any reason. If you decide for your child not to join or to drop out, you will not lose any health care services you are entitled to at the Hospital. Study staff will update you in a timely way about new information that might affect your decision to stay in the study.

Alternatives. You can choose to accept or decline the participation of your child in the study.

Why Your Child Has Been Chosen: We are testing persons including children from households selected because they keep pigs and some that do not keep pigs but have/or do not have other animals in Kiambu and Kisumu county. We are also testing persons working in pig slaughterhouses at any level.

Procedure: If you choose for your child to be in this study, we will draw 5 ml of blood (a teaspoon) for children 13-17 years and 2-3 mls for children 2-12 years from the vein in his or her arm. We will also collect a swab sample from the nose and the back of the mouth (throat) if your child has a cough or running nose. The blood and swab sample will be tested for germs of the influenza virus, or other disease-causing germs at the KEMRI/CDC lab in Nairobi. Tests may show us that your child may have been sick with influenza before or is sick with it now. A small number of blood and swab samples will be sent to CDC in Atlanta, Georgia U.S.A and/or other laboratories abroad. Researchers at these laboratories will do the test again to see if they get the same test results. The remaining amount of the sample will be stored in the freezer for possible testing for other germs in future. No human genetic testing, HIV and/or tuberculosis testing will be done on the sample**.** We will also ask you and your child questions for 30 minutes. Neither of you have to answer the questions if you do not want to.

Handling of specimens:

We will test the samples collected from your child at the KEMRI/CDC laboratory in Nairobi, Kenya and at other laboratories abroad, as not all tests can be carried out in

Kenya. We would also like to ask if we can store these samples to do more tests at a later time.

Confidentiality: Only staff involved in the study implementation will be allowed to work with your child's samples and to see your child's information. All samples will be coded at collection and will bear no information that can identify your child.

To enhance confidentiality, special codes on the information will be used and will be stored in secure study offices. For this study, each blood sample and naso/oropharyngeal swab will be labeled only with a barcode and a unique tracking number to protect your child's confidentiality. Personnel at the storage facility and testing laboratories will not know your child's identity, or the volunteer ID code assigned to your child for the study.

Risks. Taking swab samples from the nose cause temporary discomfort and might rarely cause temporary bleeding from irritation of the lining of the nose. Taking swab samples from the back of the mouth can cause a gagging sensation. Drawing blood can cause brief discomfort. Rarely, it might cause bleeding and bruising. Serious injury from taking swabs and blood are very rare. In addition, it is possible that other people will find out that your child participated in this study. There may be unknown or unforeseen risks associated with study participation.

Benefits. There will be no direct benefit for you or your child for participating in this study. In addition, information obtained from this study may help the Ministry of Health decide when and how much influenza disease occurs. Any information obtained from these tests that might be important for your health, will be communicated to you through project staff.

Contact Persons: If you have concerns regarding, injuries please contact Dr Eric Osoro on 0722216391. If you have concerns regarding your rights in being in the study, please contact the Ethics Review Committee, Kenya Medical Research Institute (KEMRI), P.O. Box 54840-00202, GPO, Nairobi. Telephone 0202722541 or 0722205901 or 0733400003.

Consent

This study has been explained to me. I have asked the questions I had. I have been informed that it is my free choice for my child to participate and if my child joins the study, I can drop out at any time without any penalty.

If you agree for your child to participate, please sign/thumb print here

_____Date: _____/_____/_____

Name of Participant _____

Witness signature (when needed, e.g. if participant cannot read)

Name of Witness _____Date: ____/_____/_____

Study Staff member who obtained this consent _____Date: _____/_____/_____

Appendix IV: Assent form for children aged 12-17 years old -- English

Prevalence of Zoonotic Swine Influenza Viruses at Household and Slaughterhouse Level in Kenya

Introduction. We are asking you to give samples for a study about what germs cause illnesses in people in this area. We want to find out how big a problem these germs are and how to treat them. The compound head for your house has already agreed to be part of this study. Today we are just asking you if you will give some samples to find out what germs may be causing problems to both humans and animals.

The specimens we want to get are these: If you agree to be in this study we will draw 5 ml of blood (a teaspoon) your arm. We will also collect a swab sample from the nose and the back of the mouth (throat) if you have a cough or running nose. If you agree we will return after 10 weeks and collect another blood sample and swab from you.

There will be no direct benefit to you from being in this study. However, there could be benefits to other people if we are able to determine those at high risk.

Risks from being in this study: Taking swab samples from the nose cause temporary discomfort and might rarely cause temporary bleeding from irritation of the lining of the nose. Taking swab samples from the back of the mouth can cause a gagging sensation. Drawing blood can cause brief discomfort. Rarely, it might cause bleeding and bruising. Serious injury from taking swabs and blood are very rare.

Benefits. You will not receive any benefit from this study. In addition, information obtained from this study may help the Ministry of Health decide when and how much influenza disease occurs. Any information obtained from these tests that might be important for your health, will be communicated to you and your parents through project staff.

To give samples today is your free choice. If you do not want to, that decision will not harm you in any way. If you do not want to, nobody will be mad at you. If you agree to give samples, but then change your mind, you can stop at any time.

We have already asked your parents/guardian about this and they said it was okay to ask you if you wanted to do this.

Will you be a part of this study and give samples? □ Yes □ No

In case your specimens are not completely used during this study, we or other investigators may be interested in studying similar diseases. The samples may be shared with other investigators at other institutions abroad including CDC Atlanta and University of Minnesota for 10 years. However, additional ethical approval will be sort from KEMRI prior to any tests in future on these specimens.

Please check below if you agree or do not agree for future use of your specimens.

Do you agree to having your blood specimen and nasal/oropharyngeal swab stored for future research (NOT to be used for any research on HIV or TB)? Initial one choice: YES _____ NO _____

Name of child (Print) _____

Date_____Child Signature (Signature or mark of consent) _____

Study Staff member obtaining this consent

Name _____

Date_____ _____

Appendix V: Household Questionnaire

SECTION A. GENERAL INFORMATION [FILL IN CAPITAL LETTERS]

A1. Household ID (From list):		A2.Date of interview: (dd/mm/yy)	
A3. Enumerator initials:			
A4. County	A5. Sub-location		
A6.1= Kiambu		A8.HH geographic coordinates id	

A9. Is this a pig owning household? ☐Yes ☐No

A10. Was this household sampled during the first phase in September 2013? ☐Yes ☐ No

SECTION B. HOUSEHOLD DEMOGRAPHICS

ENTER INFORMATION IN TABLE BELOW ON ALL HOUSEHOLD MEMBERS INCLUDING FARM WORKERS CURRENTLY LIVING (WITHIN LAST WEEK) IN THE HOUSEHOLD.

B1. Household pMember No.	B2. Position in the Household (in relation to household head)	B3. Age (years)	B4. Age in months(If less than 1 year)	B5. Sex	B6. Highest level of formal education completed	B7. Primary occupation (Pick one)	B8. Work tasks related to poultry and pigs husbandry (check all that apply)
	1=Household Head 2=Spouse of Household Head 3=Son/Daughter 4=Sibling 5=Father/Mother 6=Nephew/niece 7=Grand (son/daughter)	(Years)	Months	1=Male 2=Female	0= Child 1=No formal education 2 = Primary 3 = Secondary 4 = Post secondary 5 = Other	1. Works on farm/Farmer 2. Salaried off farm nonskilled 3. Salaried off farm skilled 4. Student 5. Housewife 6. Other(specify)	1=Slaughtering 2.Butchering 3. Cleaning barns 4 = herding/ 5=Feeding animals 6=Other 7=None

	8=Herdsman/woman 9= House help 10=Other (Specify above)						8= Don't know

B9. Relation of the person interviewed to the household head:

☐Household head ☐Spouse ☐Son/Daughter ☐Relative ☐Caretaker

B10. Do you own/keep animals in this household/farm? ☐Yes ☐ No

B11. If yes, how many animals do you own?(complete the table below)

Animal type	Total number
Cattle	
Sheep	
Goats	
Pigs	
Chicken	
Ducks	
Geese	
Turkeys	
Dogs	
Cats	
Others please specify_____	
Others please specify_____	

B12. Do you own/keep poultry or pigs in another farm apart from here?

☐Yes ☐ No ☐ Don't know

B13. If yes, how many farms? _____

B14. If owning poultry, do your poultry mix/interact with wild birds?

☐Yes ☐ No ☐ Don't know

B15. How many pig farms are within 1 km of your household? _

☐ None ☐ 1-3 ☐ >3 ☐ Don't know

B16. How many poultry farms (including backyard poultry) are within 1 km of your household?

☐ None ☐ 1-3 ☐ >3 ☐ Don't know

B17 Have you ever kept/owned pigs on this farm in the last 1 year?

☐Yes ☐ No

B18. Do you currently have pigs on the farm?

☐ Yes, IF YES GO TO SESTION C ☐ No IF NO SKIP TO PART 2

C PIG RELATED QUESTIONS – to be asked if the farm owns/keeps pigs

C1. How many of each of the different age groups of pigs do you have on the farm?

Age group	Total number
Piglet- From birth to weaning	
Growers-Any pig between weaning and sale or transfer to the breeding herd or sold for slaughter	
Weaners The permanent separation of a sow and piglets.	
Finishers Grower pigs over 70 kg live weight.	
Sows Any breeding female that has been served and is on the farm	
Boars Any male pig over 6 months and intended for use in the	

breeding herd	
Others(specify)	

C2. How many years have you kept pigs on the farm?

☐ < 1 year ☐ 1-3years ☐ >3 years ☐ Don't know

C3. How many people (including family members and workers) interact (feed, play with, clean, maintain pigsty, treat) with pigs regularly on the farm? _____

C4. Specify the household members who interact with pigs?

☐Children (<15 years) ☐Workers ☐Husband ☐Wife ☐Other family members

C5. Have you introduced any new pigs on the farm in the last 30 days?

☐Yes ☐ No

C6. If yes please complete the table below for the introduced pigs

Age group	Total number
Piglet	
Growers	
Weaners	
Finishers	
Sows	
Boars	
Others(specify)	

C7. Do you quarantine (separate) the new pigs prior to mixing them with your herd?

☐Yes ☐ No ☐ Don't know

C8.If yes, how often do you quarantine?

☐Always

☐Sometimes

☐Occasionally

☐Rarely

C9. How many days do you quarantine?

