PROFILE OF LUNG PATHOLOGY AT AUTOPSY IN CHILDREN UNDER THE AGE OF FIVE YEARS DYING FROM SEVERE ACUTE RESPIRATORY INFECTIONS (SARI) AT KENYATTA NATIONAL HOSPITAL

BY

DR. JOHN MATHAIYA NJAU (MBChB – UoN)

H58/69006/2011

A dissertation submitted as partial fulfillment of the requirement for the degree of Master of Medicine in Human Pathology at The University of Nairobi

DECLARATION

I, **Dr. John Mathaiya Njau** declare that this dissertation is my original work under the guidance of the supervisors below and to the best of my knowledge it has not been submitted to the University of Nairobi or any institute of higher learning.

Signature: _____ Date: _____

Principal Investigator

SUPERVISORS' APPROVAL

This dissertation has been submitted for examination with our approval as university supervisors.

1. **Prof. Rogena E. A.**
   Associate professor, Anatomic Pathology Thematic Unit,
   Department of Human Pathology,
   University of Nairobi, Kenya.
   Signature: _____ Date: _____

2. **Dr. Gachie A. K.**
   Consultant Pathologist,
   Department of Laboratory Medicine,
   Kenyatta National Hospital, Kenya.
   Signature: _____ Date: _____

3. **Dr. Walong E. O.**
   Lecturer, Department of Human Pathology,
   University of Nairobi, Kenya.
   Signature: _____ Date: _____

DEDICATION

I dedicate this work to all my immediate family members for their overwhelming believe in me, unconditional support and understanding during the whole study period.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude for the great support extended to me by the following institutions and individuals that made this study possible;

1. My family for their unrelenting support, patience and encouragement throughout my study,
2. Ministry of Health - Government of Kenya for sponsoring my post graduate studies,
3. CDC through KEMRI for sponsoring the study,
4. My supervisors; Prof. Rogena, Dr. Gachie and Dr. Walong for their guidance, support, patience and constructive criticism throughout the study,
5. Decedent's guardians/next-of-kin and their families for their noble contribution to the study,
6. The entire Paediatric Respiratory Etiology Surveillance Study (PRESS) team (Dr. Henry Njuguna the PI, Co-PIs, KNH/UoN pediatricians & pathologists, Study Coordinator, Study surveillance officers, Counselor, Laboratory staff from KNH, UoN, KEMRI & CDC, KNH Farewell home staff) for the excellent teamwork and determination for making the entire study successful,
7. US based paediatric pathologists; Prof. Drucilla Roberts (Massachusetts General Hospital) and Prof. Corinne Fligner (University of Washington Medical Center),
8. Special thanks to Dr. Sherif Zaki, chief of infectious disease pathology branch CDC Atlanta for his wise counsel, guidance, material support and collaboration,
9. Staff members and my fellow postgraduate colleagues in the department of Human pathology for their great support and encouragement.

LIST OF ABBREVIATIONS

AdV – Adenovirus

AIDS – Acquired Immunodeficiency Syndrome

ARI – Acute Respiratory Infection

ART – Antiretroviral Therapy

BAL – Bronchio-alveolar Lavage

CDC – Centre for Disease Control and Prevention

CMV – Cytomegalovirus

COD – Cause of Death

COPD – Chronic Obstructive Pulmonary Disease

DAD – Diffuse Alveolar Damage

DALYs – Disability Adjusted Life Years

ELISA – Enzyme Linked Immunosorbent Assay

GAVI – Global Alliance for Vaccines and Immunization

GMS – Gomori Methamine Silver

HBoV – Human Bocavirus

HE – Hematoxylin and Eosin

Hib – Haemophillus influenza type b

HIV – Human Immunodeficiency Virus

HMPV – Human Metapneumovirus

HRV – Human Rhinovirus

IHC - Immunohistochemistry

IMCI – Integrated Management of Childhood Illnesses

KDHS – Kenya Demographic and Health Survey

KEMRI – Kenya Medical Research Institute

KNH – Kenyatta National Hospital

LRTI – Lower Respiratory Tract Infections

MDG – Millennium Development Goals

MITS – Minimally Invasive Tissue Sample

NCAPD – National Coordinating Agency for Population and Development

NP – Nasopharyngeal

OP – Oropharyngeal

PAS – Periodic Acid Schiff

PCP – Pneumocystis Carinii Pneumonia

PI – Principal Investigator

PiV – Parainfluenza virus

PM – Postmortem

PRESS – Paediatric Respiratory Etiology Surveillance Study

RNA – Ribonucleic Acid

RSV – Respiratory Syncytial Virus

rt-RT-PCR – Real Time Reverse Transcriptase Polymerase Chain Reaction

SARI – Severe Acute Respiratory Infections

SARS – Severe Acute Respiratory Syndrome

SOP – Standard Operating Procedure

TAC – Taqman Array Card

TB – Tuberculosis

UoN – University of Nairobi

UNICEF – United Nations Children's Fund

URTI – Upper Respiratory Tract Infection

WHO – World Health Organization

LIST OF TABLES AND FIGURES

CONTENTS

ABSTRACT

Background: Respiratory diseases alone cause an estimated 20% of deaths in children under the age of five attributed to mainly by severe acute respiratory infections, a cause of death that is treatable. Therefore these deaths are, in large part, preventable. The challenge is to know the cause of death, and the co-factors contributing to the death, in the preventable cases. The gold standard of obtaining accurate information on the cause of death is a post-mortem examination.

Objective: To determine the leading causes of fatal respiratory disease and describe the pathological features in the lungs at autopsy among Kenyan children less than five years who died in KNH.

Design: Hospital based prospective descriptive study.

Setting: Kenyatta National Hospital Paediatric wards and Farewell home, University of Nairobi histopathology laboratory and KEMRI-CDC laboratories.

Study population: Decedent children aged 1 to 59 months meeting the SARI case definition of acute respiratory infection and required hospitalization at the time of hospital admission.

Methodology: After death, informed written consent was obtained from next of kin and clinical autopsy conducted using a standardized procedure to collect tissue and fluid samples. Fresh lung tissue was obtained using sterile techniques by both minimally invasive autopsy procedure and open lung biopsy during autopsy. The lung biopsies obtained were prepared for histopathology evaluation and molecular diagnostic tests using PCR technology.

Results: A total of 33 (51.6%) males and 31 (48.4%) females underwent autopsy. The median age (IQR) was 9.5 months (4 – 13.5) with 91.6% of the children aged less than 24 months.

43 (67.2%) children were malnourished with 28 (43.75%) of them being severely malnourished. 12 (18.75%) children had congenital anomalies and trauma was evident in 7 (10.9%) children five of them having head injuries.

At histopathology, acute pyogenic pneumonia was the most observed lung disease pattern at 39.3% followed by interstitial pneumonia at 29.9% mainly due to viral infections and *Pneumocystis jirovecii* pneumonia. Diffuse alveolar damage (DAD) was seen in 21.5% as a

result of acute lung injury and aspiration pneumonia identified in four cases (0.4%), whereas chronic granulomatous inflammation due to *Mycobacterium tuberculosis* was observed in two cases (0.2%)

Klebsiella pneumonia, *Streptococcus pneumoniae* and *Escherichia coli* were the most common bacterial isolates; others included *Staphylococcus aureus, Haemophilus influenza* and Enterobacteriaceae. The commonest viral pathogens identified were RSV and CMV at 14.1% and 12.5% respectively. Fungal infections were evident in 20.3% of the cases with PCP seen in 7 (10.9%) children who had features of immunosuppression. Other fungal agents identified included candida species affecting 4 (6.25%) children and aspergillus species in 2 (3.1%) children.

40 cases were reviewed to compare minimally invasive core needle lung biopsies versus open lung biopsies at autopsy there was concordance on major diagnostic finding at 65% (26 cases), however 14 cases (35%) were discordant. ($p = 0.017$)

Conclusion: Acute pyogenic pneumonia was the commonest cause of SARI deaths in children, significantly due to *Klebsiella pneumoniae*, *Streptococcus pneumoniae* and *Escherichia coli* infections. There was higher than expected opportunistic infections due to PCP and CMV as important causes of SARI deaths. Malnutrition and congenital malformations mainly CHD are major risk factors associated with SARI deaths with a significant number of children having life threatening physical trauma. There was positive concordance of major histopathologic findings with minimally invasive tissue samples (MITS) and full autopsy samples.

1.0 INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

This study focuses on paediatric acute respiratory infection (ARI). There are a variety of definitions available for what constitutes ARI although all relate to the clinical manifestation of an infection of the airways.

The World Health Organization defines ARI as a "presumed pneumonia" (1), concentrating mainly on infection of the lower respiratory tract and producing guidelines for clinical diagnosis of pneumonia in absence of radiology.

Simoes et al. described ARI as infection of the upper and/or lower respiratory tract. The upper respiratory tract consists of the airways from the nostrils to the vocal cords in the larynx, including the paranasal sinuses and middle ear. The lower respiratory tract covers the continuation of the airways from the trachea and bronchi to the bronchioles and the alveoli (2).
In literature, ARI is also often defined by clinical parameters. For example, Regamey et al. defined ARI as "more than two days with cough or wheeze, together with fever of above 38°C, acute rhinitis, otitis media or pharyngitis" (3).

In this study, the definition of ARI will be in keeping with that described by Simoes et al. with ARI constituting any infection of the respiratory tract, both upper and/or lower.

1.2 CLINICAL MANIFESTATIONS OF ARI

Classification of ARI can be further subdivided based on anatomy and clinical manifestation i.e. upper respiratory tract infections (URTI) and lower respiratory tract infections are infections (LRTI).

In reality many ARIs involve more than one anatomical location, especially in the upper respiratory tract. The further classifications and diagnoses are described as follows.

1.2.1 Upper Respiratory Tract Infections

These are infections which occur proximal to the entrance of the airways i.e. from the nostrils and mouth to the trachea, and including the paranasal sinuses and the middle ear. Usually these infections are mild but can progress to the lower respiratory tract or cause long term complications. This group can be further subdivided by clinical diagnosis which includes rhinitis, sinusitis, ear infections, acute pharyngitis/tonsillopharyngitis, epiglottitis and laryngitis/croup (2). Often a number of these clinical diagnoses are present at any one time.

1.2.2 Lower Respiratory Tract Infections

These are infections at any point past the trachea to the terminal end of the respiratory tract at the level of the alveoli. The main diagnoses are bronchiolitis, pneumonia and viral induced wheeze/asthma (2). In general a LRTI is more severe than URTI with increased likelihood of mortality.

1.2.2.1 Pneumonia

Of all clinical presentations of ARI, pneumonia is most associated with mortality. Both bacteria and viruses can cause pneumonia. Clinically URTI often precedes pneumonia. Symptoms of pneumonia include fever, rigors, malaise, cough and dyspnoea (4). The cough can be productive of sputum of a purulent colour. Clinical signs include dyspnoea, chest in-drawing, dullness to chest percussion, crackles and/or wheeze on auscultation (5). Very severe cases may present with convulsions or coma, organ failure may be present.

Approximately 13% of pneumonia cases are severe enough to require hospitalization (6). Of all the pneumonia cases occurring in countries with high incidence, 8.7% are severe enough to be life threatening (7). Severe pneumonia in childhood is associated with increased long-term respiratory morbidity and disease burden (8) and is more fatal than non severe disease (9). Understanding the epidemiology of severe pneumonia has been identified as a pressing priority for public health research (10).

Risk factors for pneumonia have been classified into three groups: definite (most evidence consistently pointing to the role of the risk factor); likely (most evidence consistently pointing to the role, but with some opposing findings; or scarce but consistent evidence of the role) and

possible (with sporadic and inconsistent reports of the role in some contexts (7). Risk factors for more severe disease include malnutrition, HIV or other immunosuppressive disease, household smoking and cessation of breastfeeding before 6 months of age (11). Studies on the risks factors for severe pneumonia are few in the literature and done more than a decade ago (12, 13).

1.2.2.2 Bronchiolitis

Bronchiolitis describes "an acute respiratory illness that affects infants and young children with coryza and low-grade fever that progresses over a few days to cough, tachypnoea, hyperinflation, chest retraction and widespread crackles, wheezes or both" (14). Bronchiolitis is a common clinical syndrome in young infants with hospital admission rates of 30 per 1000 for children younger than 1 year (14). This condition appears almost exclusively in children less than 1 year old, with the majority treated with supportive therapy.

The most common causative pathogen of bronchiolitis is human respiratory syncytial virus (RSV). RSV is detected in 42 – 75% of children presenting with bronchiolitis (16, 17, 18). Other pathogens have also been detected such as AdV, influenza, PiV, hRV, hMPV, coronavirus and human bocavirus (16).

1.2.2.3 Episodic viral wheeze / Asthma

Wheeze is caused by reduction in airway cross section of airway or increase in airway compliance (19). There is clinical overlap between viral induced wheeze of infancy and childhood asthma. Wheeze is the predominant clinical sign in both and in the majority of cases there is preceding URTI before wheeze appears 1 – 2 days later. One third of the children with episodic viral wheeze as a young child will go on to develop atopic asthma (20). Overlap is also seen between episodic viral wheeze and bronchiolitis, with 75% of children admitted to hospital with acute viral bronchiolitis in the first 4 months of life going on to develop subsequent episodic wheeze with viral respiratory infection (19). Episodic viral wheeze is a series of discreet episodes of respiratory distress characterized by wheeze whereas asthma later develops into a chronic disease with exacerbations of sudden deterioration in airway function, day-to-day variation in airway function and fixed or persistent airway obstruction (19).

Risk factors for development of asthma and episodic viral wheeze include maternal smoking in pregnancy, history of maternal wheeze, prematurity and reduced lung function in the neonatal period (19).

1.3 THE EPIDEMIOLOGY OF ACUTE RESPIRATORY INFECTIONS IN CHILDREN

1.3.1 The global burden of ARI

Globally, more than 10 million children die each year. Majority of these children die from preventable causes and most occur in 42 countries that are classified as poor countries (1). Six of these countries account for 50% of worldwide deaths in children younger than 5 years, and the rest of the 36 countries the remainder 40% (1).

ARIs represent a large worldwide burden in paediatric mortality and morbidity. Children under five years may experience three to six episodes of ARIs annually regardless of their socio-economic status (2). WHO data recognizes ARI primarily pneumonia as the largest identifiable cause of mortality in children under 5 years, accounting for 19% of deaths, (i.e. one in five under five deaths worldwide) yet inadequate attention is paid to this disease (21, 22).

Overall incidence of pneumonia worldwide is estimated to be over 151 million cases per year, of which 7 – 13% require hospital admission (23). Pneumonia kills more children than any other illness, more than AIDS, malaria and measles combined making it the leading cause of deaths in children aged less than five years (2, 24) which is also higher than both malnutrition and malaria deaths in this age group (1). This equates to over 2 million deaths per year, with some opinions suggesting this may be an underestimate due to misdiagnoses such as malaria and variable definitions of ARI being used for classification (25, 26). Many pneumonia deaths are in malaria prevalent regions where pneumonia mortality is frequently misclassified as malaria (11).

Pneumonia is highlighted as one of the primary targets to reduce worldwide mortality in children under five as part of the Millennium Development Goal (MDG) (11). It is estimated half of these deaths could be prevented through vaccination and even more could be treated with inexpensive antibiotics, highlighting the necessity to confront this condition globally (27).

ARI morbidity is also significant worldwide. WHO estimates ARI to cause over 94 million disability adjusted life years (DALYs) worldwide in all age groups (28). The majority of

pneumonia cases do not result in mortality and in developing countries more than 25% of children will suffer pneumonia each.

1.3.2 The burden of ARI in children in Africa

The burden of mortality is significantly higher in the developing world with most of ARI mortality being in Africa and South East Asia and the majority of deaths occurring in 42 countries of the world (26, 29). Reasons for this include lower vaccination coverage and reduced access to healthcare (2).

Throughout the last decade, deaths due to ARI remained constant or even rose in parts of both Asia and Africa. Estimates indicate that over two million children die each year from ARI with about 70% of these deaths occurring in Africa and South East Asia (2).

The incidence of pneumonia in children under the age of five years is 0.29 episodes per child-year, which equates to 151.8 million cases annually in developing countries, a further 4 million cases occur in developed countries. Fifteen countries contribute 74% of the world's annual pneumonia cases (2).

The mortality rates of children under the age of five years in most developing countries ranges from 60 to 100 per 1000 live births, one fifth of these deaths are due to pneumonia (21). Half the world's deaths due to pneumonia in children under five occur in Africa (30). In sub-Saharan Africa, the estimated proportion of death in children in this age group attributed to pneumonia is 17 – 26% (1).

Mortality is especially high in the first six months of life. This makes it an important area of overlap with newborn health. Acute ARIs are also involved in a large proportion of deaths due to measles and HIV/AIDS (2).

1.3.3 The Kenyan scenario of ARI in children

Kenya is one of the 42 countries that account for 90% of all under-five deaths in the world. Findings of the 2003 Kenya Demographic and Health Survey (KDHS) reveal that one in every nine children born dies before age five, mainly of acute respiratory infection, diarrhoea, measles, malaria, and malnutrition (31).

