DISEASE BURDEN OF CONGENITAL RUBELLA SYNDROME AT FOUR REFERRAL HOSPITALS IN ZAMBIA

By

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A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF MEDICINE IN PAEDIATRICS AND CHILD HEALTH

UNIVERSITY OF ZAMBIA

LUSAKA

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DECLARATION

I hereby declare that this dissertation represents my own work and has not been presented either wholly or in part for a degree at the University of Zambia or any other university.

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APPROVAL

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ABSTRACT

Background: Congenital Rubella Syndrome (CRS) characterised by heart, eye and hearing defects usually affects an unborn baby when the mother is infected with Rubella virus in the first trimester of pregnancy. It is vaccine preventable and has been eliminated in certain parts of the world. In developing countries like Zambia, CRS unfortunately still carries a significant but unknown morbidity and mortality burden. We conducted a combined survey for retrospective and prospective occurrences at referral hospitals in Lusaka, Copperbelt and Southern provinces of Zambia to assess the burden of CRS in the three provinces.

Methods: Data for 2010 to 2015 was obtained for the three provinces at University Teaching Hospital in Lusaka, Arthur Davison Children's and Kitwe Central Hospitals on the Copperbelt, and Livingstone Central Hospital in Southern province. This included age and date on diagnosis, location, sex, type of diagnosis (laboratory or clinical), and associated manifestations (cataracts, congenital heart disease, etc.). Estimated incidence was calculated based on observed cases and Central Statistical Office population data for the provinces.

Results: A total of 36 CRS cases (clinically confirmed) were identified. The median age was 9.5 months. About 47% (17/36) of the children were tested for Rubella specific IgM with only 31% (11/36) having valid results. About 19% (7/36) had Laboratory confirmed CRS with 43% (3/7) of these confirmed retrospectively and 57% (4/7) prospectively. The commonest clinical features were congenital cataracts, congenital heart disease (patent ductus arteriosus) and microcephaly. The commonest combined clinical features were congenital heart disease, congenital cataracts and microcephaly (14% {5/36}). Incidence was calculated for 2014 only as it had complete data. The incidence of CRS per 1, 000 live births was 0.13 for Lusaka, 0.06 for Copperbelt and 0.01 for Southern province.

Conclusion: CRS is real and still remains a problem in Zambia causing significant morbidity. It is underestimated in Zambia partly due to poor clinical assessment and record keeping.

Recommendations: Increased multidisciplinary collaboration among specialties, improved documentation of clinical details on patient case records, integration of Rubella specific IgM testing of patients' blood with quantitative IgG testing, Isolation of CRS patients and increased sensitisation of health workers.

Key words: Zambia, congenital rubella syndrome, incidence, developing countries.

DEDICATION

This work is dedicated to my family for their patience, love and sacrifice. No words can describe the gratitude in my heart. You are the wind beneath my wings.

ACKNOWLEDGEMENTS

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ABBREVIATIONS AND ACCRONYMS

ABR	:	Automated Brain Responses
ADCH	:	Arthur Davidson's Children's Hospital
AIDS	:	Acquired Immune Deficiency Syndrome
CDC	:	Centres for Disease Control and Prevention
CHD	:	Congenital Heart Disease
CHARGE	:	Coloboma of eye, Heart disease, Atresia of the choanae, Retardation of growth, Genito-urinary abnormalities, Ear abnormalities
CHOPS	:	Cognitive impairment, Heart defects, Obesity, Pulmonary abnormalities, Short stature/Skeletal abnormalities
CRI	:	Congenital Rubella Infection
CRS	:	Congenital Rubella Syndrome
DHS	:	Demographic Health Survey
ENT	:	Ear Nose and Throat
GAVI	:	Global Alliance for Vaccines and Immunisations
HIV	:	Human Immunodeficiency Syndrome
Ig	:	Immunoglobulin
KCH	:	Kitwe Central Hospital
LAB	:	Laboratory
LCH	:	Livingstone Central Hospital
MMR	:	Measles Mumps and Rubella Vaccine
MR	:	Measles Rubella Vaccine
MMRV	:	Measles Mumps Rubella and Varicella Vaccine
OPD	:	Out Patient Department
PCR	:	Polymerase Chain Reaction
PDA	:	Patent Ductus Arteriosus
PID	:	Primary Immune Deficiency
RCV's	:	Rubella Containing Vaccines
RNA	:	Ribonucleic Acid
SPSS	:	Statistical Package for the Social Sciences
TORCH	:	Toxoplasmosis, Other infections including HIV, Rubella, Cytomegalovirus, Herpes simplex

UTH	:	University Teaching Hospital
VSD	:	Ventricular Septal Defect
WHO	:	World Health Organisation

CHAPTER ONE

1.1. INTRODUCTION

Rubella is a viral infection caused by *Rubella* virus. Humans are the only known hosts. It usually presents with a rash and fever (WHO, 2003, Principles and Practice of Clinical Virology, 5th Edition, 2004). The virus is a single stranded RNA virus from the *togavirus* family being an exclusive member of *rubivirus* genus. The other genus under the *togaviruses*, *alphavirus,* includes *equine encephalitis* viruses and *o'nyong' nyong'* viruses (Principles and Practice of Clinical Virology, 5th Edition, 2004).

The infection itself is usually mild and self-limiting but when an expectant mother acquires the infection around conception and in early pregnancy (up to 16 weeks), this may result in abortions, foetal demise and Congenital Rubella Syndrome (CRS). CRS may include deafness, blindness, congenital heart defects, microcephaly and bone radiolucencies (WHO, 2009, Lanzieri TM et al, 2004). Sensorineural deafness is the most common manifestation of CRS (Cooper LZ et al, 1969, Mcintosh ED et al, 1992).

Approximately 110 000 cases are estimated to occur annually in Africa (Goodson JL et al 2011, WHO, 2009). Countries like Ethiopia and Gambia are estimated to be of low disease burden with less than 10 percent contribution to the burden. Countries like Zambia and Uganda are of medium disease burden with 10 percent to 25 percent contribution to the disease burden. Nigeria and Ghana are estimated to have more than 25 percent contribution to the disease burden being termed high burden countries (WHO, 2009).

The world Health Organisation (WHO) in accordance with its mandate to provide guidance to member states on health policy matters aims to eradicate CRS by 2020 and with partner support introduce rubella containing vaccines (RCV's) into Expanded Programmes of immunisations (EPI's) worldwide (WHO, 2009). WHO recommends that countries without rubella vaccination such as Zambia assess the burden of Rubella and CRS (WHO, 2009).

At the four hospitals under consideration, children were diagnosed with CRS but active surveillance was not routinely done i.e. blood collection for Rubella Specific IgM and case notification. Estimates were projected using laboratory data of positive blood

samples for Rubella IgM (Goodson JL et al, 2011) and were said to be an underestimation.

1.2. STATEMENT OF THE PROBLEM

CRS research to document disease burden at the four sites in Zambia was yet to be done. CRS burden estimation and Laboratory confirmation should be routinely done. While the condition is notifiable, there was no empirical evidence of its clinical picture and demographic profile. It is also imperative that patients be appropriately referred.

1.3. STUDY JUSTIFICATION

This study will contribute baseline data on the morbidity burden of CRS. Baseline data will facilitate impact assessment after introduction of rubella vaccination. It is important that such data be actively analysed to note disease patterns of CRS before and after the vaccine is introduced.

1.4. RESEARCH QUESTION

What is the disease burden of Congenital Rubella Syndrome at four referral Hospitals (University Teaching Hospital, Arthur Davidson's Children's Hospital, Kitwe Central Hospital and Livingstone Central Hospital) in Zambia?

1.5. GENERAL OBJECTIVE

To determine the disease burden and profile the management of CRS at four referral Zambian Hospitals: University Teaching Hospital (UTH)- Lusaka, Lusaka Province: Kitwe Central Hospital (KCH)- Kitwe, Copperbelt province: Arthur Davidson Children's Hospital (ADH)- Ndola, Copperbelt Province and Livingstone Central Hospital (LCH)- Livingstone, Southern Province.

1.6. SPECIFIC OBJECTIVES

1.6.1. To estimate the number of clinically confirmed CRS cases at the study sites.

1.6.2. To estimate the number of laboratory confirmed CRS at the study sites.

1.6.3. To describe clinical features and demographic profiles associated with CRS at the study sites.

1.6.4. To retrospectively profile the management of CRS.

1.6.5. To calculate incidence of CRS by province.

CHAPTER TWO

2.1 LITERATURE REVIEW

The precise incidence of rubella globally is unknown (Morice A et al, 2009, WHO, 2009). Variations in vaccination programmes, lack of surveillance and inability to confirm suspected cases are just some of the reasons attributed. Developing countries are more at a loss because they are dependent on donor assistance for sustainability of vaccination programmes and huge costs associated with surveillance and diagnosis of diseases such as CRS result in prioritisation of available resources towards high prevalence diseases such as Pneumonia and Tuberculosis (Reef SE et al, 2009). WHO however estimates the worldwide incidence of Rubella at 0.1 to 0.2 per 1000 live births (WHO, 2003).

Of the six WHO regions: The Americas, Africa, South-East Asia, Eastern Mediterranean, Western Pacific and Europe, the Americas was the first to introduce Rubella vaccination in 1969 (Castillo-Solórzano C et al, 2011, Nelsons textbook of Paediatrics, 19th Edition, 2011). After the introduction of the vaccine, the incidence of rubella decreased by more than 99 percent. At that time the vaccine was a single dose administered to children between the ages of nine months and fifteen months. An outbreak in 1989 to1991, led to the introduction of an additional dose given at fifteen months of age making the rubella vaccine a two dose vaccine (Le Baron CW et al, 2009). This second dose introduction led to a drastic drop in the incidence of Rubella from 0.45 per 100 000 to 0.1 per 100 000 (Castillo-Solórzano C et al, 2011). In 2010, Centres for Disease Control and Prevention (CDC) announced that Rubella had been eliminated in the region of the Americas (CDC, 2010). In the region of Europe, WHO had set an elimination goal of 2015 (WHO, 2009, Vyse AJ et al, 2002). This has however not been achieved due to cases being reported mainly in Poland. The population at risk in Poland are men as the vaccine campaigns were targeted at women in the reproductive age group to prevent them having children with CRS. This approach has left the men at risk of contracting and hence transmitting Rubella (WHO, 2016). WHO has set a world wide elimination goal of 2030 (WHO, 2009).

Point prevalence estimates from serological surveys throughout Africa among adults generally estimate the continental prevalence of rubella to range from 1 to 29 percent.

(Goodson JL et al, 2011). Urban transmission rates are higher than those in rural settings (Gilani Z, 2013). This can be explained by the overcrowding which is typical of the African urban setting resulting in uninterrupted transmission during an outbreak.

When Rubella immunisation is targeted at girls or women of child bearing age, the incidence of CRS declines (Menegolla IA et al, 2011). This strategy does not however lead to elimination and aggressive campaigns toward elimination target immunising the whole population (Peltola H et al, 2008). Immunising the whole population is however very costly (Peltola H et al, 2008) and not sustainable in the developing world. A cheaper public health approach toward elimination of vaccine preventable diseases is childhood immunisation. A 20 to 30 year elimination goal involves a one dose immunisation schedule and regular follow up campaigns or two dose immunisation schedules. A 10 year elimination programme involves the 20 to 30 year campaign activities and in addition, a speed up campaign targeting all children, adolescents and adults (WHO, 2003).