☐1-3 day

☐4 to 7 days

☐7 or more days

C10. Do you sell pigs? ☐Yes ☐ No

C11. If yes, have you sold any pigs in the last 30 days? ☐Yes ☐ No

C12. If yes, how many were sold in the past 30 days?

Age group	Total number

Piglet	
Growers	
Weaners	
Finishers	
Sows	
Boars	
Others(specify)	

C13. To whom do you sell your pigs (CHOOSE ALL THAT APPLY)?

☐Slaughterhouse ☐Neighbors ☐Market for live animals

☐To a buyer ☐Never sell only for family consumption

☐Other (PLEASE SPECIFY): _____

C14. How do you sell your pigs?

☐Live ☐Dressed (dead)?

C15. If you sell them dressed, where are they slaughtered?

☐On the farm, in a slaughterhouse facility ☐On the farm, in the open

☐Off farm slaughter

C16. If pigs are sold to a buyer, does the buyer come with pigs from other farms to your farm when he is collecting pigs?

☐ Yes ☐ No

C17. If yes, how often does he come with other pigs?

☐ Always ☐ Sometimes ☐ Occasionally ☐ Rarely

C18. How are your pigs housed?

☐ Closed confinement

☐ Open pens

C19. What is the floor type in the pig house?

☐ Dirt, ☐ Concrete, ☐, Wood

C20. What bedding do you use for the pigs?

☐ None, ☐ Saw-dust/ Wood-shavings, ☐ Dry grass

☐ Straw ☐Other

C21. What type of feed do you use for your pigs? (CHECK ALL THAT APPLY)

☐ Commercial ((pelleted/ground) ☐ Hotel/Market waste/scraps

☐ Vegetable waste/scraps from the farm ☐ Grazing

☐ Grain (if so which_____)

☐ Other? please list _____

C22. Do you deworm or provide supplements for your pigs?

☐Yes ☐ No

C23. During the past 3 months, were any pigs on the farm sick?

☐Yes ☐ No ☐Don't Know

C24. If yes, how many pigs were sick? _____

C25. Did any pig die of the illness?

☐Yes ☐No

C26. If yes, how many died? _____

C27. What are the common signs of sickness among pigs on the farm?

☐Loss of appetite ☐Coughing and/or running nose ☐Diarrhoea

☐ Others (specify)|_____

C28. Do you vaccinate you pigs against any diseases? ☐Yes ☐ No

C29. If yes, which ones? List them _____ ☐Don't know

C30. Do your pigs mix/interact with poultry? ☐Yes ☐ No ☐ Don't know

C31. Do your pigs mix/interact with wild birds? ☐Yes ☐ No ☐ Don't know

C32. Do your pigs mix/interact with wild animals? ☐Yes ☐ No ☐ Don't know

D: For the investigator

D1. Were any samples taken from the animals on this farm? ☐Yes

☐ No

D2. If yes, record the animal type and number of animals sampled.

Animal type	Sampled (Yes/No)	Total number sampled
Pigs		
Chicken		
Ducks		
Geese		
Turkeys		

Appendix VI: Individual Household Individual Questionnaire

Farm member/worker questionnaire

E1. Household ID_____

E2. Participant ID _____

E3. Date of sampling_____

Were you sampled during the first phase in September 2013? ☐ Yes ☐ No

E4. Sex ☐Male ☐ Female

E5. Date of Birth (DD/MM/YY)

E6. Age in Years_____

E7. What is the highest level of education you have completed?

☐ Child/student ☐No formal education ☐ Primary

☐ Secondary ☐ Post secondary

☐ Other (specify)_____

E8. What type of work have you engaged in the last 30 days? *Check all that apply.*

☐Child/student ☐Unemployed ☐Farm owner / worker,

☐Office worker ☐Slaughterhouse worker ☐Business worker

☐Other _____

E9. IF FARM WORKER, which animals have you worked with?:

☐Poultry ☐Pigs

☐Others (specify) _____

I: Respiratory illness history

I2. In the last 3 months have you had an illness with cough or sore throat or running nose)? ☐Yes ☐ No ☐ Unknown

I3. If yes, estimated # episodes last 3 months ☐1 ☐ 2 ☐ 3 ☐ 4 ☐ ≥5

I4. Estimated # episodes last 30 days ☐1 ☐ 2 ☐ 3 ☐ 4 ☐ ≥5

I5. Do you currently having any of the following symptoms (, cough or sore throat or running nose)?

☐Yes ☐ No

I6. If yes, check all that apply. IF YES TO ANY OF THE SYMPTOMS, AN NP/OP SWABS WILL BE COLLECTED FROM THE PARTICIPANT

Symptom No. of Days since onset

☐Fever (or history of fever) _____

☐Cough _____

☐Sore throat _____

☐Running nose _____

I9. In the last 3 months has anyone in your household been ill with a respiratory illness (running nose and cough or sore throat)? ☐Yes ☐ No ☐ Unknown

I10. If yes, number of household members with respiratory illness in the last 3 months

☐1 ☐ 2 ☐ 3 ☐ 4 ☐ ≥5

I11. Estimated number of household members with respiratory illness in last 30 days ☐1 ☐ 2 ☐ 3 ☐ 4 ☐ ≥5

I12. In the last 3 months has anyone in your household been hospitalized due to a respiratory illness?

☐Yes ☐ No

I13. In the last 3 months has anyone at work been ill with a respiratory illness (fever and cough or sore throat)? ☐Yes ☐ No ☐ Unknown ☐Don't work away from home

I14. In the last 3 months has anyone at work been hospitalized due to a respiratory illness?

☐Yes ☐ No ☐ Unknown

J: Occupational exposureJ1. Below is a list of animals that you may have been exposed to this year as **part of your work. Exposure** only includes times where you had at least one day with at least 30 consecutive minutes of contact. Please indicate the average number of hours per day of exposure on a normal working day and the average number of animals per day. **For children <18 years old, consider work to mean your responsibilities or chores.*

Animal type	Hrs per day of work	Avg # of animals per day
☐Chickens, broilers		
☐Chickens, layers		
☐Chickens, kienyeji		
☐Ducks		
☐geese		
☐Turkeys		
☐Pigs		
☐Horses		

☐None (END THE QUESTIONNAIRE IF SELECTED)

J2. What type of activities do you perform with poultry or pigs? (TICK ALL THAT APPLIES).

☐Feeding/watering birds

☐Feeding /watering pigs

☐Slaughter of birds

☐Slaughter of pigs

☐Transporting birds

☐Transporting pigs

☐Examining and treating birds

☐Other (specify)_____

J3. In the last 12 months, while working with animals,

Items	Frequency		Type
Eye protection	☐Always	☐Never	☐Goggles
	☐Sometimes	☐Not sure	☐Glasses
			☐Other____
Mask	☐Always	☐Never	☐Dust mask
	☐Sometimes	☐Not sure	☐Filtered mask
			☐Surgical mask
			☐Other_____

Clothing	☐Always ☐Sometimes	☐Never ☐Not sure	☐Aprons ☐Coveralls ☐Outer garments ☐Other_____
Footwear	☐Always ☐Sometimes	☐Never ☐Not sure	☐Disp. boots ☐Washable boots ☐Sneakers ☐Sandals ☐Other_____
Gloves	☐Always ☐Sometimes	☐Never ☐Not sure	☐Disposable latex/vinly ☐Cloth ☐Leather ☐Other
Hand washing	☐Always ☐Sometimes	☐Never ☐Not sure	☐Water only ☐ Water with soap

For the investigator

WERE ANY SAMPLES TAKEN FROM THIS PARTICIPANT?

☐Yes ☐ No

Appendix VII: Slaughterhouse Individual Questionnaire

A1. Participant (individual) ID number: _____

A2. Slaughterhouse (select one)

☐ Uthiru

☐ Bondo

☐ Kisumu slaughter slab

A3. Date of sampling___ / ___ / ___ (day/month/year)

A4. Were you sampled during the first phase in September 2013? ☐ Yes ☐ No

A5. Age (Years) _____

A6. Sex: ☐ Male ☐ Female

A7. How many people including children do you live with at home?

Age	Number in the household
Children <5 years	
Children 5-15 years	
>15 years	

A8. What is the highest level of education you have completed?

☐ No formal education ☐ Primary ☐ Secondary ☐ Post secondary

☐ Other (specify)_____

A9. What is your primary occupation?

☐Works fulltime at the slaughterhouse (greater than half the working time in a month)

☐ Works part-time at the slaughterhouse (less than 50% time in a month)

☐Pig trader

☐Pig farmer

☐Other (specify) _____

A10. What is your position at the slaughterhouse? (Check all that apply)

☐ I am a casual worker at the slaughterhouse

☐ I deliver pigs to the slaughterhouse (i.e., trader)

☐ I have my own business or farm

☐ I supervise or manage others here at the slaughterhouse

☐ Other (specify)_____

A11. How long have you engaged in the pig trade or worked at this slaughterhouse?

☐ less than 1 year ☐ 1-3years ☐ > 3 years

A12. What activities do you engage in at the slaughterhouse? (CHECK ALL THAT APPLY)

☐ Deliver pigs from farms ☐ Always ☐ Sometimes ☐ Never

☐ Offload pigs at the slaughterhouse ☐ Always ☐ Sometimes ☐ Never

☐ Middleman for live pigs ☐ Always ☐ Sometimes ☐ Never

☐ Clean the live pig stalls ☐ Always ☐ Sometimes ☐ Never

☐ Stun the live pigs ☐ Always ☐ Sometimes ☐ Never

☐ Skinning, Evisceration, splitting ☐ Always ☐ Sometimes ☐ Never

☐ Sell pig meat at the slaughter house ☐ Always ☐ Sometimes ☐ Never

☐ Sell pig offal at the slaughterhouse ☐ Always ☐ Sometimes ☐ Never

☐ Sell snacks at the slaughterhouse ☐ Always ☐ Sometimes ☐ Never

☐ Supervisor at the slaughterhouse ☐ Always ☐ Sometimes ☐ Never

☐ Other (please specify)_____

A13. Do you own or keep other animals at home? ☐ Yes ☐ No

If yes, which animals do you own?

☐ Poultry

☐ Dogs and cats

☐ Cattle, sheep or goats

☐ Other (specify)_____

A14. Have you ever kept/owned pigs at home in the last 1 year?

☐Yes ☐ No

A15. Do you currently keep pigs at home?

☐Yes ☐ No

A16. Do you normally see sick pigs in your work?