According to reports from the Central Bureau of Statistics and the National Coordinating Agency for Population and Development (NCAPD) in Kenya, the infant mortality rate increased from about 60 per 1,000 in 1990 to 74 in 1998 and 77 in 2003, while under-five mortality continued to increase from about 90 per 1,000 in 1990 to 112 in 1998 and 115 in 2003. This is a reversal in trend after global initiatives to improve child health caused a decline in infant and child mortality in Kenya in the 1970s and 1980s (31).

However, according to KDHS 2008/2009 report indicates that both the infant mortality rate and under-five mortality rate dropped slightly to 52 per 1,000 live births and 74 deaths per 1,000 live births respectively. This was just a slight improvement as this implies that one in every 19 children born in Kenya dies before his or her first birthday, while one in every 14 children does not survive to age five (31).

Kenya is currently ranked among the 15 countries with the highest estimated numbers of deaths due to clinical pneumonia, the mortality rate being 50.3 per 10, 000 under fives per year (2). In Kenya, pneumonia is the second leading cause of death among children under the age of five years and causes 16% of deaths in the age group. In 2008 the country had 6,185,800 children under the age of five years, 111,000 of them are estimated to have died, 16% (n=30,000) of them died of pneumonia (30). In most of the health facilities in Kenya, pneumonia in children under the age of five years is currently diagnosed using Integrated Management of Childhood Illness (IMCI) criteria in public health facilities (32).

1.4 CAUSATIVE PATHOGENS OF ACUTE RESPIRATORY INFECTIONS

Literature indicates that ARIs could be caused by either or a combination of both bacterial (e.g. *Streptococcus pneumoniae* and *Haemophilus influenza*) or viral agents (such as influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, and adenovirus). In some cases no known cause can be found in a patient presenting with ARI.

1.4.1 Bacterial Pathogens

Bacterial infection is an important cause of many types of ARI. Research into bacterial causes of ARI has been ongoing for a greater length of time than viral studies, mainly due to the techniques of culturing bacteria for investigation.

The most common bacteria detected in paediatric airways is *Streptococcus pneumoniae* (pneumococcus). It was first characterised more than a century ago and has always been linked with respiratory disease (33). Pneumococcus is commonly found in the upper respiratory tract where it is not associated with disease, however spread to the lower airway can result in severe clinical manifestations of ARI especially pneumonia (33). It is also associated with meningitis and septicaemia, leading to an estimated worldwide mortality of between 700,000 and 1 million children each year (34). Similar to other pathogens of ARI these deaths are significantly higher in the developing world with the majority being in Africa and Asia (34).

Significant developments in treatment and prevention of invasive pneumococcus disease have been made in recent year. Development of a vaccine (firstly polysaccharide and more recently developed into conjugate) and its utilisation worldwide have reduced mortality, with estimates suggesting proportions of deaths from pneumococcus has declined from 36% to 26% worldwide (34). Newer versions of the conjugate vaccine are also being introduced with varieties of vaccine preparations available that target different serotypes of pneumococcus, continued research is ongoing to the impact of vaccination strategy and whether elimination of common strains may allow more unusual serotypes to the airway (35).

Although pneumococcus is the most common bacterial pathogen involved in paediatric ARI, Hib is also a common pathogen. Together pneumococcus and *Haemophilus Influenzae* are estimated to be involved in 50% of childhood pneumonia in the developing world (5). *Haemophilus Influenzae* is estimated to cause 371,000 childhood deaths worldwide being involved in the pathology of pneumonia and also epiglottitis (36). Similarly to pneumococcus however a conjugate vaccine has been developed since the 1990s (after the polysaccharide vaccine showed only modest impact in the 1980's) which has shown promising results worldwide. The *Haemophilus Influenza* conjugate vaccine (Hib) has demonstrated to have high efficacy and is cost effective (36). Vaccination for both pneumococcus and *Haemophilus Influenzae* has been highlighted as high priorities for widespread use in the developing world in order to achieve the

millennium goals of paediatric mortality reduction. Use of Hib vaccine is more widespread worldwide than pneumococcus having been recommended by the WHO in 2005 and pneumococcus in 2008 (37).

Many other bacteria have been associated with ARI, especially pneumonia. Infections such as the fungi *Pneumocystis jirovecii* or the bacteria *Mycoplasma pneumoniae, Chlamydia trachomatis* and *Chlamydophila pneumoniae* have all been shown to be involved with paediatric ARI with comparatively less known than the two most common pathogens. The impact of these atypical bacterial pathogens varies in different studies on ARI and also with conditions the child may have such as HIV (5). Research into these less common pathogens is important as the more common bacteria have shown treatment and vaccination strategies to be an effective method of targeting bacterial disease.

1.4.1.1 Histopathology of Bacterial infections

The different bacterial pathogens are generally diagnosed by cultures of sputum or other respiratory secretions and do not generally require biopsy unless specific culture and sensitivity assays are unsuccessful on sputum samples. The histopathologic hallmark of the process is the presence of acute inflammatory exudates filling the alveolar spaces admixed with fibrin and often with necrosis of alveolar walls. Tissue Gram stains will usually highlight the organisms (38).

1.4.2 Viral Pathogens

Viral pathogens are more difficult to detect and culture leading to late discovery of pathogens. However, since the establishment of viral isolation, a number of studies have indicated that viruses are important causes of upper and lower respiratory tract diseases in infants and young children and are therefore of public health importance (39, 40).

The role of viruses in the aetiology of ARI in developing countries especially Africa is not well studied. The information on virus isolates associated with ARI is important in designing appropriate ARI control strategies including patient management, vaccination programmes, antimicrobial and antiviral therapy.

1.4.2.1 Influenza Virus

Influenza virus is an enveloped, single stranded negative RNA virus of the family Orthomyxoviridae. Influenza viruses are divided by groups with the three types being influenza A, influenza B and influenza C. All influenza A viruses express haemagglutinin (H) and neuraminidase (N) antigens upon their surface which are used to sub classify the virus with 16 H subtypes (H1-16) and 9 N subtypes (N1-9) (41).

The influenza A group of viruses are by far the most common and are responsible for the seasonal epidemics of influenza infections (42). The genome of all influenza viruses are highly plastic and susceptible to point mutations, this is demonstrated in influenza A viruses that regularly show changes in their H and A molecules. These mutations change seasonally altering the ability of the virus to evade the hosts immune system, this phenomenon is described as "antigenic drift" (42).

However periodically dramatic alterations in influenza A antigens are observed, usually every few decades. Significant changes in antigen expression results in increased susceptibility of the population to influenza infection and results in distinctly different antigenic character of influenza virus termed "antigenic shift" (42). This characteristic mutation in antigens has also allowed influenza to pass between species in the past with avian and swine transmission leading to novel influenza subtypes.

Most recently, in the spring of 2009, antigenic shift was once again observed in influenza virus of H1N1 subtype when a novel influenza virus (H1N1) appeared in Mexico causing cases of severe viral pneumonitis (43). This strain rapidly spread throughout the world and by June 2009 the WHO declared that this novel influenza virus had reached the level of global pandemic (44, 45). This newly formed virus was thought to be a product of four previously known strains of influenza A one from humans, one from birds and two from swine origin (46). Early on in the pandemic it was shown that children were more likely to suffer with infection and over sixty percent of cases in the US were patients under the age of eighteen (47). The severity of the infection has also been found to be greater in children. An age of less than five years being a high risk factor for severe illness, and risk was especially high if under two years of age (48).

In summary influenza represents one of the most common and also one of the most variable viral respiratory pathogens involved in ARI. Its predictable seasonal nature has led to developments in vaccination and prevention which have helped to reduce burden. However significant shifts in antigen structure can lead to varying clinical presentations and severity of this pathogen, most recently demonstrated by the novel H1N1 pandemic.

1.4.2.2 Parainfluenza Viruses

Parainfluenza viruses (PIV) are enveloped single stranded RNA viruses of the family Paramyxoviridae (49). There are four separate types of PIV designated PIV-1, PIV-2, PIV-3 and PIV-4. PIV is detected in between 9% and 30% of childhood ARI presentations and causes both URTI and LRTI clinical manifestations (50).

There is some variation in clinical presentations between PIV types. PIV-1 commonly presents as croup in childhood and can be detected in up to 50% of children presenting to hospital with the condition (50). PIV is also commonly detected in bronchiolitis, although in much smaller amounts than RSV, being detected in 10 – 15% of cases with the most common subtypes being PIV-1 and PIV-3 (50).

1.4.2.3 Respiratory Syncytial Virus

Human Respiratory Syncytial Virus (RSV) is a member of the paramyxovirus family (51). The single stranded RNA genome is contained within a lipid envelope with surface glycoproteins (14). There are two major strains of RSV, A and B. Some studies have reported RSV A strain being responsible for more severe bronchiolitis (51). RSV is one of the most highly prevalent pathogens in childhood respiratory disease and can present as bronchiolitis, pneumonia, otitis media, rhinitis or sinusitis. The most common presentation is bronchiolitis, being detected in over 70% of children hospitalised with bronchiolitis (14, 16).

1.4.2.4 Adenovirus

Adenoviruses (AdV) are non-enveloped DNA viruses of the family Adenoviridae first discovered in 1953 (49). They most commonly present as respiratory infections but can cause conjunctivitis and gastroenteritis (52). They are responsible for 5 – 10% of respiratory tract infections in children (49, 53). In 2010 there were 53 known subtypes of AdV although this is expected to rise (53).

1.4.2.5 Rhinovirus

Human rhinovirus (hRV) belongs to the family of viruses picornaviridae along with poliovirus, hepatitis A and enterovirus (49). This family of viruses shares a non-enveloped structure with single stranded RNA genome (ssRNA). It was first discovered in 1950s during research into the cause of the common cold (52).

In recent years, research brought a different perspective on hRV infection. hRVs can reproduce in and infect the lower respiratory tract and cause pneumonia (54). hRVs are also the commonest cause of asthma exacerbations in the young and COPD exacerbation in adults (55, 56).

1.4.2.6 Bocavirus

Human bocavirus (hBoV) was described in 2005 by a team in Sweden (57, 58). It belongs to the family parvovirus and is the first of such viruses to be discovered in humans. Despite its recent discovery hBoV appears to have an important role in ARI, with antigen studies showing almost all children have been exposed by the age of five years (57). Exact prevalence of hBoV reported in ARI has varied with it being described in 2.9-19% of ARI (43).

1.4.2.7 Human Metapneumovirus

Human Metapneumovirus (hMPV), discovered relatively recently in 2001, is of the paramyxovirus family and is the first virus within this family that has been shown to infect non-avian hosts (57). hMPV is difficult to grow in culture making it difficult to analyse its pathogenesis. It was discovered in nasopharyngeal aspirates of children with respiratory infection in whom no other pathogen could be discovered. It has been reported that hMPV is present in between 2% and 20% of ARI and antibodies to HMPV are present in 100% of children by 5 years of age, which some believe indicate that all children are mildly or sub-clinically infected by this pathogen (58).

Clinically hMPV can present as both URTI and LRTI, although LRTI is more common in children under the age of one year, being one of the most common causes of bronchiolitis second only to RSV (57, 58).

1.4.2.8 Histopathology of viral infections

Viral infection of the pulmonary tract may manifest in a variety of histological ways with several possible patterns of lung injury, including diffuse alveolar damage (DAD), acute bronchitis, organizing pneumonia, and diffuse interstitial pneumonia. The histopathologic diagnosis of specific viral infections, however, can be made only by recognizing their characteristic cytopathic effects, including nuclear and cytoplasmic alterations as seen on hematoxylin- and eosin-stained histologic slides on routine light microscopy. Several specific antibodies have been developed for use by immunohistochemistry to identify several common pulmonary viral pathogens (59).

The most common pattern of lung injury owing to viruses is diffuse alveolar damage (DAD). However, this is a very nonspecific injury pattern in the lung that can be caused by many other insults, including infections by bacteria, mycobacterial and fungal pneumonias, various forms of lung toxicity, drugs and inhalation of toxic fumes. A careful search for viral cytopathic effect as well as correlation with the clinical history is indispensible for correct interpretation. The commonest cytopathic effect caused by viruses in the lungs is the presence of cytopathic or intranuclear inclusions within the infected cells. However, some viral infections affecting the lungs such as influenza virus and severe acute respiratory syndrome (SARS) coronavirus do not produce any inclusions that are discernible on routine histologic examination (60).

1.4.3 Fungal Pathogens

Fungal infections in immune deficient pediatric patients are becoming more frequent because effective treatment permits longer survival of these patients. At risk for opportunistic fungal infections or disseminated endemic fungal infections are those children who have received transplants, prescribed immunosuppressive and chemotherapeutic agents, HIV-infected patients and premature infants. The diagnosis of fungal infection versus colonization is a frequent problem that has important therapeutic implications for these patients (61, 62).

Candidiasis

Candidiasis is caused by yeast that is normal commensal organism in the respiratory, gastrointestinal, and genitourinary tract or normal individuals. In immunocompromised states, these organisms become pathogenic and invade tissues with the most common organism is

Candidiasis albicans (38). Immunocompromised children are at high risk of invasive candidiasis infection. Once Candida organisms gain access to the blood, secondary seeding into all organs can occur. Common localizations include the liver and spleen in neutropenic hemato-oncologic patients (61).

Aspergillosis

Aspergillosis is an infection caused by a mold, aspergillus and the most common types are *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger* (60). Invasive aspergillosis is a disease that occurs in severely immunocompromised patients, including patients with prolonged neutropenia, hematopoietic stem cell and solid organ transplant recipients, patients with AIDS, premature newborns and patients with chronic granulomatous disease. The disease most frequently involves the respiratory tract and the signs and symptoms include fever, cough, dyspnea and hemoptysis (61).

Cryptococcosis

Cryptococcosis is a systemic-opportunistic mycosis caused by inhalation of spores of two species of the encapsulated yeast-like organism, *Cryptococcus neoformans* and *Cryptococcus gattii*, which cause infection in immunocompromised individuals and in immunologically normal hosts, respectively (60, 61). Most susceptible to infection are patients with T-cell deficiencies. The spectrum of disease ranges from asymptomatic pulmonary lesions to disseminated infection with meningoencephalitis. After the emergence of AIDS, cryptococcal infections have become much more common (61).

Histopathology of Fungal infections

Histologic examination often permits a rapid diagnosis and will allow the pathologist to assign the organism to a group or genus (38). Definitive speciation can be obtained later and confirmed by culture, immunonohistochemistry or PCR. Although some fungi are visible on hematoxylin and eosin (H&E), special stains such as periodic acid-Schiff (PAS), and silver stains such as Gomori methamine silver (GMS) or Grocott stains are usually needed for proper identification (63, 64, 65).

Fungi may be seen in the lungs as yeast (round spherules), hyphae (long tubular structures) and pseudohyphae (long tubular structures with regularly spaced constrictions). Fungal infections can

cause several patterns of tissue damage in the lungs including necrotizing and nonnecrotizing granulomas, cavity lesions acute bronchopneumonia and airway disease (60, 63). The morphology of the organism can be a helpful clue for diagnosis.

1.5 ARI IN HIV/AIDS

Two highly prevalent conditions in the developing world, especially Africa, are malaria and human immunodeficiency virus (HIV). Both of these are associated with increased severity of ARI and it has been reported that malaria may cause underreporting of ARI mortality due to misdiagnosis (25).

Immunodeficiency is a well documented cause of increased ARI susceptibility with the most common reason worldwide being HIV. Globally, there are also approximately 2.3 million HIV-infected children, living predominantly in sub-Saharan Africa (66). HIV infected children in Africa experience a higher incidence of pneumonia and tuberculosis, poorer response to treatment, and higher mortality from pneumonia and tuberculosis than HIV uninfected children. Although improved access to antiretroviral therapy (ART) has markedly reduced this morbidity and mortality, less than 50% of those who need it currently access ART, and even on ART it appears that they continue to experience more respiratory morbidity than HIV negative children (67).

The major identified aetiologic agents are bacteria (*Streptococcus pneumoniae, Haemophilus influezae type B*, Staphylococcus and viral (Respiratory syncytial virus). In addition HIV infected children commonly have gram negative bacterial pneumonia and opportunistic organisms such as *Pneumocystis jiroveci* and cytomegalovirus, sometimes with polymicrobial infection (68).

Tuberculosis (TB) is also a major cause of respiratory morbidity and mortality both among HIV infected and uninfected children. In a Zambian postmortem study, *Mycobacterium tuberculosis* occurred in 18% of HIV-infected and 26% of HIV-uninfected children, and was the second most commonly identified cause of death in children older than 1 year (68).

The increasing antimicrobial resistance observed in HIV infected children is of concern as it poses treatment difficulties for physicians due to the fact that immunocompromised patients have relative inability to clear infections (69).

