A CASE-CONTROL STUDY OF RISK FACTORS FOR SEVERE INFLUENZA AMONG PERSONS AGED 5 YEARS OR MORE IN A RURAL COMMUNITY IN BONDO DISTRICT, KENYA

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DECLARATION

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

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DEDICATION

This work is dedicated to my beloved wife Beatrice Achieng and my two children Branley Ope and Rawlings Kagumba

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I wish to express my gratitude to the residents of Lwak community who agreed to participate in the study and the village reporters who guided the community interviewers in navigating the villages while tracing the study participants. Many thanks to the community interviewers who had to work out of the normal working hours to get the selected case patients, conduct interviews and more importantly explain the study to the inquisitive community members.

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LIST OF ABBREVIATIONS

CD4 cells	Cluster of differentiation 4 cells
CDC	Centers for disease control
EIA	Enzyme immunoassays
ELISA	Enzyme linked immunosorbent assay
ERC	Ethical review committee
HIV	Human immunodeficiency virus
IFA	Immunofluorescent assay
KDHS	Kenya demographic health survey
KEMRI	Kenya medical research institute
OR_{MH}	Mantel-Haenzel odds ratio
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
RT PCR	Reverse transcription polymerase chain reaction
SSC	Scientific steering committee

DEFINITIONS OF TERMS USED IN THE STUDY

Animals kept in the house: This is when the participant was living with the animal in the same house.

Biomass fuel: This is fuel from charcoal or firewood.

Case: A case was any patient aged 5 years old or more, who suffered severe acute respiratory disease and had a nasopharyngeal and/or oropharyngeal swab testing positive for influenza virus by RT-PCR.

Chronic co-morbidity: A participant was considered as having a chronic co-morbidity if s/he reported having chronic lung disease, chronic heart disease, diabetes mellitus, asthma or any known chronic ill health.

Close contact to animals: This is when a participant was within one meter away from the animal referred to.

Exposure to an animal: A participant was considered exposed to an animal if s/he was living in a household owning the animal.

Exposure to indoor smoke: A participant was considered exposed to indoor smoke if s/he was living in a house where either cooking was done inside the house using firewood or the family burnt cow dung in the house.

Neighbourhood control: A neighbourhood control any person aged 5 years old or more, who had not suffered severe acute respiratory disease requiring admission to hospital in the last one year and was living in proximity to the case patient.

Passive smoking: This is a situation where a non smoker is exposed to cigarette smoke from a smoker.

Severe acute respiratory disease: This is when a person aged 5 years or more presents with cough or shortness of breath or

difficulty in breathing or pleuritic chest pain and any one of the following

- Oxygen saturation <90% or
- Fever >38.0^{0}C

Severe influenza: This is an acute respiratory disease caused by influenza and severe enough to cause hospitalization, respiratory failure or death.

Sick animals: The animals were considered to be sick if any household member knew that the animal referred to were sick from either a known or an unknown disease.

Size of household: The number of persons living in a household.

ABSTRACT

Influenza causes severe morbidity and mortality. It is also responsible for high rates of absenteeism, societal disruption and economic loss. There is still not enough evidence of the groups that can benefit from influenza vaccination in Kenya. This is in contrast to the developed countries where this information is available and is continuously being updated every year. Similarly there is scanty information on the risk factors for influenza in developing countries.

A matched case control study was conducted to identify the risk factors for severe influenza among persons aged 5 years or more in a rural community in Kenya. Cases of influenza were confirmed by real time reverse transcription polymerase chain reaction and controls were matched to case by place of residence. A standardized questionnaire was administered to all study participants and conditional logistic regression used to identify independent risk factors.

A total of 26 cases and 78 neighborhood controls were enrolled. On univariate analysis there was no significant increased risk of influenza among those exposed to indoor smoke [OR_{MH} 1.82, 95% confidence interval (CI) 0.64-5.18, p value 0.3538]. In multivariate analysis there was an increased risk of severe influenza among young adults (less than 30 years old) [adjusted odds ratio (aOR) 40.15,

95% CI 4.42-364.85, p value 0.0010] and those living in household owning cows (aOR 6.76, 95% CI 1.38-33.10, p value 0.0184).

Young adults are at high risk of severe influenza and should be prioritized for influenza prevention and control activities including vaccination depending on the strains in circulation. There is need to conduct further studies to evaluate the role of cows in predisposing individuals to severe influenza

CHAPTER ONE

INTRODUCTION

1.1 Background

Influenza is a disease characterized by acute onset of fever, myalgia, headache, sore throat and cough (Megan *et al.*, 2006 and Mandell *et al.,* 2005). It is caused by influenza viruses which are enveloped RNA viruses with segmented genomes belonging to the family *Orthomyxoviridae* (Stephenson, 2002 and Reid, 2003). The influenza viruses were first isolated in human in 1933 (Nakajima, 2006), although they are known to have caused disease outbreaks in humans earlier than this.

There are three influenza virus types, which are influenza type A, B and C. The evolutionary hosts for influenza viruses are birds, although most of them do not cause disease in birds. The virus can also survive in water. Influenza A and B viruses are the types that cause epidemics in human beings (Wright *et al.,* 2001), whereas influenza C normally causes mild respiratory symptoms and is associated with minor sporadic outbreaks.

Influenza A virus is further categorized into subtypes depending on the antigenic properties of the surface glycoprotein haemagglutinin (H) and neuraminidase (N). There are over 15 subtypes of influenza A while only three are known to cause widespread epidemics, H1, H2 and H3 in man (Wong *et al.,*

2006). Some influenza type A subtypes can cause serious disease in birds; especially poultry (e.g. subtype H5 and H7.) Influenza type B virus is not further subdivided. The surface glycoproteins are capable of stimulating an immune response.

Frequent mutations of the genes on the surface glycoprotein of influenza A or B virus usually results in emergence of variants of the viruses (Stephenson *et al.,* 2002 and Fiore *et al.,* 2007). The two important types of antigenic variation are the antigenic drift and the antigenic shift. Antigenic drift involves change in the H and N antigens in the host. This is thought to be as a result of mutation in the RNA strands resulting in changes in the amino acid sequence of the H and N antigens. New influenza variants result from these antigenic drifts. Influenza B undergoes less antigenic drift than influenza A (CDC, 2006). Antigenic shift results in a complete change in the virus and is as a result of genetic reassortment of the RNA segments that control H and N antigens when two different subtypes replicate in the same host cell simultaneously. The change involves the H antigen and may or may not involve the N antigen at the same time (Pereira, 1979). The RNA strand may come from either human or animal circulating influenza viruses. The new influenza virus is given a subscript to differentiate it from the previously circulating virus.

As a result of the continued changes in the surface glycoprotein of the virus, there is commonly an outbreak of influenza every year since people are usually

not fully immune to the altered antigenic strains of the virus circulating annually. Large pandemics of influenza have resulted from the more dramatic antigenic shifts that create a new virus to which few people in the population have any immunity. Besides the high morbidity and mortality caused by influenza, it is also responsible for high rates of absenteeism, societal disruption and economical loss (Stephenson *et al.,* 2002 and Nichol *et al.,* 2005). It is therefore necessary to identify the risk factors for influenza in order to determine effective prevention and control measures.

1.2 Study justification

Influenza is known to cause annual seasonal outbreaks and it has been associated with pandemics in the past. Severe influenza results in hospitalization, respiratory failure and even death. Influenza causes ½ million deaths annually and the deaths increase tremendously during pandemics. For example during the 1918 pandemic, over 30 million deaths due to influenza were reported. Influenza is also responsible for high rates of hospitalization, for example in the United States; over 200,000 people are hospitalized due to influenza annually.

Currently there is a worldwide alert on the possibility of influenza pandemic. As at 28th February 2008, avian influenza has infected 371 people killing 235 worldwide (WHO, 2008). To date all the initial cases of H5N1 in human have had close contact to sick or dead poultry.

In East Africa and in Kenya specifically there are no published reports of the burden of influenza. Surveillance for influenza in Kenya has recently been started in various areas in the country. Preliminary results from this surveillance suggest that influenza virus is in circulation throughout the country. Examples of the ongoing surveillance in Kenya are the Influenza Surveillance Study in Bondo District, Kenya (SSC protocol #1147), which hopes to describe the burden of influenza in the district and the Kenya influenza sentinel surveillance which is ongoing in all provincial hospitals. As at the time of writing this thesis there has been no official report on the burden of influenza in Kenya from these surveillance studies.

There is still sparse data on the groups that are at high risk for influenza and can benefit from influenza vaccine in most of Africa, including Kenya. This is in contrast to the developed countries where the risk factors of influenza have been clearly characterized (Fiore *et al.,* 2007). The elderly, pregnant women, smoking and those with chronic co-morbidity have been shown to be at high risk of severe influenza in the developed countries. Studying the risk factors which are associated with influenza will help in prioritizing the high-risk groups for interventions in order to reduce their risk of influenza. Possible interventions may include reduction of modifiable risk factors and vaccination after strain characterization of the circulating influenza virus. Such information

will be useful in policy decisions regarding influenza that will be considered by the Kenya Ministry of Health.

1.3 Objectives

1.3.1 General objective

The **general objective** of the study is to determine the risk factors associated with severe influenza.

1.3.2 Specific objectives

The **specific objectives** of the study are:

i. To determine the prevalence of chronic co-morbidities among severe influenza patients
ii. To determine the effect of exposure to indoor smoke on the risk of severe influenza.
iii. To determine the effect of exposure to domestic poultry on the risk of severe influenza.

1.4 Hypothesis

Null hypothesis: There are no identifiable risk factors associated with influenza.

Alternate hypothesis: There are identifiable risk factors associated with severe influenza.

CHAPTER TWO

LITERATURE REVIEW

2.1 History of influenza

The influenza virus was first isolated in swine in 1930 (Taubenberger *et al.,* 2001) while in human it was isolated in 1933 (Nakajima, 2006); at that time there was little progress in the understanding of the virus due to limited diagnostic methods. After the development of molecular biological methods, there was significant progress in influenza virus research (Nakajima, 2006), especially with the development of polymerase chain reaction (PCR). The virus is known to have been responsible for pandemics even as early as 1890 (Rasolofonirina, 2003) and 1918 (Reid *et al.,* 2003) although it was not isolated during these outbreaks. Samples taken from people who died during these earlier pandemics contained small pieces of genetic material derived from influenza virus (Garcia-Sastre *et al.,* 2006). Through genetic sequencing, it has been possible to determine the influenza subtype that was responsible for these pandemics (Reid *et al.,* 1999; 2000). A number of archeoserological analyses of age related haemagglutinin inhibition seroprevalence have been performed using sera collected from 1930s to 1960s and these have helped in clarifying the influenza subtype that was circulating around that time.

Frequent mutations of the genes on the surface glycoprotein of influenza A or B usually results in the emergence of variants of the virus. The first type of genetic variation is "antigenic drift" which involves the gradual change or drift in the H and N antigens of the virus currently circulating within a host population. This variation is thought to be the accumulation of point mutations within the RNA strands that result in changes in the amino acid sequences of the H and N antigens. Some of these mutations are enough to alter the antigenic character of the virus and allow it to recirculate within a population without being inhibited by antibodies to previous strains (Couch *et al.,* 1983).

The second and more dramatic genetic variation is referred to as "antigenic shift". This involves a complete change in the antigenic character of the influenza virus. These shifts come about from the re-assortment of the RNA segment(s) controlling the H and N antigens. The new RNA segments can come from human or animal influenza viruses previously circulating. This change involves at least the H antigen; the N antigen may or may not change at the same time (Pereira, 1979). The new viral subgroup is given a different subscript to differentiate it from the previous one (e.g., H1→H2, N1→N2).

Through the process of antigenic drift and shift, the influenza virus has been continuously evolving, resulting in annual influenza outbreaks and occasional influenza pandemics. The 1918 influenza pandemic has been determined to have been caused by influenza type A sub type H1N1, while the 1957 pandemic

was due to H2N2, and the 1968 pandemic was due to H3N2 (Taubenberger *et al.,* 2001). Currently, there is a fear of a potential pandemic if the avian influenza virus H5N1 which was first identified in humans in 1997 in Hong Kong (Pareek *et al.,* 2007 and Juckett, 2006) becomes easily transmissible among humans (WHO, 2005 and Sellwood *et al.,* 2007). H5 can infect humans, mostly from direct contact with infected birds. However, in rare cases H5 has been documented to have been transmitted from person to person, but this mode of transmission has not yet become efficient in humans.

2.2 Diagnosis and treatment of influenza

Influenza is transmitted from an infected person to an uninfected person through droplets by sneezing or coughing (Simonsen *et al.,* 1998) or by hand contaminated with respiratory secretions (Tellier, 2006). The incubation period is 1 to 4 days.