☐ Yes ☐ No ☐ Don't know

A16a. If yes, please indicate which months you commonly see pigs that are sick (check all that apply)

Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

A16b. IF NO PATTERN IN SICK PIG OBSERVATIONS HAVE BEEN NOTED - TICK THIS BOX ☐

A17. Have you had contact with a sick pig in the last one month?

☐Yes ☐ No ☐ Unknown

A17a. If Yes, what were the clinical signs observed?

☐Loss of appetite ☐Coughing and/or running nose ☐Diarrhoea

☐ Others(specify)_____

Respiratory illness history

A18. In the last 3 months have you ever developed a respiratory illness (cough or sore throat or running nose)? ☐Yes ☐ No ☐ Unknown

IF YES, estimated # episodes last 3 months ☐1-2 ☐ 3-5 ☐ >5

Estimated # episodes last 30 days ☐1-2 ☐ 3-5 ☐ >5

A19. Are you currently having any of the following symptoms (cough or sore throat or running nose)? ☐Yes ☐ No IF YES, CHECK ALL THAT APPLY

Symptom	Days since onset
☐Cough	_____
☐Sore throat	_____
☐Running nose	_____

IF YES TO ANY OF THE ABOVE SYMPTOMS, A NP/OP SWABS WILL BE COLLECTED FROM THE PARTICIPANT

A20. In the last 3 months has anyone in your household been ill with a respiratory illness with fever and cough or sore throat? ☐Yes ☐ No ☐ Unknown

A20a.*If yes,* number of people in your household in the last 3 months

☐1-2 ☐ 3-5 ☐ >5

A21. Estimated number of people in your household last 30 days

☐1-2 ☐ 3-5 ☐ >5

A22. In the last 3 months has anyone in your household been hospitalized of a respiratory illness?

☐Yes ☐ No ☐ Unknown

A23. In the last 3 months has anyone at work been ill with a respiratory illness (fever and cough or sore throat)? ☐Yes ☐ No ☐ Unknown

IF YES, number of people last 3 months

☐1-2 ☐ 3-5 ☐ >5

A24. Estimated number of people in last 30 days

☐1-2 ☐ 3-5 ☐ >5

A25. In the last 3 months has anyone at work been hospitalized due to a respiratory illness?

☐Yes ☐ No ☐ Unknown

FOR THE INTERVIEWER: QUESTIONNAIRE BEING ADMINISTERED TO?

☐SH manager/supervisor ☐Trader ☐Slaughterhouse worker

☐Farmer ☐Other

_____(specify)

FOR SLAUGHTERHOUSE MANAGERS/SUPERVISORS ONLY

B1. For those who work directly with live pigs, how many pigs are sold at this slaughterhouse?

Pig type	Average Daily Sales Between holidays	Average Daily Sales During holidays	If sales increase, state which holidays result in increases
Mature pigs	Live	Live	
	Dressed[1]	Dressed	

B2. How many days do pigs typically stay in the slaughterhouse prior to slaughter?

_____

B3. Average no. of days_____

B4. Range no. of days_____

B5. Do people delivering the pigs use protective clothing?

☐ Yes, all the time

☐ Sometimes,

☐ No, never

B6. IF YES or SOMETIMES, what clothes? (CHECK ALL THAT APPLY)

☐ Overalls

☐ Gloves

B7. If yes to gloves, what kind of gloves are used?

☐Cloth/leather gloves

☐ disposable gloves

☐ Other _____ (specify)

B8. Do you clean any equipment in the slaughterhouse?

☐ Yes ☐ No ☐ Sometimes

B9. IF YES, what equipment gets cleaned? (SELECT ALL THAT APPLY)

☐ Slaughter equipment

☐ Holding pens

☐ Slaughterhouse floor/space

☐ Other _____ (specify)

B10. IF YES, how often is the equipment cleaned?

☐ Daily ☐ Twice Daily ☐ Weekly ☐ Monthly

☐ Other (specify) _____

B11. Do you disinfect equipment at the slaughterhouse? ☐ Yes ☐ No ☐ Sometimes

B12. IF YES, what equipment gets disinfected? (Select all that apply)

☐ Slaughter equipment (such as knives?, captive bolt, pith rods?)

☐ Holding pens

☐ Slaughterhouse floor/space

☐ Other _____ (specify)

B13. IF YES, how often is the equipment cleaned and disinfected?

☐ Daily ☐ Twice Daily ☐ Weekly ☐ Monthly

☐ Other (specify) _____

B14. What chemical do you use for disinfection? _____

B15. Is there a time when the slaughterhouse is empty of pigs? ☐ Yes ☐ No

B16. IF YES, please describe when/why)_____

B17. If you have a sick pig, what do you do with it? (CHECK ALL THAT IS DONE)

☐ Keep it in the pens/home and treat ☐ Always ☐ Sometimes ☐ Never

☐ Slaughter and sell at full price ☐ Always ☐ Sometimes ☐ Never

☐ Slaughter and sell at discounted price ☐ Always ☐ Sometimes ☐ Never

☐ Slaughter for home consumption ☐ Always ☐ Sometimes ☐ Never

☐ Others (specify)_____

B18. If you have a pig die before it is stunned for slaughter, what do you do with it?' CHECK ALL THAT APPLY

☐ Discard in the trash ☐ Always ☐ Sometimes ☐ Never

☐ Bury it (beyond the normal trash disposal) ☐ Always ☐ Sometimes ☐ Never

☐ Sell at full price ☐ Always ☐ Sometimes ☐ Never

☐ Sell at discounted price ☐ Always ☐ Sometimes ☐ Never

☐ Self consumption ☐ Always ☐ Sometimes ☐ Never

☐ Offer to others for free ☐ Always ☐ Sometimes ☐ Never

☐ Others (specify)_____

B19. Are visitors allowed to enter the slaughter areas? ☐ Yes ☐ No

B20. If yes, how often ☐ Always ☐ Sometimes ☐ Never

B21. Do you require that visitors who enter the slaughter area wear protective gear? ☐ Always ☐ Sometimes ☐ Never

B22. If **ALWAYS OR SOMETIMES**: What kind of protective gear is used? (CHECK ALL THAT APPLY)

☐ Protective clothing

☐ Gloves

B23. If yes to gloves, what kinds of gloves are used?

☐ Cloth/leather gloves

☐ disposable gloves

☐ Other _____

B24. Are footbaths in use? ☐ Always ☐ Sometimes ☐ Never

B25. If **ALWAYS OR SOMETIMES**: What kind of footbaths is used? (CHECK ALL THAT APPLY)

☐ Dry ☐ Wet

B26. Do you and other slaughterhouse employees wash hands with soap:

☐Before handling pigs? ☐ Always ☐ Sometimes ☐ Never

☐After handling pigs? ☐ Always ☐ Sometimes ☐ Never

B27. Are slaughterhouse employees allowed to smoke on the premises? ☐ Yes ☐ No

FOR PIG TRADERS/FARMERS ONLY

C1.Do you always use the same vehicle/van to transport pigs to the market?

☐Yes

☐No

☐Not applicable

IF YES

C2. Who owns the vehicle/van?_ ☐ Self/family member ☐ Hired ☐ Employer

C3. How many pigs do you typically transport at a given time? _____

C4. Who do you buy pigs from? Check all that apply

☐Farmers ☐ Always ☐ Sometimes ☐ Never

☐Other traders ☐ Always ☐ Sometimes ☐ Never

☐Own ☐ Always ☐ Sometimes ☐ Never

☐Not applicable

C5. If in Uthiru, from which county do the pigs you bring for slaughter come from?

☐Kiambu ☐Nairobi ☐Others

C6. If in Bondo or Kisumu, where do the pigs you bring for slaughter come from?

☐Within the county ☐Outside the county

C7. Do you mix pigs from different farms while transporting them to the slaughterhouse?

☐Yes ☐No ☐Not applicable

C8. Do you transport other live animals to the slaughterhouse together with the pigs?

☐ Yes ☐ No

C9. IF YES, please list the animal types_____

C10. If Yes, how frequently do you transport pigs with other animals?

☐Almost Always ☐Frequently, ☐Sometime ☐Never)

C11. How many days on average does it take from the time you collect the first batch to the time you finally deliver them to the slaughterhouse?

☐Same day

☐1-2 days

☐>3 days

☐Don't know

C12. Does the seasonality of pig supply/demand vary through the year ☐Yes ☐No

IF NO SEASONALITY IS SEEN IN DEMAND/SUPPLY OF PIGS THROUGH THE YEAR HAS BEEN OBSERVED-- TICK THIS BOX ☐

C13. If you have a sick pig , what do you do with it? CHECK ALL THAT APPLY

☐ Keep it in the pens/home and treat ☐ Always ☐ Sometimes ☐ Never

☐ Slaughter and sell at full price ☐ Always ☐ Sometimes ☐ Never

☐ Slaughter and sell at discounted price ☐ Always ☐ Sometimes ☐ Never

☐ Slaughter for home consumption ☐ Always ☐ Sometimes ☐ Never

☐ Others (specify)_____

C14. If you have a pig die before it is slaughtered (in the farm or during transport), what do you do with it?'CHECK ALL THAT APPLY

☐ Discard in the trash ☐ Always ☐ Sometimes ☐ Never

☐ Bury it (beyond the normal trash disposal) ☐ Always ☐ Sometimes ☐ Never

☐ Sell at full price ☐ Always ☐ Sometimes ☐ Never

☐ Sell at discounted price ☐ Always ☐ Sometimes ☐ Never

☐ Self consumption ☐ Always ☐ Sometimes ☐ Never

☐ Offer to others for free ☐ Always ☐ Sometimes ☐ Never

☐ Others (specify)_____

For the investigator

C15. Were any samples taken from this participant? ☐Yes

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

KEMRI/RES/7/3/1 **17th June 2013**

TO: **DR. KARIUKI NJENGA**
PRINCIPAL INVESTIGATOR

THROUGH: **DR. JOHN VULULE**
THE DIRECTOR, CGHR
KISUMU

Dear Sir,

RE: SSC PROTOCOL NO. 2557: REVISED (*RE-SUBMISSION*): PREVALENCE OF ZOONOTIC SWINE INFLUENZA VIRUSES AT HOUSEHOLD AND SLAUGHTERHOUSE LEVEL IN KEMYA

This is to inform you that the Ethics Review Committee (ERC) reviewed the document listed above and is satisfied that the issues raised at the 214th meeting held on 23rd April, 2013 have been adequately addressed.

The study is granted approval for implementation effective this **17th day of June 2013**. Please note that authorization to conduct this study will automatically expire on **June 16, 2014**. If you plan to continue with data collection or analysis beyond this date, please submit an application for continuing approval to the ERC Secretariat by **May 6, 2014**.