1.5.1 Histopathology of Mycobacterium infections

The pathologic findings in TB are mainly related to the destructive tissue reaction at the sites of the infection. The most common reaction pattern is characterized by large, confluent necrotizing granulomas that show peripheral palisading of histiocytes, epithelioid histiocytes and frequent Langhans-type multinucleated giant cells (70).

A miliary pattern characterized by small epithelioid granulomas can also be observed. The lesions can sometimes be characterized by scattered microscopic granulomas and isolated Langhans giant cells distributed in the peribronchial location. The lesions contain bacilli mostly in the necrotic centre but they can also be identified within epithelioid histiocytes and multinucleated giant cells (70).

The organisms are best identified on acid-fast stains; Ziehl-Neelsen will stain the small beaded and thin bacilli bright red. Fluorescent staining, immunoperoxidase and PCR methods can be used to demonstrate the organism in tissues (71).

1.6 OTHER RISK FACTORS FOR ARI

There are a number of recognized risk factors that increase both the probability of suffering ARI and the likelihood of severe infection. Recognized risk factors of both bacterial and viral infections have led to development of a targeted approach to prevention of disease.

Frequency of ARI correlates closely with age, children being much more likely to be infected than adults. It is estimated that children suffer 6 – 8 respiratory infections a year compared to 2 – 4 in adults (72). Majority of these infections are mild and confined to the upper airway, resolving without presentation to a healthcare professional. However, each one of these infections has the potential to progress into a more severe clinical presentation or systemic illness. There are also observable differences in children that occur with age, with infants under two years being at especially high risk of severe LRTI due to the small calibre of their airways and their naivety to viral infections once maternal antibody has waned (73).

Previous respiratory and cardiovascular pathology is also associated with increased risk of severe ARI. In children conditions such as asthma or cystic fibrosis can increase the likelihood of respiratory infection, whereas in adulthood chronic obstructive pulmonary disease (COPD) can increase risk of hospitalization with ARI (2, 74). Pulmonary hypoplasia is recognized as a risk

factor for severe viral infection, especially RSV disease in infancy; it also correlates closely with prematurity which also is recognized to be associated with ARI (75).

Worldwide distribution of ARI severity is also skewed towards the developing world, especially Africa and Asia. It is estimated 70% of ARI burden is represented by the developing world with the majority of pneumonia mortality worldwide being present in 42 countries (2, 76). There are multiple reasons for this including lower levels of vaccination and reduced availability of hospital care (2, 77). Vaccination levels for Hib and Pneumococcus are lower in the developing world due to problems with cost and distribution; however international organizations such as the WHO and the Global Alliance for Vaccines and Immunization (GAVI) in recent years have aimed to make these more accessible (37).

Malnutrition is also an important factor which is more prevalent in the developing world and is associated with increased severity of ARI and increased risk of mortality in children (78). In the developing world it is also much more prevalent to cook with biomass fuels in an enclosed indoor setting, which is associated with increased risk of ARI (79).

Lifestyle factors can also affect a child's risk of ARI. The most recognized factor is parental smoking, both during pregnancy and infancy. It has been shown that parental smoking is associated with increased frequency of respiratory infections as well as higher prevalence of adolescent asthma, wheeze, middle ear infections and reduced lung function (80). In the post-natal period environmental tobacco exposure causes airway release of pro-inflammatory cytokines and induces hyper-reactivity (80, 81). A second suggested explanation is prenatal smoking being associated with other ARI risk factors such as prematurity and reduced birth weight (80). A household environment with postnatal smoking is also associated with respiratory infections and asthma in the absence of prenatal smoking (80).

Finally certain combinations of respiratory infections have been shown to produce more severe ARI in children. These findings are much debated in the literature but one combination that is agreed upon is influenza infection in the presence of pneumococcus infection (82). The interaction of respiratory pathogens increasing risk of severe ARI is important as it further highlights the need for full assessment of pathogens present during ARI.

1.7 POST MORTEM STUDIES IN CHILDREN

The causes of death differ substantially from one country to another, highlighting the need to expand understanding of child health epidemiology at a country level rather than in geopolitical regions (81). Other key issues include the importance of under-nutrition as an underlying cause of child deaths associated with infectious diseases, the effects of multiple concurrent illnesses, and recognition that pneumonia and diarrhoea remain the diseases that are most often associated with child deaths (22, 84). A better understanding of child health epidemiology could contribute to more effective approaches to saving children's lives (83).

Presently, an autopsy is the accepted gold standard for the determination of the cause of death. There are two types of autopsies/postmortem examination;

1. Hospital/Clinical autopsy; this is made at the behest of the medical personnel or relatives of the deceased.
2. Medico-legal autopsy; this is mandated by the law.

An autopsy is important in providing the following;

1. Establishing the cause of death,
2. Assisting in determining the manner of death,
3. Comparing the antemortem and postmortem findings,
4. Producing vital statistics for public health monitoring and evaluation, and
5. Provide a good index of the quality of patient care, both in terms of the accuracy of clinical diagnosis and the quality of treatment given.

Several studies done (Kumar et al., Brodlie et al. and Elder et al.) have shown that new diagnosis is found at autopsy in 29 to 79% of autopsies done on children 0 to 60 months of age (85, 86, 87).

Kumar et al. conducted a study on the usefulness of autopsies in children from 1984 to 1993. They concluded that, autopsy can provide additional information in more than one third of pediatric deaths. Pediatric autopsy continues to provide clinically significant data and remains a valuable tool in modern pediatric practice (88).

Dalal et al. carried out an autopsy study of paediatric deaths aiming to help in a better understanding of causes of deaths in neonates, infants and children. They concluded that, autopsy is an important clinical tool providing useful information to the physician, but there are few published reports available on pediatric autopsies and hence the need to conduct more studies on paediatric autopsies (89).

In 2010, 2315 children died at the KNH. Of the 485 autopsies done in the same year, only 6% were on children (0 – 18 years) with children under 5 years making up 2.8% of the total (90).

Karau et al. carried out a study on the knowledge, attitude and practice of bereaved parents and healthcare providers towards autopsies in children under-five years at KNH in 2011 and concluded that 79% of the bereaved parents had adequate knowledge as regards autopsy with a positive attitude being significantly associated with the level of education. 67.4% of the bereaved parents were not asked to consent to an autopsy on their deceased child. Parents with higher understanding of autopsy were more likely to consent. All health care providers had a positive attitude to autopsy, however the main reason for not obtaining consent for autopsy were due to lack of formal training in obtaining consent and the failure to obtain autopsy results in timely manner (91).

They concluded that rate of paediatric autopsies at KNH needs to be improved and this can be well achieved by counseling the bereaved parents on both the need for autopsy as well as demystifying potential barriers to consent including religious and traditional beliefs. Health care providers need to be equipped with counseling and consent obtaining skills and by encouraging their participation in autopsies (91).

Autopsies play a crucial role in the development of science and the practice of medicine as they generate accurate vital statistic, provide pathological descriptions of new diseases and offer powerful tools for education, quality assurance and in research.

2.0 STUDY JUSTIFICATION

According to WHO/UNICEF estimates, 46% of the world's under-five mortality occurs in Sub-Saharan Africa. Respiratory disease is the number one cause of hospitalization and death in African children, with an estimated 35 million cases (compare to industrialized countries 1.6 million cases) and over one million deaths annually. Out of every two children that die in the world from pneumonia, one is an African child (92).

Many children suffer with respiratory infections with significant morbidity, cost to the society due to parental work absence, cost due to hospitalizations, and educational morbidity due to school absences, but do not die. Why some children die from their respiratory infections and others do not is likely complex but identifiable variables are known, for example air pollution is known to increase the frequency of childhood asthma hospitalizations and the severity of respiratory infections (93).

Despite its importance in regard to morbidity as well as childhood mortality, the epidemiology and pathogenesis of ARI, particularly in Africa, remains understudied and consequently underappreciated. If we can identify these variables that can be obviated, then we may be able to decrease this category of childhood morbidity and mortality (94).

The target of MDG 4 is to "Reduce by two thirds, from 1990 to 2015, the under-five mortality rate". Efforts to improve child survival can be effective only if they are based on reasonably accurate information about the causes of childhood deaths (95).

Accurate information on specific diseases in African children dying from respiratory illnesses is scarce. There is a need to obtain such data to place into perspective the relative importance of specific diseases as causes of mortality in African children. Knowledge of the causes of childhood respiratory diseases in a community is important in development of practical diagnostic, therapeutic, and prophylaxis protocols, and for epidemiological surveillance and control (68).

Empiric management can be effective only if data about disease frequency, morbidity, and mortality are available. Since autopsies are the only way to obtain information on the actual causes of death, the principal investigator undertook an autopsy study to find out the cause of

death and define the aetiological range of fatal acute pulmonary infections in Kenyan children in Kenyatta National Hospital, assess relations between these diseases and other risk factors associated with these deaths.

The scope of this study has enormous public health ramifications, and importantly, provides a rational foundation for developing, implementing, and monitoring prophylactic and therapeutic interventions.

3.0 RESEARCH QUESTION

What are the pathological lung changes at autopsy in children aged 1 to 59 months old dying from SARI at Kenyatta National Hospital?

3.1 BROAD OBJECTIVE

1. To describe the pathological features of the lungs at autopsy in children aged 1 to 59 months dying of SARI at Kenyatta National Hospital.

3.2 SPECIFIC OBJECTIVES

1. To determine the organisms associated with pediatric respiratory deaths at Kenyatta National Hospital.
2. To assess relations between the SARI and other risk factors associated with these deaths.
3. To compare minimally invasive lung tissue sampling with full post-mortem lung biopsy histopathology evaluation and detection of pathogenic organisms.

4.0 MATERIALS AND METHODS

4.1 STUDY DESIGN

This was a hospital based prospective descriptive study of pediatric respiratory deaths meeting the SARI case definition.

4.2 STUDY AREA

The study was conducted at Kenyatta National Hospital located in Nairobi which is Kenya's main National Referral and Teaching Hospital. It also serves the population of Nairobi and its environs as a Provincial/Level 5 Hospital. The study was mainly centered in KNH paediatric wards, KNH farewell home, UoN histopathology laboratory and KEMRI-CDC laboratories.

4.3 STUDY DURATION

The study was conducted from September 2014 to February 2016.

4.4 STUDY POPULATION

The study population included all children who died at KNH aged 1 to 59 months and met the SARI case definition.

4.4.1 Case definition

SARI was defined as an acute respiratory infection with:

- History of fever or measured fever ≥ 38° C,
- And cough,
- Onset of symptoms within the last 14 days ,
- And requires hospitalization.

4.4.2 Inclusion criteria

1. Decedent 1 to 59 months of age who met SARI case definition at the time of hospital admission.
2. Decedent whose next of kin provided written consent for autopsy and specimen collection.
3. Decedents whose autopsy interval was within 48 hours but at least less than seven days, provided that the body was refrigerated within 48 hours of death.

4.4.3 Exclusion criteria

1 Decedents less than one month or greater than five years of age.

2 Deaths that did not meet the SARI case definition upon admission to the hospital.

3 Decedents without informed consent.

4.5 SAMPLE SIZE DETERMINATION

The sample size was determined by using the formula with finite population correction (Daniel, 1999) as follows;

$$n = \frac{\mathrm{NZ}^2\ \mathrm{p}(1-\mathrm{p})}{\mathrm{d}^2(\mathrm{N}-1) + \ \mathrm{Z}^2\mathrm{p}(1-\mathrm{p})}$$

Where:

n = Required sample size

N = Population size in this case 200 being the sample size of the main paediatric autopsy study,

Z = Critical value for a 95% confidence interval = 1.96,

p = Expected proportion (in proportion of one) 0.5, and

d = Estimated level of precision in this case is 10%.

Using this formula;

$$n = \frac{200(1.96)^2\ 0.5(1-0.5)}{(0.1)^2(200-1) + (1.96)^2 0.5(1-0.5)}$$

$$n = 65.103$$

$n = 65$ cases

Sampling Method

All deceased children who met SARI criteria in order of reception at KNH farewell home and body well preserved according to the standard operating procedure.

4.6 METHODOLOGY

This study was build upon a pre-existing sentinel surveillance system for hospitalized Severe Acute Respiratory Illness (SARI) and influenza that was already in place. In this surveillance system, consenting patients meeting the WHO SARI case definition verbally completed a structured enrollment questionnaire.

For SARI patients that died while hospitalized for their illness, and for whom consent was obtained from the family, the principal investigator performed minimally invasive and standard complete autopsies, and also collected post-mortem specimens that were used to establish the causes and etiologies of death. Molecular diagnostic testing of aseptically collected specimens utilizing Taqman Array Card (TAC) and conventional real time RT-PCR technologies were used to identify any pathogens that may have been associated with mortality.

In quality specimens whereby no pathogens were identified, the principle investigator collaborated with viral and bacterial pathogen discovery laboratories at CDC/Atlanta in an effort to further detect the presence of novel pathogens.

4.7 POST-MORTEM EXAMINATION

4.7.1 Post Mortem Procedures

Surveillance officers followed the SARI cases prospectively until discharge from the hospital, or death. At time of outcome (in this case death) a follow-up questionnaire was used to collect data on treatment, routine laboratory tests, radiological investigations, final diagnosis by attending clinician and date of death.

At the time of death, the decedent child's next of kin were consoled and counseled by a trained grief counselor. At an appropriate time as determined by the grief counselor, the details of the study was explained to the parent or guardian, information about the study in the appropriate language was given, and an informed written consent to perform the post mortem studies was obtained from the next of kin by a pediatric resident or the principal investigator (Appendix 4). Some of the parents or guardians were allowed time to consult any relatives present and come back with questions. When the parents or guardians declined to give consent, the main reasons provided were recorded.

For the subset of SARI cases that die within the hospital, the body was wrapped in sterile gowns, packed and a special identity tag attached in the ward. The body was collected within 6 hours of death, transported to the mortuary and refrigerated immediately at 4 to 8°C. Post-mortem was conducted within 24 hours from the time consent was given.

Post-mortem specimens were collected according to the protocols in the department of Human Pathology UoN/KNH. Specimen preparation and testing took place at KNH/UoN and the KEMRI/CDC laboratories.

The post-mortem procedures were carried out in three phases,

1) A nasopharyngeal swab specimen was collected by the principal investigator.
2) Transthoracic percutaneous core needle biopsies were collected from each lung via the dependent – dorsal aspect at supraclavicular regions, and the mediastinal region between the second and forth rib for histopathology, and prepared for testing using the molecular diagnostic testing process described below.
3) The full post-mortem followed a standardized procedure (Appendix 5). Photos of organ were taken to record gross pathological features using a digital camera. In addition two sections from the upper, middle and lower zones of each lung as well as one section each from lymph nodes in the left and right hilum were taken for histopathology. Additional sections were taken as indicated from the gross examination. The fresh lung tissue was divided into two specimens of at least 1 cc each and prepared for histopathology and testing using the molecular diagnostic testing
4) Autopsy findings, both gross and histopathology, were recorded on a data capture sheet. Relevant information including the clinical notes and past medical records for the deceased was obtained from the hospital records department. Anonymzed and abstracted data from the SARI data collection form was made available to the principal investigator to aid in pathological interpretations.

4.7.2 Post-mortem laboratory testing procedures

1) Nasopharyngeal swab, core needle biopsies and lung autopsy specimens were separately tested at the CDC/KEMRI Laboratories using the Version 4 Taqman Array Card (TAC) which uses PCR technology to simultaneously target 13 bacterial (*Streptococcus*

pneumonia, Streptococcus pyogenes, Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, Pneumocystis jirovecii, Legionella spp, Mycoplasma pneumonia, Chlamydia pneumonia, Klebsiella pneumonia, Bordetella pertussis, Moraxella catarrhalis, Mycobacterium tuberculosis) and 7 viral (Influenza A, B, C, Parainfluenza 1,2,3,4, Respiratory Syncytial Virus (RSV), Rhinovirus, Enterovirus, Human metapneumovirus (HMPV), Coronavirus 1,2,3,4) targets. To provide an added validation to selected TAC findings, BAL specimens were also be tested using traditional real-time reverse transcription PCR (rt-RT-PCR) tests for influenza A and B, RSV, adenoviruses, parainfluenza 1-3, and human metapneumoviruses, and to detect selected bacterial targets including S. *pneumonia*e and *M. tuberculosis*.

2) Dry blood sample was collected during autopsy for HIV ELISA test.
3) Histopathology samples were processed at KNH/UoN histopathology Laboratories following the standard operating procedures (SOPs) for tissue preparation, blocking and mounting.
   a) Special slides recommended for the study were used; clear labeling of the slides was done
   b) Staining of the microscopic sections was done with freshly prepared haematoxylin and eosin stains. Quality of stain was confirmed before mounting of the slides.
   c) After the principal investigator reviewed the histopathological features and diagnosis of the cases, two blinded supervisors independently confirmed these findings. In the event of conflicting opinions among the two pathologists, a third blinded pathologist was consulted. In the event of lack of consensus among the three pathologists, then the diagnosis with majority consensus was accepted.
   d) Data was carefully entered into the respective data collection forms, avoiding mix-ups and transcription errors.