Influenza infected patients will complain of acute onset of fever (Reece, 2007), headache, shivering, dry cough, general malaise, myalgia or dry throat. Gastrointestinal symptoms may occur but these are usually limited to children. Uncomplicated influenza is self-limiting and lasts approximately seven days. However, cough and weakness may last for another few weeks. More severe disease can result from primary viral or secondary bacterial pneumonia (Seki *et al.,* 2007). Influenza complications generally result from secondary bacterial infections in the lower respiratory tract that results in pneumonia.

Upon infection with influenza, the virus replicates in the upper respiratory tract and can therefore be detected in the respiratory epithelia and secretions. Various laboratory techniques can be used to detect influenza infection. Such techniques involve virus isolation, viral antigen or nucleic acid detection.

Maximum titer of virus is present on day 2 and 3 after onset of symptoms, although the virus is generally detectable from day 1 to day 5 of illness. The types of respiratory specimen suitable for the detection of the virus include throat swabs, nasal washes, nasopharyngeal aspirates, tracheal aspirates, and bronchoalveolar lavage (Fiore *et al.,* 2007).

Diagnosis of influenza is laboratory-based. Isolation of influenza virus by culture is the "gold standard" in the diagnosis of influenza (Krafft *et al.,* 2005). Detection of viral antigens is done through the use of antibody specifically directed at viral antigens; such methods include enzyme-linked immunosorbent assay (ELISA), enzyme immunofluorescent assay (EIA) and indirect fluorescent antibody (IFA). These methods allow for rapid detection of the virus in respiratory specimens. Reverse transcription polymerase chain reaction (RT-PCR) amplifies and identifies specific segments of the influenza viral genome and is the latest and most sensitive method of diagnosing acute influenza infection.

Detection of viral specific antibodies requires the testing of acute sera and convalescent sera taken at least 10 days apart and detecting a rise in titre of more than 4 fold. Such tests include haemagglutination inhibition test and complement fixation tests.

Drugs for the treatment of influenza are highly effective especially if treatment is initiated early (Kawai *et al.,* 2005). There are two classes of drugs available for the treatment of influenza, which are M2 ion channel inhibitors and neuraminidase inhibitors (Hayden *et al.* 2006). The M2 ion channel inhibitors are only effective against influenza A. Examples of M2 ion channel blockers are amantadine and rimantadine. Rimantadine is better tolerated by patients but not necessarily more effective while amantadine causes gastrointestinal and central nervous system side effects. Recent studies indicate that there is increasing influenza resistance to the adamantanes (amantadine and rimantadine) (Bright *et al.,* 2005; 2006 and Wong *et al.,* 2006). New drugs (neuraminidase inhibitors) recommended for treatment are oseltamivir and zanamivir (Kawai *et al.,* 2006). Treatment with these drugs should be commenced within 48 hours of the disease onset but ideally within 12 hours. Resistance may occur during treatment with oseltamivir (not zanamivir) (Reece, 2007) and fortunately zanamivir is effective in situations where oseltamivir is resistant (Ong *et al.,* 2007). Chemoprophylaxis can also be taken during influenza outbreaks (Stephenson *et al.,* 2006).

2.3 Public health importance of influenza

Globally

Influenza is a serious health problem worldwide (Donatelli *et al.,* 2003). Influenza causes seasonal outbreaks annually and sometimes pandemics (Lynch *et al.,* 2003). It is estimated that influenza causes approximately half a million deaths globally every year (Donatelli *et al.,* 2003 and Deyde *et al.,* 2007). In the United States influenza causes more than 30,000 deaths annually (Reid *et al.,* 2003; Lynch *et al.,* 2003 and Deyde *et al.,* 2007). During pandemics the mortality due to influenza is even higher. The 1918 pandemic, the largest in history, killed more than 30 million people (Garcia-Sastre *et al.,* 2006; Tumpey *et al.,* 2005 and Taubenberger, 2006). In the earlier influenza outbreaks, such as that of 1918, higher case fatality ratios were observed amongst young adults (Simonsen *et al.,* 1998), as opposed to the recent outbreaks where a higher case fatality ratio is found amongst the elderly patients (American College of Physicians, 2006).

In the United States hospitalization due to influenza is estimated at more than 200,000 annually (Lynch *et al.,* 2003 and Thompson *et al.,* 2004). The rates of influenza associated with hospitalization is highest among children less than 5 years of age and adults >75 years of age (Hayden *et al.,* 2006).

In the United States 5-20% of the population is infected with influenza virus annually (Deyde *et al.,* 2007). Influenza causes high rates of absenteeism from

work and school, increased medical consultation and economic loss (Stephenson *et al.,* 2006 and Nichol *et al.,* 2005).

Africa.

The aetiological agents of respiratory illnesses in Africa remain largely unknown due to inadequate laboratory and financial resources (Hazlett *et al.,* 1988). As a result there is limited information on influenza in Africa. Some countries in Africa have however reported influenza outbreaks. One such outbreak of influenza A occurred in a police residential college in Pretoria, South Africa, where the overall attack rate was 34% (Besselaar *et al.,* 2004). In Madagascar, between 1975 and 2002 a total of 12 epidemics of flu were confirmed by viral isolation (Rasolofonirina, 2003). During the most recent outbreak that occurred in 2002, the majority of the cases lived in the rural areas, and children and adults 60 years and older were the most affected (WHO-GOARN investigation team, 2002).

There are limited data from a few studies that have been done on influenza in Africa. Only 6.7% of the samples from sentinel surveillance data in Madagascar were positive for influenza, out of which 72.5% were positive for influenza A, while 27.1% were influenza B (Rabarijaona *et al.,* 2003). In a study done in Cote d'Ivoire between January 2003 and December 2004, 12.8% of the nasal secretion samples analyzed were positive for influenza and most of them were isolated in June to October (Akoua-Koffi *et al.,* 2007). This study contradicts

the previous thought that in the tropics influenza is endemic and more than one period of activity may occur in a given year as opposed to the temperate regions where influenza is epidemic and occurring during winter (Dosseh *et al.*, 2000).

In Africa, avian influenza (H5N1) in humans has been documented in Egypt (45 cases) and Nigeria (1 case) (Centers for Disease Control and Prevention, 2007).

Kenya

There are no published studies on the magnitude of influenza in Kenya. However it is thought that in East Africa the frequency of infection with influenza virus is correlated with the population density and movement of people which encourages the importation and exportation of the virus from one area to another (Montefiore *et al.,* 1970).

2.4 Risk factors for influenza

Among adults, the elderly (>65 years of age) are at an increased risk of influenza related death (Fiore *et al.,* 2007 and Thompson *et al.,* 2004). In children those less than one year old are at an increased risk of influenza (Katz *et al.,* 2007), with the highest morbidity and mortality observed among those younger than 6 months (Shah *et al.,* 2007).

Co-morbidities are known to increase the risk for influenza (Katz *et al.,* 2007; Apisarnthanarak *et al.,* 2007 and Keren, 2006). Those with chronic lung disease, cardiovascular disease and diabetes are at high risk of influenza complications (Dosseh *et al.,* 2000; Davis *et al.,* 2006 and López-de-Andrés *et al.,* 2007). Influenza related death is more common among persons with cardiovascular disease (Davis *et al.,* 2006). Influenza is also known to trigger asthmatic attacks (Zhu *et al.,* 2007).

Pregnant women are at an increased risk of influenza (Dodds *et al.,* 2007) and its complications (Schanzer *et al.,* 2007 and Cox *et al.,* 2006). Smoking cigarettes is known to increase the risk of respiratory infections including influenza (Arcavi *et al.,* 2004; Murin *et al.,* 2005 and Kark *et al.,* 1982). However, it is not known whether exposure to biomass smoke increases the risk of influenza.

There is no specific study designed to investigate the risk of influenza amongst HIV positive individuals. However, in an influenza outbreak setting it was found that those with HIV were at a higher risk of developing complications (Boschini *et al.,* 2006). It is thought that HIV infected individuals are at a high risk of developing influenza and as a result influenza vaccination is currently recommended for persons infected with HIV irrespective of their immune status (Gallagher *et al.,* 2007). Following influenza infection or vaccination there was

no significant increase in HIV viral load or a decrease in CD4 cell count (Skiest *et al.,* 2003).

Influenza is known to infect wild and domestic poultry, pigs, horses and other mammals (Park *et al.,* 2007). Exposure to wild birds may increase the likelihood of influenza infection. However, in the case of avian influenza H5N1, risk factors independently associated with infection in human are preparing sick or dead poultry for consumption, having sick or dead poultry in the household and lack of indoor water source (Dinh *et al.,* 2006).

2.5 Prevention and control of influenza

Vaccination is the main stay of prevention of influenza (Fiore *et al.,* 2007 and Nichol, 2006). Antiviral drugs used for prophylaxis and treatment of influenza are adjunct to vaccination but not substitutes to annual vaccination (Fiore *et al.,* 2007). Advising on frequent hand washing and improved respiratory hygiene have been demonstrated to reduce respiratory diseases, but not adequately studied to determine if they reduce transmission of influenza virus (Fiore *et al.,* 2007). Few data are available to assess the effect of community level respiratory mitigation strategies, such as closing of schools, avoiding gatherings and use of masks on reducing virus transmission during typical seasonal influenza epidemics (Fiore *et al.,* 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

The study was conducted among a rural population who live within the catchment area of Lwak Mission Hospital. Lwak Mission Hospital is located within Bondo district in Western Kenya as shown in Figure 1. Lwak Mission Hospital is located within the Demographic Surveillance Site (DSS) of Centres for Disease Control. This site was chosen because of two reasons. Firstly, it was the site where influenza surveillance had been ongoing and it was therefore possible to have a list of patients who had suffered severe influenza. Secondly being located within the DSS area, it was possible to trace the participants since their homes were well known.

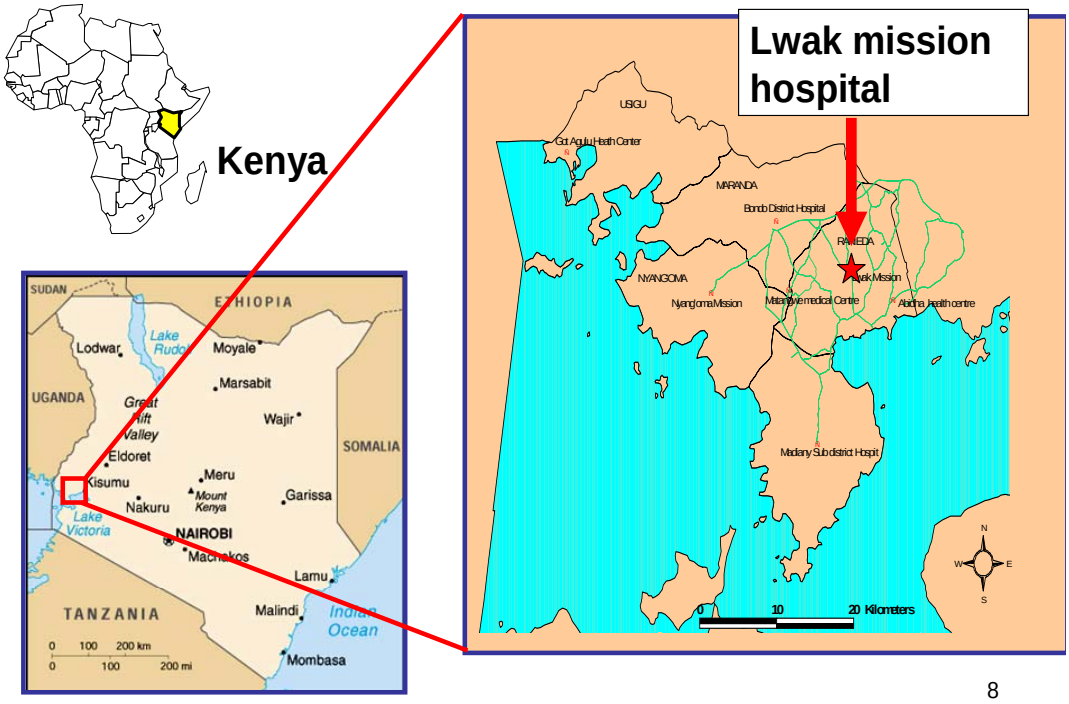

Figure 1: Map showing the location of Lwak mission hospital in Bondo district, Kenya

3.2 Study population

The population that live within the catchment area of Lwak Mission Hospital is predominantly rural, living mostly on subsistence farming and fishing. It is estimated that 25,000 people live within this area. The catchment of the hospital was taken as the area within less than five kilometre radius from the hospital. The five kilometre radius was chosen because a recent study showed that most

people in Kenya access clinical services that is within 5 km radius from their residence (Noor *et al.,* 2003). Beyond the five kilometre radius utilization of clinical services substantially reduces. The area is culturally homogenous with majority of the population being Luo. The area is highly impoverished with more than seventy percent of the population living below the poverty line (Central Bureau of Statistics *et al.,* 2004). The area also has a high HIV prevalence rate (2003, >10% men, >20% women aged 13-34 years, CDC unpublished data). The study population was therefore persons living within the catchment of Lwak Mission Hospital.