It has however been noted that there is an increase in the incidence of CRS in regions of low immunisation coverage (Schoub B et al, 2009). This was seen in Costa Rica (Morice A et al, 2003, Jimenez G et al, 2007), Greece (WHO, 2003) and South Africa (Schoub B et al, 2009). In South Africa, the vaccine was being provided in the private sector leading to a shift in the epidemiology of Rubella i.e. an increase in the number of CRS cases. On this premise, WHO recommends vaccination introduction only in countries with high coverage i.e. 80 percent to be exact. The Zambian Demographic Health Survey of 2009 estimated Zambia's immunisation coverage at 85 percent and thus qualified the country for RCV introduction. The Zambian Ministry of Health with partner support e.g. Global Alliance for Vaccines and Immunisation (GAVI), had planned to introduce RCV's at that time and finally RCV's were introduced to the public sector in September 2016. The introduction of RCV's started with an initial accelerated approach characterised by mass immunisation of children between the ages of 9 months to 15 years old with Measles, Rubella vaccine (MR). This was followed by the integration of the vaccine (MR) into the EPI with the vaccine being given at 9 months and 15 months of age respectively.

Rwanda, was the first country in sub-saharan Africa to incorporate the Rubella Vaccine into its EPI. Vaccination of infants in Rwanda commenced early in 2014 (WHO, 2014).

WHO recommends that ''Burden of Congenital Rubella'' be assessed using retrospective case record review which can be supplemented by tracing Rubella specific IgM blood results for countries where routine surveillance is done (World Health Organisation: "Recommended standards for surveillance of selected vaccine-preventable Diseases,'' 2003).

In Costa Rica Jimenez G et al measured the ''burden'' of CRS by calculating an expected incidence using reported cases of Rubella in women of reproductive age (15-45) using modelling analysis during epidemic and endemic years.

In Zambia, there are very few published studies on Rubella and/or CRS. A study was conducted by Theresa Watts et al in 1980 looking at the sero-prevalence of expectant mothers attending antenatal clinic in urban Lusaka. From the enrolled number of approximately 200 women, the study found that about 80 percent had antibodies to Rubella reflecting previous exposure to infection by the wild type virus as these mothers were not immunised. This meant the remaining 20 percent were susceptible to being infected and hence potentially transmitting Rubella to their unborn babies.

WHO has set up sentinel laboratories around the world. The WHO sentinel laboratory in Zambia started Rubella surveillance in 2002. Samples analysed in the laboratory include those referred for Measles IgM testing. If found negative for measles, the samples are tested for Rubella. This is in line with the WHO recommendation that Rubella surveillance runs parallel with successful measles programmes. This is cost effective and is possible because of the marked clinical overlap between the two conditions (Vyse A J et al, 2010). Between 2002 & 2009, 16 cases of infants with laboratory confirmed CRS were detected in Zambia (Goodson JL et al, 2011). In this study by Goodson et al, the authors conducted Medline searches for Rubella seroprevalence assays and analysed regional measles surveillance data submitted to WHO. In Zambia data supplied by the WHO sentinel lab revealed a total of 647 Rubella cases of which 336 occurred in females. Of these 336 females, 54 belonged to the reproductive age group and hence were potential transmitters of the virus to their unborn babies. This study did not sub analyse to see the regions where these women were coming from as they were interested in the CRS case aggregated data.

A study done in rural Zambia (Gilani Z et al 2013) concluded that prevalence rates of Rubella in rural Zambia are actually less than what was documented in previous surveys in similar settings e.g. Watts T et al. In This Ph.D. dissertation paper submitted to John Hopkins University, Gilani examined the age structure and spatial characteristics of people's susceptibility and immunity to Measles and Rubella vaccines in rural Choma District of the Southern province of Zambia in 2008-2009. The study involved collecting dried blood spots from 632 participants which were analysed for Measles and Rubella specific IgG antibodies. Incidence was then estimated using a catalytic compartmental model. The observed Rubella seropositivity was 50% generally while that among pregnant women was 77%.

There is published documentation of four case reports of laboratory confirmed CRS at the University Teaching Hospital, UTH in Lusaka, Zambia (Mazaba-Liwewe et al, 2013). Those were neonates who on admission were noted to have had characteristic CRS features and a diagnosis made. The diagnosis was then confirmed by serology (i.e. Rubella specific IgM) in the WHO sentinel laboratory at the UTH.

In 2012, an outbreak of suspected Measles occurred in Zambia. The outbreak occurred at a time when the Ministry of Health had introduced a second dose of measles vaccine at eighteen months of age. In addition, a measles campaign targeting all children up to fifteen years of age was embarked upon. The public attributed the appearance of symptoms of rash and fever to the vaccine. Notably, there was a school outbreak at *Mpelembe* Secondary School in Kitwe town, of Copperbelt Province which documented 53 cases. According to verified reports from the WHO sentinel laboratory, blood samples revealed the infection was in fact Rubella.

2.2. Rubella virus infection

2.2.1. Pathogenesis

Rubella infection is spread by aerosol droplets with replication occurring in the respiratory epithelium of the nasopharynx. The virus then migrates to the regional lymph nodes (sub-occipital, posterior cervical and posterior auricular chains) where further replication occurs. During this incubation period (fourteen to twenty one days), a viremia ensues and the patient may complain of fever, headache, malaise, anorexia, injected conjunctiva and rhinorrhoea (Principles and Practice of Clinical Virology, 5th

Edition 2004, Nelsons textbook of Paediatrics, 19th Edition 2011). These symptoms might be accompanied by a maculopapular rash, one to five days after their onset, lasting five days and starting on the face extending caudally. The rash might pose a diagnostic challenge in dark skinned people as it is usually faint and might be absent in up to fifty percent of cases (Principles and Practice of Clinical Virology, 5th Edition 2004). The infection can be dismissed as a common cold. *Forchheimer* sign is noted when the soft palate has petechiae (Nelsons textbook of Paediatrics, 19th Edition 2011).

The period of maximal infectivity is five days before the appearance of the rash to six days after the rash disappears. After the period of lymph node involvement, a viremia ensues leading to transplacental infection to the developing foetus. The exact mechanism leading to anomalies in the foetus is unknown but tissue destruction via vasculitis is the proposed mechanism. The virus is postulated to induce a direct cytopathic effect. Tissue destruction is also thought to result by apoptosis induced by an unidentified rubella specific protein (Principles and Practice of Clinical Virology, 5th Edition 2004).

2.2.2. Clinical presentation

Defects may be; *Ophthalmic*: Cataracts, Microphthalmia, Glaucoma, Pigmentary Retinopathy and Chorioretinitis (Vijayalakshmi P et al, 2002), *Auditory*: Sensorineural Deafness and/or *Cardiac:* Patent Ductus Arteriosus, Peripheral Pulmonary Artery Stenosis, or Ventricular Septal Defects (Lanzieri TM et al, 2004, Cooper LZ et al, 1969, Gupta JD et al, 1975).

Other manifestations include microcephaly, hepatosplenomegaly, hepatitis, thrombocytopenia, meningoencephalitis and radioluncencies of long bones (Lanzieri et al, 2004).

Late onset manifestations include: Type 1 diabetes mellitus, autism, developmental delay, and sub-acute sclerosing pan-encephalitis as is seen in measles (Nelsons textbook of Paediatrics, 19th Edition 2011).

The main differential for Rubella is Rubeola (Measles). Other differentials include scarlet fever, infectious mononucleosis, kawasaki disease and coryza (Nelsons textbook of Paediatrics, 19th Edition 2011).

There are also several infectious and non-infectious disorders which manifest with ophthalmic, auditory and/or cardiac defects associated with microcephaly, mental retardation etc. These should be considered in the differential of CRS and hence the need for lab confirmation. In cases where the IgM for Rubella is negative other congenital infections should be evaluated for particularly Toxoplasmosis, Cytomegalovirus and Enteroviruses. Non-infectious differentials for CRS include Visceral Myopathy, Hypomyelia Leukodystrophy, Chromosome 1p deletion, Noonan, CHARGE and Digeorge syndromes.

2.2.3. Laboratory diagnosis

Definitive diagnosis of Rubella is made by demonstrating Rubella specific IgM antibody in blood (Gupta JD et al, 1975). This occurs within two weeks of acute infection. At about four weeks there begins a switch to IgG (Gupta JD et al 1975). Virtually all Infants born with CRS have positive IgM at birth and about sixty percent at six months and about thirty percent are still positive at twelve months (WHO, 2009). Unlike IgG, IgM does not cross the placenta and thus serum detection indicates recent exposure to Rubella virus (Principles and Practice of Clinical Virology, 5th edition 2004).

An alternative means of CRS lab diagnosis is demonstrating a four-fold rise in Rubella specific IgG. Since neonates have some passively transferred IgG, demonstrating this rise reflects the neonate being infected (Beasley RP et al, 1969, Nelsons textbook of Paediatrics, 19th Edition 2011, WHO, 2011).

Viral culture demonstrates the virus in nasopharyngeal swabs collected during active infection. Newer methods include molecular techniques like Polymerase Chain Reaction (PCR). The major challenge with PCR is the cost which makes it impractical for routine use but has usefulness in research (WHO, 2003).

2.2.4. Treatment

There is no antiviral therapy for rubella as the infection is self-limiting with management being symptomatic. Management of CRS however can be challenging as it is multidisciplinary (Principles and Practice of Clinical Virology, 5th edition 2004) involving the Paediatrician, Ophthalmologist, Neurologist, Cardiologist, Cardiothoracic surgeon, Audiologist/ Audiometrist and Developmental intervention Specialists. The

outcome of these neonates largely depends on the severity of the defects usually cardiac and neurological contributing the gravest consequences. A 25 year follow-up of CRS patients in Australia revealed average intelligence and normal society integration after specialist intervention of eye and ear anomalies (Principles and Practice of Clinical Virology 5th edition 2004). Management of CRS is however costly and the small cost & benefit of vaccination far outweigh that of treatment (Lanzieri et al, 2004).

2.2.5. Prevention

Vaccination has been the basis of elimination in the WHO, regions (Bart KJ et al, 1985, WHO, 2000). The vaccine is a live attenuated vaccine like measles, mumps, varicella, yellow fever and polio (WHO, 2011). Most are based on an RA 27/3 strain. Other strains are based on the *Takashi, matsuura* and TO-336 strains used in Japan and the BRD-2 strain used in China (Beasley RP et al, 1969). There are four available preparations of the vaccine: a stand-alone vaccine and combination RCV's. Divalent measles & rubella (MR), trivalent measles, mumps and rubella (MMR) and a quadrivalent measles, mumps rubella and varicella (MMRV) {The cooperative group 2000}.

There is more than 95 percent response to a single dose of rubella vaccine (Tischer A et al, 2000, Beasley RP et al, 1969). Vaccine induced immunity is lifelong though some studies have demonstrated low antibody levels in a few individuals at 20 year follow-up (Davidkin I et al, 2008, O' Shea S et al, 1988, WHO, 2011). The indication for second dosing for measles and mumps results in a second dose of the rubella as well in the trivalent RCV. For the 5 percent of individuals who fail to seroconvert after vaccinations, concurrent infections and pre-existing maternal antibodies are implicated (Crovari P et al, 2011).

Re-infection, a rare phenomenon is defined as a significant rise in antibody concentration in an individual with pre-existing antibodies. If re-infection occurs in the first trimester of pregnancy in a previously infected woman, the likelihood of CRS is said to be low (Elias E et al, 1972).