4.8 QUALITY ASSURANCE

- Standard operating procedures were developed and all personnel were trained to ensure systematic collection and analysis of the required specimen (Appendices 4 and 5).
- Sterile procedures were employed throughout to prevent any contaminants from the wards where the bodies were wrapped in sterile drapes, storage under refrigeration at between 2°C and 8°C.
- Aseptic technique was maintained at autopsy during prosection and sample collection for molecular studies.
- All reagents were prepared according to the manufacturer's instructions and controls used appropriately.

4.9 DATA COLLECTION INSTRUMENTS

Data was collected using predesigned questionnaire and report forms.

4.10 DATA MANAGEMENT AND ANALYSIS

- Data on the decedents was abstracted from the patient's file using coded questionnaire bearing a unique identification number by the research assistant.
- All specimens (nasopharyngeal /oral-pharyngeal swabs, trachea-bronchial lavage, needle biopsy, and autopsy specimens) were also assigned the patient's unique identification number so that they are linkable to the epidemiological data collected in the wards.
- Data was first stored in hard copy registers, kept safely & later summarized in using netbooks & finally entered into an excel spread sheet in a password protected computer.
- All statistical tests were performed at 5% level of significance (95% confidence interval) using SSPS version 20.0 software.
- Univariate analysis involved frequency distribution for categorical variables & descriptive statistics (mean, median, SD).
- Categorical variables (e. g. gender) were presented using charts & frequency distribution tables.
- A P-value of < 0.05 was considered to be statistically significant.

4.11 ETHICAL CONSIDERATIONS

1. Permission was obtained from the KNH Ethical and Research committee (KNH-ERC) and the Department of Human Pathology, University of Nairobi and the study undertaken after formal approval.
2. The purpose of the study was carefully explained to the decedent children's next of kin(s) with a view to obtaining written consent prior to enrollment in the study. (Appendix 3)
3. Strict confidentiality was observed throughout the entire study period, held in trust by participating investigators, research staff and the study institutions. The Study participants were given study identification numbers and no personal identification data were recorded. No Information concerning the individual study findings was released to any unauthorized third party without prior written approval of the study institutions or the Ethics Research Committee.
4. After each autopsy, the decedent's next of kin(s) were briefed on the preliminary cause of death and within one month after conducting various tests, the next of kin were contacted and notified in details the actual cause of death and issued with a post mortem report.

5.0 STUDY RESULTS

Between the months of September 2014 to December 2015, a total of 945 respiratory illness cases were identified at KNH in the department of paediatrics. Out of these 200 (21%) deaths due to respiratory infections were recorded.

Of the parents or guardians of these 113 (56.5%) children were reached out and underwent grief counseling. The remaining 87 parents/guardians did not undergo grief counseling due to various reasons among them being that the body was transferred from hospital before the study team could set up a counseling appointment or they could not be reached on phone or the appointment was missed without any explanation.

Of the 113 parents/ guardians that underwent counseling, 64 (56.6%) consented for autopsy. The most common stated reason for consenting for an autopsy in this group was to confirm the cause of death (80%). The other reasons given included to obtain information that may influence future pregnancies and early childhood care (10%) and also to help other children.

A total of 64 children who died at KNH and whose parents/guardian had consented for autopsy were recruited into the study.

Decedents' Biodata

There were 34 (53.1%) males and 30 (46.9%) females giving a male to female ratio of approximately 1:1. The age of the descedent children recruited ranged from 2 to 48 months.

Table 1: Age and sex distribution of deceased children at the time of death

Age group (months)	No. of cases	Percentage	Sex Distribution	
			Males	Females
< 12	43	67.2%	25	18
12 – 23	15	23.4%	6	9
24 – 35	3	4.7%	1	2
36 – 47	1	1.6%	1	0
≥ 48	2	3.1%	1	1
Total	64	100%	34	30

91.6% of the cases were below 24 months of age with 67.2% of all the cases being aged less than 12 months and median age (IQR) was 9.5 months (4 – 13.5).

Duration of hospital stay of deceased children

The length of hospital stay prior to death for this group of children ranged from a few hours to more than 14 days. Duration of hospital stay, median (IQR) was 3 days (1 – 4).

Table 2: Duration of hospital admission for the deceased children

Length of hospital stay (Days)	Frequency	Percentage
< 1	11	17.2%
1 – 3	32	50%
4 – 7	10	15.6%
8 – 14	5	7.8%
> 14	6	9.4%
Total	64	100%

The duration of death to post mortem examination, median (IQR) was 3 days (2 – 4).

Nutritional status assessment

At autopsy, all children had their weights and MUAC taken including the standard athropometric measurements. 43 (67.2%) were found to be malnourished with 28 (43.75%) of the children being severely malnourished.

Table 3: Malnutrition classification

Malnutrition Severity	MUAC (cm)	Frequency	Percentage
None	>13.5	14	21.88%
At risk	12.5 to 13.4	7	10.94%
Moderate	11.5 to 12.4	15	23.44%
Severe	<11.5	28	43.75%
Total		64	100%

Other risk factors assessed

All children demonstrated a respiratory pathology upon examination of the internal organs that mainly included diffuse lung consolidations, several cases had pleural effusion and one case had multiple tubercles and a lung abscess.

12 (18.75%) children had congenital anomalies of which 9 of them had congenital heart diseases mainly septal defects.

Trauma was also evident in 7 (10.9%) children with 5 of them having head injuries while the remaining two; one had a pelvic injury and the other had right upper limb gangrene due to prolonged tourniquet application. Documentation of these cases was also done for medical legal purposes.

Histopathology findings

Lung biopsies were collected aseptically by minimally invasive tissue sampling method through transthoracic percutaneous core needle biopsy and also open lung biopsy for both histopathology examination and pathogen detection.

On microscopy the lungs demonstrated different patterns as shown below with some cases having a mixed picture.

Table 4: Histopathology patterns of the lungs biopsies

Histomorphological patterns observed	Frequency	Percentage
Acute pyogenic pneumonia	42	39.3%
Interstitial pneumonia	32	29.9%
Diffuse alveolar damage	23	21.5%
Aspiration pneumonia	4	0.4%
Atelectasis	4	0.4%
Granulomatous inflammation	2	0.2%
Total	107	100%

Table 5: Leading pathogens detected on lung specimen: histopathology and TAC

Histopathology		TAC	
Pathogen	**Frequency**	**Pathogen**	**Frequency**
Enterobacteriaceae	10	*K. pneumoniae*	10
RSV	9	*S.pneumoniae*	8
S.pneumoniae	8	PCP	7
K, Pneumoniae	8	*H.influenza*	7
CMV	8	*S.aureus*	7
PCP	5	Enterobacteriaceae	6
Candida	4	RSV	5
Adenovirus	3	HMPV	4
M. tuberculosis	2	*E. coli*	3
Aspergillus	2	*M. tuberculosis*	2
PIV 3	2	Adenovirus	2
PIV 1	1	PIV 3	2
Total	62	Total	63

Comparison of minimally invasive tissue sample and complete autopsy lung biopsy

40 cases were randomly selected and lung histopathology reviewed by two separate blinded pathologists. 36 (90%) transthoracic percutaneous core needle biopsies obtained through minimally invasive tissue sampling showed adequate pulmonary tissue for diagnosis. Other non-pulmonary tissues in biopsies included heart, liver, lymph node, thymus, fat and skeletal muscle.

Consensus diagnosis was reached on 39 out of the 40 cases in terms of specimen adequacy. There was concordance on major diagnostic finding at 65% (26 cases) however 14 cases (35%) were discordant. ($p = 0.017$)

6.0 AUTOPSY PHOTOGRAPHS

Plate 1: Minimally invasive lung tissue sampling procedure

(1a) shows transthoracic percutaneous core needle biopsy through the supraclavicular route to collect apical lung biopsy whereas (1b) shows the anterior chest wall route to collect middle and lower lobes biopsy.

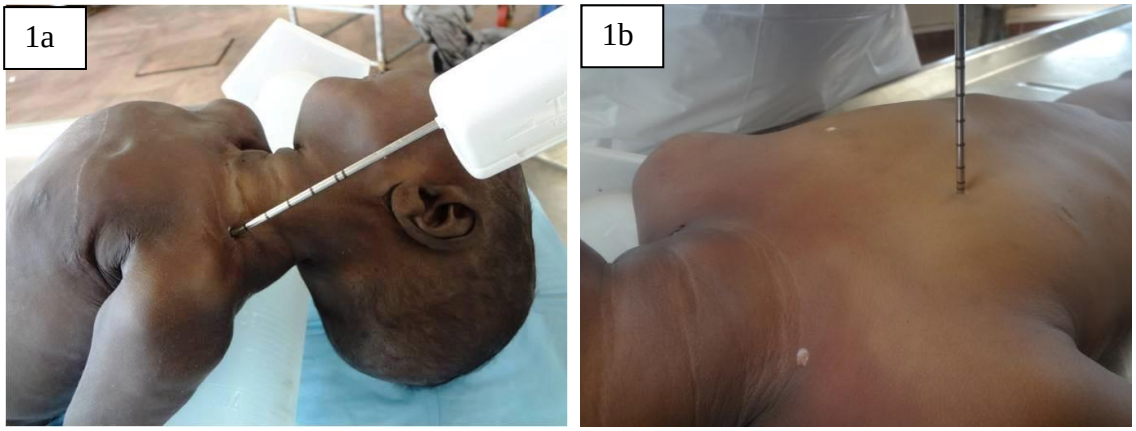
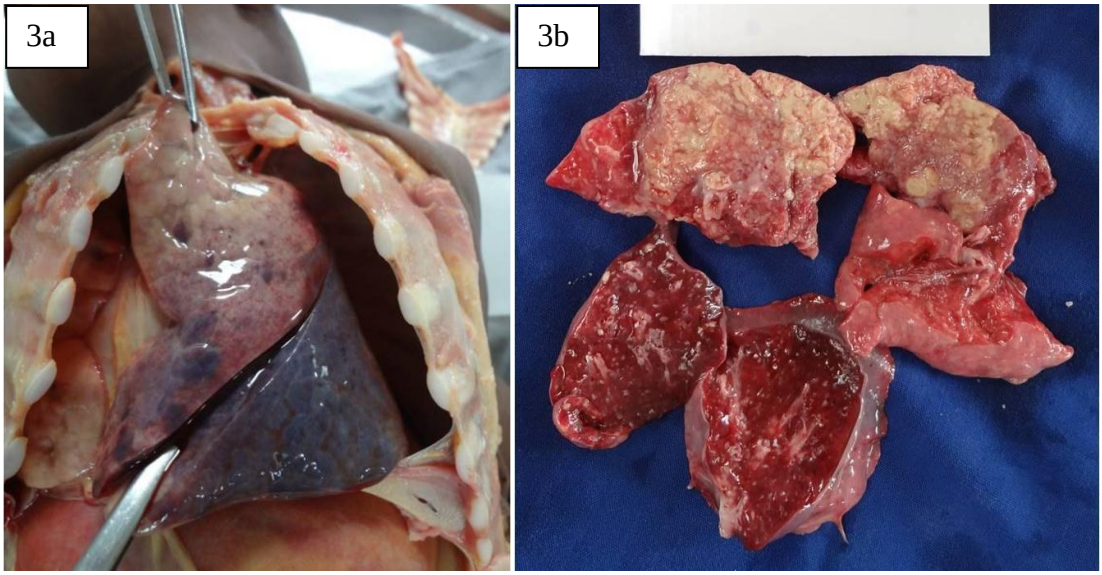


Plate 2:

(2a) Autopsy procedure in progress with supervisor and (2b) shows open lung biopsy specimen collection for histopathology evaluation.

Plate 3:

(3a) Demonstrates sterile lung biopsy specimen collection procedure for TAC and (3b) shows a sectioned lung with numerous tubercles in the upper lobe ready for collection of specimen for histopathology evaluation.



7.0 HISTOPATHOLOGY PHOTOMICROGRAPHS

Plate 4: Acute Pyogenic Pneumonia

(4a) Acute bronchopneumonia owing to bacteria shows filling of alveolar spaces with abundant neutrophils (x40) as (4b) demonstrates gram negative rods in same tissue. (X100) (4c) GMS stain demonstrates budding candida species as superimposed infection and (4d) highlights Enterobacteriocae species ((X40) by immunohistochemistry technique.

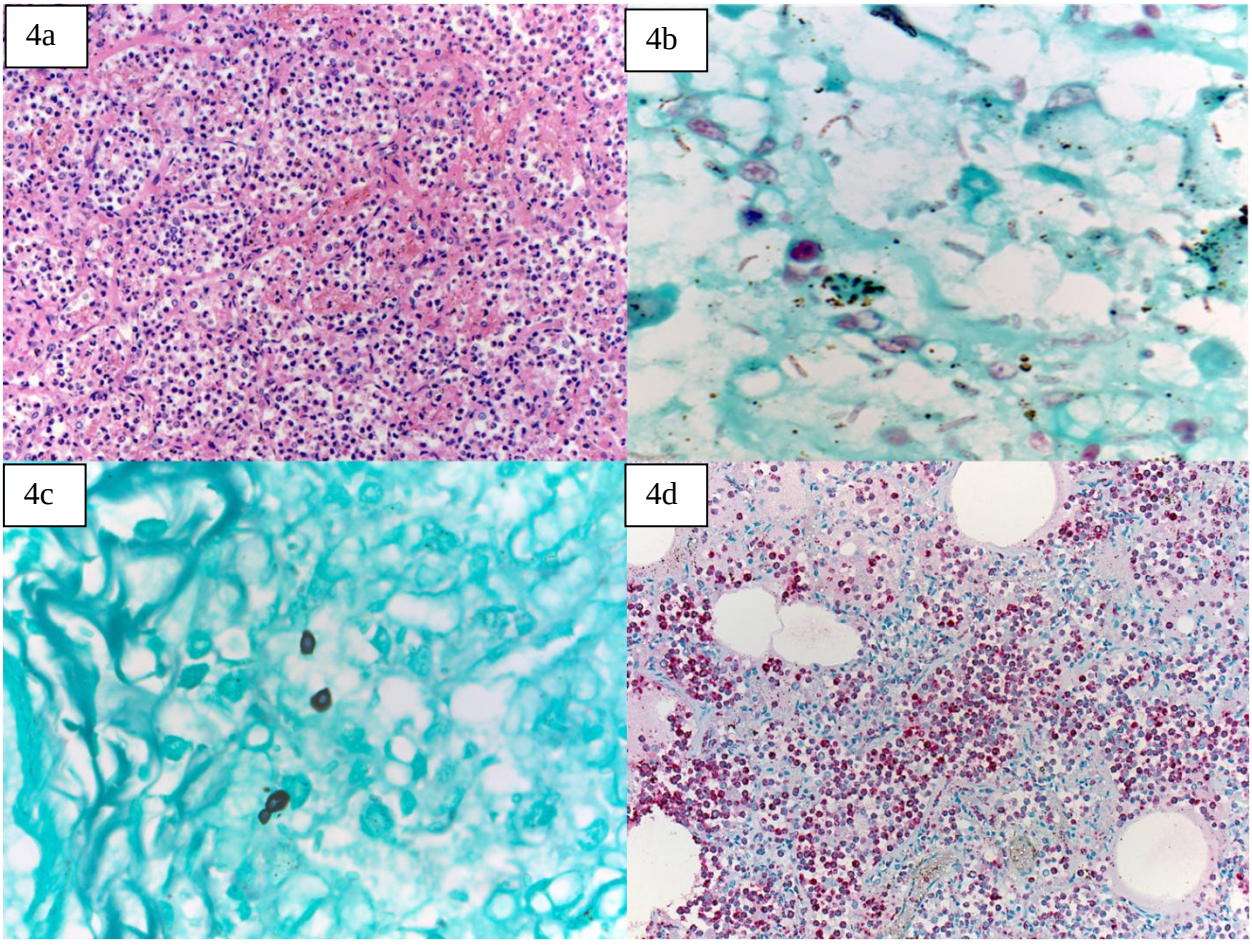


Plate 5: Interstitial Pneumonia

(5a) HE (x40) shows extensive interstitial pneumonitis characterized by marked lymphocytic infiltrate in the interstitium. (5b) HE (X100) Interstitial pneumonia with intra-alveolar foamy eosinophilic exudates and a large cell with a large round basophilic intranuclear inclusions characteristic of *Pneumocystis carinii* pneumonia and CMV respectively. (5c) GMS stain (X400) highlighting the organisms of PCP within the alveolar exudates. The cysts are crescent-shaped forms. (5d) immunohistochemical stain (X100) with antibodies against *Pneumocystis carinii* shows positive staining round or crescentric-shaped forms within the alveolar spaces.