3.2.1 Inclusion criteria

Any person living within the catchment area of Lwak Mission Hospital and aged five years old or more was eligible for enrolment into the study.

3.2.2 Exclusion criteria

Participants who refused or could not give informed consent were excluded. Similarly, participants who were known to have permanently moved out of the study area were excluded.

3.3 Study design

A matched case control study was conducted. Each control was matched to the case by place of residence and all study participants were residents of Bondo

district. Since severe influenza is a rare disease, in order to increase the power of the study, three neighborhood controls were selected for each case patient. All the cases were drawn from Lwak Mission Hospital.

i. **Case definition:** A case was defined as any patient aged 5 years old or more, who suffered severe acute respiratory disease and had a nasopharyngeal and/or oropharyngeal swab testing positive for influenza virus by RT-PCR.

Severe acute respiratory disease was defined as any person aged 5 years or more presenting with cough or shortness of breath or difficulty in breathing or pleuritic chest pain and any one of the following

- Oxygen saturation <90% or
- Fever >38.0^0C

Influenza can cause mild illness leading to frequent outpatient consultations. In such cases the patient presents with influenza like illness (ILI) symptoms. This study did not look for the risk of mild influenza but rather severe influenza. Severe influenza was defined as acute respiratory disease caused by influenza and severe enough to cause hospitalization, respiratory failure or death.

ii. **Neighbourhood control definition:** A neighbourhood control was defined as any person aged 5 years old or more, who had not suffered

severe acute respiratory disease requiring admission to hospital in the last one year and was living in proximity to the case patient.

A suitable control was one who had not been hospitalized due to a respiratory illness, and one year was chosen to remove any possibility of misclassification of a severe influenza patient being enrolled as a control. From the foregoing, it is apparent that it is possible for persons who had suffered ILI that was not severe enough to require hospitalization could be included as controls in the study. In this community surveillance for ILI had not started and therefore it was impossible to know who had suffered ILI with laboratory confirmed influenza. In case persons with non severe ILI were included as controls, it could only reduce the strength of the association.

3.4 Sample size determination

Sample size was determined using the formula of Fleiss (Fleiss, 1981) as outlined below.

$$n_1 = \frac{\left[z_{\alpha/2}\sqrt{(r+1)\bar{p}\bar{q}} + z_{1-\beta}\sqrt{rp_1q_1 + p_2q_2} \right]^2}{r(p_1 - p_2)^2} \qquad n_2 = r \times n_1$$

Variable	Case-control study	value
n1	Number of cases	
n2	Number of controls	
zα/2	z-score for two-tailed test based on α level	1.96
z1-β	z-score for one-tailed test based on β level	0.84
r	controls : cases	3
p1	proportion of cases with exposure	
q1	1 – p1	
p2	proportion of controls with exposure	0.5
q2	1 – p2	0.5
OR	Odds ratio	4

3.5 Parameters used in the calculation of sample size

Since several exposures were going to be tested it was assumed that the prevalence of any of the risk factors in the community was 50%. The probability of erroneously finding an association between any risk factor and severe influenza when none exists in reality (type I error) was taken as 0.05. The probability of erroneously not finding an association between the risk factor and influenza when one exists in reality (type II error) was taken as 0.2

(power of the study is 80%). The odds ratio that was considered important to detect was an odds ratio of 4, which was therefore used in the calculation of the sample size. Since infection with severe influenza is a rare condition and we wanted to maximize the power of the study, 3 controls were enrolled for each case. The marginal benefit in efficiency in enrolling more than 3 controls is small.

The calculated sample size was 30 cases and 90 neighbourhood controls. Assuming that 10% of the selected participants may not be traced or the questionnaires may be spoilt the sample size was adjusted to 33 cases and 99 neighbourhood controls. This gives a total sample size of 132 participants to be interviewed.

It is important to note that the prevalence of the exposure in the community could vary from one risk factor to another. The power of the study will be able to detect different odds ratios depending on the prevalence of each particular risk factor in cases and controls as shown in Table 1.

Table 1: Sample size calculations

Prevalence of risk factor in controls	Prevalence of risk factor in cases	Odds ratio	Number of controls	Number of cases	Total participants
10%	34.0%	4.64	99	33	132
10%	35.5%	4.95	90	30	120
10%	38.0%	5.52	78	26	104
20%	47.5%	3.62	99	33	132
20%	49.0%	3.84	90	30	120
20%	51.5%	4.25	78	26	104
30%	59.0%	3.36	102	34	136
30%	61.0%	3.65	90	30	120
30%	63.0%	3.97	78	26	104
40%	69.0%	3.34	102	34	136
40%	71.0%	3.67	90	30	120
40%	73.5%	4.16	78	26	104
50%	79.0%	3.76	99	33	132
50%	80.0%	4.00	90	30	120
50%	82.5%	4.71	78	26	104

3.6 Sampling method

Between 6th March 2006 and 3rd August 2007, a total of 27 severe influenza positive patients were eligible for entry into the study. All the patients who were positive for influenza were selected to participate in the study. Patients who refused to participate in the study and who permanently moved out of the study area were excluded.

The neighbourhood controls were selected by spinning a bottle at the door of the case patient and walking in the direction in which the bottle pointed, skipping the first homestead and randomly selecting one control from all eligible controls in the next homestead. The second neighbourhood control was selected by walking in the same direction, skipping the next homestead and randomly selecting from all the eligible members in the next homestead. The third control was selected using the same procedure as outlined above.

3.7 Data collection tools

Data was collected using a structured questionnaire. The questionnaires were converted into scannable forms. The questionnaires were field tested in the study area before data collection commenced. The responses were entered in ink on the paper based questionnaires.

3.8 Data collection

Data collection involved conducting interviews with enrolled cases and controls. If the participant was a minor, then the parent was interviewed. After establishing the eligibility for enrolment and tracing the participant, the study purpose, risks and benefits was explained and a written consent obtained from each and every participant or guardian. A standard informed consent form was available in both English (Appendix 3) and Dholuo (Appendix 5). It was administered in the language of choice to the participant. If the participant was a minor or was unable to give consent then a legally authorized representative provided informed consent on behalf of the participant. The legal representative was any closest living family member, in most cases the parent.

Training of the interviewers took three days and the topics covered during the training included the following

- Background information on pneumonia, seasonal and pandemic influenza
- Enrolment procedures for the study
- Enrolment of cases
- Enrolment of controls
- Possible bias that must be avoided
- Consent seeking from study participants
- Interviewing techniques

The trained interviewers collected information from both cases and controls using a similar semi structured questionnaire. The information included demographic characteristics and risk factors for influenza.

3.9 Study variables

Among cases, exposure history was required for the period before the patient became ill. For controls the exposure history was required for a similar period before the case (that was matched to that particular control) became ill. The questions asked from the study participants are as indicated in appendix 8.

The socio economic status of the study participants was measured using the technique of principal component analysis (Vyas *et al.,* 2006). The proxy measures of socioeconomic status that were used included education, occupation of head of household and spouse, and asset ownership (TV, radio, lantern, sofa sets, bicycle, cows, sheep, chicken and ducks).

3.10 Statistical analysis

The questionnaires were scanned, data cleaned and stored in a Microsoft Access database. Data analysis was done using the statistical software, EPI INFO version 3.2.2. Data analysis was done by stratifying on case control sets to obtain a matched odds ratio, which was used as a measure of the strength of association and 95% confidence interval around the odds ratio calculated. Statistical test of significance that controlled for matching were done using

Mantel-Haenzel chi square or with Fisher's test if the expected frequencies in the cells were less than five. Stratified analysis was done to look for effect modification and confounders. For the exposure variables that were continuous, the means were compared using paired *t*-test or with Wilcoxon rank sum test if the population distribution was not normal and the variances were significantly unequal.

Variables that achieved a level of significance at p value 0.10 and those that have been reported in previous literature as possible risk factors were included in the multivariable conditional logistic regression analysis model and eliminated through the stepwise backward elimination method. The variable with the highest p value was eliminated first, and the model rerun. This process was continued until only significant variables remained in the model. Interaction between significant variables was assessed by including a product term in the model. Interaction was determined to exist if the p value for the product term was < 0.05.

3.11 Ethical considerations

Approvals were obtained from the following institutions to conduct the study.

- Jomo Kenyatta University of Agriculture and Technology
- Centre for Vector Biology Clinical Research, scientific steering committees.

- KEMRI national scientific steering committee.(KEMRI SSC # 1237)
- KEMRI National Ethical Review Committee (Appendix 2)
- CDC institutional review board (CDC IRB #5138).

A written informed consent was obtained from all study participants before interview. The consent forms provided information on risks, benefits to the patient and an assurance of confidentiality.

CHAPTER FOUR

RESULTS

4.1 Demographic characteristics of the study participants

Out of the available 27 severe influenza confirmed patients, 26 cases were enrolled into the study. One case could not be found because he had migrated from the area. Seventy eight (n=78) controls matched to the place of residence were identified to each case and therefore a total of 104 participants were interviewed. Fourteen (54%) cases had tested positive for influenza B while twelve (46%) cases were positive for influenza A. During the years 2006 and 2007, the predominating influenza type in circulation from July to August was influenza A, whereas influenza B predominated from December through to March as shown in Figure 2.

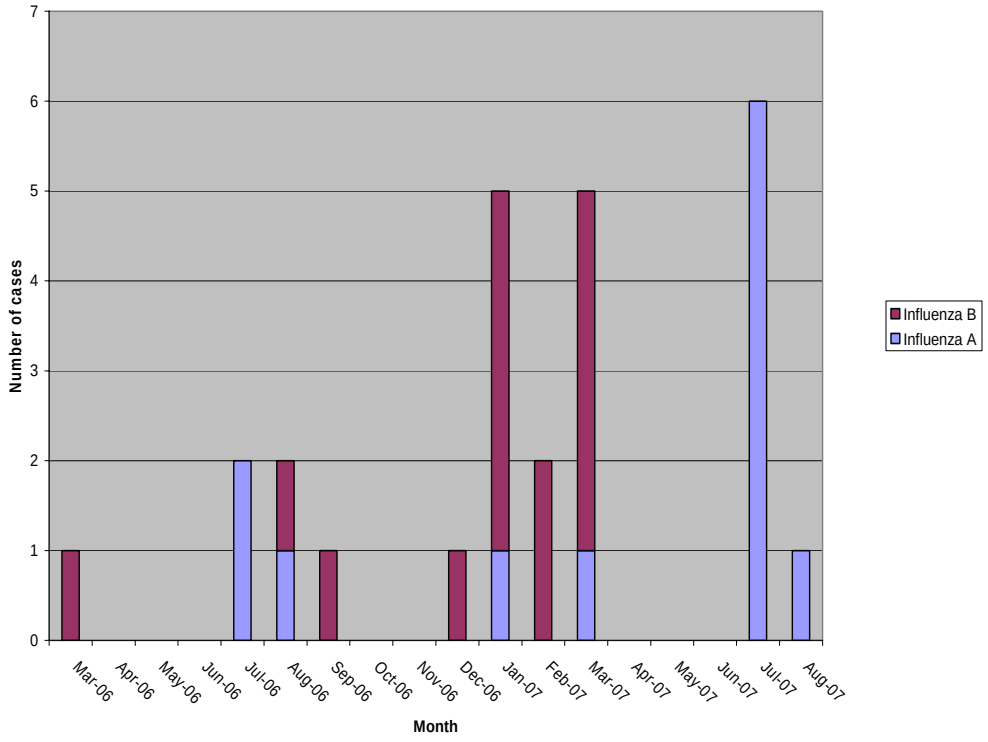

Figure 2: Monthly influenza virus type in circulation in Lwak, Bondo District

Out of the one hundred and four participants, seventy four (71%) were females while thirty (29%) were males as shown on Figure 3. The mean age of the participants was 36 years (standard deviation of 20 years). The youngest enrolled participant was 6 years old while the oldest participant was 95 years old. The age distribution of the study participants (Figure 4) shows that, the cases were younger than the controls. Ninety (87%) participants did not go beyond primary school level of education which is consistent with KDHS finding in the rural area. Twenty six (25%) participants were single, while fifty

eight (56%) and twenty (19%) participants were married and widowed respectively. The demographic characteristics of the cases and the controls are shown in Table 2. There were significant differences in age, gender, level of education and marital status of cases compared to controls. However, no significant differences were noted in the socioeconomic status and religion of the cases compared to controls. The major religions of the study participants were Catholics and Protestants. There were no other religions like Muslims or Hindus in this community.