The vaccine is generally well tolerated with mild side effects such as pain, redness and induration at the site of injection. Low grade fever, rash, irritability, lymphadenopathy, myalgia and parasthesias are documented. Arthritis has been reported in adolescents

mainly women who received the vaccine two weeks post vaccination (Crovari P et al 2011). Causal association between MMR vaccine and thrombocytopenia, parotitis, febrile convulsions and limb complaints has been made by Cochrane review of thirty one controlled trials. MMRV, the quadrivalent RCV however carried an excess risk of febrile seizures (4.3/10000) {Lieberman JM et al, 2006}.

Contraindications to RCV's include: 1) Pregnancy: This is a theoretical risk. Reviews of more than 1000 women who were unknowingly vaccinated during early pregnancy have shown not one single case of CRS reported. This theoretical risk however has led to a recommendation that women intending to fall pregnant should delay pregnancy for at least one month after the time of receiving rubella vaccine. In the event that a woman discovers she is pregnant soon after receiving a RCV, abortion is not recommended. 2) Allergy or history of hypersensitivity to neomycin and gelatine is a contraindication as these are components of RCV's. 3) HIV infection is not in itself a contraindication to vaccination but advanced infection or AIDS is as the risk of severe infection resulting from vaccination is high. 4) Congenital immune disorders or Primary Immunodeficiency Syndromes. 5) Malignancy (Lieberman J M et al, 2006).

Administration of immunoglobulins or other antibody containing blood products should be deferred for three to eleven months after vaccination with the MMR vaccine as these can neutralise the vaccine and hence render it ineffective especially the measles component. If the need to administer antibody containing blood products is urgent then the patient should be revaccinated after eleven months. If the monovalent Rubella vaccine is to be used and an individual received blood products, it is prudent to wait three months before vaccination. Blood product administration should be deferred for at least two weeks after monovalent Rubella vaccine administration unless of course the indication is dire (Kutler B J et al, 2006).

3. METHODOLOGY

3.1. STUDY DESIGN

This study was a cross-sectional study with both retrospective and prospective components. This methodology was adopted because of the condition's rarity.

Retrospective: Involved reviewing files / case records five years (October 2009 to October 2014) back for children who were seen with a diagnosis of CRS. As CRS can present with heart, eye and hearing defects, files were specifically looked for in the Cardiac, Ophthalmology, ENT, Neonatal, Paediatric Neurology and OPD sections of the above Hospitals.

Prospective: Infants were enrolled when they presented to the study sites meeting the inclusion criteria for clinically confirmed CRS. This started in October of 2014 through to October 2015.

3.2. TARGET POPULATION

Retrospective: All children seen in the last five years (October 2009 to October 2014) who were diagnosed as having CRS or whose documented features met the CRS criteria were enrolled into the study.

Prospective: Infants who were seen at the study sites and suspected to have CRS were recruited into the study.

3.3. STUDY SITES

The four referral hospitals, UTH, ADH, KCH and LCH are located in Lusaka, Ndola, Kitwe & Livingstone towns respectively. ADH is Zambia's only dedicated children's hospital with a bed capacity of around 550. KCH has a total bed capacity of around 630. KCH and ADH serve as the tertiary referral institutions in the copper belt region. LCH has a bed capacity of 235 and receives referrals from southern province and the southern part of western province. UTH, Zambia's ultimate referral hospital has a 1655 bed capacity. Patients enrolled in this study were recruited from the Ophthalmology, Cardiac, Neonatal, ENT & Paediatric OPD sections of the above Hospitals**.**

3.4. ELIGIBILITY

3.4.1 Inclusion Criteria

Case definitions (World Health Organisation: "Protocol for CRS sentinel surveillance." African Region, 2009)**:** An infant is said to have **clinically confirmed CRS** if a **trained** clinician examines and confirms or identifies **two majors** OR **one major and one minor** clinical signs as listed below:

MAJORS: Cataract(s) and/or Congenital Glaucoma; Congenital Heart Disease; loss of Hearing; Pigmentary Retinopathy.

MINORS: Purpura; Splenomegaly; Microcephaly; Mental Retardation; Meningoencephalitis; Radiolucent bone disease; Jaundice with history of onset within 24 hours after birth.

A laboratory confirmed CRS case is a clinically confirmed CRS case whose blood test for Rubella specific IgM test is positive.

Retrospective: Children were enrolled into the study if they had a diagnosis of CRS made within the last five years (October 2009 to October 2014) regardless of their age and the clinical features documented in their files or case records met the CRS case definition for clinically confirmed Congenital Rubella Syndrome. (See below) The reason that the children older than 1 year were enrolled was that in sub-Saharan Africa, children may only come into contact with a trained clinician after the age of 1 year but will still have the initial features e.g. patent ductus arteriosus or cataracts for which history can help ascertain whether cataracts for example are acquired versus congenital as these would not have been corrected.

Prospective: Infants were eligible to be enrolled into the study if they were suspected to have CRS and were enrolled if they met the CRS case definition for clinically confirmed CRS and Parental / Guardian consent was sought.

3.4.2. Exclusion Criteria

Retrospective: Children whom on closer scrutiny of their file/case record did not meet the case definition for clinically confirmed CRS were not enrolled into the study.

Prospective: Infants were excluded when a trained clinician refuted the diagnosis of CRS or if after being evaluated met the inclusion criteria but parents or guardians

declined to consent. Children one year and older at the time of presentation and meeting the case definition for clinically confirmed CRS were not enrolled into the study prospectively but their details were entered retrospectively.

3.5. SAMPLE SIZE

The prevalence formula below was used to calculate the sample size with the following assumptions made:

3.5.1. Prevalence crudely matches the incidence with rare conditions.

3.5.2. Estimated prevalence of 0.01% based on the lower value of WHO estimated worldwide incidence of Rubella at 0.1 to 0.2 per 1000 live births (WHO, 2003).

3.5.3. An average of two cases present to Zambian health care facilities per year. This is extrapolated from the fact that between 2002 & 2009, 16 cases of infants with laboratory confirmed Rubella cases were detected in Zambia (Goodson et al, 2011, WHO, 2011).

Prospective:

$N = \{Z^2 \times P (1-P)\} / (E)^2$

N = sample required

Z =Z statistic =1.96 (95% C I)

P= expected prevalence: 0.01% which is 0.0001

E= confidence interval: 0.05

Therefore $N = (1.96)^2 \times 0.01(1-0.01)$

$\qquad (0.05)^2 \quad = 15$

Factoring in a 5% refusal to participate: 15-(0.05 x 15) = 14

Prospective sample size = 14

Retrospective: 2 cases per year (Goodson et al, 2011) and surveillance since 2012. 12 years (2002 to 2014). 2 x 12 =24 assuming all the children are still being followed up.

Assuming one fifth of the projected number has either improper documentation or misplacement of files (24- {0.2×24}) =19

Retrospective sample size = 19.

Total calculated sample size {Retrospective and Prospective (15+19)} = 33.

3.6. SAMPLING METHODS

A census approach was undertaken to try and identify the maximum number of children with CRS. In addition to trying to identify as many affected children as possible, the retrospective component also aimed to profile the management that was undertaken for these children i.e. whether they were appropriately referred (fourth specific objective). Appropriate referral was whether all the noted clinical features were appropriately referred to the respective specialist(s).

Retrospective: An Initial visit to the study sites to carry out a five year case/file review (October 2009 to October 2014) for CRS cases was done in October 2014. Five years was adopted because in addition to adhering to WHO CRS surveillance, Ministry of Health, outpatient files are retrievably stored for five years. This comprised of reviewing records retrieved in ophthalmology, cardiac, ENT, neonatal, general paediatric and neurology clinics, speech & hearing centres & Special schools at the sites as earlier described. In each section, the registers were searched for the names and file numbers of any children who were seen with the diagnosis of CRS. The accompanying file number was then used to specifically look for that file. Once the file/case record was traced, it was scrutinised by the principal investigator for the clinical details that were documented to see if the child fitted in the case definition for **clinically confirmed CRS** after which they were entered into the data base and assigned a unique identification number.

Ophthalmology: All files for children with congenital eye problems were identified and scrutinised for documented features that would classify the child as having CRS.

Cardiology: Files of children with congenital heart disease: Patent Ductus Arteriosus, Peripheral Pulmonary artery stenosis, complex congenital heart disease or Ventricular Septal Defects were scrutinised.

ENT: Files of children with primary deafness were scrutinised.

Neonatology: Anomaly registers, case record indices and ward progress books were scrutinised for compatible features.

Speech and Hearing Centre and UTH Special School: Sight and hearing impaired children were targeted with the accompanying school medical record reviewed. In the speech and hearing centre, it was challenging to trace the accompanying files and the register did not document the other features e.g. cataracts. A few case records (multi-disciplinary assessment forms) that we managed to get in the special school for the previous five years particularly at UTH had a clear aetiology other than CRS (cerebral palsy) automatically excluding these children. The other hospitals did not have speech and hearing centres or special schools.

Outpatient registers: Diagnoses of congenital malformations, cataracts, blindness, deafness or heart disease were sought in an attempt to trace the accompanying file. This was in order to see if the final diagnosis was CRS.

The childs details (demographics, clinical features, maternal age, history of maculopapular rash in pregnancy, gestational age as well as whether the Rubella was laboratory confirmed or not) were then entered into the CRS suspected case investigation form (Appendix A). The CRS was further classified as **laboratory confirmed CRS** if the blood test was positive for Rubella specific IgM. If the blood was collected and no result was documented on file, an attempt was made to trace the result. The file was also scrutinised to assess whether the infant was appropriately referred in view of the features that were documented on file, for example a child with Congenital Heart Disease to the Cardiologist or one with cataracts to the ophthalmologist.

Prospective: During the initial visit in 2014, sensitisation was done to reorient clinicians & raise the index of suspicion for CRS cases by way of clinical meetings at the study sites. Recruitment of infants meeting the inclusion criteria at the study sites then followed. Each study site had a focal point clinician to examine the infants suspected of having CRS. At UTH the PI, a paediatric registrar was the focal point clinician whilst the other three sites had paediatricians as focal point clinicians. When the clinician confirmed that the infant met the inclusion criteria (that the infant had clinically confirmed CRS), then the infant was considered eligible for the study. For Infants considered eligible for the study, Parents and / or guardians were provided with

full information regarding what Rubella is, its cause, differentials, presentation (including CRS), complications and prevention. The study was explained in simple language which was translated according to parent/guardian preference and their consent sought.

Upon obtaining written consent by way of signature or thumb print, the infant's data was then entered into a WHO CRS investigation form with a unique identification number. One millilitre of venous blood was then collected in a labelled plain specimen bottle for rubella specific IgM testing. For centres other than UTH, the sample was temporarily stored in the respective hospitals main laboratories awaiting transportation as per WHO standard operating procedure (WHO "Protocol for CRS sentinel surveillance." African Region, 2009). The specimens were eventually taken to the WHO sentinel laboratory (Virology laboratory at UTH). Results were entered on the forms and communicated to attending doctors then subsequently parents and / or guardians by the principal investigator. It would have been ideal for Rubella specific IgG to have been concurrently tested but unfortunately the study relied on routine Rubella surveillance and was not financially powered to do so. IgG would have been useful particularly for those whose IgM was consistently negative to prove rubella infection or refute the CRS diagnosis if both IgM and IgG were negative.

3.7. DATA MANAGEMENT

The initial sensitisation ensured reorientation of the clinicians with the diagnosis of CRS.

Retrospective: For the children whose diagnosis of CRS was documented on their case records or whose clinical features met the clinical classification of CRS, data was transferred onto the WHO surveillance form by the principal investigator, PI. These forms were then transported to the WHO sentinel laboratory in sealed envelopes where they were entered into the CRS data base.