Any unanticipated problems resulting from the implementation of this protocol should be brought to the attention of the ERC. You are also required to submit any proposed changes to this protocol to the SSC and ERC prior to initiation and advise the ERC when the study is completed or discontinued.

You may embark on the study.

Yours faithfully,

EAB

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI ETHICS REVIEW COMMITTEE

Appendix VIII: Study Approvals

Ethical Review Committee approval

Animal Care and Use Committee Approval

KENYA MEDICAL RESEARCH INSTITUTE

Centre for Virus Research, P.O. Box 54628 - 00200 NAIROBI - Kenya
Tel: (254) (020) 2722541, 2713349: 0722-205901, 0733-400003; Fax: (254) (020) 2726115
Email: cvr@kemri.org

KEMRI/ACUC/ 03.04.13 **10th April 2013**

Dr. Kariuki Njenga
IHAHP, CDC
Kenya

Dr. Njenga,

RE: Animal use approval for "Prevalence of Zoonotic Swine Influenza Viruses at Household and Slaughterhouse Level in Kenya" protocol

The KEMRI animal care and use committee acknowledges the resubmission of the above mentioned protocol addressing the issues raised earlier.

The committee has established that the collection of specimens from the various listed species of domestic animals is necessary in attaining the study objectives.

It has also been confirmed that permission to obtain specimens from animals will be done with the owners consent and the study is being carried out in conjunction with the Department of Veterinary Services as stated in the letter **RES/GEN.VOL.XII** dated 12th February 2013.

The committee grants you the approval to proceed with your study after obtaining all the other necessary approvals that may be required.

The committee wishes you all the best in your work.

Yours sincerely,

Dr. Konongoi Limbaso
Chairperson KEMRI ACUC

In Search of Better Health

Ministry of Health Administrative Approval

MINISTRY OF HEALTH

Telegrams: "MINHEALTH", Nairobi
Telephone Nairobi 0202717077
Fax: 2714130
Email: pphs@health.go.ke
When replying please quote

AFYA HOUSE
CATHEDRAL ROAD
P O Box 30016
NAIROBI

31st July, 2013

TO WHOM IT MAY CONCERN

APPROVAL TO CONDUCT RESEARCH ON ZOONOTIC SWINE INFLUENZA IN KENYA

The Ministry of Health and the Ministry of Agriculture, Livestock and Fisheries in collaboration with Kenya Medical Research Institute (KEMRI) and the Centers for Disease Prevention and Control (CDC) are planning to conduct a study on zoonotic swine influenza in Kenya.

Swine influenza is a respiratory illness of pigs which can also cause disease in humans. The purpose of the study is to determine the prevalence and incidence of zoonotic swine influenza infections in humans at household and swine slaughter house levels. It is expected that findings from this study will provide information for developing control programs for zoonotic influenza and form a basis of expanding the influenza surveillance system.

The study is will be conducted in two phases with the first phase in September and December 2013.

I have noted that the ethical aspects of the study have been reviewed and approved by the Kenya Medical Research Institute (KEMRI)

I have therefore given approval for the conduct of the study.

DR. S.K.SHARIF, MBS, MBChB, M.Med. DLSTMH, MSc.
DIRECTOR PUBLIC HEALTH & SANITATION

Appendix IX: Publications

Publication 1

Osoro et al. Environmental Health and Preventive Medicine (2019) 24:53
https://doi.org/10.1186/s12199-019-0808-6

Environmental Health and Preventive Medicine

RESEARCH ARTICLE Open Access

Seroprevalence of influenza A virus in pigs and low risk of acute respiratory illness among pig workers in Kenya

Eric Mogaka Osoro[1*], Shirley Lidechi[2], Doris Marwanga[2], Jeremiah Nyaundi[2], Athman Mwatondo[3], Mathew Muturi[4], Zipporah Ng'ang'a[1] and Kariuki Njenga[5]

Abstract

Background: Influenza A viruses pose a significant risk to human health because of their wide host range and ability to reassort into novel viruses that can cause serious disease and pandemics. Since transmission of these viruses between humans and pigs can be associated with occupational and environmental exposures, we investigated the association between occupational exposure to pigs, occurrence of acute respiratory illness (ARI), and influenza A virus infection.

Methods: The study was conducted in Kiambu County, the county with the highest level of intensive small-scale pig farming in Kenya. Up to 3 participants (> 2 years old) per household from pig-keeping and non-pig-keeping households were randomly recruited and followed up in 2013 (Sept-Dec) and 2014 (Apr-Aug). Oropharyngeal (OP) and nasopharyngeal (NP) swabs were collected from participants with ARI at the time of study visit. For the animal study, nasal and oropharyngeal swabs, and serum samples were collected from pigs and poultry present in enrolled households. The human and animal swab samples were tested for viral nucleic acid by RT-PCR and sera by ELISA for antibodies. A Poisson generalized linear mixed-effects model was developed to assess the association between pig exposure and occurrence of ARI.

Results: Of 1137 human participants enrolled, 625 (55%) completed follow-up visits including 172 (27.5%) pig workers and 453 (72.5%) non-pig workers. Of 130 human NP/OP swabs tested, four (3.1%) were positive for influenza A virus, one pig worker, and three among non-pig workers. Whereas none of the 4462 swabs collected from pig and poultry tested positive for influenza A virus by RT-PCR, 265 of 4273 (6.2%) of the sera tested positive for virus antibodies by ELISA, including 11.6% (230/1990) of the pigs and 1.5% (35/2,283) of poultry. The cumulative incidence of ARI was 16.9% among pig workers and 26.9% among the non-pig workers. The adjusted risk ratio for the association between being a pig worker and experiencing an episode of ARI was 0.56 (95% CI [0.33, 0.93]), after adjusting for potential confounders.

Conclusions: Our findings demonstrate moderate seropositivity for influenza A virus among pigs, suggesting the circulation of swine influenza virus and a potential for interspecies transmission.

Keywords: Acute respiratory illness, Influenza A virus, Pig workers, Zoonoses

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Full list of author information is available at the end of the article

Background

Influenza A viruses pose a significant risk to human health because of their wide host range (swine, birds, horses, dogs, cats, sea mammals) and capacity to reassort into novel viruses that can cause serious epidemics or pandemics [1]. Pigs are believed to play an important part in the evolution of viruses of pandemic potential because of their inherent ability to allow replication of swine, avian, and human influenza viruses and potential to have mixed infections [2–4]. For example, the 2009 influenza A H1N1 pandemic virus that was associated with 150,000–570,000 deaths globally was the product of re-assortment of circulating human influenza and avian influenza strains with pigs suspected as the mixing vessel [5].

Pig-to-human and human-to-pig influenza (reverse zoonosis) virus transmission events have been documented in North America, Europe, Asia, and Africa [6–11]. Severe disease following these zoonotic events has been reported in persons with chronic medical conditions, although most such infections are mild or subclinical [12, 13]. Reverse zoonosis of influenza virus is considered an important source of swine influenza virus (SIV) diversity which reduces the efficacy of vaccines to SIV in pigs [14].

The transmission of influenza viruses between pigs and humans is not only associated with occupational and environmental exposures, but also with the virus evolution and emergence of novel transmissible strains capable of infecting humans and spreading from person to person that can lead to pandemics [6, 8, 15].

Studies have shown evidence of infection with newly emerging SIVs as well as higher prevalence of SIVs among persons whose occupation involves close contact with pigs [6, 16]. Findings from a preliminary study in pigs from a Kenyan slaughterhouse revealed an overall influenza A seroprevalence of 15%, including > 12% seroprevalence of the pandemic 2009 H1N1 influenza virus, suggesting transmission of influenza viruses from humans to pigs [17].

The growing demand for pig products in Kenya has resulted in rapid growth in intensive small-scale pig farming [18]. Although pig workers in such livestock production systems may be exposed to swine influenza viruses, no studies on the occupational exposure risks to influenza viruses have been documented in the country.

Here, we conducted a longitudinal study to determine the association between occupational exposure to pigs and the occurrence of ARI and influenza A virus infection. We monitored ARI among the humans to determine its utility in detection of influenza virus infections among pig workers. We also assessed the farming practices associated with high risks of influenza virus transmission among pig keepers.

Methods

Study area

The study was conducted in Kiambu County in Kenya (Fig. 1), a county that has the largest proportion of intensive small-scale pig farmers in Kenya [18]. Within the county, we selected two sub-counties that have the highest number of pig farms and selected households based on whether they kept pigs or not.

Pig-keeping households were selected by systematic random sampling from a comprehensive list of pig farmers in the area prepared by the local veterinary officers. Two to three non-pig-keeping households were selected from the neighbourhood of each selected pig-keeping household.

Study design and sampling

The participants from pig-keeping and non-pig-keeping households were enrolled in September 2013 and April 2014, with a follow-up visit conducted 12 to 14 weeks after enrollment. Concurrent cross-sectional sampling of pigs and poultry in enrolled households was conducted at enrollment and follow-up visits.

All members above 2 years of age in selected households were eligible for enrollment. In each household, up to 3 persons were randomly selected. At enrollment, a questionnaire was administered and nasopharyngeal (NP) and oropharyngeal (OP) swabs collected from participants who met the acute respiratory illness (ARI) case definition.

The participants were then visited after 12 to 14 weeks to administer the follow-up questionnaire as well as collect NP/OP swabs if the participant had ARI at the time of follow-up. Pig exposure was defined as cleaning barns, feeding, or slaughtering pigs as part of routine daily activities (> 3 times a week) for the month preceding the study interview. Poultry exposure was similarly defined for those working with poultry.

Participants with pig or poultry exposures were classified as pig workers and poultry workers, respectively. The determination of pig or poultry exposure status was made during enrollment. Acute respiratory illness (ARI) was defined as an illness with a history of fever or cough lasting less than 10 days.

Animal samples were collected from pigs and poultry on the farm at enrollment and follow-up. Pig nasal swabs and sera were collected from all age groups including piglets, weaners, growers, finishers, sows, and boars. The number of pigs sampled was proportionate to herd size with all animals sampled from small herds (< 10 pigs) and up to 15 animals sampled from herds with > 10 pigs.

For poultry, sera and oropharyngeal swabs were collected from up to 3 animals each of chickens, ducks, turkeys, and geese present on the farms.