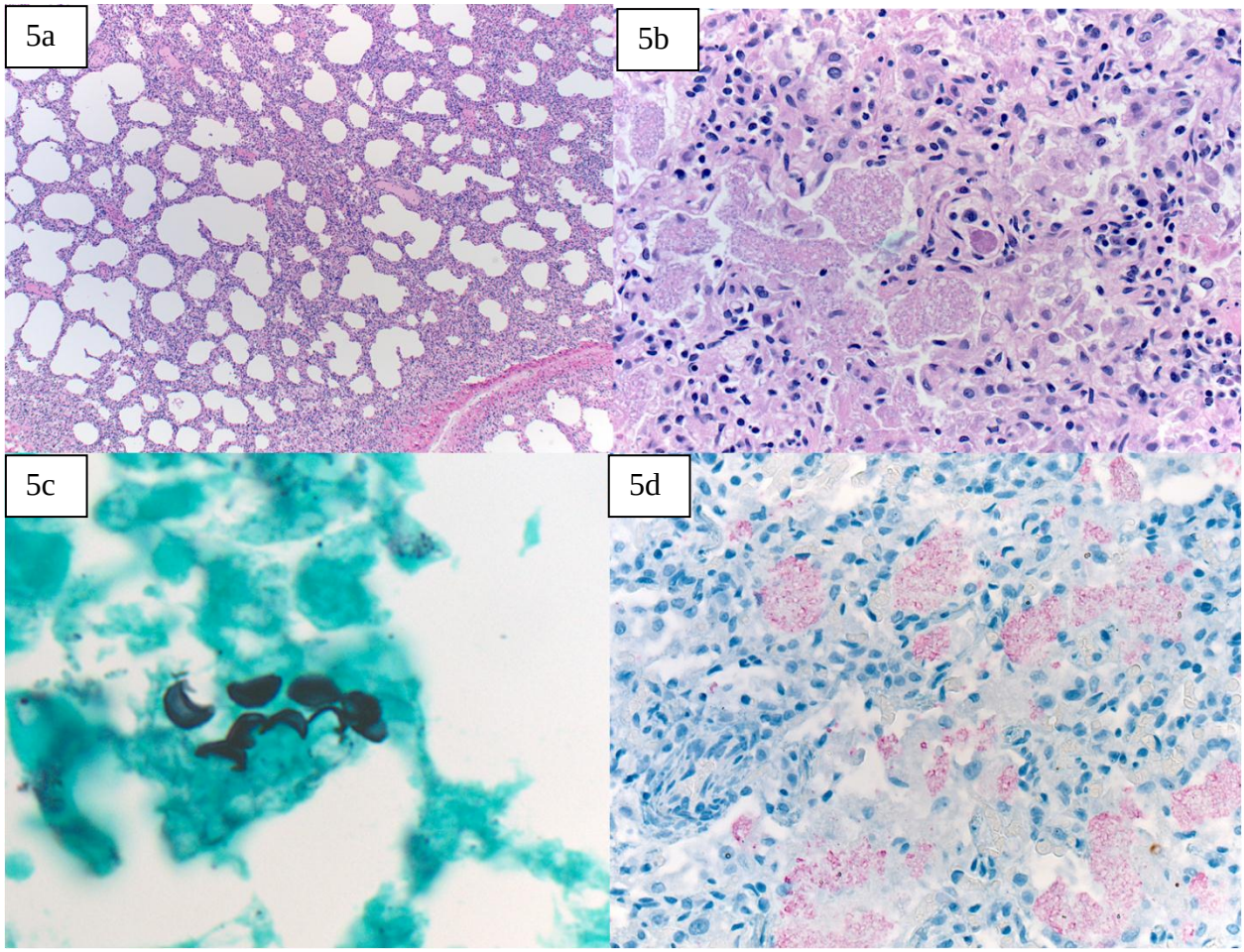


Plate 6: Necrotizing Pneumonia

(6a) HE (X40) core needle lung biopsy demonstrating extensive pulmonary oedema and focal necrotizing granulomatous pneumonia, (6b) HE (X100) shows extensive necrosis of lung tissue with hyphae elements and (6c) at a higher magnification (X400) there are numerous hyphae in a necrotic background. (6d) GMS Stain (X400) highlighting numerous septate hyphae some branching at right angles with fruiting bodies and conidial heads characteristic of Aspergillus species.

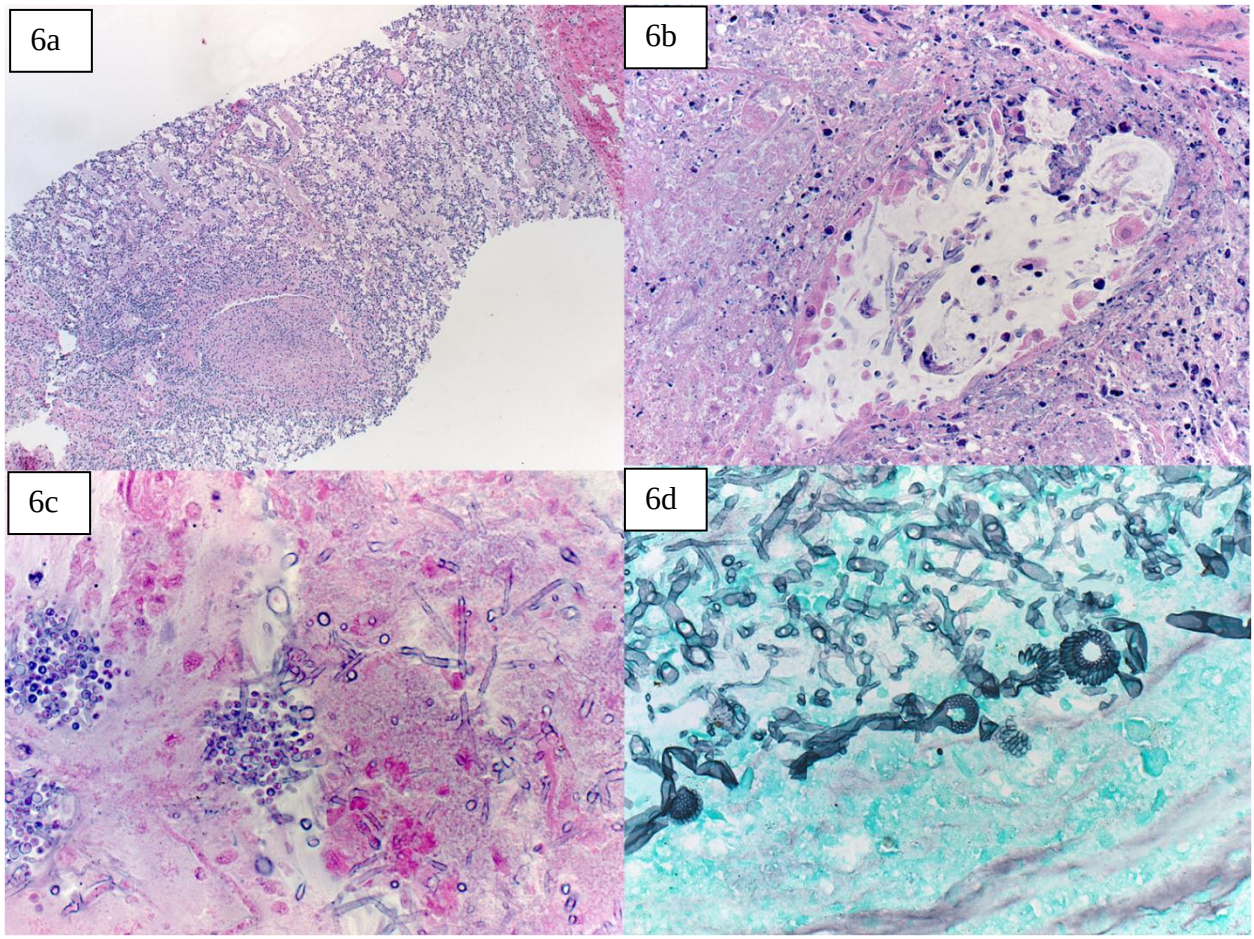


Plate 7: Granulomatous Pneumonia

(7a) HE (X40) Core needle biopsy of the lung demonstrating granulomas and (7b) at higher magnification (X100) of the granuloma showing chronic granulomatous inflammation characterized by the presence of Langhan type multinucleated giant cells, epithelioid cells and lymphocytes. (7c) Ziehl Neelsen stain (X1000/Oil-immersion) demonstrating Acid-Alcohol Fast bacilli. (7d) Immunohistochemical stain (X400) with antibodies against mycobacterium species positively identifies pulmonary *Mycobacterium tuberculosis* (staining purple).

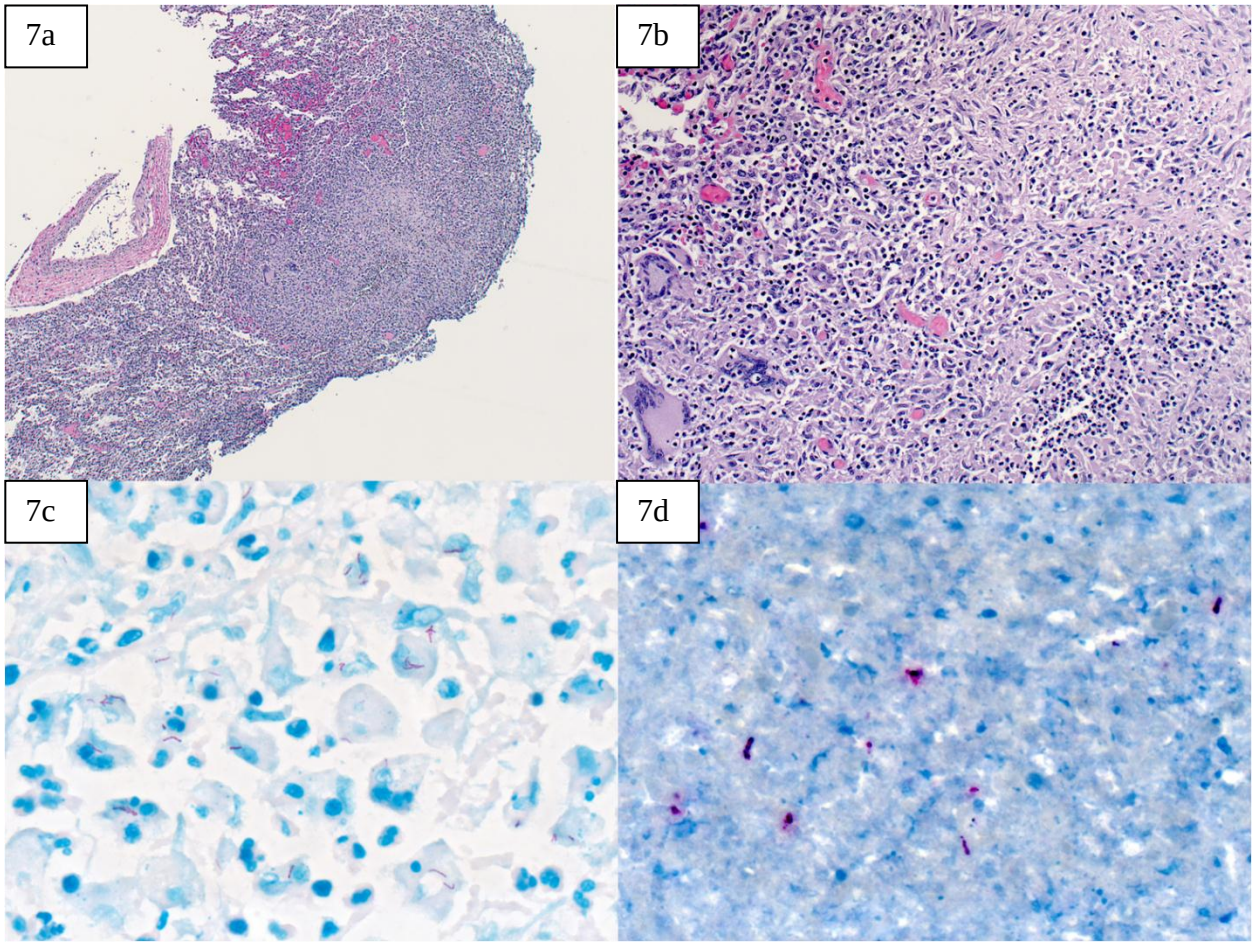
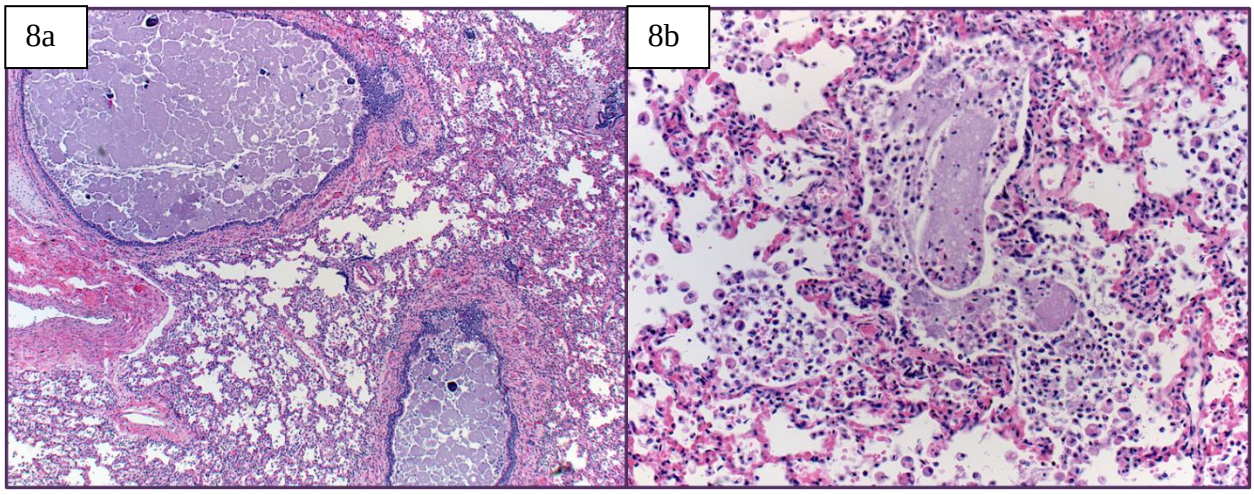


Plate 8: Aspiration Pneumonia

(8a) HE (X40) Lung tissue demonstrating milk formula within the bronchioles and (8b) at higher magnification (X100) showing milk formula in the alveolar spaces with marked mononuclear cells infiltrate comprising lymphocytes and alveolar macrophages that extend to the interstitium characteristic of the early phase of aspiration pneumonia.



8.0 DISCUSSION

The high mortality attributed to pneumonia in childhood has been reported in numerous publications, but surprisingly there is very little systematic analysis of pneumonia deaths in hospital in developing countries (96, 97).

In this study 945 respiratory illness cases were identified at KNH in the department of paediatrics over a period of 16 months. Out of these 200 (21%) deaths due to respiratory infections were recorded. However these were not the only deaths that occurred as some were missed out during surveillance especially during weekends or on public holidays.

Determining the etiology of pediatric pneumonia is challenging given the difficulty in obtaining sterile cultures from the site of infection and the lack of diagnostic reference standards (98).

First, it is difficult to obtain tissue specimens from the infection site in the lung. At present, pneumonia diagnosis relies on the assessment of clinical samples such as blood or sputum using standard culture-based microbiological techniques (99).

Additional samples are likely to be more representative of the diseased area, such as induced sputum, bronchoscopic lower respiratory tract washings or direct lung needle aspirates. Frequently, none of these antemortem specimens are able to be collected from cases of very severe pneumonia in which the patients die during or soon after admission, leaving an important gap in our knowledge of the causes of fatal pneumonia (98).

A second factor that introduces complications into the microbiological workup of pneumonia is the large number of organisms (which may or may not be pathogenic) inhabiting the upper respiratory tract, which is often the site of sampling in children with pneumonia (100). For example, S. pneumoniae, the most common global cause of bacterial pneumonia in children, is also carried in the nasopharynx of the majority of children. This lowers the specificity of PCR tests or other tests that are conducted on samples such as sputum or nasopharyngeal culture that originate from or pass through the upper respiratory tract (101). Standard clinical and laboratory diagnostics will therefore fail to identify a causative pathogen in a significant proportion of cases of pneumonia (102).

Postmortem examination of fatal pneumonia cases increases the overall proportion of cases with a definitive diagnosis, and importantly, provides information that increases our understanding of the causes of fatal pneumonia (68).

Autopsy data are usually difficult to obtain in research studies because of acceptability to relatives. In this study, parents/guardians of the113 children that died due SARI underwent grief counseling but only 64 (56.6%) consented for autopsy. The remaining 49 (43.4%) declined which is in keeping with a study done by Karau et al. in 2012 conducted a study at KNH and found out that 55.7% of the bereaved parents had a positive attitude towards autopsy compared to 44.3% who had negative attitude (91).

There were varied reasons given by parents/guardians for declining to consent for autopsies but majority did not want to see the body mutilated or cut (45%), 30% felt it was religiously or culturally unacceptable and 20% had a feeling that the diagnosis should have been established when the child was alive and therefore they didn't see the benefit of autopsy. 5% however felt that the child was too young for autopsy (91).