Prospective: Once the patient was recruited, the WHO surveillance form was filled in. At UTH, the PI would fill in the form and then transport it to the laboratory manager at the sentinel laboratory. For the other sites, the form was filled in by the focal point clinician who then emailed a scanned copy to the PI at UTH. The emailed scanned form was then printed and then entered into the database. Once results were available, the laboratory manager would then enter them into the database. The laboratory manager

ensured limited access to the database through password protection and restricted lab access and hence assured patient confidentiality. The WHO surveillance form ensured uniformity and participant identification was made possible by the use of unique numbers.

3.7.1. Statistical Analysis

The Dependent variables were:

- Laboratory confirmed CRS.
- Clinically confirmed CRS

The Independent variables were**:**

- Child's age
- Infant's sex
- Residential area
- Head circumference.
- Maternal age
- Parity
- History of maculopapular rash in pregnancy and gestational age at which it occurred.
- Appropriate referral

Data was analysed using Microsoft excel 2013. The data was purely descriptive therefore no major analytical statistics were used. Tables and graphs were used to describe the data whilst means and medians were used to describe some proportions.

3.8. ETHICAL ISSUES

Ethics approval from the Research Ethics Committee ERES was sought. Permission was sought from Ministry of Health to carry out the research at the study sites. Written consent was obtained by either signature or thumb print after explanation of the study and procedure in simple language was done to the parents and / or guardians. Parents/Guardians were fully informed that they were free to decline and this did not affect the infant's treatment. They were also informed that there was no financial or material reward provided for participating in the study.

3.8.1. Confidentiality

The information in the study underwent shared confidentiality as need for notification and appropriate referral arose.

3.8.2. Patients Advantages

Participating in the study ensured appropriate referral for management of complications of infants with CRS as most of the complications require specialist management. It also meant more time spent on educating/explaining the condition of the child to parents / caregivers.

3.8.3. Patients Disadvantages

Parents/Guardians were informed of the risk involved in this study which was mainly pain from the needle prick during collection of the one ml blood specimen for Rubella specific IgM.

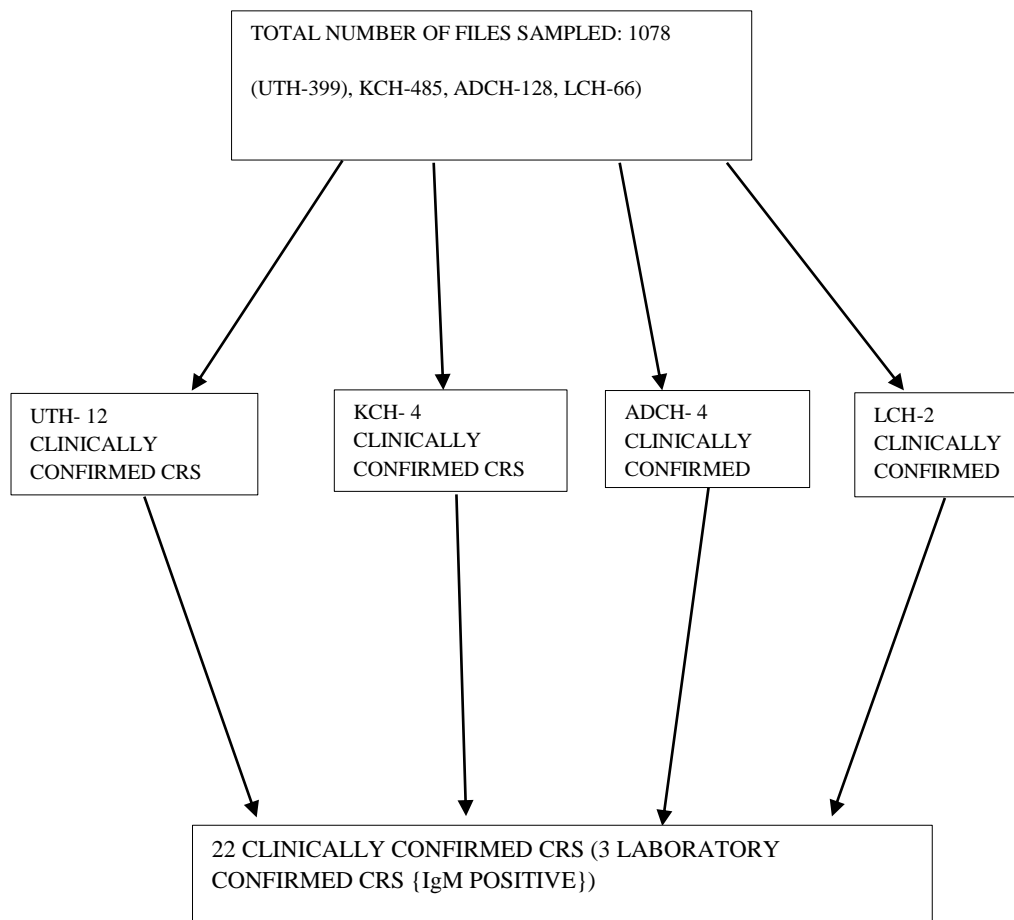### 3.8.4. Community Advantages

Parents/Guardians were informed that the information obtained from the study will be used as baseline data for Rubella vaccination.

This study also resulted in Increased CRS awareness among health care personnel and participants. The baseline data generated will help in trend analysis in terms of pre and post vaccination epidemiology.

4. RESULTS

There were total of 36 children identified with clinically confirmed CRS in the period 2009 – 2015, 22 were identified retrospectively from the hospital clinical records and 14 were enrolled prospectively upon meeting enrolment criteria. The flow chart below summarises the retrospective study process.

```
┌─────────────────────────────────────────────┐
│ TOTAL NUMBER OF FILES SAMPLED: 1078          │
│                                              │
│ (UTH-399), KCH-485, ADCH-128, LCH-66)        │
└─────────────────────────────────────────────┘
```

| UTH- 12 CLINICALLY CONFIRMED CRS | KCH- 4 CLINICALLY CONFIRMED CRS | ADCH- 4 CLINICALLY CONFIRMED | LCH-2 CLINICALLY CONFIRMED |