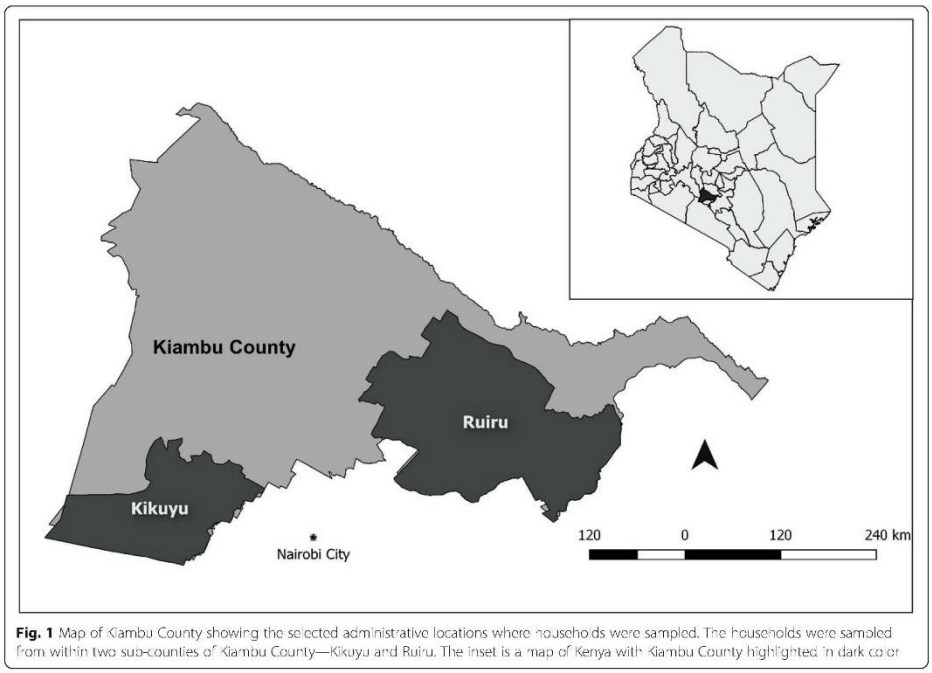

Fig. 1 Map of Kiambu County showing the selected administrative locations where households were sampled. The households were sampled from within two sub-counties of Kiambu County—Kikuyu and Ruiru. The inset is a map of Kenya with Kiambu County highlighted in dark color

Sample size

The primary outcomes on the human component of the study were the prevalence of influenza A virus infection and the number of ARI episodes reported during the follow-up period.

The sample size was calculated assuming 8% prevalence of acute respiratory illness among pig workers (exposed), and 2% among non-pig workers (unexposed) translating to a sample size of 394 participants with 99 in the exposed and 295 in the unexposed group (exposed to unexposed ratio of 1:3) [19, 20].

In the animal study, the primary outcome was the detection of influenza A virus infection and seroprevalence of influenza A virus. A minimum sample size of 392 pigs was determined based on an expected seroprevalence of 15% and a design effect of two at 95% confidence level.

Sample collection and testing

The NP and OP swabs were collected from each enrolled human participant that met the case definition of ARI at the time of sampling. The two swabs were put together in a cryovial viral transport media (VTM), temporarily stored in a cool box at 2–8 °C, and later in the day transported to the Kenya Medical Research Institute (KEMRI) laboratory in Nairobi and stored at − 80 °C until testing. Serum, and nasal or OP swabs were collected from each pig and poultry. Each animal swab was separately placed in a cryovial containing VTM, temporarily stored in a cool box at 2–8 °C and transported to KEMRI laboratory in Kisumu for storage at − 80 °C until testing.

Animal serum was tested for antibodies against influenza A viruses using the IDEXX® ELISA (FlockChek AI MultiS-Screen Ab Test Kit®, Westbrook, Maine), following the manufacturer's instructions [21, 22]. A seropositive herd was defined as any household with at least one IgG antibody positive pig.

Human NP/OP samples were tested for viral RNA by real-time reverse transcriptase polymerase chain reaction (RT-PCR) using CDC primers and probes for influenza A and influenza B viruses [22, 23]. The RNA was extracted using the QIAamp RNA extraction kit (Qiagen Inc., Valencia, CA) following the manufacturer's instructions. Cutoff for positivity was read at cycle threshold (C_T) values ≤ 40. Positive and negative controls were used to validate the test assay.

Subtyping was attempted for influenza A positive swabs for seasonal human influenza, avian, and swine

influenza [24]. Animal nasal and OP swabs were screened for influenza A virus by RT-PCR using the CDC protocol for influenza A virus detection that targets the matrix gene [23].

Data collection and analysis

Standardized questionnaires on smartphones were administered to all participants to collect data on demographics, clinical symptoms, and exposure to risk factors including specific activities with reference to pig and poultry raising, transportation, slaughtering, and dressing. For animals, data on herd demographics and risk factors (age, species present on the farm, herd size, species raised, and husbandry practices) were collected.

We used R Statistical Software (version 3.5.1) for data cleaning and analysis [25]. Descriptive statistics were determined for socio-demographic and other characteristics comparing pig workers and non-pig workers. Categorical variables were compared using chi-square test and Fisher's exact test while continuous variables were compared using the Student t test. The cumulative incidence for ARI was calculated as the number of episodes reported by participants divided by the total number of participants.

Crude risk ratios were determined for the initial assessment of the association between pig exposure and episodes of ARI. We applied the generalized linear mixed model (GLMM) using the Poisson distribution to adjust the risk ratio between pig exposure and ARI for clustering and potential confounding. We assessed for overdispersion before applying the Poisson distribution where a p value of < 0.05 would indicate overdispersion. The predictor variables (fixed effects) included in GLMM to predict the occurrence of ARI episodes were pig workers, age, sex, occupation, education level completed, reported chronic disease, and poultry exposure. We accounted for clustering at household and individual level (repeat ARI episodes) by including the variables as random effects in the mixed model. The GLMM was done using the lme4 package in R statistical software where the estimation is based on the maximum likelihood [26].

Model selection was conducted using stepwise selection using Akaike information criterion and Bayesian information criteria measures where lower values suggest a better model fit [27].

The adjusted risk ratio and the 95% confidence intervals were then computed and a p value of < 0.05 considered statistically significant.

Ethical considerations

The study was approved by the KEMRI Scientific and Ethics Review (Protocol # SSC 2557), KEMRI Animal Care and Use Committee, the CDC Institutional Review Board and National Institute of Health, and Division of Microbiology and Infectious Diseases review board. Informed consent was obtained from all participants. Assent and parental permission were obtained for minors.

Results

Household characteristics

A total of 634 households with 2175 persons were enrolled, of which 488 (77.0%) households participated in the follow-up visit. From 170 pig-keeping households, 373 (61.9%) participants were enrolled, of which 204 (54.7%) had a follow-up visit. From 464 non-pig-keeping households, 764 (48.6%) participants were enrolled, of which 421 (55.1%) had a follow-up visit (Fig. 2). The average number of participants enrolled per household was 2.2 and 1.7 for pig-keeping and non-pig-keeping households, respectively. There were no significant differences in sex, age, or education level completed of household heads between the pig-keeping and non-pig-keeping households.

Nearly all (97.9%) pig-keeping households reared other animals, significantly higher than 73.1% in non-pig-keeping households ($p < 0.001$). Among pig-keeping households, the median number of pigs was 13 (range 1 to 200) and 54.2% had reared pigs for at least 2 years. A total of 2066 pigs were sampled, of which 1118 (63.2%) were female and nearly half (48.6%) were either finishers or growers (Fig. 3). Besides pigs, the majority of the pig-keeping farms reared poultry (83.3%).

Individual characteristics

Of 1137 participants enrolled, 625 (55%) had a follow-up visit and included in the analysis (Fig. 2). There were no significant differences in sex, occupation, and highest education level completed between the participants who received follow-up visits and those lost to follow-up ($p > 0.05$). The demographic characteristics and pig worker status of the 625 participants who had follow-up visits are presented in Table 1. Among the 172 pig workers, 92.4% were residents of pig-keeping households, 55.2% were male, and the 21–40 years age group accounted for 45% of the participants. About 80% of the pig workers reported their occupation as farming, 80% were also poultry workers with 2.3% reporting no formal education (Table 1). Non-pig workers were mostly female (57.2%), about one third (34.4%) were between 21 and 40 years old and 54.1% were farmers (Table 1). Human influenza vaccination in the previous 12 months was reported by two pig workers and one non-pig worker.

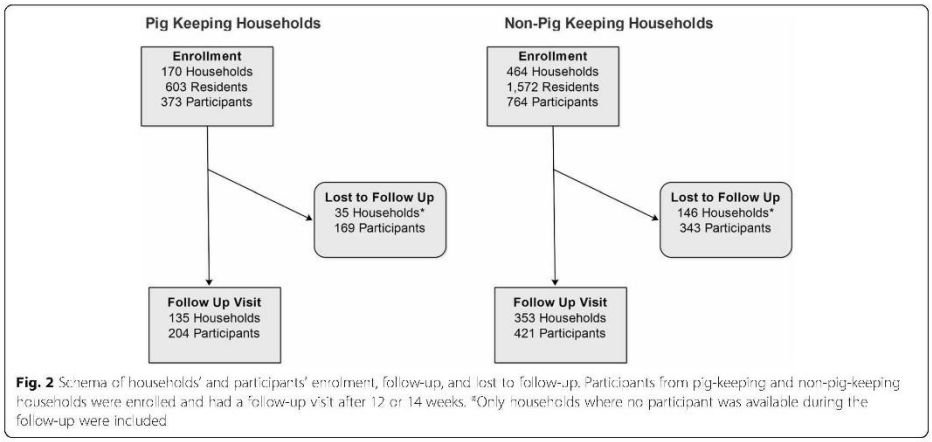

Fig. 2 Schema of households' and participants' enrolment, follow-up, and lost to follow-up. Participants from pig-keeping and non-pig-keeping households were enrolled and had a follow-up visit after 12 or 14 weeks. *Only households where no participant was available during the follow-up were included

Human and animal influenza A virus results

A total of 130 swab samples were collected from human participants who reported ARI either at enrolment or during follow-up with only five participants having samples collected at both enrolment and follow-up visits.

Among 91 samples from participants who completed the follow-up visit, 24 (26.4%) were from pig workers and 67 (73.6%) from non-pig workers. Four (3%) human swabs tested positive for influenza A by RT-PCR: one from a pig worker and three from non-pig workers. The positive samples could not be subtyped.