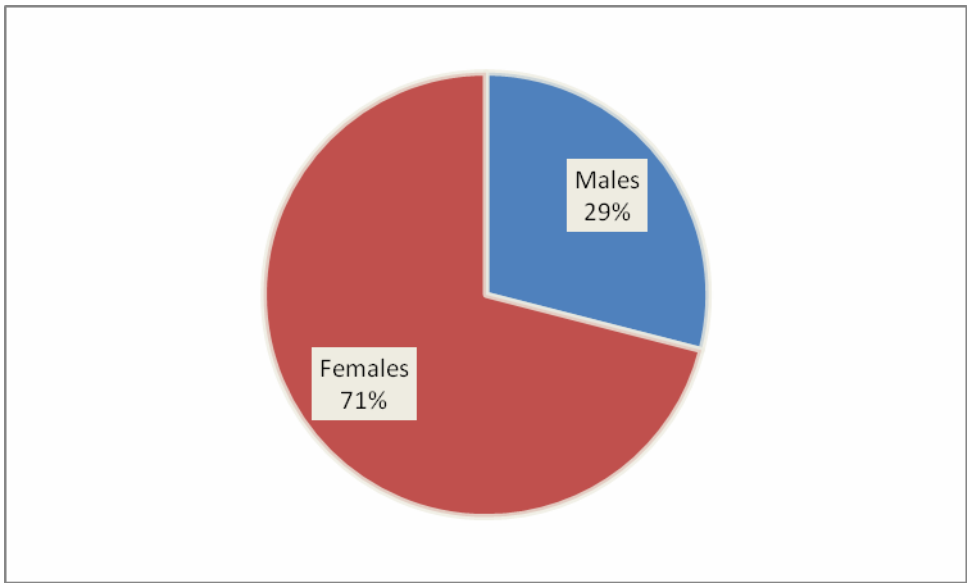

Figure 3: Sex distribution of the study participants

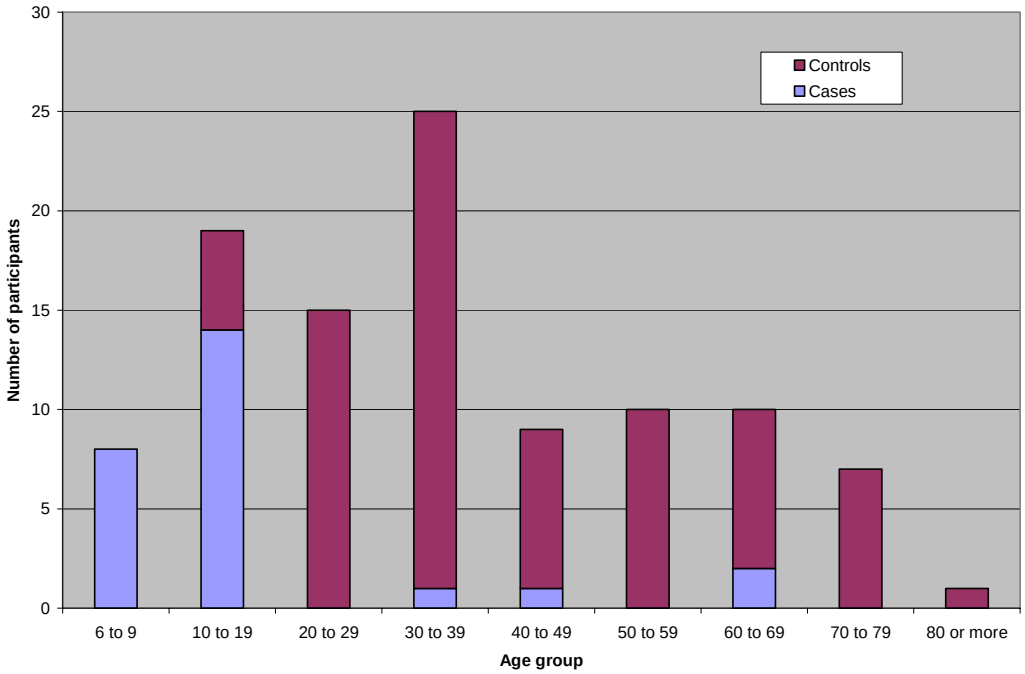

Figure 4: Age distribution of the study participants

Table 2: Demographic characteristics of cases and controls

Factor	Cases n = 26	Controls n = 78	P value
Mean age in years (SD)	17 (17)	42 (18)	<0.0001
Sex			0.0062
Male	13 (50.0%)	17 (21.8%)	
Female	13 (50.0%)	61 (78.2%)	
Education			0.0458
None	1 (4.0%)	10 (12.8%)	
Primary	25 (96.2%)	54 (69.2%)	
Secondary	0	12 (15.4%)	
Post secondary	0	2 (2.6%)	
Socioeconomic status			0.3140
Low SES	8 (32.0)	48 (61.5)	
Middle SES	12 (48.0)	23 (29.5)	
High SES	5 (20.0)	7 (9.0)	
Marital status			<0.0001
Single	20 (76.9%)	6 (7.7%)	
Married	4 (15.4%)	54 (69.2%)	
Widow	2 (7.7%)	18 (23.1%)	
Religion			0.3402
Catholic	8 (32.0%)	13 (16.9%)	
Anglican	5 (20.0%)	9 (11.7%)	
Baptist	3 (12.0%)	19 (24.7%)	
Nomiya	5 (20.0%)	19 (24.7%)	
Other	4 (16.0%)	17 (22.1%)	

4.2 Risk factors

4.2.1 Demographic risk factors

The mean age of severe influenza cases was significantly lower than the mean age of the controls (17 years vs. 42 years, p value <0.0001). The univariate analyses of the demographic risk factors for severe influenza are shown in Table 3. There was a significant association between age and severe influenza. Persons younger than 30 years of age were forty seven times more likely to suffer from severe influenza than those who are older than 30 years of age. On univariate analysis, there was a statistically significant association between gender and severe influenza. Males were five times more likely to develop severe influenza than females (OR_{MH} 4.7, 95% CI 1.5-14.2, p value 0.0075). Those with primary level of education and above were three times more likely to develop severe influenza than those without any education, although this relationship was not statistical significant (OR_{MH} 3.33, 95% CI 0.44-25, p value 0.37). There was a statistically significant relationship between marital status and severe influenza. Single persons were fifty five times more likely to develop severe influenza than those who are married or widows (OR_{MH} 55, 95% CI 6.76-447, p value <0.0001). Catholics were three times more likely to develop severe influenza than Protestants, although this relationship was not statistical significant (OR_{MH} 2.52, 95% CI 0.77-8.24, p value 0.1682).

Table 3: Demographic risk factors

Risk factor	Cases N (%)	Controls N (%)	OR_{MH} (95% CI)	P value
Age in years				
Older (≥30)	4 (15.4)	58 (74.4)	1.00	
Young (<30years)	22 (84.6)	20 (25.6)	47.00 (4.88-452.32)	<0.0001
Gender				
Females	13 (50.0)	61 (78.2)	1.00	
Males	13 (50.0)	17 (21.8)	4.67 (1.53-14.23)	0.0075
Level of education				
No education	1 (3.8)	10 (12.8)	1.00	
Primary & above	25 (96.2)	68 (87.2)	3.33 (0.44-25.48)	0.3692
Marital status				
Married/widow	6 (23.1)	72 (92.3)	1.00	
Single	20 (76.9)	6 (7.7)	55.00 (6.76-447.43)	<0.0001
Religion				
Protestants	17 (68.0)	64 (83.1)	1.00	
Catholics	8 (32.0)	13 (16.9)	2.52 (0.77-8.24)	0.1682

4.2.2 Chronic co-morbidities

In this study, participants who reported having chronic lung disease, chronic heart disease, diabetes mellitus, asthma, or any known chronic ill health were

classified as having chronic co-morbidity. The prevalence of chronic co-morbidities was much higher among cases (54%) than the controls (30%). There was a significant association between the presence of chronic co-morbidities and severe influenza as shown in Table 4. Those with chronic co-morbidities were seven times more likely to develop influenza than those without co-morbidities (OR_{MH} 7.33, 95% CI 1.69-31.84, p value 0.0113). Chronic lung disease was more prevalent among cases (15%) than controls (4%). Even though those with chronic lung disease were six times more likely to suffer from severe influenza this association was not statistical significant (OR_{MH} 5.50, 95% CI 0.94-32.09, p value 0.1083). The numbers of persons with diabetes or chronic heart disease were too few to evaluate the risk of severe influenza.

Table 4: Chronic co-morbidity

	Cases	Controls		
Risk factor	N (%)	N (%)	OR_{MH} (95% CI)	P value
Chronic co-morbidity				
No	12 (46.2)	55 (70.5)	1.00	
Yes	14 (53.8)	23 (29.5)	7.33 (1.69-31.84)	0.0113
Chronic lung disease				
No	22 (84.6)	75 (96.2)	1.00	
Yes	4 (15.4)	3 (3.8)	5.50 (0.94-32.09)	0.1083

4.2.3 Alcohol consumption

Only a small proportion of the study participants consumed alcohol (13.5%). The prevalence of alcohol consumers was higher among the controls (16.7%) than the cases (3.8%). The mean number of bottles of alcohol drunk by the controls was higher than the cases (2 vs. 1, p value 0.5486) and the controls drunk alcohol on more days in a week than the cases (4 vs. 1, p value 0.2416) as shown in Table 5. No significant association was found between alcohol consumption and the risk of severe influenza (OR_{MH} 0.17, 95% CI 0.02-1.61, p value 0.0516).

Table 5: Alcohol consumption

Risk factor	Cases	Controls	P value
Mean number of bottles of alcohol in a day (SD)	1 (0)	2 (1)	0.5486
Mean number of days in week consuming alcohol (SD)	1 (0)	4 (2)	0.2416

4.2.4 Size of household and age of children living in household

There was no significant association between the size of the household and the risk of severe influenza. The mean size of household was 6 and 5 for cases and controls respectively (p value 0.1020). There was no association between having children less than five years old in the household and severe influenza as

shown in Table 6. Having children less than two years old in the household seemed protective although the relationship it was not statistical significant.

Table 6: Age of children living in household

Risk factor	Cases N (%)	Controls N (%)	OR_{MH} (95% CI)	P value
Children < 5 years old				
No	8 (30.8)	31 (39.7)	1.00	
Yes	18 (69.2)	47 (60.3)	1.50 (0.57-3.94)	0.5737
Children <2 years old				
No	18 (69.2)	41 (52.6)	1.00	
Yes	8 (30.8)	37 (47.4)	0.52 (0.21-1.30)	0.0956

4.2.5 Smoking

Only a small proportion of the study participants were smokers (8.7%). Among smokers, the mean number of cigarette sticks smoked per day was higher among cases than controls although this was not statistically significant (10 vs. 4, p value 0.1762). No association between smoking and severe influenza was found as shown in Table 7. Passive smoking is a situation where a non smoker is exposed to cigarette smoke from a smoker. Although the prevalence of exposure to passive smoke was higher among cases (11.5%) than controls

(7.7%), there was no significant association between exposure to passive smoking and severe influenza.

Table 7: Cigarette smoking

Risk factor	Cases N (%)	Controls N (%)	OR_{MH} (95% CI)	P value
Smoking				
No	25 (96.2)	70 (89.5)	1.00	
Yes	1 (3.8)	8 (10.3)	0.29 (0.03-2.94)	0.1444
Passive smoke				
No	23 (88.5)	72 (92.3)	1.00	
Yes	3 (11.5)	6 (7.7)	1.60 (0.36-7.16)	0.8415

4.2.6 Indoor smoke

The common source of cooking fuel in this community was firewood. All the cases and almost an equivalent proportion of the controls used firewood for cooking. Only a few controls 2.6% used charcoal for cooking while none of the cases used charcoal for cooking. All the study participants were using biomass fuel (firewood or charcoal) for cooking which is consistent with the practice among rural communities in Western Kenya. Although the mean number of hours spent by the controls in the cooking area was significantly longer than the cases (2.88 vs. 1.63 hours, p value 0.0012), the prevalence of cooking inside the house was almost similar between the cases (68.0%) and the controls (64.7%).

Those who were exposed to smoke from cooking fuel for less than 2 hours in a day were four times more likely to suffer from severe influenza than those who were exposed for longer hours. There was no significant association between cooking inside the house and severe influenza as shown in Table 8. Similarly there was no significant difference in the prevalence of burning cow dung in the house between the cases (15.4%) and the controls (11.8%). Subsequently there was no significant association between exposure to burning cow dung in the house and severe influenza. One was considered exposed to indoor biomass smoke if he was living in a house where either cooking was done inside the house using firewood or the family burnt cow dung in the house. Although those who were exposed to any indoor smoke were two times more likely to suffer severe influenza than those who were not exposed, this relationship was not statistically significant (OR_{MH} 1.82, 95% CI 0.64-5.18, p value 0.3538).