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┌─────────────────────────────────────────────┐
│ 22 CLINICALLY CONFIRMED CRS (3 LABORATORY    │
│ CONFIRMED CRS {IgM POSITIVE})                │
└─────────────────────────────────────────────┘
```

Flow chart showing Retrospective study process.

CHARACTERISTICS OF THE STUDY CHILDREN

The majority of children (55%) enrolled retrospectively presented to the hospital when they were above 24 months. The youngest child in this age range was 2 years (28 months) whilst the oldest was 6 years (80 months). Table 1 shows the age distribution of the study children retrospectively identified.

Table 1. Age distribution (Retrospective group)

AGE RANGE (Months)	PROPORTION OF CHILDREN	MINIMUM AGE (Months)	MAXIMUM AGE(Months)
0-6	9% (2)	1	4
6-12	18% (4)	7	10
12-24	18% (4)	12	18
Above 24	55% (12)	28	80

All children recruited prospectively were below 12 months and 71% of these fell in the age category below 6 months. The youngest child recruited was 2 weeks old.
Table 2 shows the age distribution of the study children prospectively recruited.

Table 2. Age distribution (Prospective group)

AGE RANGE (Months)	PROPORTION OF CHILDREN	MINIMUM AGE (Months)	MAXIMUM AGE(Months)
0-6	71% (10)	0	4
6-12	29% (4)	6	11

There were more female {70% (25/36)} than male children {30% (11/36)} in the study. Figure 1 shows the sex distribution of the total study population.
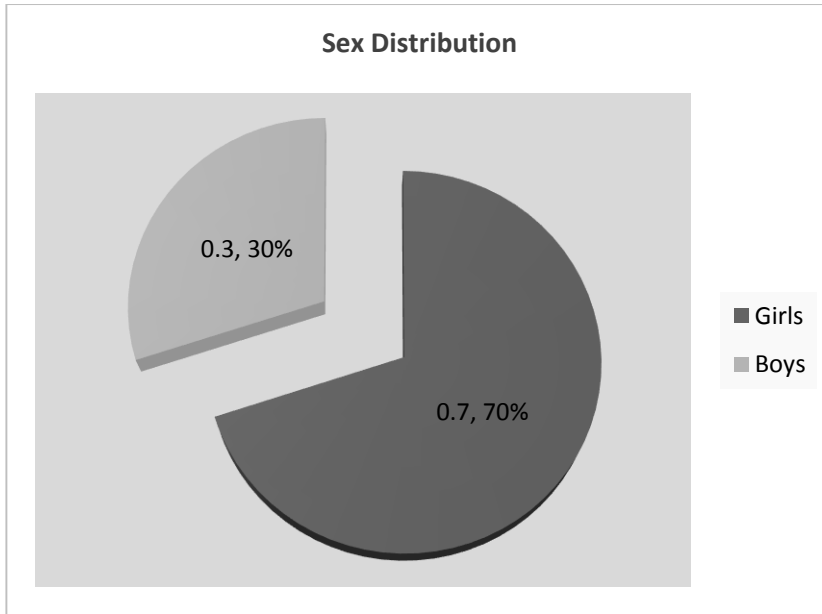
Figure 1 Sex Distribution of total population

Maternal age was poorly documented in the case files as only 28% (10/36) of the children had maternal age documented. Majority of maternal ages documented were for those children recruited prospectively. The mean maternal age for the whole group was 22.3 years.

The majority of children were identified in Lusaka province {69% (25/36)}, followed by Copperbelt province {25% (9/36)}, and Southern province {6% (2/36)}. There were 69% (25/36) children identified at the UTH, 11% (4/36) were identified at Kitwe Central, 14% (5/36) were identified at Arthur Davidson, and 6% (2/36) were identified at Livingstone Central Hospital. Table 3 shows the children recruited at the different health facilities while Figures 2 and 3 show the different proportions recruited to the Retrospective and Prospective studies respectively.

Table 3, Health facility population distribution

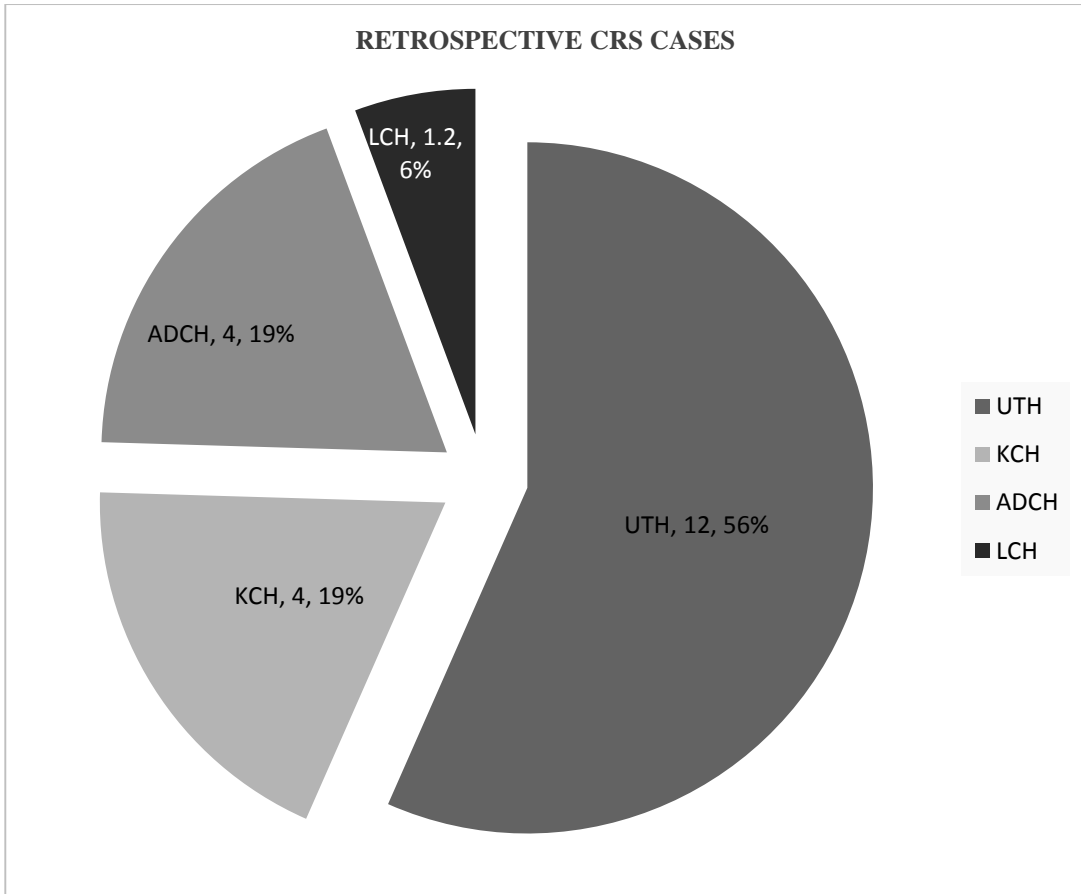| HOSPITAL	RETROSPECTIVE GROUP	PROSPECTIVE GROUP	TOTAL
UTH	12	13	25 (69%)
KCH	4	0	4 (11%)
ADCH	4	1	5 (14%)
LCH	2	0	2 (6%)

Figure 2. Retrospective clinically confirmed CRS cases by health institution
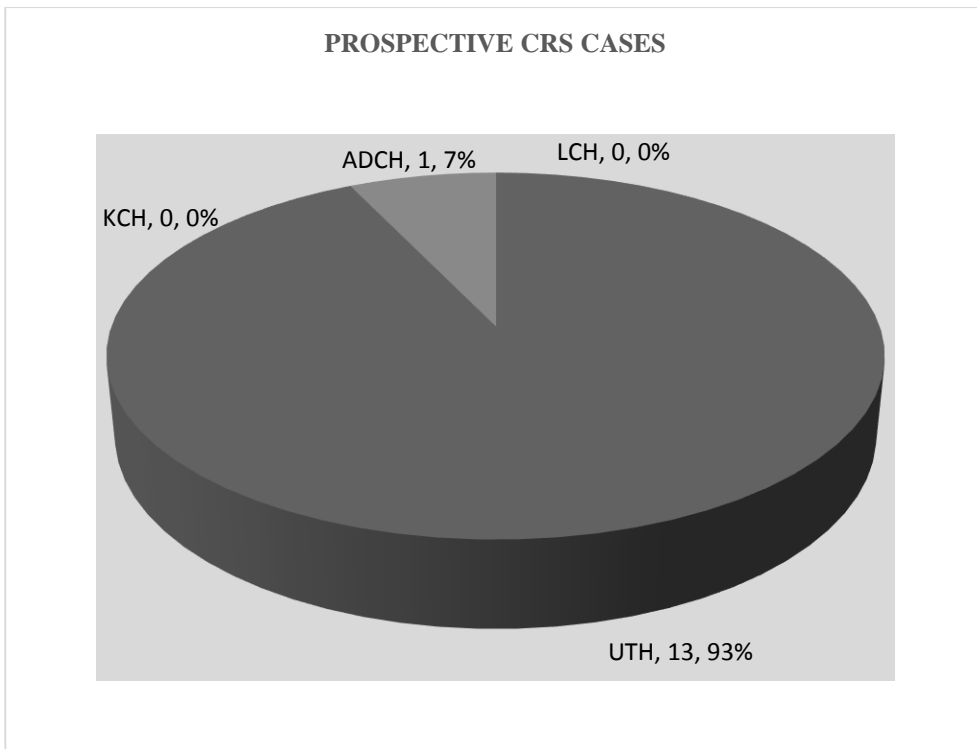

Figure 3. Prospective clinically confirmed CRS cases by health Institution.

Table 4 shows the Rubella IgM test results within the two study groups. The retrospective group had few IgM requisitions or documented results. Within the retrospective group there were a total of 18% (4/22) IgM requisitions, 14% (3/22) with positive rubella test results, 5% (1/22) with negative results. Within the prospective group there were a total of 100% (14/14) IgM requisitions, 29% (4/14) resulting in positive rubella test results and 29% (4/14) with negative results. About 43% (6/14) of requests resulted in the lab stating that the results were either missing or that there was lab error in result processing.

Table 4. Rubella IgM test results and study group tabulation

STUDY GROUP	POSITIVE IgM (LAB CONFIRMED)	NEGATIVE IgM	LAB ERROR	IgM NOT REQUESTED /DOCUMENTED
RETROS-PECTIVE	14% (3/22)	5% (1/22)	0	18/22 (81%)
PROS-PECTIVE	29% (4/14)	29% (4/14)	43% (6/14)	0

Of the 7 children with positive Rubella IgM, 3/7 children were identified retrospectively with no documentation on the exact age of confirmation on their files and the rest (4/7) identified prospectively. Of the prospective group 75% (3/4) belonged to the under six months category whilst 25% (1/4) belonged to six to twelve months category. Figure 4 refers.
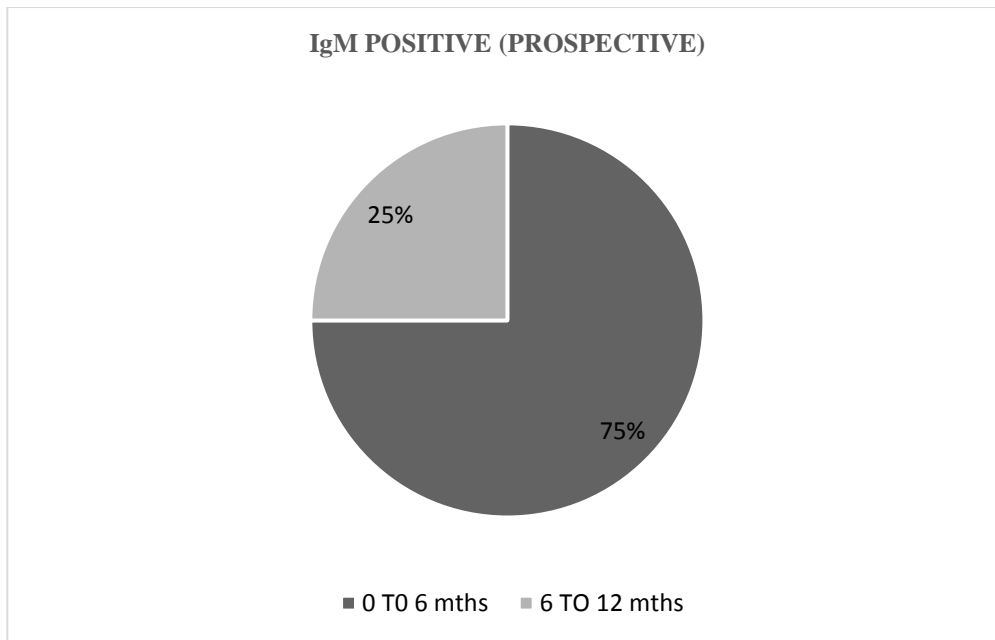
Figure 4. Positive Rubella IgM and Age category (Prospective cases)

All of the children (36) in the study had clinically confirmed CRS. There were a total of 19% (7/36) with laboratory confirmed CRS. Among the children in the prospective group, 29% (4/14) had laboratory confirmed CRS, whilst 14% (3/22) of the children in the retrospective group had laboratory confirmed CRS (Figure 5).
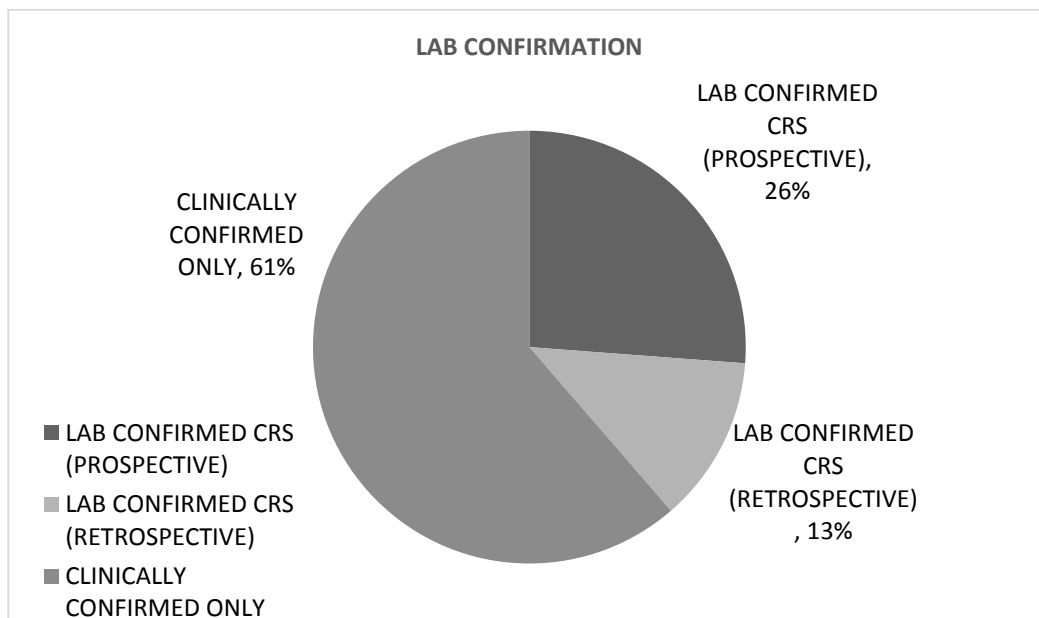

Figure 5. CRS Laboratory confirmation.

Most of the children, 93% (13/14) in the prospective group had echocardiography done whilst 59% (12/22) in the retrospective group had echocardiography done. Overall there were 61% (22/36) of children with confirmed Congenital Heart Disease (CHD), 31% (11/36) with no CHD, and the status was undetermined for 8% (3/36). The ones with undetermined status had a systolic murmur on auscultation but echocardiography was not done to confirm what lesion they had (Figure 6). Of the 22 children with CHD, 68% (15/22) had PDA. There were 9% (2/22) with pulmonary stenosis, 9% (2/22) with VSD and 14% (3/22) with complex congenital cardiac disease. All 3 patients with complex cardiac disease had lesions that had VSD and PDA in addition to other lesions (Figure 7).
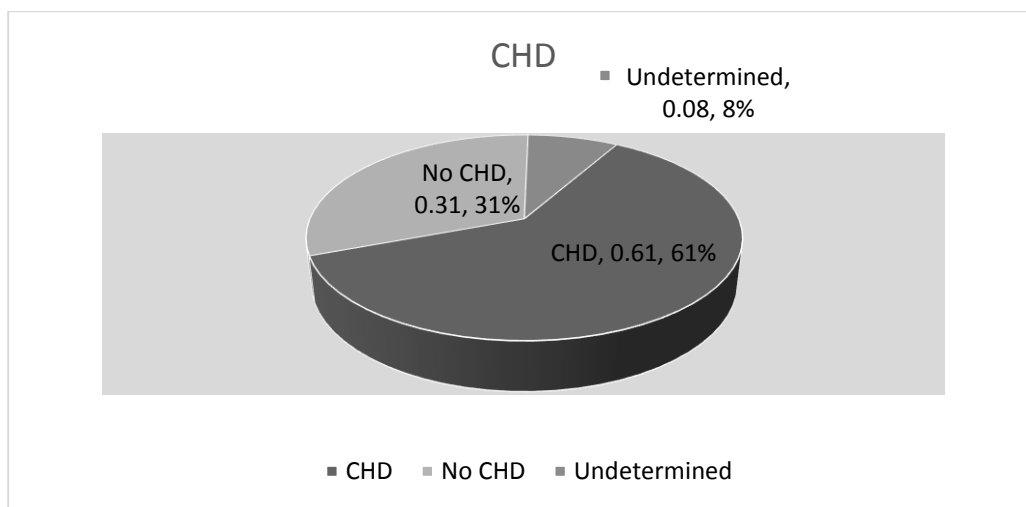

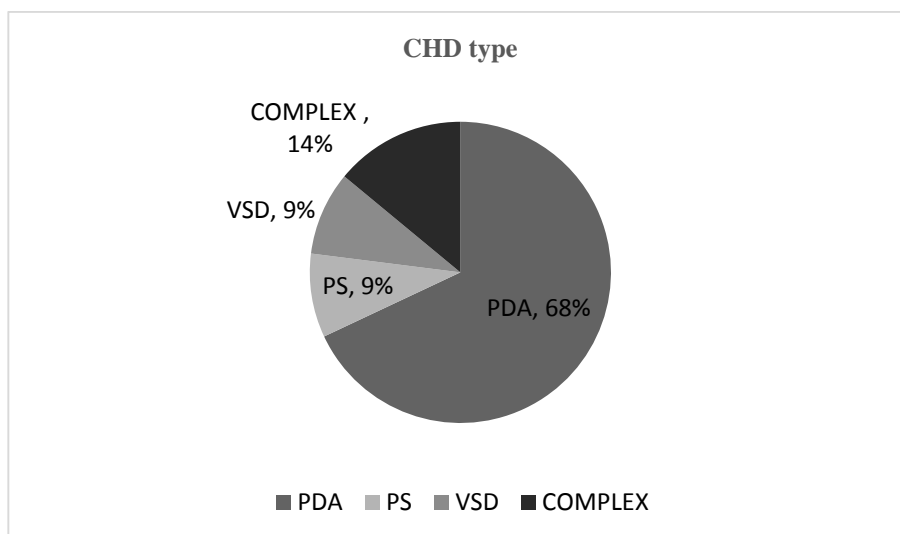**Figure 6. Congenital Heart Disease (frequency).**

Figure 7. Congenital Heart Disease (type).

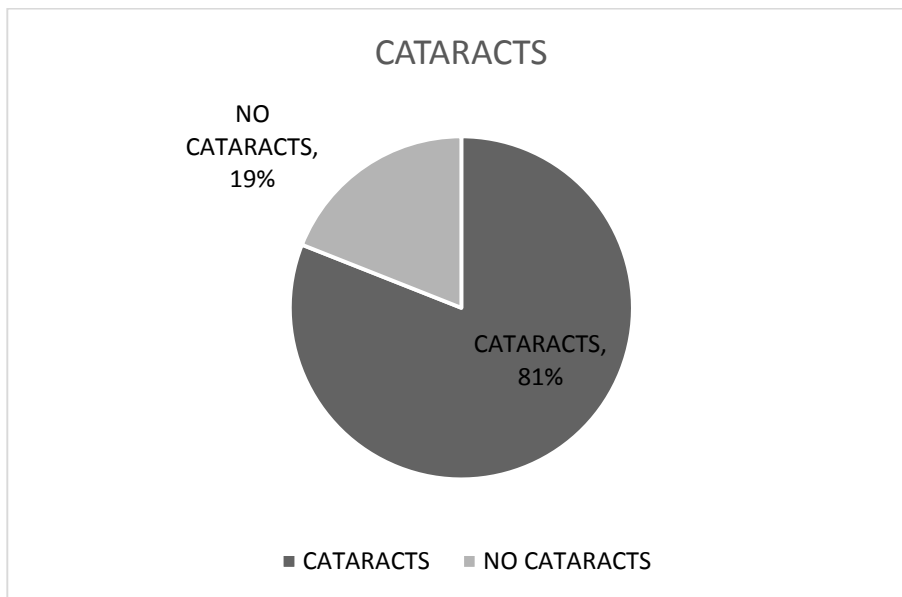There were a total of 81% (29/36) children with cataracts and 19% (7/36) without cataracts, (Figure 8).

Figure 8. Cataracts in the study population

There was only one child with pigmentary retinopathy and only 11% (4/36) of the children had hearing impairment. Of those with hearing impairment, 14% (3/22) were from the retrospective group and 7.1% (1/14) from the prospective group (Figure 9).

About 39% (14/36) of the children had microcephaly. In the prospective group they comprised 57% (8/14) and in the retrospective group, 27% (6/22) (Figure 9).

There were only 6% (2/36) children with meningoencephalitis (both from the retrospective group). The children with meningoencephalitis were clinically diagnosed, one neonate had reduced consciousness and seizures with cerebrospinal fluid showing cellular and metabolic alterations. The other was an infant who had altered mentation, seizures, fever, cataracts and suspected PDA (Echocardiography was not done). Lumbar puncture was not performed on the second infant. Mental retardation was identified in 25% (9/36) children retrospectively. For the children with ''mental retardation'' all of whom were identified retrospectively, there was no formal mental testing documented on file but the clinicians on assessment had noted a below average peer matched performance of response to basic questions and wrote ''global developmental delay'' in 5 patients and ''mental retardation'' in 4 patients. There was