A total of 4462 nasal and oropharyngeal swabs from animals were collected; 2173 (48.7%) from chicken, 2066 (46.3%) from pig, 126 (2.8%) from ducks, 56 (1.3%) from geese, and 41 (0.9%) from turkey. None of the swabs was positive for influenza A virus by RT-PCR. A total of 4273 serum samples were collected from the animals, including 2283 (53.4%) from poultry and 1990 (46.6%) from pigs. Overall, 265 (6.2%) of the animal sera were positive for influenza A virus by ELISA, including 230 of 1990 (11.6%) pig sera and 35 of 2283 (1.5%) poultry sera. Among poultry, the seropositivity for influenza A was 3.3% for geese, 2.9% for ducks, 1.4% for chicken, and 0% for turkeys.

Fifty-eight (34.1%) of the pig-keeping households had at least one seropositive pig during the sampling points. The median number of seropositive pigs among these households was two (range 1 to 10).

Association between pig workers and acute respiratory illness

We examined the risk of occurrence of ARI by pig worker status during the follow-up period. Overall, a total of 151 episodes of ARI were reported from 116 participants, giving a combined cumulative incidence of 24.2%. Three-quarters (87) of the participants with ARI

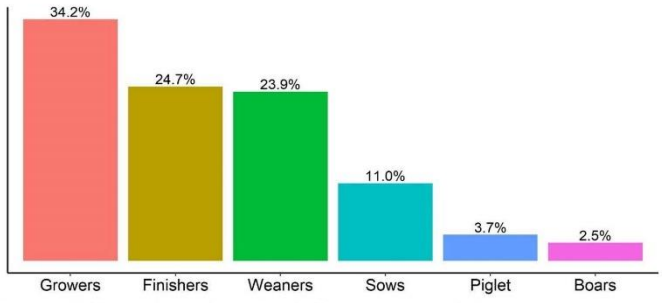

Fig. 3 Proportion of pigs sampled ($n = 2066$) in pig-keeping households by maturity status, Kiambu, 2013–2014

Table 1 Demographic and other characteristics of study participants by pig worker status, Kiambu, 2013–2014

Variable	Pig workers (*N* = 172), *n* (%)	Non-pig workers (*N* = 453), *n* (%)	*p* value
Pig-keeping household			
Yes	159 (92.4)	45 (9.9)	< 0.001
No	13 (7.6)	408 (90.1)	
Follow-up month			
Sept–Dec	95 (55.2)	224 (49.4)	0.229
Apr–Aug	77 (44.8)	229 (50.6)	
Sex			
Female	77 (44.8)	259 (57.2)	0.007
Male	95 (55.2)	194 (42.8)	
Age in years, mean (SD)	40.3 (15.7)	39.6 (18.6)	0.646
Age group, years[a]			
Below 10	0 (0.0)	20 (4.4)	0.005
10 to 20	15 (8.8)	67 (14.9)	
21 to 40	77 (45.0)	155 (34.4)	
41 to 60	56 (32.7)	144 (31.9)	
Above 60	23 (13.5)	65 (14.4)	
Highest level of education completed[a]			
No formal Education	4 (2.3)	13 (3.0)	0.615
Primary	68 (39.8)	173 (39.3)	
Secondary	68 (39.8)	157 (35.7)	
Post-secondary	31 (18.1)	97 (22.0)	
Occupation[a]			
Business	4 (2.5)	49 (13.5)	< 0.001
Farmer	135 (82.8)	196 (54.1)	
Office Worker	10 (6.1)	33 (9.1)	
Unemployed	14 (8.6)	84 (23.2)	
Poultry worker[a]			
Yes	136 (79.5)	250 (55.4)	< 0.001
No	35 (20.5)	201 (44.6)	
Use tobacco[a]			
Yes	16 (9.3)	34 (7.5)	0.566
No	155 (90.7)	419 (92.5)	
Reported chronic disease[a]			
Yes	23 (13.4)	81 (17.9)	0.214
No	149 (86.6)	371 (82.1)	

[a]Variable has some missing data. *SD* standard deviation

reported only one episode, 26 (22.4%) reported two episodes, and three (2.6%) reported between three and five episodes.

Among pig workers, there were 29 ARI episodes (cumulative incidence of 16.9%) while among non-pig workers there were 122 ARI episodes (cumulative incidence 26.9%). On bivariate analysis, pig workers had a 47% lower risk of having ARI compared to non-pig workers with an unadjusted risk ratio (RR) of 0.53 (95% CI [0.33, 0.84]).

A Poisson generalized linear mixed model was used to adjust the RR for potential confounding against age, sex, poultry exposure, education, month of sampling, occupation, and reported chronic disease. The adjusted RR for pig workers was 0.56 (95% CI [0.33, 0.93]), indicating that pig workers had a 44% lower risk of having ARI compared to non-pig workers (Table 2).

The model also showed that participants from households where members had reported acute respiratory illness in the previous 3 months had three times higher risk of reporting ARI.

Participants who had completed primary or secondary education had about a 60% lower risk of developing ARI compared to those without formal education (Table 2).

Assessing risky practices that promote transmission at the human-animal interface

To assess risky practices associated with the transmission of the influenza virus at the human-animal interface, we restricted the analyses to pig-keeping households. The majority (88%) of the households had pens with concrete floors, with 30% of them using sawdust for beddings.

While the majority of the households fed the pigs with commercial feeds, about 60% of the households also used scraps/wastes from the household or the market.

Less than half (46%) of the households separated new pigs before allowing them to mix with the rest of the herd, and 11% of the households had added new pigs to the herds within the month preceding the interview. Among households which practised quarantine for new pigs, about half (52.5%) quarantined for 3 days or less. Almost three-quarters of the pig-keeping households did not vaccinate their pigs for any disease, and none of the households reported vaccinating the pigs against influenza. A quarter of the households reported that the pigs regularly mixed with other animals on their farm (or animals in other farms).

Overall, there was low usage of personal protective equipment by pig workers, with 96.3% using eye protection less than once a week and less than half (45.7%) using protective coveralls and aprons when working in pig pens.

However, 72.2% used footwear most of the time (> 5 times a week) and nearly all (96.9%) reported washing hands after working in the barns

Discussion

This study documents a human-animal environment in Kiambu County with robust influenza virus circulation, demonstrated by > 6% seropositivity of the virus among

Table 2 Multivariate Poisson generalized mixed-effects model for the association between occurrence of acute respiratory infection and pig worker status, Kiambu, 2013–2014

Exposure/potential confounder	Crude RR (95% CI)	Adjusted RR (95% CI)	*p* value for adjusted RR
Pig worker	0.53 (0.33, 0.84)	0.56 (0.33, 0.93)	0.025
Age in years	1.00 (0.99, 1.01)	0.99 (0.98, 1.00)	0.172
Reported chronic disease	1.48 (0.93, 2.35)	1.52 (0.88, 2.62)	0.134
Education level completed			
Primary	0.53 (0.25, 1.12)	0.35 (0.15, 0.86)	0.022
Secondary	0.4 (0.19, 0.86)	0.31(0.12, 0.78)	0.013
Post-secondary	0.59 (0.27, 1.33)	0.41 (0.16, 1.08)	0.071
Household member with ARI in previous 3 months	3.13 (2.03, 4.83)	2.97 (1.78, 4.93)	< 0.001

the pig and poultry populations including almost 12% in the pig population.

Similarly, we found 3% of humans with acute respiratory illness positive for influenza A virus by RT-PCR. These findings agree with other studies, including a Kenyan study that reported 15% influenza virus prevalence among pigs and other studies elsewhere in Africa and Asia reporting as high as 67% influenza virus seroprevalence among swine in live markets [28–31].

Since swine influenza vaccination was not practiced by the farmers in our study, the level of seropositivity suggests exposure to circulating virus. The 1.4–3.3% prevalence of influenza virus among the poultry species (chicken, ducks, and geese) reported in our study support the finding that Kiambu County is an environment of animal influenza virus circulation.

Our finding of 3% influenza positive ARI human cases is lower than a community study in Romania that reported 13% influenza A virus positive samples among ARI cases by PCR [32, 33]. The low number of PCR positives among ARI cases could be due to sampling a healthy population who did not have active infection at the time of sampling or had a subclinical infection. The follow-up time per participant was about 3 months and seasonality of influenza could account for the relatively low number of positive cases.

We found that pig workers had about half the risk of having ARI compared to non-pig workers. This could be due to healthy worker effect where persons who work closely with pigs have systematic differences associated with occurrence of ARI compared to the non-pig exposed [34].

For example, pig workers had a lower proportion of < 20-year-olds compared to non-pig workers (9% vs 19%, respectively). Our study finding is contrary to other studies that showed pig workers have higher odds of respiratory illness compared to non-pig workers [35, 36]. However, these were cross-sectional studies conducted in high-income countries to assess chronic respiratory health among farmers. It is likely that serology results from our study would have been higher in pig workers, or at least comparable with those of non-pig workers. However, attempts to carry out serology in humans were unsuccessful.

Our finding of lower risk of ARI among pig workers suggest that monitoring ARI in the general population would likely miss potential zoonotic events. Zoonotic influenza events are likely to first appear among those working or exposed to pigs or poultry, mostly young adults. With the reported levels of exposure to swine influenza among pigs in our study, including the pig workers in the influenza surveillance could enhance efforts to detect early zoonotic influenza events.

Focused surveillance in an occupationally exposed group offers a potentially cost-effective mechanism to monitor trends of influenza, including influenza zoonotic events. A number of innovative and affordable approaches such as mobile-based surveillance could be applied to offer the needed early warning mechanism to identify increases in acute or severe respiratory episodes in this group [28].

In the mixed-effects model for occurrence of ARI, having a household member with an episode of ARI in the previous 3 months was an independent risk factor. These findings are consistent with the known transmission of pathogens associated with ARI through close contact [37].

When we assessed the farming practices associated with increased transmission of zoonotic influenza, we found that pig-keeping households also kept poultry (mixed animal farming), and majority did not quarantine newly introduced pigs before mixing with the herd [30]. In addition, there was inadequate use of personal protective equipment while working in pig pens. Studies have documented that lack of quarantine and uncontrolled movement between farms, and poor usage of personal protective equipment are risk factors for influenza transmission [29, 31]. These transmission-promoting practices could be due to the lack of knowledge and facilities for biosecurity measures.

This study had several limitations. We had a loss to follow-up of 45% of the enrolled participants. While there were no differences in age or education level between those lost to follow-up and those retained, they could have had different experiences on ARI, influenza A positivity and farming practices.