Table 8: Exposure to indoor smoke

Risk factor	Cases N (%)	Controls N (%)	OR_{MH} (95% CI)	P value
Cooking inside the house				
No	8 (32.0)	24 (35.3)	1.00	
Yes	17 (68.0)	44 (64.7)	1.37 (0.40-4.65)	0.8361
Burning cow dung in the house				
No	22 (84.6)	67 (88.2)	1.00	
Yes	4 (15.4)	9 (11.8)	1.25 (0.38-4.10)	0.9569
Duration of exposure to smoke from cooking fuel				
2 or more hours	11 (44.0)	61 (79.2)	1.00	
< 2 hours	14 (56.0)	16 (20.8)	3.92 (1.45-10.61)	0.0045

4.2.7 Households owning animals

4.2.7.1 Chicken

Severe influenza patients owned substantially more chicken than the controls (mean number of chicken 12 vs. 8, p value 0.0108). Similarly the proportion of

households of the cases keeping chicken was higher than the controls (100% vs. 89.7%, p value 0.0910) as shown in Table 9. Those who were exposed to sick or dying chicken were about twice more likely to suffer severe influenza but this was not statistically significant (OR_{MH} =1.79, 95% CI 0.72-4.44, p value 0.2922). In this study, the animals were considered sick, if any household member knew that the referred animals to, were sick from either a known disease or an unknown disease. Keeping chicken in the house or having close contact was not significantly associated with the risk of severe influenza. In this study, close contact was defined as being less than one meter away from the animal referred to.

Table 9: Exposure to chicken

Risk factor	Cases N =26	Controls N =78	OR_{MH} (95% CI)	P value
Mean number owned (SD)	12 (9.7)	8.0 (6.8)		0.0108
Household keeping chicken				
No	0	8 (10.3)		
Yes	26 (100.0)	70 (89.7)		0.0910
Sick or dying chicken				
No	13 (50.0)	50 (64.1)	1.00	
Yes	13 (50.0)	28 (35.9)	1.79 (0.72-4.44)	0.2922
Keeping chicken in the house				
No	9 (34.6)	25 (32.1)	1.00	
Yes	17 (65.4)	53 (67.9)	0.90 (0.37-2.21)	0.6326
Close contact with chicken				
No	3 (11.5)	16 (20.5)	1.00	
Yes	23 (88.5)	62 (79.5)	1.88 (0.52-6.71)	0.4751

4.2.7.2 Ducks

Out of the one hundred and four study participants, ninety three (89.4%) did not keep ducks. Households of severe influenza patients owned more ducks than the controls (1 vs. 0, p value 0.0942). Those who owned ducks were about two and a half times more likely to have severe influenza although this relationship was not statistically significant as shown in Table 10. Exposure to sick or dying ducks, however, was not associated with severe influenza. Although those who kept ducks in the house or had close contact with ducks were twice more likely to suffer from influenza, both relationships were not statistically different.

Table 10: Exposure to ducks

Risk factor	Cases N =26	Controls N =78	OR_{MH} (95% CI)	P value
Mean number owned (SD)	1 (2)	0 (1)		0.0942
Household keeping ducks				
No	21 (80.8)	72 (92.3)	1.00	
Yes	5 (19.2)	6 (7.7)	2.50 (0.76-8.19)	0.2230
Sick or dying				
No	26 (100.0)	77 (98.7)		
Yes	0	1 (1.3)		0.7500
Keeping ducks in the house				
No	24 (92.3)	75 (96.2)	1.00	
Yes	2 (7.7)	3 (3.8)	2.00 (0.33-11.97)	0.7963
Close contact with ducks				
No	23 (88.5)	73 (93.6)	1.00	
Yes	3 (11.5)	5 (6.4)	1.80 (0.43-7.53)	0.6831

4.2.7.3 Cows

Sixty two (59.6%) of the one hundred and four study participants owned cows in the household. Those who owned cows were five times more likely to develop influenza than those who did not own cows (p value 0.0023). Exposure to sick or dying cows was not associated with severe influenza as shown in Table 11. Those who had close contact with cows were twice more likely to suffer from severe influenza than those who did not have contact with cows, this relationship did not differ significantly. It was not possible to evaluate whether keeping cows in the house was a risk factor since all the study participants kept cows outside the houses.

Table 11: Exposure to cows

Risk factor	Cases N =26	Controls N =78	OR_{MH} (95% CI)	P value
Mean number owned (SD)	6 (5)	2 (4)		0.0003
Household keeping cows				
No	3 (11.5)	39 (50.0)	1.00	
Yes	23 (88.5)	39 (50.0)	5.29 (1.62-17.22)	0.0023
Sick or dying				
No	21 (80.8)	63 (80.8)	1.00	
Yes	5 (19.2)	15 (19.2)	1.00 (0.25-4.00)	0.7518
Close contact				
No	10 (38.5)	42 (53.8)	1.00	
Yes	16 (61.5)	36 (46.2)	1.88 (0.74-4.63)	0.2695

4.2.7.4 Other animals

All study participants kept cats, sheep and dogs outside the house which is consistent with the practice among rural communities in western Kenya. There was no statistically significant relationship between ownership, contact or exposure to sick or dying dogs, cats or sheep and the risk of developing severe influenza.

4.3 Independent risk factors

All the variables that attained a p value of 0.10 or less and factors that are known to be associated with influenza were entered into multivariable conditional logistic regression analysis model. The variables in Table 12 have been presented in the relevant sections of the results and it shows all the variables that were included in the multivariate model.

Table 12: Summary of the results from univariate analysis, indicating the factors that were included in the multivariate model

Risk factor	Cases N =26	Controls N =78	OR_{MH} (95% CI)	P value
Mean number of ducks owned (SD)	0.9 (2.1)	0.3 (1.3)		0.0942
Mean number of chicken owned (SD)	12.3 (9.7)	8.0 (6.8)		0.0108
Young (<30 years)	22 (84.6)	20 (25.6)	47.00 (4.88-452.32)	<0.0001
Gender (male)	13 (50.0)	17 (21.8)	4.67 (1.53 - 14.23)	0.0075
Less than 2 hours exposed to kitchen smoke	14 (56.0)	16 (20.8)	3.92 (1.45-10.61)	0.0045
Drinking alcohol	1 (3.8)	13 (16.7)	0.17 (0.02-1.61)	0.0516
Child < 2 years old in the household	8 (30.8)	37 (47.4)	0.52 (0.21-1.30)	0.0956
Household keeping cows	23 (88.5)	39 (50.0)	5.29 (1.62-17.22)	0.0023
Chronic medical condition	14 (53.8)	23 (29.5)	7.33 (1.69-31.84)	0.0113
Smoking	1 (3.8)	8 (10.3)	0.28 (0.01-2.41)	0.1444

On multivariate analysis the significant independent risk factors for severe influenza was age of the patient and living in a household owning cows as shown in Table 13. Persons younger than 30 years were forty times more likely to suffer from severe influenza than those older than 30 years in age. Persons living in households owning cows had a seven fold increased risk of severe influenza. The other variables were not a significant independent risk factor by multivariate logistic regression analysis. No significant interaction between the age of patient and the ownership of cows was found when the product term was included in the model (p value 0.1703).

Table 13: Factors significantly associated with severe influenza on multiple logistic regression

Variable	Adjusted OR	95% CI	P-value
Younger than 30 years	40.15	4.42 - 364.85	0.0010
House hold owning cows	6.76	1.38 - 33.10	0.0184

CHAPTER FIVE

DISCUSSION

This study suggests that young adults less than 30 years old are at a high risk of severe influenza. This finding is similar to earlier influenza outbreaks like in the 1918 influenza pandemic where the younger population suffered severe disease (Simonsen *et al.,* 1998). This is in contrast to the more recent influenza outbreaks where the elderly are the ones who suffer severe influenza (Fiore *et al.,* 2007 and Simonsen *et al.,* 2007). In the more recent avian influenza H5N1 outbreak, the younger age group suffered severe disease with a mean age of the cases being 19 years (Smallman-Raynor *et al.,* 2007), similar to the mean age of cases in this study which was 17 years. A likely explanation is that the younger age groups were immunologically naive, having not suffered influenza episodes in the past and had not received influenza vaccination and thus they suffered severe disease. The older adults were protected from the disease since they had acquired some immunity from previous influenza infections. Another possibility is that the elderly did not access the hospital in this community and so were not being diagnosed as often as younger adults. In this setting the younger age group may therefore benefit from influenza vaccination.

Since the objective of this study was to determine the risk factors (including demographic risk factors) for severe influenza no attempts were made to make

the controls similar to cases in the demographic characteristics. A neighbourhood control was selected randomly from among all eligible controls in order to ensure that each potential control had an equal probability of being selected. As a result on univariate analysis there was a significant association between marital status and severe influenza. Single persons were at a higher risk of severe influenza than those who were married or widows. This finding was dismissed since marital status is strongly correlated with the age of the participant, as those who are younger in age had not had the opportunity to get married. Therefore, being single was not a risk factor per se but rather the age of the person that played an important role.

Influenza is known to infect birds, pigs, humans and other mammals like dogs, horses and ferrets. Aquatic birds are thought to be the source of all influenza A virus in other species like the ones mentioned above (Alexander *et al.,* 2000) and transmission of influenza A virus between human and animals can occur (Alexander *et al.,* 2000). Human influenza A virus infection has been demonstrated in dairy cows by rising titres to influenza A and it usually results in loss of milk production (Crawshaw *et al.,* 2007). However attempts to isolate the virus from nasal mucus and or swab samples from the cows have been unsuccessful (Graham *et al.,* 2002). In this study living in households owning cows was an independent risk factor for severe influenza. Those who lived in households owning cows were at a higher risk of severe influenza than those that did not own cows. Moreover cases owned a significantly higher number of

cows than the controls. This study suggests that cows may play a role in influenza transmission or there is a risk factor that is strongly correlated with ownership of cows, which is responsible for the increased risk of severe influenza. However, since no serological data was taken from the cows, there is no conclusive information whether they were infected with influenza A, moreover molecular studies are necessary to determine if there are similarities between the virus that caused disease in human and the one isolated from cattle. Even if they were infected, no conclusive statements can be made about the mechanism of transmission hence the mode of transmission of influenza in cattle remains largely unknown.

Some studies have shown that influenza epizootics occurring in various non-human animals sometimes coincide with epidemics of influenza in humans (Easterday, 1980). In some animals influenza infection mimics influenza infection in humans, while in others there are no signs of the disease (Easterday, 1980). Results obtained from this study showed that there was no significant association between severe influenza and exposure to sick or dead cows. There was no association between the number of hours of contact or the frequency of contact with cows and severe influenza; suggesting that the intensity of personal contact was not an important risk factor for severe influenza. Further studies to evaluate the association between cows and influenza in this setting are therefore necessary.

Several studies have shown that smoking is a risk factor for influenza (Arcavi *et al.,* 2004; Murin *et al.,* 2005 and Kark *et al.,* 1982). A study done among the Israeli military showed that smoking was also a risk factor for severe influenza (Noor *et al.,* 2003). In this study there was no significant association between smoking and severe influenza. However, the prevalence of smoking in the population was too low in the community and thus a larger sample size is required to detect a significant association. Biomass fuel was commonly used in this rural community for cooking. Although exposure to biomass smoke may be associated with influenza, results obtained from this study showed that it had no significant association with severe influenza.

There was no association between the drinking of alcohol and severe influenza. It is important to note that there are possibilities that the participants underreported alcohol consumption. Drinking of alcohol is closely correlated with smoking and probably that is why both factors did not achieve statistical significance. Besides during data collection it was not taken into account the type of alcohol that one drank, this may lead towards a tendency of obtaining a null relationship. Perhaps the strength of alcohol that one consumes may be associated with severe influenza, therefore, further evaluation of the association between severe influenza and alcohol consumption is required.

Overcrowding is a risk factor for contagious respiratory diseases. In this study there was no significant association between severe influenza and the size of the

household. This indicates that overcrowding may be a risk factor for influenza but is not sufficient enough to be associated with severity of the disease. The presence of children less than five years old in the household tended to be a risk factor while the presence of children less than two years old in the household tended to be protective for severe influenza, although both factors did not achieve statistical significance in this study. A possible explanation would be that older children interact more with other people than young ones and can therefore acquire influenza infection elsewhere and contribute towards influenza transmission within the household. On the other hand younger children interact less with other people besides the household members.

Previous studies have reported that chronic co-morbidities are significantly associated with severe influenza (Dosseh *et al.,* 2000; Davis *et al.,* 2006 and López-de-Andrés *et al.,* 2007) and this is in agreement with the results from this study on univariate analysis where chronic co-morbidity was significantly associated with severe influenza. However on multivariate analysis no statistical significant differences were shown. There was a strong confounding effect by age since those with chronic co-morbidities were more likely to be older than those without chronic co-morbidities. The skewed age distribution of the study participants (cases being young while controls being old) will most likely lead to an underestimation of the strength of association.

Study limitations

There was a significant difference in the demographic characteristics of the cases and controls. Notably, the cases were young adults, while the controls were older. This makes the comparison of several exposures between the cases and the controls not feasible. Since the objective was to determine all potential risk factors for severe influenza including demographic risk factors; the controls were not matched to case for age, as doing so would make it impossible to evaluate age as a possible risk factor. As a result, age was a strong confounding factor in the study since several other exposure variables that were investigated were also associated with age, such variables included smoking, exposure to biomass smoke, presence of a chronic co-morbidity and marital status. Because of the extreme difference in ages of the cases and the controls, the confounding effect of age was difficult to control for in the multivariate analysis. In order to evaluate other risk factors that were age dependent better, either individual or frequency matching of controls to cases on age would have been required in the study design.