A study done by McHaffie et al. ascertained that the main reasons for declining an autopsy were the dread of the child being mutilated or subjected to further invasion (103). A similar study in Nigeria by Oluwasola et al. looked at the reasons for which family members would refuse to give consent for autopsy on a deceased relative. The highest ranked reason was fear of mutilation of the body, while others included delay in funeral and the deceased being considered too young (104).

At the time of death, 91.6% of the cases were below 24 months of age with 67.2% of all the cases being aged less than 12 months. According to WHO statistics the highest levels of infection occur in children less than two (2) years of age, the peak age at risk is the second six (6) months of life and vulnerable to fatal infection as the body's immunity is still developing (6).

In some developing countries, as for example Southern India, 50% of infants have been colonized by *Streptococcus pneumoniae* by 2 months of age and 80% are carriers by the age of 6 months. A study in South Africa showed that the prevalence of carriage of *Streptococcus pneumoniae* was 30%, 44%, 51% and 61% in children aged 6 weeks, 10 weeks, 14 weeks and 9

months, respectively. In industrialized countries, carriage occurs on the average at about six months of age (105).

Among the SARI death cases, 67.2% were malnourished children. This probably reflects malnutrition as a risk factor for severe pneumonia and its association with high mortality. Available evidence shows prevalence of pneumonia among the severely malnourished is as high as 72%, that pneumonia occurs 19 times more commonly in malnourished children and that pneumonia deaths among the severely malnourished is 27 times higher than in the well nourished (106).

Studies in children have shown that most of the defense mechanisms are altered in severe PEM. This lowers their resistance to infection and as such infections are not only frequent but also occur with greater severity contributing to the high mortality rates seen in these patients (107).

It is also known that secretory antibodies of IgA class play an important role in the protection of mucosal surfaces against infective agents. This local immunity is dependent of systemic immunity. In children with severe malnutrition, secretory IgA levels in duodenal fluid, saliva, tears and nasal secretions are significantly reduced. This alteration accounts for the increased incidence of mucosal infections such as pneumonia and diarrhoeal diseases seen in these malnourished children (108).

In this study, it was noted at autopsy 12 (18.75%) children had congenital anomalies of which 9 of them had congenital heart diseases mainly septal defects. Cardiac and pulmonary pathophysiologies are closely interdependent, which makes the management of patients with congenital heart disease (CHD) all the more complex. Pulmonary complications of CHD can be structural due to compression causing airway malacia or atelectasis of the lung (109).

Respiratory tract infection in children with CHD is an important cause of morbidity and mortality including respiratory failure, prolonged mechanical ventilation, and hospitalization. These patients often have many contributing factors that place them at increased risk for respiratory tract infection. Respiratory viruses including respiratory syncytial virus (RSV), human metapneumovirus, and influenza are commonly seen (110).

Children with hemodynamically significant congenital heart disease (HS-CHD), as well as premature infants are at high risk for severe RSV diseases. Mortality rates for CHD patients hospitalized with RSV have been reported as about 24 times higher compared with those without RSV infection (111).

In a study of 2613 children under 24 months old with CHD, bronchiolitis was the commonest cause of hospitalization (54.1%). Respiratory Syncitial Virus was the most common agent identified and children receiving adequate RSV prophylaxis had a 58.2% reduction in RSV related hospitalization rate. The benefits of early corrective surgery after RSV infection may not outweigh the risks of surgery (109, 112).

Trauma was also evident in the study group involving seven (7) (10.9%) children with five (5) of them having head injuries majority of them being managed for meningitis. More recent epidemiologic studies have identified other subsets of patients at high risk for acquiring nosocomial bacterial pneumonia. Such patients include children, persons above 70 years of age; persons who have endotracheal intubation and/or mechanically assisted ventilation, a depressed level of consciousness (particularly those with closed-head injury), or underlying chronic lung disease; and persons who have previously had an episode of a large-volume aspiration (113).

The diagnosis of etiology in severe pneumonia remains a challenging area. Postmortem lung tissue potentially increases the sensitivity of investigations for identification of causative pathogens in fatal cases of pneumonia and can confirm antemortem microbiological diagnoses (114).

Tissue sampling allows assessment of histological patterns of disease and ancillary immunohistochemical or molecular diagnostic techniques. It may also enhance the recognition of noninfectious conditions that clinically simulate acute pneumonia (114).

An etiological diagnosis of pneumonia can be made using histology, where pathogens are indicated by characteristic tissue changes such as granulomatous inflammation in tuberculosis or viral inclusion bodies; specific organisms can be identified using morphology and histochemical or immunohistochemical staining (115).

In addition, autopsy can reveal lung conditions that clinically simulate acute infectious pneumonia, such as malaria, lymphocytic interstitial pneumonia (LIP), noninfectious pneumonia (due to aspiration) or acute heart failure (114).

In this study, causes of lung disease were diverse, and multiple pathological findings were common in many children. The most frequent major diseases diagnosed on histopathology were acute pyogenic pneumonia (including bronchopneumonia, lobar pneumonia, and abscess) 39.3%, Interstitial pneumonia, 29.9% was the second most common picture attributed to mainly by viral infection and *Pneumocystis jirovecii* pneumonia.

Because the lung reacts to various types of injuries in a similar fashion regardless of the aetiology the changes observed in acute lung injury are nonspecific in this case giving a picture of diffuse alveolar damage (DAD), 21.5% due to the severity of the infections (114).

Aspiration pneumonia was observed in 4 cases 0.4% whereas chronic granulomatous, inflammation due to *Mycobacterium tuberculosis* was observed in two cases (0.2%) whereby one was bearing typical necrosis, abscess and multiple tubercles with acid-fast bacilli present.

Infections may produce ‘‘final common pathway’’ appearances in the lung, such as consolidative pneumonia, interstitial inflammation, or diffuse alveolar damage, which are not specific to a particular pathogen. However, the diagnostic sensitivity of autopsy tissue can be increased by the use of special stains (such as Gram, Giemsa, Ziehl-Neelsen or silver stains), immunohistochemistry or molecular pathology techniques to identify different pathogens as causative agents of infectious lung diseases (114, 116).

Klebsiella pneumonia, Streptococcus pneumoniae and *Escherichia coli* were the most common bacterial isolates; others included *Staphylococcus aureus, Haemophilus influenza* and *Pseudomonas aeruginosa*. A similar range of bacterial pathogens has been shown by other studies. The WHO Bulletin 2008 report identified *Streptococcus pneumoniae* and *Haemophilus influenzae, Staphylococcus aureus* and *Klebsiella pneumoniae* as the major bacterial causes of childhood pneumonia. Contrary to our findings, WHO mentioned *Haemophilus influenzae* as an important cause in developing countries. This report also cited older data to show that pneumococcus as responsible for 30–50% cases, H. influenzae type b for 10–30% cases, followed by *Staphylococcus aureus* and *Klebsiella pneumoniae* (22).

A systematic review in India of mortality risk in children with pneumonia identified 16 studies, and reported that among severely malnourished children, *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli*, and *Haemophilus influenzae* are the common organisms in that order (117).

A lung aspirate study done at KNH by Hatimy et al. in 1999 had 10 different bacterial isolates which were cultured of which 82.1% were gram-negative and 17.9% were gram-positive organism. *Klebsiella pneumoniae* was the most predominant organism isolated (118). This was shown to be more prevalent in malnourished children who may be more susceptible to gram-negative infection (70%).

Similarly, in a Nigerian study to determine bacterial pathogens in malnourished children with pneumonia, *Klebsiella pneumoniae* was the most commonly identified organism from lung aspirates (39.4%). The other organisms were *Staphylococcus aureus* and *Escherichia coli* forming 30.3% and 8.8% respectively (119).

In this study, the commonest viral pathogens identified through both histopathology and TAC were RSV and CMV at 14.1% and 12.5% respectively. The others include Human metapneumovirus (HMPV), Adenoviruses, and parainfluenza viruses which are similar to most of the studies carried out on community acquired pneumonias (120).

Viruses are responsible for 30–67% cases of CAP, and are the most common in children less than 2 years. The most frequently identified are respiratory syncytial virus (RSV) isolated in 13–29% and rhinovirus (3–45%) either in combination with bacteria or alone (120).

Other viruses responsible for pneumonia comprise adenovirus (1–13%), influenza (4–22%) and parainfluenza virus (3–10%), rhinovirus (3–45%), human metapneumovirus (5–12%), human bocaviruses (5–15%). The less common are enterovirus, varicella-, herpes- and cytomegalovirus (120).

RSV is one of the most highly prevalent pathogens in childhood respiratory disease and can present as bronchiolitis, pneumonia, otitis media, rhinitis or sinusitis. Risk factors for increased severity include prematurity (associated with ten-fold increase in risk), congenital heart disease,

immunosupression and neurodisability. Its virulence is mainly linked to its action on the immune system (120).

The immune response to RSV is atypical compared to other respiratory viruses. Symptoms begin 3-5 days after inoculation. It is highly contagious virus that can be transmitted through direct contact with respiratory secretions and indirect inoculation from contaminated surfaces. Once infected the immune system activates T-helper cells that react with expression of cytokines. Some research has shown that the immune response to RSV is consistent with T helper-2 (Th-2) class of T-helper cell activation such as interleukin-4 (IL-4) and interleukin-5 (IL-5) and interlukin-13 (IL-13) (120).

A second distinguishing feature of immune response to RSV is the ability to re-infect, with the same strain being able to infect the same host time after time. This is of interest as the immune memory response to viruses usually prevents re-infection. The exact reason for this is unknown, it has been hypothesised that RSV inhibits CD8+ T cells and so the immune systems memory response is ineffectual. This explanation is debated however with Chang et al. suggesting this feature is not specific to RSV and so may not give adequate explanation (120).

CMV infection was also prevalent among the viral pathogens being present in 12.5% of the cases. CMV is a herpes virus that can produce life-threatening pulmonary infections in immunocompromised hosts (121).

Although effective antiviral therapy for CMV is available timely diagnosis remains a major challenge for this condition, particularly in the pediatric population where CMV often presents with atypical patterns of lung infection (122).

The diagnosis of CMV pneumonia can be even more difficult in situations when there is no high-index of suspicion, for instance, in patients that do not have an underlying immunodeficiency syndrome (i.e., HIV-infected patients) (122).

Indeed, a detailed clinical characterization of CMV lung infection in this age group is critical to select patients that may warrant invasive procedures to obtain lung samples (i.e., pediatric bronchoscopy or open lung biopsy) and may benefit from prompt CMV therapy before confirmatory testing is available (122).

Fungal infections were evident in 20.3% of the cases with PCP seen in 7 (10.9%) children who had features of immunosupression. The other fungal agents were also seen in the same category of children comprised of candida species affecting 4 (6.25%) children and aspergillus species in 2 (3.1%) children.

A Zambian necropsy study done in children by Chintu et al. showed *Pneumocystis carinii* pneumonia as the sole finding was present in only 15 (26%) of 58 cases. It was also common with CMV infection and was present in 25 patients three of whom also had tuberculosis, *Pneumocystis carinii* pneumonia and shock lung occurred in ten cases (68).

PCP is strongly associated with immunosuppression and continues to be a major cause of death among HIV-infected infants and children in the developing world. Autopsies done in Africa revealed PCP in 16% of children who died with HIV/AIDS during 1992 and 1993, in 29% of those who died during 1997 and 2000, and in 44% of those who died during 2000 and 2001 (68, 123, 124).

The single most important factor in susceptibility of HIV-infected patients of all ages to PCP is the status of cell-mediated immunity of the host (125). Severe compromise, reflected by a marked decrease in CD4 T lymphocyte (CD4) cell count and percentage, is the hallmark of high risk for PCP. Coinfection with other organisms such as cytomegalovirus (CMV) or pneumococcus has been reported in HIV-infected children. Children with dual infections may have more severe disease leading to death (126, 127, 128).

Minimally invasive core needle lung biopsies versus open lung biopsies at autopsy.

From a diagnostic standpoint, the ideal way to determine the etiology of pneumonia is to obtain a specimen directly from the location of the infection (i.e. the lung). Of the 40 cases reviewed, there was concordance on major diagnostic finding at 65% (26 cases), however 14 cases (35%) were discordant. ($p = 0.017$)

Examples of successful postmortem studies from countries such as Zambia and the Ivory Coast confirm the practical feasibility of doing open autopsy studies on pneumonia. However, their principal disadvantage is low consent rates; for example, only 25% of families approached in Lusaka consented to open autopsy following the death of their child. They also depend on

finding experienced pathologists at the study sites who can examine autopsy material adequately, with appropriate laboratory, microbiological and clinical support (68).

Transthoracic percutaneous needle biopsy offers several potential advantages to open autopsy. It allows tissue collection for microbiological and histopathological diagnosis with ancillary tests.

Taking a biopsy confined to the lung may also allay some concerns regarding consent, as it is rapid, will not delay burial arrangements, is minimally invasive and leaves little mark on the body.

The principal disadvantage of this approach is sampling error. Studies of needle biopsy as an alternative to open autopsy have reported variable success, and depending on the organ sampled, the success rate can be as low as 50% of the total biopsies. Sampling error can lead to disparities between the resultant tissue diagnoses compared with an open autopsy. If multiple biopsy sites are used, then the sampling of other tissues such as the spleen or body fluids such as CSF or urine can be performed, which may increase the value of a microbiological diagnosis on the lung biopsy tissue, without requiring open autopsy (129, 130, 131).

9.0 STUDY LIMITATION

1. The study experienced several interruptions due to financial problems thereby delaying recruitment of study participant, and subsequently delay in sample processing.
2. HIV is a major risk factor of SARI, unfortunately most of the samples had not been tested to correlate the findings.

10.0 CONCLUSION

1. Most children in the study died from preventable or treatable infectious diseases including malnutrition with acute pyogenic pneumonia as the commonest cause of death from pulmonary disease in children, significantly due to *Klebsiella pneumoniae* and *Streptococcus pneumoniae* infections.
2. There was higher than expected opportunistic infections due to PCP and CMV including other viral infections notably RSV as important causes of death in under fives.
3. Malnutrition and congenital malformations mainly congenital heart diseases are major risk factors complicating SARI and leading to deaths.

4. A significant number of children have life threatening physical trauma in a background of SARI.
5. There was positive concordance of major histopathologic findings of lung biopsy specimen obtained by minimally invasive tissue sampling and full autopsy techniques.

11.0 RECOMMENDATIONS

1. Most of the deaths were associated with acute pyogenic pneumonia and therefore antibiotic management should be modified in light of the commonest pathogens in this region, high multidrug resistance and drug sensitivity test carried out.
2. Higher than usual opportunistic infection such as CMV and PCP should raise a high index of scaled up clinical management of SARI and prevention strategies such as vaccination programs also require to be factored.
3. Attempts should be made to bring back routine autopsy within KNH and other health institutions for surveillance of killer diseases and bereaved parents need to be counseled on the need for autopsy on their deceased child as well as educating them on the potential barriers to consenting for autopsy.
4. Minimally invasive autopsy procedure using transthoracic percutaneous core needle biopsy technique proved an effective way of obtaining lung biopsy at autopsy in SARI cases and therefore should be adopted especially in scenarios where potential barriers to the ‘‘gold standard’’ of open autopsy are encountered as it is easy, fast and more acceptable.

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APPENDICES

APPENDIX 1: SARI SURVEILLANCE CASE FOLLOW-UP QUESTIONNAIRE

Patient ID number: _____ Date: _____ Interviewer's initials: _____

PART ONE: Follow-up Questions

1. Was the patient tested for HIV during this admission?
   Yes□ No□
2. If yes, what was the HIV test result?
   Positive □ Negative□
3. Did the patient receive antibiotics during this admission other than anti TB drugs?
   Yes□ No□ If yes, name of antibiotic?
4. Was TB therapy started during this admission?
   Yes□ No□ Not Applicable□
5. Did the patient take anti-viral drugs during this admission? Yes□ No□
6. Did the patient receive oxygen during this admission?
   Yes□ No□
7. Did the patient receive mechanical ventilation during this admission?
   Yes□ No □ If yes, no. of days on mechanical ventilation? _____
8. Was the patient admitted to ICU during this admission?
   Yes□ No □ If yes, number of days admitted in ICU_____
9. Fill in the following investigation results where applicable
   Hemoglobin level _____g/dl
   White blood cell count _____x 10^9/L
   Neutrophil count_____ x 10^9/L
   Lymphocyte count_____ x 10^9/L
   Erythrocyte sedimentation rate _____mm/h
   Chest x-ray report: Normal findings□ Abnormal findings □
   If abnormal, are chest x-ray findings consistent with pneumonia?
   □ Yes No□

PART TWO: Final Outcome

10. What is the discharge diagnosis? _____
11. Date of final outcome? _____
12. What was the final outcome?
    □ Discharge
    □ Death
    □ Refused hospital treatment/absconded
    □Transferred to another hospital facility, specify Name of facility _____
    _____

APPENDIX 2: CONSENT INFORMATION FORM FOR POSTMORTEM STUDY

Kindly receive our condolences on the death of your child. He/She had an illness affecting his/her lungs which may have led to his/her death. Lung illnesses are one of the leading causes of sickness and death in our country. We would like to find out the cause of the lung illness that may have led to his/her death and this can only be best achieved by carrying out a post mortem procedure.