Although the study period included 9 months of the year, follow-up period for each participant was about 3 months. It is also likely that not all ARI episodes were reported due to participant recall bias. A longer and more frequent follow-up and with serological testing would allow for sampling to account for seasonality of influenza infections as well as subclinical cases. Another limitation is that we were not able to conduct hemagglutination inhibition (HI) tests to determine the influenza A virus strains circulating among pigs. However, a study in Kenya in 2012 [38] reported 72% of seropositive pigs had influenza virus (A/H1N1/pdm09) by HI, findings that could reflect the influenza virus strains among pigs in our study.

Conclusion

Our study documents moderate seropositivity among pigs for influenza A virus, suggesting circulation of swine influenza virus and therefore a potential for interspecies transmission. Swine workers had a lower risk of ARI compared to non-swine workers. While serological studies among swine workers may be a better approach to quantify the risk of zoonotic influenza infection, focused syndromic surveillance in this population offers an important early warning system for such zoonotic events in Kenya.

Abbreviations

ARI: Acute respiratory illness; CDC: Centers for Disease Control and Prevention; CI: Confidence interval; ELISA: Enzyme-linked immunosorbent assay; GLMM: Generalized linear mixed model; KEMRI: Kenya Medical Research Institute; NP: Nasopharyngeal; OP: Oropharyngeal; RT-PCR: Real-time reverse transcriptase polymerase chain reaction; SD: Standard deviation; SIV: Swine influenza viruses; VTM: Virus transport media

Acknowledgements

We thank the Ministry of Health, Ministry of Agriculture, and Fisheries; the County Government of Kiambu; and Kenya Medical Research Institute for their participation in the implementation of the study. We acknowledge Penina Munyua and Clayton Onyango from the Centers for Disease Control and Prevention—Kenya, for their technical assistance during the implementation of the study.

Funding statement

This work was funded with funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Department of Health and Human Services, contract no. HHSN266200700007C and Core Funding of the Global Disease Detection Division of US Centers for Disease Control and Prevention.

Disclaimer

The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the official position of the National Institute of Allergy and Infectious Diseases or US Centers for Disease Control and Prevention or the Government of Kenya.

Authors' contributions

EO, KN, and ZN contributed to the conceptualization and design of the study. EO, MM, AM, and DM contributed to the sample and data collection. SL, JN, and KN contributed to the laboratory testing. EO and DM contribued to the data analysis. EO wrote the manuscript draft. EO, MM, AM, DM, SL, JN, KN, and ZN contribute to reviewing and editing the manuscript. All authors read and approved the final manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Ethics approval and consent to participate

The study was approved by the KEMRI Scientific and Ethical Review Committee and the Animal Care and Use Committee, and all participants gave informed consent before enrolment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests

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Received: 15 April 2019 Accepted: 2 August 2019
Published online: 17 August 2019

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Publisher's Note

Publication 2

Osoro et al. BMC Res Notes (2019) 12:628
https://doi.org/10.1186/s13104-019-4667-4

BMC Research Notes

RESEARCH NOTE Open Access

Detection of pandemic influenza A/H1N1/pdm09 virus among pigs but not in humans in slaughterhouses in Kenya, 2013–2014

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Abstract

Objective: We conducted four cross-sectional studies over 1 year among humans and pigs in three slaughterhouses in Central and Western Kenya (> 350 km apart) to determine infection and exposure to influenza A viruses. Naso-pharyngeal (NP) and oropharyngeal (OP) swabs were collected from participants who reported acute respiratory illness (ARI) defined as fever, cough or running nose. Nasal swabs and blood samples were collected from pigs. Human NP/OP and pig nasal swabs were tested for influenza A virus by real-time reverse transcriptase polymerase chain reaction (PCR) and pig serum was tested for anti-influenza A antibodies by ELISA.

Results: A total of 288 participants were sampled, 91.3% of them being male. Fifteen (5.2%) participants had ARI but the nine swabs collected from them were negative for influenza A virus by PCR. Of the 1128 pigs sampled, five (0.4%) nasal swabs tested positive for influenza A/H1N1/pdm09 by PCR whereas 214 of 1082 (19.8%) serum samples tested for Influenza A virus antibodies. There was higher seroprevalence in colder months and among pigs reared as free-range. These findings indicate circulation of influenza A/H1N1/pdm09 among pigs perhaps associated with good adaptation of the virus to the pig population after initial transmission from humans to pigs.

Keywords: Swine, Human, Influenza A virus, Surveillance

Introduction

Influenza A viruses circulate widely in animals, including birds, humans, pigs, and other mammals, and frequently cause severe epidemics and pandemics that affect both animals and humans [1–4]. The most recent influenza pandemic was in 2009, which was caused by a novel pig-origin influenza A virus resulting in > 500,000 human deaths globally [5]. A common mechanism of emergence of novel influenza viruses is acquisition of new antigenic material during an inter-species transmission event [3, 6].

Pigs, long believed to be a mixing vessel for inter-species influenza virus transmission, can be a source of swine influenza infection to humans occupationally exposed to them [7]. Pig slaughterhouses present a particularly prime environment for pig-to-human transmission of influenza A viruses, and with increasing pig farming in low biosafety and biosecurity settings in Kenya, the level of human exposures to swine influenza viruses has increased [8]. Few studies on influenza virus transmission at the human-animal interface have been conducted in sub-Saharan Africa, including one in a Kenyan pig slaughterhouse that detected A/H1N1/pdm09 among pigs, suggesting introduction from humans [9].

To mitigate the severity of influenza pandemics, early detection through syndromic surveillance in humans is key [3]. Even though human influenza surveillance in Kenya has improved by targeting acute respiratory illness at sentinel sites, there is no emphasis on people occupationally exposed to pigs or birds. Here, we conducted a series of cross-sectional studies among human and pigs in three pig slaughterhouses to determine infection and exposure to influenza A viruses.

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Main text

Methods

We conducted four cross-sectional studies over a period of 1 year among humans and pigs in three slaughterhouses in Kiambu (Uthiru slaughterhouse), Kisumu and Siaya (Bondo slaughterhouse) counties (Fig. 1). Kiambu County in central Kenya features farms that have intensive pig production system while farms in Kisumu and Siaya counties in Western Kenya employ extensive pig production systems. These contrasting pig production systems represent varying degrees of contact between humans and pigs.

Sample size and sampling

All pig slaughterhouse workers, farmers and traders who visited the slaughterhouses to deliver pigs or to purchase meat, animal health personnel and others working in the slaughterhouses were requested to participate in the study. For pig sampling, an estimated seroprevalence of 20% was assumed, precision level of 5% and at 95% confidence level, giving a minimum sample size of 246 per sampling period. The slaughterhouses were visited each consecutive working day for 10 days to sample the pigs. For Uthiru (Kiambu) slaughterhouse, we sampled every other pig to a maximum of 25 per day. For Kisumu and Bondo (Siaya) slaughterhouse where volume of pigs slaughtered is low, all the pigs presented for slaughter on each day of sampling were targeted for sampling.

Human and animal sample collection

Nasopharyngeal (NP) and Oropharyngeal (OP) swabs were collected from participants reporting acute respiratory illness during sampling. Acute respiratory illness (ARI) was defined as reported cough, runny nose or sore throat. The swabs were placed in cryovials with virus transport medium (VTM) and shipped to Kenya Medical Research Institute (KEMRI) laboratories in Nairobi on ice, where they were preserved at − 80 °C until testing.

Nasal swabs and blood samples were collected from pigs; nasal swabs were placed in cryovials with VTM and shipped to KEMRI laboratories in Kisumu for storage at − 80 °C until testing. Blood samples were processed for sera on the same day of collection and stored at − 80 °C

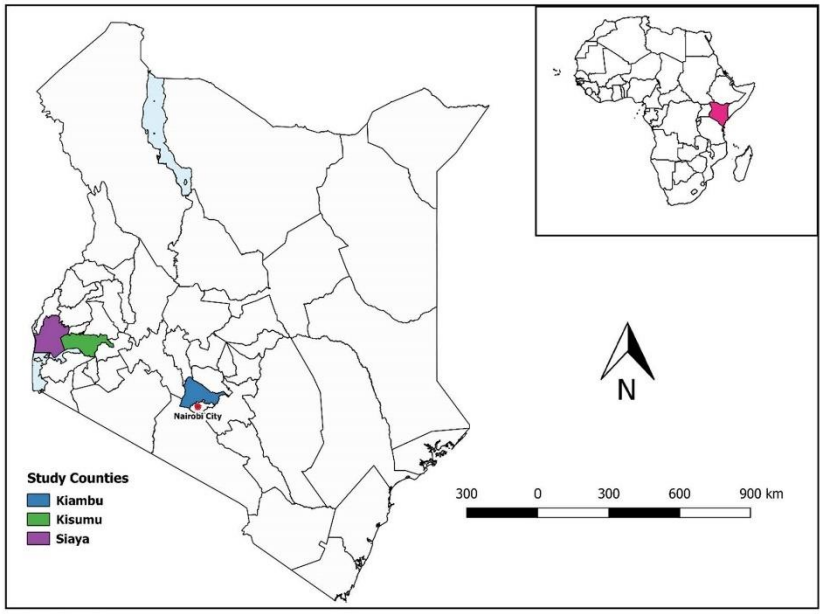

Fig. 1 Map of Kenya showing the three counties where the sampling was conducted. Inset is a map of Africa with Kenya highlighted. Map created in QGIS

until testing. All testing of animal samples was at the KEMRI laboratories.

Serological and molecular testing for influenza A virus

We used IDEXX® ELISA kit (FlockChek AI MultiS-Screen Ab Test Kit®, Westbrook, Maine) to test animal serum for influenza A virus antibodies, following manufacturer instructions. We applied an adjusted cut-off of the S/N of < 0.673 for pig sera, which had been shown to increase sensitivity and specificity [10].

We used real-time reverse transcriptase polymerase chain reaction (RT-PCR) to test human NP/OP samples for influenza A virus RNA by applying primers and probes against the matrix gene of influenza A and NS1 gene of influenza B viruses [11]. A cycle threshold (C_T) value of ≤ 40 was the cut-off for positivity. Positive and negative controls were used to validate test assay.

Pig nasal swabs were tested for influenza A virus by RT-PCR using the CDC protocol for influenza A virus detection [12]. The Influenza A sub-typing utilized oligonucleotides targeting hemagglutinin and neuraminidase genes (contemporary human pandemic H1, human AH3, AH7, AH5, N1 and N2) of swine influenza viruses [13].