Sample size

This study was limited by a small sample size. The prevalence of some of the exposure variables was too small in the study population such that there was no enough power to detect significant associations.

Selection bias

More female controls were enrolled than males because at the time the interviewers went to recruit the controls; females were more likely to be found at home. This leads to a selection bias since males do not have an equal chance of being recruited as controls. This bias may lead to an over estimation of the strength of association between the disease and several risk factors, such as gender, exposure to biomass smoke and marital status. It is however thought that in this study, selection bias did not affect the association between severe influenza and age or household ownership of cows, since the gender of the participant was not associated with age or household ownership of cows.

Among the cases, there might have been a selection bias in that younger people are more likely to travel to the clinic for care than the elderly. Either because they are frail or that they do not believe in the Western medical system, the elderly might be less likely to access the clinic, and therefore would have been less likely to have been diagnosed with influenza. This would have led to influenza being overrepresented in young adults.

Recall bias

For some study participants the interview was conducted several months after the case patient became ill. Thus they were required to recall exposures that occurred several months ago. Attempts were made to reduce this problem by ensuring that the exposures evaluated were those that do not change rapidly

over time. It was assumed that the cases were not more likely to recall the exposures than controls, thus if recall problems existed then it would affect both the cases and the controls equally. In case recall bias existed it would lead to an underestimate of the strength of association.

Interviewer bias

The interviewers were not blinded to the disease status of the study participants. There is therefore the possibility that the interviewers were probing more for risk factors among the cases than the controls. It was assumed that this did not happen in this study since the interviewers were well trained, closed ended questions were used in the study instrument and interviewers were strictly supervised during data collection. In case there was interviewer bias then it would lead to an overestimate of the strength of association.

Misclassification bias

The risk factors for influenza A may not be similar to the risk factors for influenza B. Similarly different subtypes of influenza A may have different risk factors e.g. H5N1 has different risk factors from H3N2. No differentiation was made among the influenza types or subtypes while enrolling cases. Furthermore, some of the controls may have suffered mild influenza not requiring admission, hence if mild and severe influenza share risk factors then this misclassification will lead to an underestimate of the strength of

association. However, it was believed that this was unlikely since influenza is likely an uncommon disease and it is unlikely that many controls had influenza.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1Conclusions

- Prevention and control of influenza should be targeted at the population at highest risk of severe illness. This study suggests that young adults less than 30 years old are at higher risk of severe influenza than older adults.

- Although this study did not determine whether cows were infected with influenza, the results suggested that there is an association between ownership of cows and severe influenza.

- Age was a strong confounding factor in this study; hence some important risk factors may have been eliminated in the multivariate model because of this.

- Exposure to indoor smoke from burning cow dung or cooking fuel was not a risk factor for severe influenza in this setting.

- The results of this study on univariate analysis, suggest that there is some association between severe influenza and livestock (chicken,

ducks and cows) keeping. However, some of the associations did not achieve statistical significance due to the small sample size. In a rural community where majority of the population live on subsistence farming including livestock keeping, there is a high likelihood of intense human-livestock interaction.

6.2 Recommendations

Policy:

- The policy makers should prioritize young adults for influenza prevention and control programmes including possible vaccination after characterizing the strains circulating.
- Policy makers need to provide funding for further work to determine if the strains of influenza virus that circulate in Kenya and the rest of Africa, are similar to those circulating in the northern hemisphere, which are used to determine which strains to put in the annual influenza vaccine.
- Surveillance for influenza in human should be integrated with influenza surveillance among livestock probably with collaboration among the appropriate key stakeholders such as the Ministry of Health and the Ministry of Livestock production.

Further studies:

- In future studies researchers should match the controls to cases for age and sex in the study design in order to better define other important risk factors.

Research:

- Further research is required to evaluate the association between cows and influenza in this rural community.

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APPENDICES

Appendix 1: KEMRI Scientific Steering Committee (SSC)

KENYA MEDICAL RESEARCH INSTITUTE
P.O. Box 54840, NAIROBI 00200, Kenya,
Tel: (02) 722541, Fax: (02) 720030, E-mail: kemri-hq@nairobi.mimcom.net Website: www.kemri.org

ESACIPAC/SSC/70 7th May, 2007

O. Maurice CDC

Thro'
Director,
Centre for Vector Biology Clinical Research
P.O. Box 1578
KISUMU

FORWARDED 11/5/07

Ref: SSC No: 1237-A Case Study of Risk Factors for Severe Influenza among Adults and Children in Rural Community in Bondo District, Kenya

This is to inform you that the above-mentioned proposal in which you are the PI was discussed during the 135th SSC meeting held on 2nd May, 2007. It was recommended that you revise this proposal in view of the issues raised during the meeting.

1. The null hypothesis requires re-phrasing to fit into the analytic (comparable) design expected of a case-control study.

2. This being a case study control going to test for a host of exposures, over 22; the many variables may dilute the emphasis on certain crucial variables that one requires to pay attention to.

After revising re-submit two (2) copies of the proposal to the SSC as soon as possible.

C. Mwandawiro, PhD
SECRETARY, SSC

Appendix 2: KEMRI National Ethical Review Committee (ERC)

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: kemri-hq@nairobi.mimcom.net; director@kemri.org; Website: www.kemri.org

KEMRI/RES/7/3/1 **June 21, 2007**

FROM: SECRETARY, KEMRI/National Ethical Review Committee

THROUGH: CENTRE DIRECTOR, CVBCR

TO: **Dr. Ope Maurice**

RE: **SSC No. 1237** - A case control study of risk factors for severe influenza among adults and children in a rural community in Bondo District, Kenya.

MEETING: 144th meeting of KEMRI/National Ethical Review Committee held on 19th June 2007

Thank you for your study proposal that aims primarily to determine the risk factors associated with severe influenza and as a secondary objective to investigate the relationship of influenza and HIV.

Due consideration has been given to ethical issues and you may proceed with the study.

You are responsible for reporting to the Ethical Review Committee any changes to the protocol or in the Informed Consent Document. This includes changes to research design or procedures that could introduce new or more than minimum risk to human subjects.

Respectfully,

R.C.Kithinji

R. C. Kithinji,
For: Secretary,
KEMRI/NATIONAL ETHICAL REVIEW COMMITTEE

Appendix 3: Informed Consent for influenza risk factor study

(Flesch-Kincaid readability score – 7.5) – English

Today's date	□□/□□/□□	**DSS permanent ID**	□□□□□□□□□□
Name of village			
Name of selected participant			

You (or NAME of CHILD) are already enrolled in the IEIP household morbidity surveillance study and the Demographic Surveillance System (DSS). You are provided with free medical care at Lwak hospital and a community interviewer visits your home every two weeks to ask you questions about you health status. We want to study the risks for influenza in this area. We are asking questions about possible risk factors for influenza among those who suffered and those who did not suffer from influenza.

For cases: You (or NAME of CHILD) have been picked to be part of this study because according to our records, when you recently visited the clinic at Lwak with a respiratory illness the specimen we took from your nose or throat tested positive for influenza. This study hopes to learn the risks that might have led to you suffering from influenza. We want to be able to reduce these risks for other people.

For neighborhood controls: You (or NAME of CHILD) have been picked to be part of this study because you live in the same neighborhood with someone who suffered from influenza. We chose you at random from all eligible participants who live within the same neighborhood.

For hospitalized controls: You (or NAME of CHILD) have been picked to be part of this study because you were recently seen at Lwak with a respiratory illness other than influenza. We want to compare symptoms and risk factors for influenza against those of other respiratory illnesses.

If you choose to be part of this study, it will take about 20 minutes of your time. It is your free choice to be part of your study.

What we would like to do:

If you agree to be part of this study, we will ask you some questions. The questions will be about your home, the animals you keep at home, yourself and the medical problems you (or NAME of CHILD) may be having.

For cases we are also asking your consent to use the data that was collected from you (or NAME of CHILD) during the visit to Lwak hospital. This will be used to see if any of the other diseases you were suffering from at that time might be a risk for influenza.

You (or NAME of CHILD) will still be part of the IEIP household morbidity surveillance and the DSS, which means you will still access free treatment for certain conditions at Lwak hospital and you will still be visited every two weeks by community interviewers and every 4 months by DSS interviewers. You can refuse to be part of this new study for illness and still be part of the IEIP morbidity surveillance study and the DSS.

If you agree to be part of this study, we would like to ask your permission to link the information collected in this study with the information collected in the IEIP household morbidity surveillance and the DSS.

As part of this study, we would like to find out if you (or NAME of CHILD) have HIV. We will ask if you (or NAME of CHILD) have HIV and may ask to see your written documentation of an HIV test result if you have it. If you don't know if you (or NAME of CHILD) have HIV we will offer to test you (or NAME of CHILD) for HIV. This is to see if HIV might be an important risk for influenza. Every person in this study will be asked about HIV.

A trained HIV VCT counselor will come to your home sometime in the next few weeks to explain the test, to counsel you and to ask your permission to do that test. The results of the HIV test will be kept private. I will not know the results of that test. You will get your results immediately after the test is done.

You (or NAME of CHILD) may be in the survey today and refuse the HIV test when the counselor comes to your house.

Benefit from being in this study:

The results of this study might lead to understanding the risk factors for influenza and lead to future ways of preventing influenza. We will also be able to know the groups of people who can benefit from influenza vaccine.

If you agree to be part of this study there will be no need of you traveling to have an HIV test as we will be offering home testing of HIV. You will also be able to know your HIV status and lead to initiation of early treatment. If you test positive for HIV and you know it, you will have reduced chances of transmitting the virus to your partner.

Risks from being in this study:

There are no risks to you (or NAME of CHILD) in being part of this study today.

You are free to choose to be part of this study. You have the right to refuse or stop at any time. If you stop the study, you and your family members will still be part of the IEIP morbidity surveillance study and the DSS and will still be able to access free health care for certain conditions at Lwak.

The facts about you and your family from this study will be kept private as much as possible. No names will be used on any of the study reports. Should any more questions arise, if you feel like you or your family might have been harmed by being in the study, or if you want to quit the study, please contact SUPERVISOR NAME at the CDC Office in Lwak (Asembo) or Dr. Ope Maurice at the CDC office in Kisian (0572022983). If you have questions or concerns about your rights or your child's rights as a research participant or the treatment of research participants contact Dr. Monique Wasunna, the KEMRI Ethical Review Committee contact person at 020-2722541.

Consent signing:

The consent form has been explained to me and I agree (for NAME of CHILD) to take part in the study. I understand 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 my family or me.

Participant	**Name: .....**	**Signature: .....**	**Date□□/□□/□□**
Witness*	**Name: .....**	**Signature: .....**	**Date□□/□□/□□**

* Subject may sign or provide verbal consent in the presence of a witness. The witness (by his/her signature) verifies that the consent form has been accurately translated to the subject and this is the subject's signature or that he/she has provided verbal consent.

Appendix 4: Influenza risk factor study: Assent form for children 7-17 years old (Flesch-Kincaid readability score 2.8) – English

Today's date	□□/□□/□□	**DSS permanent ID**	□□□□□□□□□□
Name of village			
Name of selected participant			

We are looking to find out the factors that may be responsible for severe influenza or its complications.

For cases: You have been picked to be in the study because the specimen that was taken from your nose or throat tested positive for influenza.

For neighborhood controls: You have been picked because you live near somebody who suffered from influenza.

For hospitalized controls: You have been picked because you were hospitalized for another respiratory illness other than influenza.

You can help us out if you want. It is your choice. If you don't want to help it is OK. Nobody will be mad at you.

We are asking questions about possible risk factors for influenza among those who suffered and those who did not suffer from influenza. It will take about 20 minutes.

For cases we are also asking for your permission to use the information that was collected from you during the visit to Lwak mission hospital. We will use this to find out if any other disease that you may have been suffering from made it easier for you to suffer from severe influenza.

You will still be part of all other studies that you were involved in and still enjoy all the rights and privileges you had before.

If you agree to be in the study, we are also asking for permission to link the information collected in this study to the information that has been collected in DSS and IEIP household morbidity study.

We would also like to find out if you have HIV. If you don't know your HIV status we will offer to test you for HIV. The results of this test will be kept private. I will not know the results of this test.

A person trained in doing the HIV test will come to you in a few weeks time to do the test.

You may be in the survey today and refuse the test when this person comes.

Benefits from the study

The information we collect can help us learn more about influenza and how to control it.

Knowing your HIV status may lead to early initiation of treatment.

Risks of being in the study

There are no risks of being in the study today.

If you agree to be in the study today but change your mind later it is OK. You can stop at anytime.

We asked your parents and they said it was okay to ask if you wanted to do this. If you have any more questions, please ask your parents or me.

Will you be a part of this study ? ☐ Yes ☐ No

Name of child (Print) _____Date_____

Child Signature (Signature or mark of consent) _____

To be signed by witness:

The above statement has been read to the child who agrees to participate in the study.