What is a Post-Mortem?

A Post-Mortem (which is also called an autopsy) is the medical examination of a person that takes place after death. It may seem somewhat insensitive of us to bring up the question of a post mortem at this difficult time for you and your family but there are important reasons why we need to do so.

We intended to help you understand what is involved in a Post Mortem examination, why it can be important and to outline your choices in this regard. We want to respect your wishes in every way we can. If there is anything you don't fully understand or would like to discuss further please do not hesitate to contact a member of staff that you are dealing with in the hospital.

Why are Post-Mortem examinations carried out?

The post-mortem examination is one of the most informative investigations in medicine. It provides objective details on a person's illness or disease and on the response to any treatment given to them. The Post Mortem is an important means of establishing a medical diagnosis. A post mortem can sometimes leave questions unanswered in relation to the death but even in these situations it can provide important and valuable information for the family and the doctors.

For the bereaved family the post-mortem provides information and explanations about the cause of death but can also reveal other conditions, knowledge of which could be of benefit to other family members.

The information obtained during the examination can also greatly assist doctors by providing knowledge that can be used in their treatment of other people in the future.

Post-Mortems can also be very valuable for ongoing medical training purposes.

What does a Post-Mortem entail?

The post mortem examination is performed by a specially trained doctor called a Pathologist. A Pathologist specializes in the study of disease. During the examination the Pathologist is assisted by a Technician who is also specially trained for this purpose. The post mortem examination itself is carried out with the same care that would be taken if the deceased were having an operation.

During the Post-Mortem the body is carefully examined and any abnormalities or injuries are noted. In a full Post-Mortem examination all internal organs such as the brain, heart, lungs, and glands will take place. Incisions are made to allow the organs to be removed and studied in detail. X-rays and/or photographs may be taken during the examination.

Small portions of tissue from each organ may be kept to prepare microscopic blocks and slides. These slides are carefully examined to establish a diagnosis and they become part of the post mortem record. A small amount of blood may also be kept to facilitate biochemical, metabolic and toxicology analysis.

A limited Post-Mortem can be carried out which confines itself to those organs most likely to have been directly associated with the cause of death.

Appearance of the deceased after the Post-Mortem

Relatives are often concerned about the appearance of their loved one after the Post Mortem examination. Incisions are carefully stitched and dressed as neatly as possible and as facial disfiguration should not occur it is not usually obvious from the appearance of the deceased that a Post-Mortem has been undertaken.

Will the post-mortem affect funeral arrangements?

Every effort will be made to perform the post-mortem in a timely fashion so as not to affect or impinge upon funeral arrangement. In case you have any concern, please feel free to discuss intended funeral arrangements with any of the hospital staff you are dealing with.

What we will do

We will take some lung specimen using a needle and syringe. As explained above, we will open the chest and take samples of the lungs and other organs in the chest that may be damaged or have disease causing germs. These specimens will be sent to laboratories in KNH and KEMRI/CDC in Nairobi, and to CDC Laboratories in Atlanta, Georgia, USA for analysis so as to identify germs that may have led to your child's illness and death.

For each specimen, we will place a number that uniquely identifies the samples. This number is similar to the one on questionnaires that collected clinical data when the child was in the ward. In addition, we will collect some blood and test it for HIV and influenza.

Will the post-mortem report be available?

Post-Mortem reports can be obtained on request from the Hospital. As laboratory tests can take time to complete the report itself may not be available for some months. We will let you know the results of tests done once they are ready and the cause of death.

Benefit from being in this study:

The study will provide no direct benefit to you. In general, the study will help us to learn more about causes of lung illnesses that lead to death in our country so that we can provide better care to prevent similar deaths in the future. If you would like we will call you and tell you what we think was the cause of death for your child. It may take up to a few weeks for us to determine the final cause of death.

Risks from being in this study:

The body may have cuts on the chest and abdomen which will be sewed together after the post mortem as explained above.

How will the deceased information be protected?

No names shall appear on samples collected. Instead, numbers will be used to identify the samples. Most of the samples collected will be tested here in the hospital and at KEMRI/CDC laboratory. However, for some tests that cannot be done here, part of the samples may be sent to special laboratories in CDC in the United States.

All the study records will be kept secretly and securely. There will be people involved in the study who will need to see the deceased's health records. These people may include members of the study team, the study monitors, and members of the ethics committees that oversee the study. The information shared with the data team during data analysis will not contain names or any other personal identifying information. Reports and publications from this study will not contain decedent's name or any other personal identifying information.

What happens if I do not want a post mortem for the deceased?

You can choose not to have the deceased participate in this study. The body will still receive the usual care at the mortuary.

Will it cost anything?

It will not cost you anything to have the deceased participate in this study. We will meet the post-mortem costs, costs of any laboratory tests done and mortuary fees for a period of up to 5 days since death.

Who do I call if I have questions or problems?

- If you have questions or complaints about this study or in case you change your mind about any of the decisions you have made (although there may be a short time limit for some of these). If you wish to make changes to anything you have consented to, or wish to withdraw your consent, please contact the principal investigator on phone number 0721213088.
- If you have questions about rights as a study participant, call the Ethical Review Committee for Research in Human Subjects**.** You should contact the ethics committee if you feel you have not been treated fairly or if you have other concerns. The ethics committee contact information is:

What does your signature (or initials/mark) on this consent form mean?

Your signature (or initials/mark) on the consent form means:

- You have been informed about this study's purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to have the deceased body to be used in this study.

APPENDIX 3: POST MORTEM CONSENT FORM

I……. as the nominated next-of-kin of ……... (deceased), knowing that I am also entitled to refuse, I agree to have the deceased body take part in the study and I also give authority to have a post mortem performed on the deceased body.

Please indicate Yes or No:

I have read or had the consent information form read to me, the autopsy process has been explained to me and had a chance to ask questions which were clarified to my satisfaction. I understand that there is no obligation whatsoever to agree to the post mortem examination. ☐ **Yes** ☐**No**

I understand that it will be necessary to remove organs, or parts of organs, tissues and/or other body fluids for examination during the post mortem. ☐**Yes** ☐**No**

I understand that photography may be used during the post mortem examination ☐**Yes** ☐**No**

I understand that some tissue or organs may need to be retained for more detailed laboratory examination. ☐**Yes** ☐**No**

I understand that small amounts of organs/tissue will be retained for the purposes of preparing histological blocks and slides, which become part of the permanent post mortem record. ☐**Yes** ☐**No**

I agree to allow specimens from the deceased to be stored and possibly used after this study is over for further diagnostic, education and research purposes related to respiratory disease: ☐**Yes** ☐**No**

I agree to allow the deceased medical records to be reviewed by study staff, ethics committee members, and legal authorities: ☐**Yes** ☐**No**

I agree to specimens and data being sent outside the country for research: ☐**Yes** ☐**No**

I HEREBY CONSENT TO A POST MORTEM BEING CARRIED OUT IN ACCORDANCE WITH THE DETAILS NOTED ABOVE:

Signed:_____ Dated:_____

Relationship to deceased:_____

Name in print:_____

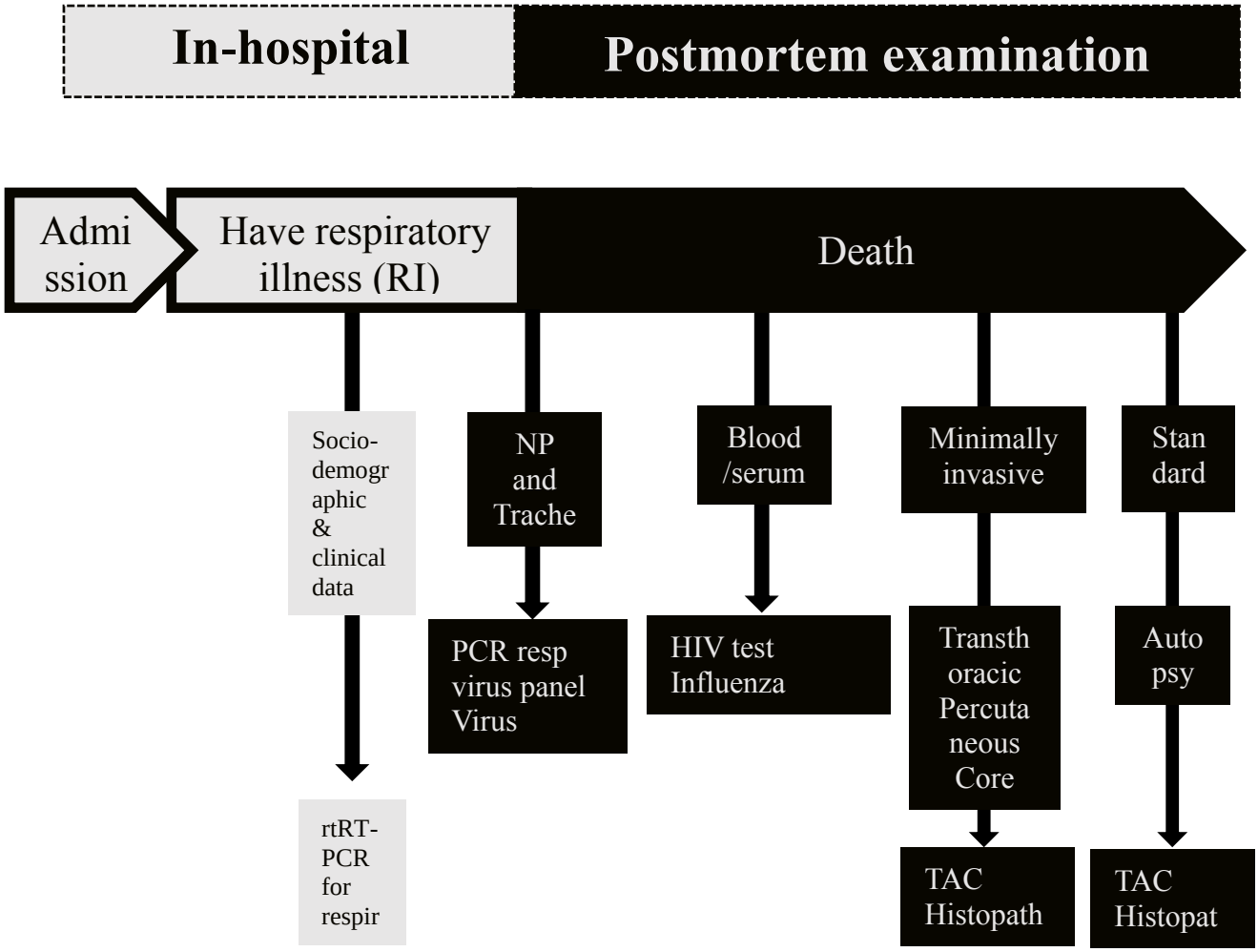
Address:_____

_____ Telephone number:_____

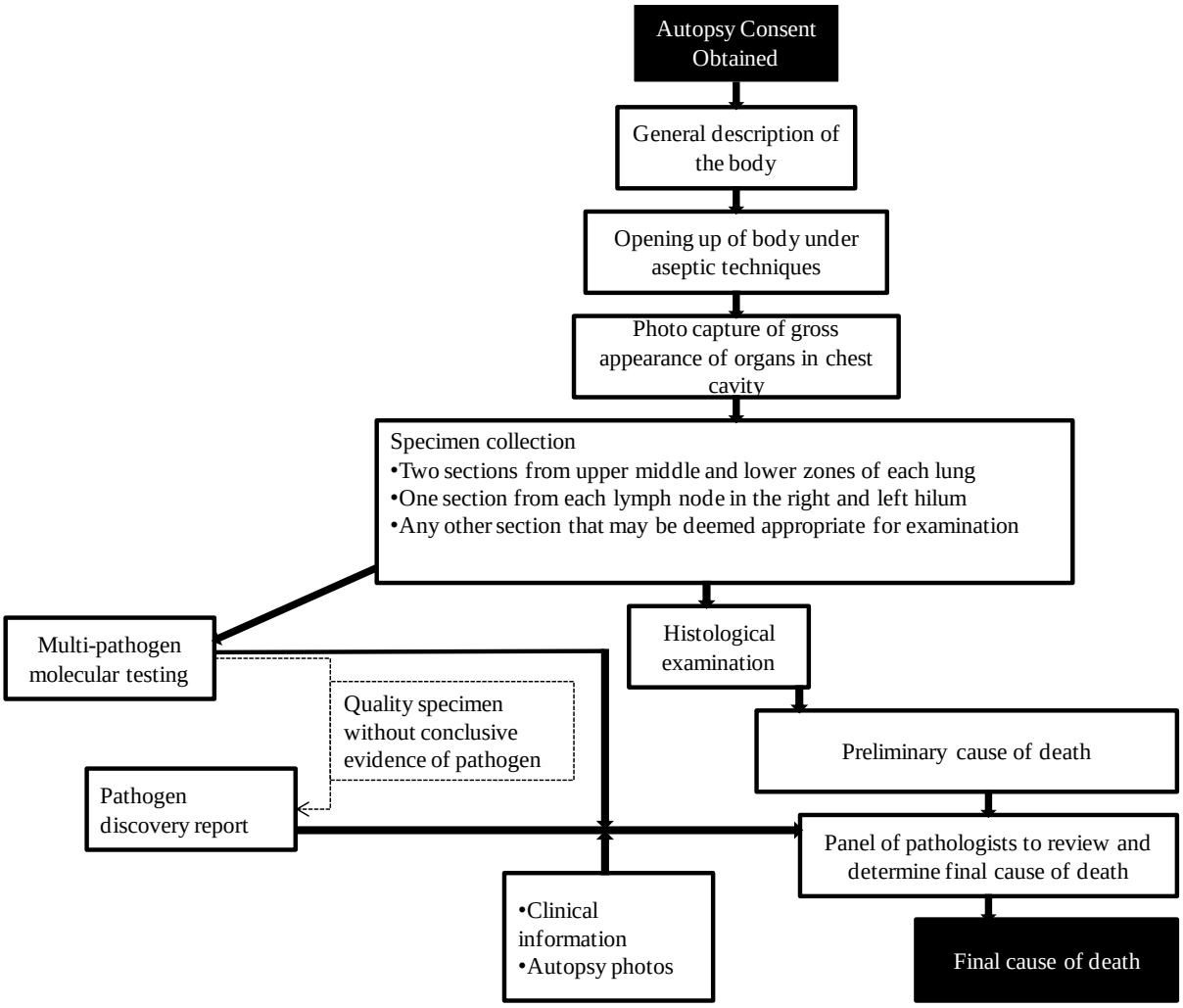
Witnessed by: _____ Date:_____

Name:_____ Designation_____

APPENDIX 4: SARI SURVEILLANCE AND AUTOPSY SPECIMENS FLOWCHART:



APPENDIX 5: PROCEDURES FOR FULL AUTOPSY



APPENDIX 6: POSTMORTEM DATA COLLECTION FORM

FULL AUTOPSY DATA COLLECTION FORM,

Preliminary Screening

Pathologist's initials: _____ , _____ , _____

Data collected by (initials): _____

Decedent's hospital IP number: _____

Autopsy case number: _____

Study enrollment number: _____

Date of admission: _____

Date of death: _____

Autopsy date: _____

Child's age (months): _____

Sex: Male □ Female □

Consent present: Yes □ No□

Body identified: Yes □ No□

Clinical summary present: Yes □ No□

1. **SECTION 1**: **External examination**
   - 1.1. Anthropometric measurements
     - 1.1.1. Weight: _____ (kg)
     - 1.1.2. Height/crown-heel length: _____ (cm)
     - 1.1.3. Crown ramp length: _____ (cm)
     - 1.1.4. Head circumference: _____(cm)
     - 1.1.5. Chest circumference: _____(cm)
     - 1.1.6. Abdominal circumference: _____(cm)
     - 1.1.7. MUAC: _____(cm)
   - 1.2. Presence of rigor mortis: □ Yes □ No
   - 1.3. Presence of lividity: □ Yes □ No
     - 1.3.1. If yes, specify location _____
   - 1.4. General signs
     - 1.4.1. Pallor □ Yes □ No
     - 1.4.2. Jaundice □ Yes □ No

1.4.3. Cyanosis □ Yes □ No
1.4.4. Finger clubbing ❑ Yes ❑ No
1.4.5. Dehydration ❑ Yes ❑ No
1.4.6. Enlarged lymph nodes ❑ Yes ❑ No

Specify location (tick/fill in where applicable)