no record of jaundice in the majority of the children as only 8% (3/36) were identified, 7% (1/14) from the prospective group and 9% (2/22) from the retrospective group. See Figure 9.

OTHER CRS CLINICAL FEATURES

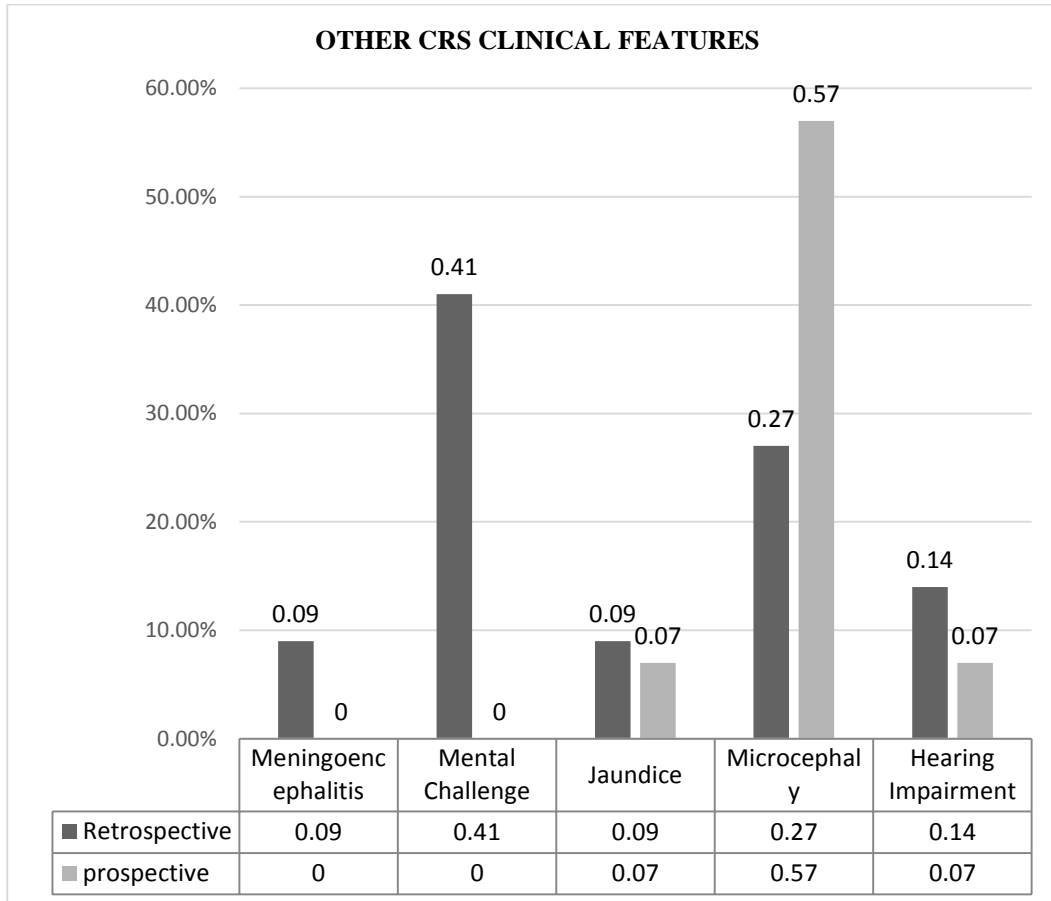|	Meningoenc ephalitis	Mental Challenge	Jaundice	Microcephal y	Hearing Impairment
■ Retrospective	0.09	0.41	0.09	0.27	0.14
■ prospective	0	0	0.07	0.57	0.07

Figure 9. Other CRS clinical features and group allocation

All the children from the prospective group were appropriately referred and not a single child from the retrospective group was appropriately referred (Figure 10).

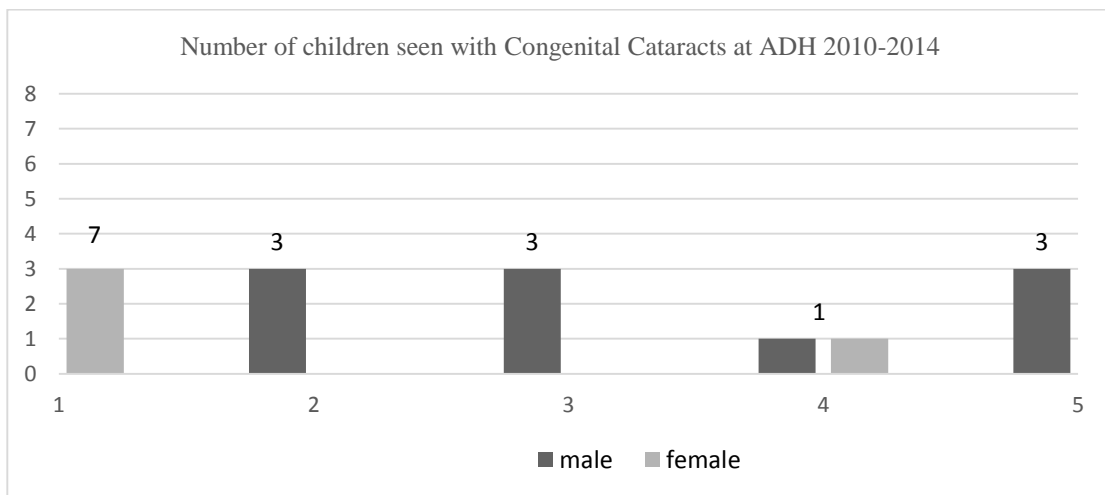**Figure 10: Appropriate referral: Proportion of study group appropriately referred**

Figure 11: Children seen with Congenital Cataracts at ADH (2009-2014).
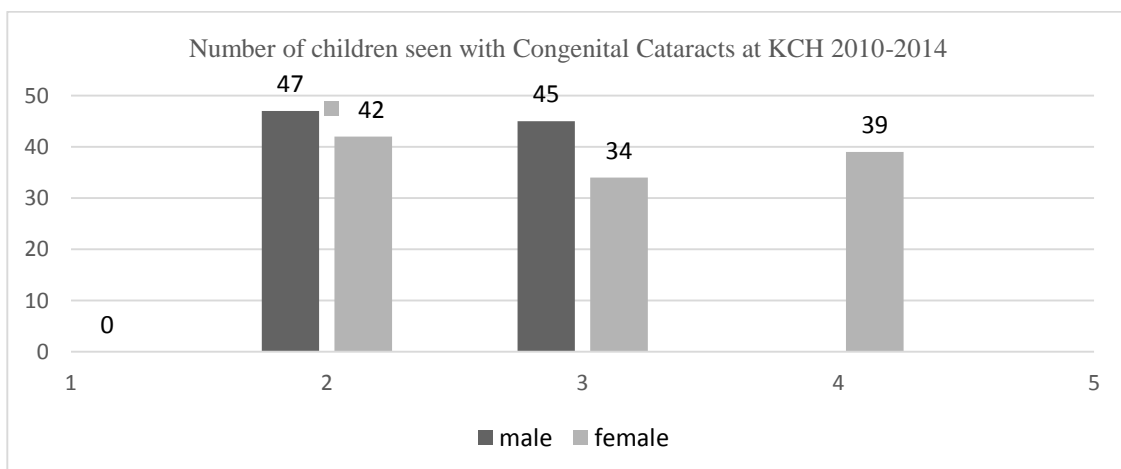
Figure 12: Children seen with Congenital Cataracts at KCH (2010-2014).

Approximately 88% of 132 of children's files with congenital cataracts sampled did not have a full systemic examination on them. Figures 13 and 14 refer.
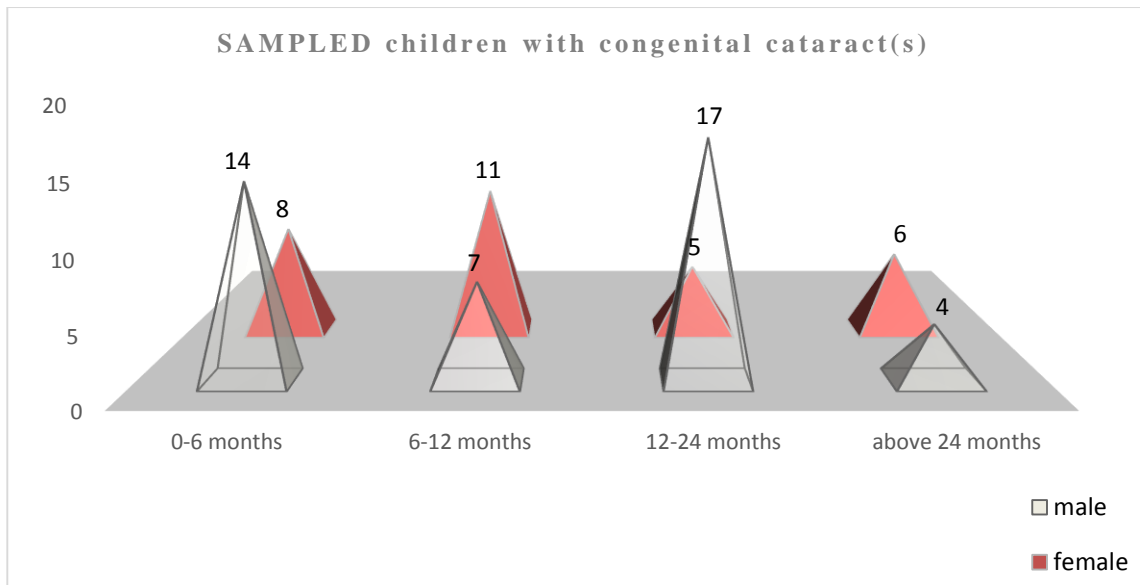
Figure 13. Age and sex distribution of randomly selected files of children with congenital cataracts
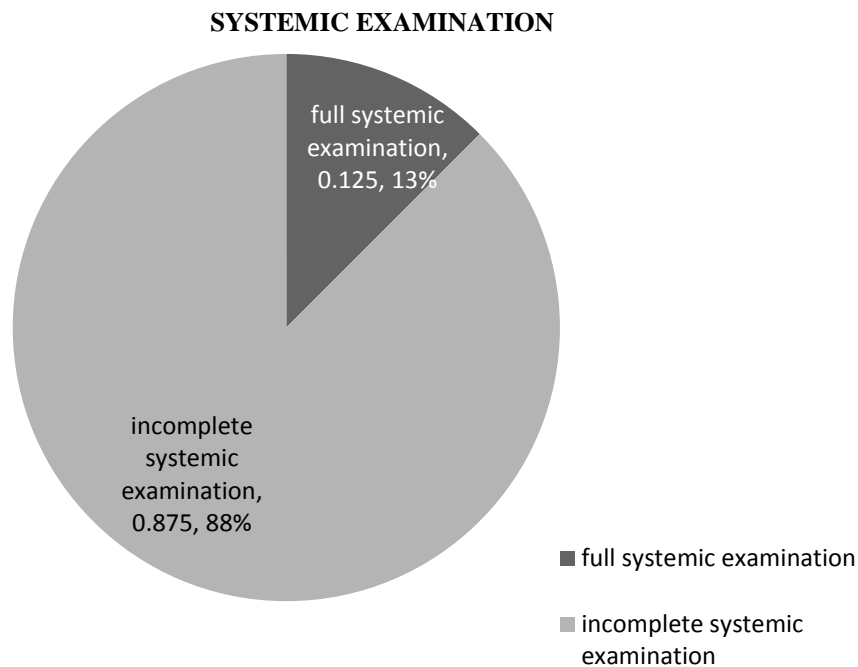
Figure 14. Proportion of sampled files of children with congenital cataracts without systemic examination.

Using figures for live births per province for 2014, a crude incidence for CRS was calculated (Table 5)

Table 5. Incidence of CRS per 1000 live births in 2014 calculated by province using live birth projections 2014 (Zambian DHS 2013)

PROVINCE	Copper belt	Lusaka	Southern
CRS CASES	9	25	2
LIVE BIRTHS	86,637	106,256	78,202
INCIDENCE	0.06	0.13	0.01

Table 6. Summary table of the clinical findings of each of the patients in the study and the corresponding status of their lab result (Bold represents prospective patients)

No:	AGE	CHD	CAT	RET	DEA	MIC	MEN	JAU	MR	IgM
1	1	VSD	N	N	N	Y	N	Y	N	Pos
2	7	PDA	N	N	N	Y	N	N	N	ND
3	17	PDA	N	N	N	N	N	N	Y	Pos
4	7	ND	N	Y	N	N	N	Y	Y	ND
5	10	ND	Y	N	N	N	N	N	Y	ND
6	13	ND	Y	N	Y	N	N	N	N	ND
7	18	U	Y	N	N	N	N	N	Y	ND
8	28	ND	Y	N	N	Y	N	N	Y	ND
9	36	ND	Y	N	N	N	N	N	N	ND
10	45	U	Y	N	N	N	N	N	Y	ND
11	48	ND	Y	N	N	N	Y	N	N	ND
12	72	U	Y	N	N	N	Y	N	N	ND
13	4	PDA	Y	N	N	N	N	N	N	ND
14	9	ND	Y	N	N	Y	N	N	Y	ND
15	12	PDA	Y	N	N	N	N	N	N	ND
16	31	PDA	Y	N	N	Y	N	N	N	ND
17	32	PS	Y	N	N	N	N	N	Y	ND
18	36	PDA	Y	N	N	N	N	N	N	ND
19	36	PS	Y	N	N	N	N	N	N	Pos
20	48	PDA	Y	N	Y	N	N	N	N	ND
21	72	PDA	Y	N	N	Y	N	N	Y	ND
22	80	PDA	Y	N	Y	N	N	N	N	ND
23	**1**	**PDA**	**Y**	**N**	**N**	**Y**	**N**	**N**	**N**	**RM**
24	**4**	**N**	**Y**	**N**	**N**	**Y**	**N**	**N**	**N**	**Pos**
25	**1**	**CCHD**	**Y**	**N**	**N**	**N**	**N**	**N**	**N**	**RM**
26	**3**	**PDA**	**Y**	**N**	**N**	**N**	**N**	**N**	**N**	**RM**
27	**0**	**N**	**Y**	**N**	**N**	**N**	**N**	**N**	**N**	**RM**
28	**0**	**N**	**Y**	**N**	**N**	**Y**	**N**	**N**	**N**	**RM**
29	**0**	**CCHD**	**N**	**N**	**N**	**Y**	**N**	**N**	**N**	**Pos**
30	**8**	**PDA**	**Y**	**N**	**N**	**N**	**N**	**N**	**N**	**Neg**
31	**7**	**PDA**	**Y**	**N**	**N**	**N**	**N**	**N**	**N**	**Neg**
32	**6**	**CCHD**	**Y**	**N**	**Y**	**Y**	**N**	**N**	**N**	**Neg**
33	**4**	**PDA**	**Y**	**N**	**N**	**Y**	**N**	**N**	**N**	**RM**
34	**11**	**PDA**	**N**	**N**	**N**	**Y**	**N**	**N**	**N**	**Neg**
35	**1**	**ND**	**N**	**N**	**N**	**N**	**N**	**Y**	**N**	**Pos**
36	**1**	**VSD**	**N**	**N**	**N**	**Y**	**N**	**N**	**N**	**Pos**

KEY

No. : Number

U: Undetermined

ND: Not done (Respective test not done)

N: No; meaning the respective clinical feature was absent

Pos: Positive IgM

Y: Yes; meaning the respective clinical feature was present

Neg: Negative

JAU: Jaundice

MR: Mental Retardation

MEN: Meningoencephalitis

RM: Result Missing

MIC: Microcephaly

Age: Age in months

CHD: Congenital Heart Disease

CAT: Cataracts

RET: Retinopathy

DEA: Deafness

5. DISCUSSION

Rubella, a vaccine preventable disease continues to be a significant health problem in the developing world (WHO, 2015). In this study, 36 patients with clinically confirmed CRS were identified. Of these 36, 14 were recruited prospectively and 22 retrospectively. This is cause for concern as it shows that there is still circulating wild type virus evidenced by the manifestation of CRS in children of mothers infected in pregnancy.

The mean ages between the Prospective and Retrospective groups differed significantly because the retrospective encompassed children who were older with the oldest child who presented to hospital aged six years (80 months) for a chest infection but on further clinical assessment, was found to have CRS.

There were more females (70 percent) than males (30 percent) in the study. This is not related to CRS itself as maternal infection is the deciding factor of CRS infection. Sex differentiation of the foetus would have occurred by the time transplacental infection occurs.

Maternal age was poorly documented on file (10/36) but for the few documented mean age was 23 years overall with 23.5 and 22.5 for the retrospective and prospective groups respectively. The youngest mothers were 16 years (prospective group) and 17 years (retrospective group). Younger mothers are particularly more likely to have an infant with CRS as they have had less time to be exposed to the wild type of Rubella virus in the absence of immunisation as compared to an older mother whose more years of life exposes them to seasonal outbreaks and hence naturally acquiring immunity (WHO, 2003).

Only one mother gave history of Maculopapular rash in their pregnancy in both groups. This was a mother aged 19 years of age who was referred to UTH from *Kalomo* District Hospital in Southern Province. She gave a history of a maculopapular rash at two months gestation. She reported that there were several reports of an itchy rash with associated sneezing and coughing in her village during the time period that she had a rash in May 2013. Literature reports that the rash may be absent in 50% of cases and

poses a further diagnostic challenge in dark skinned people (Principles and Practice of Clinical Virology, 5th Edition 2004).