Name of witness (Print) _____

Date_____Witness Signature (Signature or mark of consent) _____

Appendix 5: Informed Consent for influenza risk factor study

(Flesch-Kincaid readability score – 7.5) – Luo

Tarik ma kawuono	□□/□□/□□	**Number mar DSS**	□□□□□□□□□□
Name of village / Nying gweng			
Name of selected participant / Nying jachiwre ma oyier			

In (kata nying nyathini) ne ose ruaki e nonro mar IEIP ma ngiyo touche to gi ranyisi mag touchégi e udi, to gi nonro mar DSS. Imiyi thieth ma nono e osiptal ma Lwak kendo ja chiw penjo limiga dalani bang jumbe ariyo ka jumbe ariyo penji ewi ngimani. Wadwaro nono thuolo mar gamo athung'a motegno e gwengni. Wapenjo thuolo madi bedie e gamo athung'a motegno kuom joma athung'a motegno osetuoyo to gi joma pok otuoyo.

Ne jogo ma osetuoyo: In (kata nying nyathini) oyieri mondo ibed achiel e nonroni nikech kaluore gi ndiko marwa, ka nyocha idhi e clinic ma Lwak gi chandruok mar yueyo, gi pim mane wagolo e umi gi duondi ne ogolo ni in gi

athung'a motegno. Nonro ni, ni gi geno mar ngeyo thuolo mar hinyruok mane omiyo athung'a motegno otuoyi. Wadwaro bedo ni wanyalo geng'o thuolo mag hinyruokgi ne jomoko.

Ne jogweng mapok otuoyo: In (Kata nying nyathini) oyieri mondo ibed achiel e nonro ni nikech idak e gweng achiel gi ngato mane athung'a motegno ose tuoyo. Ne wayieri radha radha e kind ji duto mane inyalo ruaki ma odak e gweng' achiel.

Ne jogo ma oyud e osiptal: In (Kata nying nyathini) oseyieri mondo ibed achiel e nonroni nikech nyocha oneni Lwak gi chandruok korka yueyo maok athung'a motegno. Wadwaro pimo ranyisi to gi thuolo mar hinyruok e kind athung'a motegno to gi touché mamoko makelo chandruok mar yueyo.

Ka iyiero bedo e nonroni, obiro kawo thuoloni kuom dakika 20. En yieroni bedo e nonro.

Gima dwaher timo:

Ka iyie bedo achiel e nonroni, Wabiro penji penjo moko. Penjogi biro bedo ewi dalani, gik ma ipidho (Kaka jamni ,dhok, gwen, paka kata guok) in iwuon to gi chandruok mar tuo ma (Kata nying nyathini) inyalo bedogo.

Ne jogo ma osetuo, bende wakwayo thuoloni mondo wati gi weche mane wasekawo kuomi (Kata nying nyathini) e kinde mag limbe e osiptal ma Lwak. Ma ibiro ti go e ng'iyo ka achiel kuom touché mane in go e seche go nyalo bedo thuolo mar hinyruok mar athung'a motegno.

In (Kata nying nyathini) pod ibiro bedo achiel e nonro mar IEIP to gi DSS, manyiso ni pod ibiro yudo thieth ne touche moko e osiptal ma Lwak kendo pod ibiro limi bang jumbe ariyo ka jumbe ariyo gi jo chiw penjo to gi bang dweche angwen ka dweche angwen gi jo chiw penjo ma DSS. Inyalo tamori bedo e nonro mar tuoni, to pod ibed achiel e nonro mar IEIP ma ngiyo touche to gi ranyisi mag touchégi e udi, to gi nonro mar DSS.

Ka iyie bedo achiel e nonroni, dwaher kwayo thuoloni mar tudo weche ma wagolo e nonroni to gi weche ma ogol e nonro mar IEIP ma ngiyo touche to gi ranyisi mag tuochegi e udi, to gi nonro mar DSS.

Kaka achiel mar dwach nonro, dwaher ng'eyo ka in (Kata nying nyathini ni) gi kute mag ayaki. Wabiro penjo ka in (Kata nying nyathini ni) gi kute mag ayaki kendo wanyalo kwayo mondo wane andikani mar duoko mar kute mag ayaki ka

in go. Ka ikia ka in (Kata nying nyathini ni) gi kute mag ayaki to wabiro chiwoni (Kata nying nyathini) pim mar kute mag ayaki. Ma en mondo omi one ka kute mag Ayaki e thuolo maduong mar athung'a motegno.

Jahocho ma otiegi ne pim gi hocho ma ichiwruoke ewi kute mag ayaki biro biro dalani e jumbe mabirogi mondo olerni pim, hoyi kendo kwayo thuolo mari mar pim. Duoko mar pim mar kute mag ayaki ibiro kan ma opondo. Ok abi ng'eyo duoko mag pim. Ibiro yudo duoko magi mapiyo bang ka osetim pim. I (Kata nying nyathini) nyalo bedo e nonro kawuono to itamori pim mar kute mag ayaki ka jahocho obiro e odi.

Ber mar bedo e nonro:

Duoko mar nonroni nyalo kelo ngeyo matut thuolo mag hinyruok mag athung'a motegno to gi kelo yore mag geng'o athung'a motegno e kinde mabiro. Bende wabiro ngeyo grube mag dhano manyalo konyore gi chanjo mar athung'a motegno.

Ka iyie bedo e nonroni ok bibedo tiende mar dhi manyo pim mar kute mag ayaki nikech wabiro kelo pim mar kute mag ayaki e dala. Bende ibiro nyalo ng'eyo duoko mari mar pim mar kute mag ayaki kendo telo e thieth chon. Ka pim ogolo ni in gi kute mag ayaki, ibiro bedo gi thuolo mapiny mar lando kute mag ayaki ne ng'ama iriworigo.

Hinyruok mawuok e bedo e nonro:

Onge hinyruok ne in (Kata nying nyathini) kuom bedo e nonroni kawuono.

In thuolo mar yiero bedo e nonroni. In gi ratiro mar tamori kata chung' e sa asaya. Ka iweyo nonro, in kata joodi pod biro bedo achiel e nonro mar IEIP ma ngiyo touche to gi ranyisi mag tuochegi, to gi nonro mar DSS kendo pod ibiro nyalo yudo thieth ne touché moko e Lwak.

Weche ma oa kuomi to gi joodi man e nonroni ibiro kan ma opondo kaka nyalore. Onge nying ma ibiro ti go e ripod nonro moro amora.

Kapo penjo moko owuok, ka iparo ni in kata achiel kuom joodi ohiny kuom bedo e nonro kata ka idwaro weyo nonro, yie itudri gi NYING JA TAA NONRO e ofis CDC ma Lwak (Asembo) kata Dr. Maurice Ope e ofis CDC ma Kisian (0572022983).

Ka in gi penjo kata wach ewi ratiro mari kata mar nyathini kaka achiel e nonro kata thieth mar joma ni e nonro, tudri gi Dr. Monique Wasunna, the Kemri Ethical Review Committee ng'ama itudruok go e namba mar simu 020-272241.

Keto lwedo e andika mar yie:

Andika mar yie oselerna kendo ayie ni (Nying nyathi) mondo obed e nonrro. Awinjo ni an thuolo mar yiero mondo mi kik abed e nonroni sa asaya kendo wacho ni "OOYO" ok bi kelo chandruok ne jooda kata an.

Jachiwre	**Nying:…….**	**Lwedo:……**	**Tarik□□/□□/□□**
Janeno*	**Nying:……**	**Lwedo:……**	**Tarik□□/□□/□□**

* jachiwre nyalo keto lwedo kata chiwo thuolo gi dhoge e nyim janeno. Janeno (Kuom keto lwedo) lero ni andika mar yie olok e yo makare ne jachiwre kendo ma en lwet jachiwre kata jachiwreni ochiwo thuolo gi dhoge.

Appendix 6: Influenza risk factor study: Assent form for children 7-17 years old (Flesch-Kincaid readability score 2.8) – Luo

Tarik ma kawuono	□□/□□/□□	**Number mar DSS**	□□□□□□□□□
Name of village / Nying gweng			
Name of selected participant / Nying jachiwre ma oyier			

Wamanyo yore manyalo kelo athung'a motegno kata gi chandruok mage.

Ne jogo ma osetuoyo: Oyieri mondo ibed e nonro nikech gi pim mane ogol e umi ogolo ni ne in gi athung'a motegno.

Ne jogo mapok otuore gi athung'a motegno: oyieri nikech idak machiegni gi ng'ama athung'a motegno osetuoyo.

Ne jogo mapok athung'a motegno otuoyo to ne ni e osiptal: Oyieri nikech ne oruaki e wod nikech chandruok mar yueyo machielo maok athung'a motegno.

Inyalo konyowa ka idwaro. En yiero mari. Ka ok idwar konyo to ni kare. Onge ng'ama biro neno kodi marach.

Wapenjo penjo ewi yore mag hinyruok manyalore kuom athung'a motegno kuom jogo mane otuore gi athung'a motegno to gi jogo mane ok otuore. Biro kawo kar dakika 20.

Ne jogo ma osetuore bende wakwayo thuolo mari mondo wati gi weche mane ogol kuomi ekinde mag limbe e osiptal mar misen ma Lwak. Wabiro tiyo gi ma e nono kapo tuo moro nono madine tuoyi ne ochiwo thuolo mondo mi athung'a motegno tuoyi.

Pod ibiro bedo achiel e nonro mamoko mane in tiere kendo neno ber kuom ratiro magi duto to gi ber ma isebedogo ekinde mokalo.

Ka iyie bedo e nonro, bende wakwayo thuolo mari mondo mi watud weche ma wagolo e nonro ni to gi weche ma osegol e DSS gi nonro mar IEIP ma ngiyo touché.

Bende dwaher ngiyo ka in gi kute mag Ayaki. Duoko mag pimni ibiro kan mopondo. Ok abi ngeyo duoko mar pimni.

Ng'ama otiegi e timo pim mar kute mag Ayaki biro limi e jumbe mabiro mondo otim pim.

Inyalo bedo e nonro kawuono to itamori pim kaponi ngani obiro.

Ber mar nonro.

Weche ma wachoko nyalo konyowa e ngeyo matut kuom athung'a motegno to gi kaka inyalo genge. Ngeyo chalni korka kute mag Ayaki nyalo kelo dhi e thieth chon.

Hinyruok mawuok e bedo e nonro.

Onge hinyruok e bedo e nonro ma kawuono.

Ka iyie bedo e nonro kawuono to iloko pachi bange, ni kare. Inyalo weyo saa asaya.

Ne wapenjo jonyuolni to gi wacho ni ni kare ka idwaro timo ma. Ka in gi penjo mamoko, yie ipenj jonyuolni kata an.

Bende ibiro bedo achiel e nonroni? ☐ Ee ☐ Ooyo

Nying nyathi (Ndik gi nukta

madongo)_____Tarik_____

Lwedo mar nyathi (Lwedo kata ranyisi mar yie) _____

Onego oket e lwedo gi janeno:

Wach man malono osesom ne nyathi ma bende oseyie bedo e nonro.