1.4.6.1. ❑ Cervical
1.4.6.2. ❑ Submandibular
1.4.6.3. ❑ Sub-clavicular
1.4.6.4. ❑ Axillary
1.4.6.5. ❑ Inguinal
1.4.6.6. ❑ Generalized
1.4.6.7. Other , specify:_____
1.4.6.8. Describe appearance (tick/fill in where applicable)
1.4.6.8.1. ❑ Discrete
1.4.6.8.2. ❑ Matted
1.4.6.8.3. ❑ Grossly enlarged
1.4.6.8.4. Other, specify:_____

1.4.7. Skin description
1.4.7.1. Medical intervention marks (e.g. IV access) present □ Yes □ No
1.4.7.1.1. If yes, specify location:
1.4.7.1.2. Scalp IV □ Yes □ No
1.4.7.1.3. Intraosseous IV □ Yes □ No
1.4.7.1.4. Jugular IV □ Yes □ No
1.4.7.1.5. Antecubital fossae □ Yes □ No
1.4.7.1.6. Forearm IV □ Yes □ No
1.4.7.1.7. Other specify:_____

1.4.7.2. Are skin lesions present? □ Yes □ No
1.4.7.2.1.1. If yes, describe: _____
1.4.7.2.1.2. Appearance _____
1.4.7.2.1.3. location _____
1.4.7.2.1.4. Size _____
1.4.7.2.2. Any other Musculoskeletal abnormality □ Yes □ No
1.4.7.2.2.1. If yes ,specify
1.4.7.2.2.1.1. Bone fracture □
1.4.7.2.2.1.2. Rachitic rosary□
1.4.7.2.2.1.3. Delayed and widened fontanels □
1.4.7.2.2.1.4. Head bossing □
1.4.7.2.2.1.5. Feet scissoring□

1.4.7.2.2.1.6. Obvious trauma□
1.4.7.2.2.1.7. Other specify_____

1.4.8. Hair changes □ Yes □ No
If yes, describe:
1.4.8.1. □ Thin
1.4.8.2. □ Sparse,
1.4.8.3. □ Brittle
1.4.8.4. □ Easily pluckable
1.4.8.5. □ Brownish
1.4.9. Dependent edema □ Yes □ No
1.4.9.1. If yes specify location:
1.4.9.1.1. Facial edema □ Yes □ No
1.4.9.1.2. Lower limbs □ Yes □ No
1.4.9.1.3. Upper limbs □ Yes □ No
1.4.9.1.4. Sacral area □ Yes □ No
1.4.9.1.5. Other specify:
1.4.10. Nutritional status
1.4.10.1. Normal nutritional status Yes □ No □
1.4.10.2. If no, classify as below
1.4.10.2.1. Malnutrition of severe degree □ Yes □ No
1.4.10.2.2. Malnutrition of moderate degree □ Yes □ No
1.4.10.2.3. Malnutrition of mild degree □ Yes □ No
1.4.10.2.4. Childhood obesity □ Yes □ No

2. SECTION 2: Internal examination (Description of organs/tissues in-situ)

2.1. Tracheal position □ Central □ Displaced
2.1.1. If displaced, which side? □ right □ left
2.2. Thoracic cavity
2.2.1. Thymus present □ Yes □ No
2.2.2. Evidence of lung collapse? □ Yes □ No
2.3. Pleural fluid present □ Yes □ No
2.3.1. If yes,
2.3.1.1. Volume: Right chest cavity_____mls, Left chest cavity_____mls
Describe appearance
2.3.1.1.1. □ Straw colored
2.3.1.1.2. □ Purulent
2.3.1.1.3. □ Hemorrhagic
2.3.1.1.4. □ Turbid

2.3.1.1.5. □ Mucoid discharge

2.3.1.1.6. Other specify:_____

2.4. Presence of pericardial effusion □ Yes □ No

2.4.1. If yes,

2.4.1.1. Volume _____mls

Describe appearance

2.4.1.1.1. □ Straw colored

2.4.1.1.2. □ Purulent

2.4.1.1.3. □ Hemorrhagic

2.4.1.1.4. □ Turbid

2.4.1.1.5. Other specify

2.5. Abdominal cavity

2.5.1. Ascitic fluid present □ Yes □ No

2.5.2. If yes, describe:_____

2.5.2.1. Volume _____mls

Describe appearance

2.5.2.1.1. □ Straw colored

2.5.2.1.2. □ Purulent

2.5.2.1.3. □ Hemorrhagic

2.5.2.1.4. □ Turbid

2.5.2.1.5. Other

specify_____

3. SECTION 3: Internal examination (Description of eviscerated organs/tissues)

3.1. Respiratory system

3.1.1. Trachea appearance (tick/fill in where appropriate)

3.1.1.1. □ Presence of foreign bodies

3.1.1.2. □ Hyperemic

3.1.1.3. □ Edematous

3.1.1.4. Other,

describe:_____

3.1.2. Mediastinum

3.1.2.1. Are hilar nodes enlarged? □ Yes □ No

3.1.3. Lungs

3.1.3.1. Weight: Right:_____gms, Left _____gms

3.1.3.2. General appearance of the lungs

3.1.3.2.1. □ Normal pink and aerated

3.1.3.2.2. □ Congenital malformation

3.1.3.2.3. □ Obvious adhesions □ Yes □ No

3.1.3.2.4. If yes describe_____

3.1.3.2.5. Obvious pathology? □ Yes □ No

3.1.3.2.5.1. If present, describe_____

3.1.3.2.6. Anthracotic pigmentation □ Yes □ No

3.1.3.2.7. Congestion □ Yes □ No

3.1.3.2.7.1. If yes, specify location: _____

3.1.3.2.7.1.1. □ right lung

3.1.3.2.7.1.1.1. □ upper

3.1.3.2.7.1.1.2. □ middle

3.1.3.2.7.1.1.3. □ lower lobe(s)

3.1.3.2.7.1.2. □ left lung

3.1.3.2.7.1.2.1. □ upper

3.1.3.2.7.1.2.2. □ lower lobe(s)

3.1.3.2.8. □ Red hepatization □ Yes □ No

3.1.3.2.8.1. If yes, specify location: _____

3.1.3.2.8.1.1. □ right lung

3.1.3.2.8.1.1.1. □ upper

3.1.3.2.8.1.1.2. □ middle

3.1.3.2.8.1.1.3. □ lower lobe(s)

3.1.3.2.8.1.2. □ left lung

3.1.3.2.8.1.2.1. □ upper

3.1.3.2.8.1.2.2. □ lower lobe(s)

3.1.3.2.9. □ Grey hepatization □ Yes □ No

3.1.3.2.9.1. If yes, specify location: _____

3.1.3.2.9.1.1. □ right lung

3.1.3.2.9.1.1.1. □ upper

3.1.3.2.9.1.1.2. □ middle

3.1.3.2.9.1.1.3. □ lower lobe(s)

3.1.3.2.9.1.2. □ left lung

3.1.3.2.9.1.2.1. □ upper

3.1.3.2.9.1.2.2. □ lower lobe(s)

3.1.3.2.10. □ Lung abscess: □ Yes □ No

3.1.3.2.10.1. If yes, specify location:_____

3.1.3.2.10.1.1. □ right lung

3.1.3.2.10.1.1.1. □ upper

3.1.3.2.10.1.1.2. □ middle

3.1.3.2.10.1.1.3. □ lower lobe(s)

3.1.3.2.10.1.2. □ left lung

3.1.3.2.10.1.2.1. □ upper

3.1.3.2.10.1.2.2. □ lower lobe(s)

3.1.3.2.11. □ Lung cavity: □ Yes □ No

3.1.3.2.11.1. If yes, specify location:

_____

3.1.3.2.11.1.1. □ right lung

3.1.3.2.11.1.1.1. □ upper

3.1.3.2.11.1.1.2. □ middle

3.1.3.2.11.1.1.3. □ lower lobe(s),

3.1.3.2.11.1.2. □ left lung

3.1.3.2.11.1.2.1. □ upper

3.1.3.2.11.1.2.2. □ lower lobe(s)

3.1.3.2.12. □ Granulomas: □ Yes □ No

3.1.3.2.12.1. If yes, specify location:

3.1.3.2.12.1.1. □ right lung

3.1.3.2.12.1.1.1. □ upper

3.1.3.2.12.1.1.2. □ middle

3.1.3.2.12.1.1.3. □ lower lobe(s),

3.1.3.2.12.1.2. □ left lung

3.1.3.2.12.1.2.1. □ upper

3.1.3.2.12.1.2.2. □ lower lobe(s)

3.2. Cardiovascular system

3.2.1. Heart

3.2.1.1. Weight _____gms

3.2.1.2. General appearance of the heart

3.2.1.2.1. Normal □ Yes □ No

3.2.1.2.1.1. If abnormal describe

3.2.1.2.1.1.1. Grossly enlarged □ Yes □ No

3.2.1.2.1.1.2. Congenital anomalies identified? □ Yes □ No

3.2.1.2.1.1.2.1. If yes, what type?

3.2.1.2.1.1.2.2. □ Septal defects□ Yes □ No

3.2.1.2.1.1.2.3. □ Obstructive defects□ Yes □ No

3.2.1.2.1.1.2.4. □ Cyanotic defects□ Yes □ No

3.2.1.2.1.1.2.5. □ Tetralogy of Fallot □ Yes □ No

3.2.1.2.1.1.3. Evidence of infarction? □ Yes □ No

3.2.2. Major vessels

3.2.2.1. General appearance of the major vessels

3.2.2.1.1. Normal □ Yes □ No

3.2.2.1.2. Presence of thrombi □ Yes □ No

3.3. Gastrointestinal system

3.3.1. Peritoneum

3.3.1.1. General appearance of the peritoneum

3.3.1.2. Normal □ Yes □ No

3.3.1.2.1. If abnormal describe

3.3.1.2.1.1. Signs of inflammation? □ Yes □ No

3.3.1.2.1.2. If yes describe_____

3.3.2. Stomach

3.3.2.1. Signs of inflammation? □ Yes □ No

3.3.2.2. If yes describe_____

3.3.2.3. Any stomach contents? □ Yes □ No

3.3.2.3.1. If yes, describe

3.3.2.3.1.1. Food contents□ Yes □ No

3.3.2.3.1.2. Medicines□ Yes □ No

3.3.2.3.1.3. Others indicate_____

3.3.2.3.2. Estimate the stomach contents in ml_____

3.3.3. Intestines

3.3.3.1. General appearance of the intestines

3.3.3.1.1. Normal □ Yes □ No

3.3.3.1.1.1. If abnormal describe

_____

3.3.3.1.1.1.1. Signs of intestinal obstruction□ Yes □ No

3.3.3.1.1.1.1.1. If yes describe the area involved

3.3.3.1.1.1.1.1.1. Pylorus

3.3.3.1.1.1.1.1.2. Ileum

3.3.3.1.1.1.1.1.3. Caecum

3.3.3.1.1.1.1.1.4. Ascending colon

3.3.3.1.1.1.1.1.5. Transverse colon

3.3.3.1.1.1.1.1.6. Descending colon

3.3.3.1.1.1.1.1.7. Sigmoid

3.3.3.1.1.1.1.1.8. Rectum

3.3.3.1.1.1.1.1.9. Intussusception

3.3.3.1.1.1.2. Focal lesions observed □ Yes□ No

3.3.3.1.1.1.2.1. If yes describe

3.3.3.1.1.1.2.1.1. Location

3.3.3.1.1.1.2.1.2. Appearance

3.3.3.1.1.1.2.1.3. Gangrenous□ Yes □ No
3.3.3.1.1.1.2.1.4. Localized Abscess □ Yes □ No
3.3.3.1.1.1.2.1.5. Peyer patches □ Yes □ No
3.3.3.1.1.1.2.1.6. Matted lymph nodes □ Yes □ No
3.3.3.1.1.1.2.1.7. Multiple mesenteric lymph nodes □ Yes □ No

3.3.4. Liver
3.3.4.1. Weight _____gms
3.3.4.2. General appearance
3.3.4.2.1. Congested □ Yes □ No
3.3.4.2.2. Fatty change□ Yes □ No
3.3.4.2.3. Nutmeg appearance □ Yes □ No
3.3.4.2.4. Abscess seen □ Yes □ No

3.3.5. Gall bladder: Weight _____gms
3.3.5.1. Normal Yes □ No □
3.3.5.1.1. If no describe_____

3.3.6. Kidneys
3.3.6.1. Weight: Right _____gms, Left _____gms
3.3.6.2. General appearance of the kidneys
3.3.6.2.1. Normal □ Yes □ No
3.3.6.2.2. If abnormal, specify morphological anomalies
3.3.6.2.3. Cysts □ Yes □ No
3.3.6.2.4. Abscesses □ Yes □ No
3.3.6.2.5. Other specify _____

3.3.7. Adrenals
3.3.7.1. Weight: Right _____gms, Left _____gms
3.3.7.2. Morphological anomalies □ Yes □ No
3.3.7.3. If yes, describe:_____

3.3.8. Ureters
3.3.8.1. Morphological anomalies □ Yes □ No
3.3.8.2. If yes, describe:_____

3.3.9. Bladder
3.3.9.1. Weight:_____gms
3.3.9.2. Morphological anomalies □ Yes □ No
3.3.9.3. If yes,
describe:_____

3.3.10. Reproductive system
3.3.10.1. Morphological anomalies □ Yes □ No

3.3.10.1.1. If yes,
describe_____

3.4. Endocrine system

3.4.1. Thymus

3.4.1.1. Weight _____gms

3.4.1.2. Morphological anomalies □ Yes □ No

3.4.1.2.1. If yes, describe

3.4.2. Spleen

3.4.2.1. Weight _____gms

3.4.2.2. Morphological anomalies □ Yes □ No

3.4.2.2.1. If yes, describe_____

3.4.3. Pancreas

3.4.3.1. Weight _____gms

3.4.3.2. Morphological anomalies □ Yes □ No

3.4.3.2.1. If yes,
describe_____

3.4.4. Thyroid

3.4.4.1. Weight _____gms

3.4.4.2. Morphological anomalies □ Yes □ No

3.4.4.3. If yes,
describe_____

3.5. Central nervous system

3.5.1.1. CSF appearance

3.5.1.1.1. □ Clear

3.5.1.1.2. □ Hemorrhagic

3.5.1.1.3. □ Straw colored

3.5.1.1.4. □ Turbid

3.5.1.1.5. Other, describe_____

3.5.1.2. Any features of increased intracranial pressure □ Yes □ No

3.5.1.2.1. If yes describe_____

3.5.1.3. Brain weight _____gms

3.5.1.4. Morphological anomalies □ Yes □ No

3.5.1.4.1. If yes, describe

3.5.1.5. Any evidence of trauma □ Yes □ No

3.5.1.5.1. If yes, describe:

4. SECTION 4: DECLARATION OF THE PRELIMINARY CAUSE OF DEATH

Preliminary cause of death at autopsy

Disease or condition directly leading to death*

_____due to (or as a consequence of)

Antecedent causes

Morbid conditions, if any, giving rise to the above cause, stating the underlying condition last

_____due to or as a consequence of)

_____due to (or as a consequence of)

_____

Other significant conditions contributing to the death, but not related to the disease or condition causing it

_____

_____

*This does not mean the mode of dying, e.g. heart failure, respiratory failure. It means the disease, injury or complication that caused death.

APPENDIX 7: POSTMORTEM LUNG BIOPSY SPECIMEN HISTOPATHOLOGY DATA COLLECTION FORM

HISTOPATHOLOGY OF THE RESPIRATORY SYSTEM				
Study enrollment number:	KNH Autopsy No. _____			
Decedent's age:	Months_____			
Date of admission:				
Date of death:				
Date of autopsy:				
Gross findings				
Anthropometric measurements:	Actual measure	Comment(s)		
Body weight (kg)				
Height/crown rump length (neonates) (cm)				
MUAC (cm)				
Chest circumference (cm)				
Other Measurements				
General Morphological appearance	Description			
Respiratory System	Lung wt. _____ g (right) and _____ g (left)			
Histopathology findings of the Respiratory System				
Lungs	Yes	No	Core Biopsy	Describe
Diffuse alveolar damage				Hyaline membrane fibrosis
Interstitial pneumonitis				Mild, moderate,

				severe
Interstitial Fibrosis				
Bronchopneumonia				Focal, patchy, diffuse
Bronchiolitis				Focal, patchy, diffuse
Pulmonary edema				Mild, moderate, severe
Pulmonary hemorrhage				Focal, diffuse
Necrosis				
Granulomas				Necrotizing, non-necrotizing
Aspiration Material in airways				
Atelectasis				
Viral inclusions				
Suspicious for Pneumocystis jiroveci				
Other Fungi				
Bacteria				Rods, cocci
Other: thrombi etc. ….				

Other tissues/organs	Description (tick where appropriate)
Trachea/bronchi	Tracheobronchitis Necrosis Bacteria Viral inclusions Fungi Other(s) describe below
Hilar lymph nodes	Granulomas Hemophagocytosis Necrosis Follicular hyperplasia Sinus hyperplasia Proliferation of vascular channels (state site) Other(s) describe below
Heart	Myocarditis Viral inclusions Necrosis Myocyte hypertrophy Myocyte atrophy Fibrosis (state site) Other(s) describe below