Majority of patients were identified at UTH (69%) and this can be explained by the fact that it serves as the ultimate referral hospital in Zambia or that CRS is recognised and diagnosed more frequently because of the expertise available at the University Teaching Hospital. Prospectively, 93% of patients were recruited at UTH which is possibly due to the fact that it is where the principal researcher was stationed with several opportunities to re-sensitise health workers including doctors on the need for Rubella / CRS surveillance. This was followed by KCH (14%) with none recruited prospectively but 18% recruited retrospectively. The zero prospective recruitment was an unexpected finding because KCH eye unit serves as the national referral centre for paediatric cataract extraction. There is a well established Ophthalmology department strengthened by cooperating partners, Orbis international. ADCH patient contribution was 18% retrospectively and 7% prospectively and that of LCH 9% retrospectively and 0% prospectively.

All the children in the study were clinically confirmed whilst 19% had Laboratory confirmed CRS. Of the children with Laboratory confirmed CRS, 29% belonged to the retrospective group with 71% belonging to the prospective group. This supports the fact that there was more sensitisation of Health workers on need for CRS surveillance including collection of samples from clinically confirmed CRS cases for surveillance. For the children identified retrospectively with positive IgM, it was not clearly documented in their case records at what age the test was done. For the ones identified prospectively, 75% (3/4) were below six months of age with the remainder being above six months (25% {1/4}).

About 29% (4/14) of children in the prospective group had negative Rubella specific IgM results all of whom were above six months with increasing likelihood of the switch from IgM to IgG and thus having less than half the chance of IgM positivity (WHO, 2009). For the 4 patients with negative IgM, it is also possible that we could have been dealing with an alternative diagnosis. Two of them had a combination of congenital cataracts and patent ductus arteriosus. This combination can have several differentials including Senger and CHOPS syndromes. Another infant with a negative IgM from the prospective group was six months old and had critical congenital heart disease (with

VSD and PDA combination), congenital deafness, congenital cataracts and microcephaly. The differential diagnosis of this presentation includes CHARGE and Usher syndromes. The last child was eleven months old with PDA and microcephaly which also has several differential diagnoses. The fact that clinically confirmed CRS has several differential diagnoses reinforces the need for lab confirmation to be done on clinically confirmed cases. It also raises the question of whether clinically confirmed cases should be referred to by a more appropriate term such as ''clinically suspected'' CRS.

 Unfortunately, 43% (6/14) of those whose IgM was collected had laboratory error or missing results. This is a very strong and potentially avoidable limitation with a lot of room for improvement for which the study gave the appropriate feedback to the laboratory. Attempts were made to recall parents for repeat testing, three were unwilling whilst we could not get hold of two of them.

Congenital Heart Disease (CHD) was a prominent clinical feature in the study population (61%). The proportion with CHD in the retrospective group was 59% and that in the prospective group 64%. About 50% of children with CRS have CHD (Oster ME et al, 2010). The most frequent lesion in our patients was Patent Ductus Arteriosus (PDA) with 68% contribution followed by Pulmonary Stenosis (PS) {9%} and Ventricular Septal Defects (VSD) {9%}. This finding is in congruence with previous surveys which cite PDA as the most frequent lesion in CRS (Oster ME et al, 2010). It should be noted that the proportion of children with CHD could potentially be 69% (25/36) as three children on whom murmurs were auscultated did not have echocardiography done. These three children were classified as having undetermined CHD status.

The commonest clinical feature found in the study was the presence of congenital cataracts (81%). In literature eye abnormalities account for 43% of CRS clinical features (Oster ME et al, 2010). A plausible explanation for the high cataract proportion in our patients is the fact that it is the anomaly that will stare both the mother and health care worker in the face literally. Our approach in the retrospective process to identify children with cataracts in eye units/hospitals inherently skewed the number of children with cataracts in our study to be disproportionately high. This explanation is

particularly plausible because of the good eye programme at KCH with good record keeping.

There was only one child with pigmentary retinopathy, a child with the classic salt and pepper lesions seen in CRS. This child was recruited retrospectively. The proportion of children identified via ophthalmology is however a gross underestimate as evidenced by the lack of documentation of systemic examination despite there being a generic form for a systemic approach to the child with congenital cataract (Figures 12, 13 and 14). A well-documented systemic examination can aid in clinical classification of these children and subsequently, laboratory confirmation of CRS. The only near comprehensive systemic examination that was found on the few case records was the anaesthetist review prior to cataract removal. This means that for those who do not return to care, the diagnosis might have been missed.

The commonest anomaly encountered in CRS is hearing impairment (58%) {Oster ME et al, 2010, Cooper LZ et al 1969} but in this study only 4 children (11%) had deafness (Figure 8). Of these four children, three were documented retrospectively. One explanation for this is the inability of our centres to objectively assess hearing in children under two years due to not having equipment for automated brain responses ABR and Oto-acoustic- emission, OAE. Hearing screening in Zambia is however available in selected local facilities in Lusaka e.g. Beit Cure and local dissemination to colleagues about the availability of such facilities has been done.

Microcephaly was present in 39% of the total number of children in the study. (Figure 8). Literature reports that just under 25% of CRS patients have microcephaly (Rorke et al, 1973) and post-mortem studies have demonstrated it to predominantly affect grey matter with white matter being intact. There was no documented case of Hydrocephalus. It is a rare complication of CRS and documented case reports possibly attribute it to associated leptomeningitis or an associated congenital malformation (Tiwari et al 2015).

Meningoencephalitis was diagnosed in only 6% of patients (Figure 8: All from the retrospective group). Approximately 10-20% of children with CRS have Meningoencephalitis (Cooper LZ et al, 1969). The low number we found can be attributed to the challenges of obtaining CSF from children with parental apprehension being the biggest barrier. Another factor could be Health worker reluctance to CSF