Data collection and analysis

A structured questionnaire was administered to participants to collect data on sociodemographic characteristics, frequency and level of contact with pigs, influenza vaccination history and acute respiratory illness using Personal Digital Assistant devices running on a windows-based application. Data were cleaned and analyzed by the R statistical software [14]. Descriptive statistics were conducted for socio-demographic characteristic by pig exposure status. Pig exposure was defined as any person who routinely skinned pigs, stunned pigs, sold pork or offals at the slaughterhouse. Categorical and continuous variables were compared using Chi Square test (or Fishers exact test) and Student t-test, respectively. Prevalence was determined as proportion of samples positive for influenza A virus against all samples tested.

The study was approved by the KEMRI Scientific and Ethical Review Committee and the Animal Care and Use Committee and all participants gave written informed consent before enrolment.

Results

All three slaughterhouses operated for 5 days a week (Monday to Friday), receiving pigs from farms within the respective and neighboring counties. The Uthiru slaughterhouse received an average of 50 pigs per day while the Bondo and Kisumu slaughterhouses received 3 to 5 pigs per day. All pigs slaughtered in the three slaughterhouses were adults.

A total of 288 participants were sampled over the four sampling periods, 91 (31.6%) in September 2013, 43 (14.9%) in December 2013, 101 (35.1%) in May 2014, and 53 (18.4%) in September 2014. More than half (51.7%) of the participants were from Uthiru slaughterhouse.

Majority of participants were male (91.3%), and 35.4% (n = 102) of them were classified as pig exposed. The mean age for the participants was 35.5 years with a significant difference between the mean age of pig exposed (32.5 years) and non-pig exposed persons (37.2 years). Although 55.9% of all participants had completed at least secondary education, 5% of non-pig exposed had no formal education whereas all pig exposed participants had some formal education (Table 1).

Fifteen (5.2%) participants had ARI during the sampling periods. Of the 9 OP/NP swabs collected from these ARI cases, none were positive for influenza A virus.

In total, 1128 pigs were sampled (nasal swabs) for influenza testing, including 73% from Uthiru slaughterhouse. Of these, 5 pigs (0.4%) were positive for Influenza A virus RNA and all subtyped as A/H1N1/pdm09 virus. Serum was collected from 1082 pigs, 75% of them from Uthiru slaughterhouse. Of these, 214 (19.8%) pigs were positive for influenza A virus antibodies by ELISA. Samples collected in September 2014 had the highest prevalence of 37.1% (93 of 251), followed by September 2013 at 19.8% (47 of 237). Among the positive samples (n = 214), 65.4% (140) were collected in September 2013 or September 2014. Among slaughterhouses, 34.5% (30 of 87) of the samples from Bondo were seropositive, followed by 22.6% (21 of 93) in Kisumu slaughterhouse (Table 2). None of the farmers reported vaccinating their pigs against influenza.

Discussion

We found evidence of both active influenza A virus infection and widespread exposure (seropositivity) among pigs but no infection among humans in a linked human-animal study in three slaughterhouses in Kenya. Influenza virus (A/H1N1/pdm09) virus RNA and antibodies, which is associated with seasonal human influenza in Kenya, was detected in the pig samples from central and western Kenya, suggesting either persistent human to pig transmission of influenza virus (A/H1N1/pdm09) or establishment and continued circulation of influenza virus (A/H1N1/pdm09) among pig populations [15]. This finding is consistent with a similar study in Kenya where 0.5% of sampled pigs were found to have A/H1N1/pdm09 virus [16]. Between 2016 and 2018 on average, seasonal human influenza in Kenya was associated with A/H1N1/pdm09 (32.5%), human A/H3N2 (33.8%) and influenza B (30.9%) [17].

Table 1 Sociodemographic characteristics of participants by pig exposure status, 2013–2014

Characteristic	Categories	Pig exposure		Total	p-value
		Yes n (%)	No n (%)		
Sex	Female	6 (5.9)	19 (10.2)	25 (8.7)	0.212
	Male	96 (94.1)	167 (89.8)	263 (91.3)	
Age in years	Mean (SD)	32.5 (11.2)	37.2 (12.2)	35.5 (12)	0.001
Highest education level completed	No formal education	0 (0.0)	9 (4.8)	9 (3.1)	0.008
	Primary	45 (44.1)	73 (39.2)	118 (41.0)	
	Secondary	51 (50.0)	75 (40.3)	126 (43.8)	
	Post-secondary	6 (5.9)	29 (15.6)	35 (12.2)	
Occupation	Slaughterhouse worker	84 (82.4)	72 (38.7)	156 (54.2)	< 0.001
	Pig farmer	7 (6.9)	29 (15.6)	36 (12.5)	
	Pig trader	7 (6.9)	49 (26.3)	56 (19.4)	
	Other	4 (3.9)	36 (19.4)	40 (13.9)	
Sampling period	Sep, 2013	32 (31.4)	59 (31.7)	91 (31.6)	0.012
	Dec, 013	8 (7.8)	35 (18.8)	43 (14.9)	
	May, 2014	35 (34.3)	66 (35.5)	101 (35.1)	
	Sep, 2014	27 (26.5)	26 (14.0)	53 (18.4)	
Slaughterhouse	Bondo	29 (28.4)	47 (25.3)	76 (26.4)	0.210
	Kisumu	27 (26.5)	36 (19.4)	63 (21.9)	
	Uthiru	46 (45.1)	103 (55.4)	149 (51.7)	

SD standard deviation

Table 2 Seroprevalence of influenza A virus among pigs by sampling period and slaughterhouse, 2013–2014

	Samples tested	Positive	Seroprevalence (%)	95% CI
All samples	1082	214	19.8	17.5, 22.3
Sampling period				
Sep, 2013	237	47	19.8	15.3, 25.4
Dec, 2013	293	28	9.6	6.7, 13.5
May, 2014	301	46	15.3	11.7, 19.8
Sep, 2014	251	93	37.1	31.3, 43.2
Slaughterhouse				
Bondo	87	30	34.5	25.3, 44.9
Kisumu	93	21	22.6	15.3, 32.1
Uthiru	902	163	18.1	15.7, 20.7

CI confidence interval

Our study provides evidence of intense circulation of swine influenza virus among pig populations in two distinct geographical regions of Kenya, located > 350 kilometres apart, with the high average seroprevalence of 20%. The higher prevalence reported in Bondo (34.5%) and Kisumu (22.6%) slaughterhouses located in Western Kenya may be due to the free-range nature of pig production there, when compared with Uthiru slaughterhouse in the central region of the country where confined production system is practiced. Our findings also point to higher influenza transmission during the colder months (July–September) as supported by almost two-thirds of the seropositive pigs sampled during this period. In addition, all the PCR positive samples were collected during the cold season. Trends in human seasonal influenza in Kenya have also shown higher transmission during the colder months of June to August [15].

The occurrence of influenza A/H1N1/pdm09 virus in pigs has been documented in most regions of the world, including Africa where it has been reported in Kenya, Nigeria, Ghana and Cameroon [16, 18, 19]. Studies have shown that when influenza virus (A/H1N1/pdm09) circulates in local pig populations it continues to undergo antigenic changes over time [20]. The influenza A virus seroprevalence reported in our study was comparable to 17% reported in an earlier study in Kenya [16]. However, studies from other countries showed varied findings ranging from 5% in Uganda to 49% in Vietnam [21–24]. The variations in prevalence reported in the studies may be due to differences in sampling methodology (farm level vs live market vs slaughterhouses), and pig populations in the study area. The Southeast Asia region has large pig farms that likely support higher influenza virus transmission [23, 24].

In conclusion, our study reports detection of influenza virus (A/H1N1/pdm09) among pigs and high seroprevalence adding to the evidence of intense circulation among pigs from the few studies in the East

Africa region. The increasing commercialization of pig farming in Kenya, provides a suitable environment for exposure and occurrence of zoonotic events related to influenza A virus [8]. The evolution of reassortant viruses and their potential transmission to humans is unpredictable making routine monitoring at the pig-human interface a priority. Virological surveillance offers a mechanism to detect early any changes in the antigenic structure or zoonotic transmission events.

Limitations of the study

Our study had several limitations. We were not able to collect demographic data such as age, sex and farm level factors among the sampled pigs to allow for testing for associations with seropositivity. While most of the pigs brought for slaughter were mature adults, they were mostly delivered by traders who would not provide reliable farm level data on the sampled pigs. Another limitation is that we did not conduct haemagglutination inhibition (HI) tests to confirm the influenza strains circulating among pigs. However, another study in 2010–2012 [16] reported 72% of seropositive pigs had influenza virus (A/H1N1/pdm09) by HI, findings which might reflect the diversity of influenza virus among pigs in our study.

Abbreviations

ARI: acute respiratory illness; CI: confidence interval; KEMRI: Kenya Medical Research Institute; NP: nasopharyngeal; OP: oropharyngeal; PCR: real-time reverse transcriptase polymerase chain reaction; VTM: virus transport medium.

Acknowledgements

We thank the Ministry of Health, Ministry of Agriculture, and Fisheries, the County Governments of Kiambu, Kisumu and Siaya for providing administrative approval of the study. We appreciate the staff at the Kenya Medical Research Institute, Center for Global Health Research One Health program who conducted the field work. We also acknowledge Penina Munyua and Clayton Onyango from the Centers for Disease Control and Prevention-Kenya for their technical assistance during the implementation of the study.

Disclaimer

The findings and conclusions in this manuscript are those of the authors and do not necessarily represent the official position of the National Institute of Allergy and Infectious Diseases or US Centers for Disease Control and Prevention or the Government of Kenya.

Authors' contributions

EO, KN, ZN: Conceptualization and design of the study; EO, MM, AM, DM: Sample and data collection; SL, JN, KN: Laboratory testing; EO, DM: Data analysis; EO: Writing draft manuscript; EO, MM, AM, DM, SL, JN, KN, ZN: Reviewing and editing manuscript. All authors read and approved the final manuscript.

Funding

This work was funded with funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH), Department of Health and Human Services, Contract No. HHSN266200700007C and Core Funding of the Global Disease Detection Division of US Centers for Disease Control and Prevention. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study was approved by the KEMRI Scientific and Ethical Review Committee and the Animal Care and Use Committee and all participants gave written informed consent before enrolment. Written informed consent was also obtained from the animal owners for sampling of pigs and poultry.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 25 March 2019 Accepted: 19 September 2019
Published online: 24 September 2019

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