Nying janeno (Ndik gi nukta madongo)_____

Tarik_____Lwedo mar janeno (Lwedo kata ranyisi mar yie) _____

Appendix 7: Extract from IEIP household morbidity surveillance study

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INFLUENZA PROJECT- INFLUENZA CASE CONTROL STUDY

Extract from IEIP household morbidity surveillance study

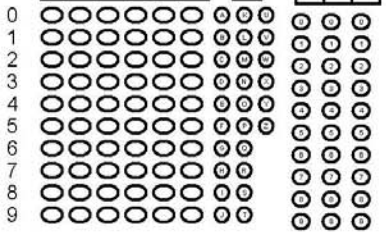
Unique identifier ☐☐☐ Participant study identity ☐☐☐ / ☐

(O = Case 1,2,3 = Control)

Date of admission ☐☐ / ☐☐ / ☐☐☐☐ *(dd/mm/yy)*

Date of dicharge ☐☐ / ☐☐ / ☐☐☐☐ *(dd/mm/yy)*

1. Case status O Case O Hosiptalized Control
2. Names of the patient
   - (a) First name ☐☐☐☐☐☐☐☐☐☐
   - (b) Juok name ☐☐☐☐☐☐☐☐☐☐
   - (c) Last name ☐☐☐☐☐☐☐☐☐☐
3. Permanent residence DSS permanent ID ☐☐☐☐☐☐ ☐ ☐☐☐



4. Sex O Male O Female
5. Date of birth ☐☐ / ☐☐ / ☐☐☐☐ *(dd/mm/yyyy)*
6. Date of Specimen collection ☐☐ / ☐☐ / ☐☐☐☐ *(dd/mm/yyyy)*
7. Viruses identified

O None	O Parainfluenza 1	O Adenovirus
O Influenza A	O Parainfluenza 2	O Respiratory syncitial virus
O Influenza B	O Parainfluenza 3	O Human metapneumovirus

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8. What was the diagnosis ? *(In case of in patient as recorded in the admission register: in case of outpatient, as recorded in the outpatient register*

Pneumonia	O Yes	Dysentry	O Yes
Upper respiratory tract infection (URTI)	O Yes	Intestinal worms	O Yes
Wheezing / bronchospasm	O Yes	Anaemia	O Yes
Otitis media	O Yes	Malnutrition	O Yes
Conjunctivitis	O Yes	Oral candidiasis	O Yes
Meningitis	O Yes	Rash / skin problem	O Yes
Diarrhea / gastroenteritis	O Yes	Scabies	O Yes
Dehydration	O Yes	Burn	O Yes
Pharyngitis / tonsillitis	O Yes	Wound / injury	O Yes
Viral syndrome	O Yes	Amobiasis	O Yes
Maraia	O Yes		

Other 1 ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Other 2 ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

9 (a) Was the patient admitted to hospital ? O Yes O No O Don't Know

9 (b) What was the discharge diagnosis ?*(As recorded in the discharge summary or the in patient file)*

Pneumonia	O Yes	Dysentry	O Yes
Upper respiratory tract infection (URTI)	O Yes	Intestinal worms	O Yes
Wheezing / bronchospasm	O Yes	Anaemia	O Yes
Otitis media	O Yes	Malnutrition	O Yes
Conjunctivitis	O Yes	Oral candidiasis	O Yes
Meningitis	O Yes	Rash / skin problem	O Yes
Diarrhea / gastroenteritis	O Yes	Scabies	O Yes
Dehydration	O Yes	Burn	O Yes
Pharyngitis / tonsillitis	O Yes	Wound / injury	O Yes
Viral syndrome	O Yes	Amobiasis	O Yes
Maraia	O Yes		

Other 1 ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Other 2 ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Appendix 8: Risk factor study questionnaire

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Influenza Project- Influenza Case Control Study
Risk Factor Questionnaire

PRE-PRINTED SECTION

Form type ☐

File number ☐☐☐☐☐☐☐ *Filenum*

1. = O HMS Adult
2. = O HMS Child
3. = O Influenza Adult
4. = O Influenza Child

Formtype

Participant study identity ☐☐☐ / ☐ (0= Case 1,2,3 = Control) *MATCHID CC*

Visit / Admission for case ☐☐ / ☐☐ / ☐☐☐☐ *(dd/mm/yyyy) applies to the case but is a reference date for the control)* *ADMDATE*

Visit date for hospitalized control ☐☐ / ☐☐ / ☐☐☐☐ *(dd/mm/yyyy)* *DISDATE*

Case status O Case O Hospitalized Control O Neighbourhood Control *CAEST*

Applies to neighbourhood controls only (otherwise leave blank)

1. Names of the patient

First name ☐☐☐☐☐☐☐☐☐☐ *FNAME*

Juok name ☐☐☐☐☐☐☐☐☐☐ *JNAME*

Last name ☐☐☐☐☐☐☐☐☐☐ *LNAME*

2. Permanent residence
DSS Permanent ID ☐☐☐☐☐☐ ☐ ☐☐☐ *DSSID*

0 1 2 3 4 5 6 7 8 9

Recorder ID ☐☐☐☐ *RECDID*

Date of interview ☐☐ / ☐☐ / ☐☐☐☐ *(dd/mm/yyyy)* *INDATE*

PART 1: DEMOGRAPHICS

1.1 (a)
1.0 Sex O Male O Female *GENDER*

Date of birth (dd/mm/yyyy) ☐☐ / ☐☐ / ☐☐☐☐ *DOB*

1.1 (b) Age ☐☐☐ *years* *AGE*

1.2 (a) What is your highest level of education attained?***(isomo ma igik e okang mane?)*** *EDUC*

O None O Primary

O Secondary O Post Secondary Education

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1.2 (b) What is the total numbers of years of education you have had ?*(Higni adi duto ma ikao kisomo)* EDUCYRS

☐☐ *years*

Family Socio-economic

1.3 (a) What is the M**ost important** income generating activity of the household administrator ?*(Ere gima wuon ot timo maduong makelo yuto engima maru)* INCADM

- O Subsistence farming *(pur matin mar chiemo)*
- O Commercial farming *(pur mangeny mar ohala)*
- O Salaried worker (e.g teacher,nurse,office) *(jatich ma ichulo osara)*
- O Small business (e.g sell maize) *(ohala matindo)*
- O Business owner (e.g duka,kiosk) *(ohandi*
- O Skilled labor (e.g carpenter, tailor,jua kali) *(Tich fundi ma isomo)*
- O Unskilled labor (e.g shamba, construction) *(Tich ma ingeyo to ok isomo)*
- O Fishing *(Lupo)*
- O Housewife *(Chi ot)*
- O Other (specify) *(mamoko)* ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ INCADMX

1.3 (b) What is the M**ost important** income generating activity of the spouse ?*(Kuom tich ma minodi / jaodi timo, ere maduong makelo yuto ?)* INCSPS

- O Subsistence farming *(pur matin mar chiemo)*
- O Commercial farming *(pur mangeny mar ohala kata maiuso mondo uyudi konyruok)*
- O Salaried worker (e.g teacher, nurse,office) *(jatich ma ichulo osara)*
- O Small business (e.g sell maize) *(ohala matindo)*
- O Business owner (e.g duka, kiosk) *(ohandi*
- O Skilled labor (e.g carpenter, tailor,jua kali) *(Tich fundi ma isomo)*
- O Unskilled labor (shamba, construction) *(Tich ma ingeyo to ok isomo)*
- O Fishing *(Lupo)*
- O Housewife *(Chi ot)*
- O Other (specify) *(mamoko)* ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐ INCSPSX

1.3 (c) **Items of ownership (mwandu ma in go)**

How many of each of the items does your household have at the moment ?

Radio(*Nyakalondo)* ☐☐ Lantern *(Taya)* ☐☐ TV ☐☐ *radio sofa lantern*

Bicycle *(Ndiga)* ☐☐ Sofa *(Komb sofa)* ☐☐ *tv bicycle*

1.4 What is your religion ? *(Din)* O Catholic O Anglican O Baptist or other Protestant RELIG

O Legio maria O Roho Israel O Seventh Day Adventist

O Nomiya

O Other (specify) *(mamoko)* ☐☐☐☐☐☐☐☐☐☐ RELIGX

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1.5 What is your marital status ? *(in gi jaodi sani)* MARITST

O Single *(Pok okenda)* O Married *(ose kenda)* O Living with partner *(Adak gi jaoda)*

O Widowed *(Chi Liel)* O Divorced *(Awe jaoda chuth)* O Separated *(Awe jaoda)*

PART 2 : RISK FACTORS

2.1 (a) Do you suffer from any known medical condition ?*(Be in gi tuo moro amora mon'gere)* MEDCON

O Yes O No O Don't know

2.1 (b) If yes, which medical condition ? *(Ka ee, en mane)*

ASTHMA
PTB
DM
CHD
CLD

O Known Asthmatic *(Kor mathung)* O Pulmonary Tuberculosis *(Kahera)*

O Chronic heart disease *(Tuo adundo)* O Chronic Lung disease *(Tuo kor)*

O Diabetes mellitus *(Tuo sukari)*

MEDNMX

O Other (specify) *(Tuoche mamoko)* [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ]

2.2 (a) Has a clinician ever recommended that you take some medication regularly ? *(Be daktari ne osendiko ni yedhe ma itiyo godo pile pile ?)* REGTRT

O Yes O No O Don't know

2.2 (b) If yes, which ones ? *(Ka e yath mane ?)*

O Bronchodilators *(Ventolin, Franol, Inhalers,Aminophyline,Adrenaline)* BRODIL

O Anti diabeties *(Insulin,glibenclamide)* ANTIDM

O Benzathine, penicillin *(2.4 BP)* BENPEN

O Asprin ASPRIN

O Anti TB drugs *(Rifater)* ANTITB

O Other *(Specify* [ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ][ ] REGTRTX

2.3 Do you drink any kind of alcoholic beverage ? *(Be imadho kongo kata gik makech ?)* ALCHL

O Yes O No O Don't know

If yes then ask (Ka ee, kongo mane ?)

2.3 (a) How many days in a week do you drink alcoholic beverages ? *(Ei juma achiel imatho kongo ndalo adi ?)* ALCHLD

[ ][ ] *(days)*

2.3 (b) On average in a night, how many alcoholic beverages *(bottles of beer, glasses of changaa*) do you drink ?
(Ei otieno achiel, imatho kongo madirom nade?) (chupa kongo, okombe changaa)

[ ][ ] *(bottles)*

ALCHLBT

[ ][ ][ ][ ][ ][ ]

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These questions refer to the period before the case patient got ill and was admitted to the hospital or to a simliar period for control patients.

2.4 (a) How many people were you living with in the same household during the period before you became ill ? *HHSZ*
(reference period) (Ji adi mane idak go e ot ka pok ne ibedo matuo ?)

☐☐ *people*

2.4 (b) Of these people how many were (*Eijogi , ji adi mane)*

(i) Children under two years of age?(*Nyithindo ma pok oromo higni ariyo ?*) ☐☐ *CHILD2YR*

(ii) Children under five years of age? (*Nyithindo ma pok oromo higni abich ?)* ☐☐ *CHILD5YR*

2.5 (a) Were you a smoker during the period before you were admitted (reference period) ? *(Be ne imadho ndawa kapok ne orwaki e wod ?)* *SMOKE*

O Yes O No O Don't know

2.5 (b) If yes how many cigarette sticks per day ? *(Ka e ndawa adi mane imadho odiochieng)* ☐☐ *sticks* *STICKS*

2.6 Was anybody you were living with in the same household at the time a smoker ? *(Be ngama ne idak godo bende ne madho ndawa e ndalo no?)* *SMOKEHH*

O Yes O No O Don't know

2.7 (a) Where was cooking done in your house at that time ? *(Ne utedo kanyee ndalo no ?)* *COKPL*

O Inside the house *(Ei ot)*

O Outside the house *(oko mar ot)*

O Both *(Gite)*

2.7 (b) What was your primary source of cooking fuel in the household at that time?*(Ango mane utiyo godo kar tedo endalo go)* *COKFEL*

O Paraffin stove *(Mafuta)* O Charcoal *(Makaa)* O Gas cooker *(Gas)* *COKFELX*

O Firewood *(Yien*

O Other (specify) *(Mamoko)* ☐☐☐☐☐☐☐☐☐☐☐☐☐☐

2.7 (c) On average, how many hours would you spend in the cooking area while the food is being cooked ? *COKHRS*
(Ka iparo,seche adi ma inyalo kawo e kar tedo seche ma itedochiemo?)

☐☐ *hours*

2.8 (a) Did you ever burn cow dung in the house?*(Bende ne iwango owuoyo e i ot)* O Yes O No O Don't know *COWDUN*

2.8 (b) If yes, how many hours in a day were you exposed to the smoke from the burning cow dung ? *COWDUNHR*
(Ka e ei odiochieng achiel seche adi ma ibete iro mar owuoyo)

☐☐ *hours*

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2.9 Did you or your household own the following animals during the period before you become ill (reference period) ?
(Be ne un gi jamni e ndalo ma ne pok ibedo matuo ?)

Animal	Number owned by house (if none indicate - 00)	Kept inside the house?	Sick or dying animals ?	Close contact or touching	Estimate number of hours of contact per week	Frequency of contact per week	
Chicken *(Gweno)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	CHIK CHIKHSE CHIKSIC CHIKCON CHIKHRS CHIKWK
Ducks *(Atudo)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	DUCK ,DUCKHSE DUCKSIC DUCKCON DUCKHRS DUCKWK
Cows *(Dhok)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	COWS COWSHSE COWSSIC COWSCON COWSHRS COWSWK
Sheep *(Rombe)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	SHEP SHEPHSE SHEPSIC SHEPCON SHEPHRS SHEPWK
Dogs *(Gwogi)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	DOGS DOGSHSE DOGSSIC DOGSCON DOGSHRS DOGSWK
Pigs *(Anguro)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	PIGS PIGSHSE PIGSSIC PIGSCON PIGSHRS PIGSWK
Cats *(Nyambura)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	CATS CATSHSE CATSSIC CATSCON CATSHRS CATSWK
Donkey *(Punda)*	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	DONK DONKHSE DONKSIC DONKCON DONKHRS DONKWK
Others specify *(Mamoko*							X1HSE X1SIC X1CON X1HRS X1WK
	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	X2 X2HSE X2SIC X2CON X2HRS X2WK
	☐☐	O Yes O No O DK	O Yes O No O DK	O Yes O No O DK	☐☐	☐☐	

2.10 (a) If female, Were you pregnant at the time you were admitted to the hospital ? *(Ka in dhako be ne iyache ndalo mane orwaki ei wuod)* *PREG*

O Yes O No O Don't know

2.10 (b) If yes, how many months pregnant were you ? *(ka e ne iyach dweche adi)* ☐☐ *Months*

PREGMTH

☐☐☐☐☐☐