collection in neonates despite it being a fairly safe procedure in this age group. A few of the younger infants recruited prospectively were treated for sepsis which could have possibly included CNS infection.

Mental retardation now termed intellectual disability (ICD 10) was diagnosed in 25% of the patients, all retrospectively recruited (Figure 9). The fact that it was not identified in the prospective group could be due to the challenge in recognising intellectual disability in infants. As more children in the retrospective group were older at diagnosis, features of intellectual disability become more apparent as learning and speech deficits. In the intellectually disabled child, hearing impairment must be ruled out. In infants the diagnosis of mental challenge is made on identifying failure of attainment of expected developmental milestones and subtle deficits are identified once the child enters preschool (American Association on Mental Retardation 2004). Given that the diagnosis of mental retardation is challenging in smaller children, the children in the prospective group should have been subjected to an age specific developmental assessment scale to objectively determine mental retardation and this was unfortunately overlooked during patient recruitment. It would be prudent to refer CRS patients to developmental intervention specialists where available for thorough assessment.

All of the infants in the prospective group were appropriately referred (100%). Referral status in the retrospective group (0%) was assessed by the management plans on file for the patients to see the appropriate specialists. For the hospitals without the appropriate specialists, escalation to the next level hospital was sought. As appropriate referral was an outcome in the prospective group, it is hence clear why there was adherence to appropriate patient referral (Figure 10).

As the only year with a complete data set was 2014, a crude incidence was calculated by looking at the province of residence of the study population. The Lusaka value of 0.13 is consistent with the postulated worldwide incidence of 0.1 to 0.2 per 1000 live births (WHO, 2013). Looking at the fore-mentioned study limitations, this is a gross underestimate and validates the need for the vaccine to be introduced into our EPI as soon as possible. The paucity of data in the retrospective clinical case records makes it difficult to completely combine the data for block analysis and discussion.

Only three children had the classic triad of CRS i.e. deafness, eye abnormalities and congenital heart disease. The fourth child with deafness did not have cardiac

examination on file and an echocardiography was not done and hence a PDA could not be ruled out. Three of these had no documentation of IgM on file and one was recruited prospectively at six months of age with a negative IgM. The differential diagnosis of such a combination is vast as an OMIM (Online Mendelian Inheritance in Man) search revealed. It includes Refsum Disease, Peroxisome Biogenesis Disorder and Jervell and Lange-Nielsen syndrome.

The commonest combined clinical features were Congenital Heart Disease, Congenital Cataracts and Microcephaly with about five children having this combination. This was followed by sixteen children having a combination of cataracts and congenital heart disease. Four children had a combination of microcephaly and cataracts and another four had a combination of mental retardation and cataracts. The combination of Congenital Heart Disease, Congenital Cataracts and Microcephaly has a wide differential diagnosis including Phenylketonuria, Williams's syndrome and certain muscular dystrophies. The combination of congenital cataracts and microcephaly is equally wide including connective tissue disorders such as Osteogenesis Imperfecta.

Interestingly one child with a positive IgM for Rubella was a one month old infant being evaluated for septicaemia and conjugated hyperbilirubinaemia and a TORCH screen revealed a positive Rubella IgM. This infant had jaundice with no cataracts. Unfortunately the infant demised before Echocardiography and Ophthalmoscopy could be done to formally evaluate for congenital heart and eye disease respectively. The polymorphic presentation of our patients and the wide differential diagnosis make it imperative that an infant who meets the case definition for clinically confirmed CRS be investigated as early as possible for lab confirmation.

CHAPTER SIX

6.1. CONCLUSION

A total of 36 children were enrolled into the study with clinically confirmed CRS. Of the 36 children identified, 61% (22/36) of these were enrolled retrospectively and 39% (14/36) prospectively. Of the children with clinically confirmed CRS, 47% (17/36) were tested for Rubella specific IgM with only 31% (11/36) having valid results. 19% (7/36) had Laboratory confirmed CRS with 43% (3/7) of these confirmed retrospectively and 57% (4/7) prospectively. The commonest clinical features were congenital cataracts (81%), congenital heart disease (61%) {PDA representing 68% of this} and Microcephaly (39%). The commonest combined clinical features were Congenital Heart Disease, Congenital Cataracts and Microcephaly (14% {5/36}). The incidence of CRS per 1000 live births by province in 2014 for Lusaka, Copperbelt and Southern Provinces were 0.13, 0.06 and 0.01 respectively.

6.2. LIMITATIONS OF THE STUDY:

The biggest limitation encountered in this study was the bulk of missing information on the files during retrospective assessment. The particular challenge was ophthalmology files where the documented findings were predominantly the eye examinations with little documentation of the paediatric assessment. At ADCH and KCH a child had two separate files: one for ophthalmology and the other for the Paediatric side. Pairing of the files was challenging making it difficult to trace the missing systemic findings of the child. This is particularly unfortunate because a study in India (Vijayalakshani et al, 2007) revealed that cataracts among children have a high sensitivity (80%) of detecting CRS in India. There is no reason that this finding cannot be extrapolated to our setting since RCVs are not part of our EPI and India had a low immunisation coverage: 42%, 30% and 5% from Delhi, Chandigarh and Goa respectively (Dewan and Gupta et al, 2012).

Attempted ENT file review revealed missed opportunity in identifying CRS because there was no clear diagnosis in the registers that would help trace files and no attempt at systemic examination documentation for the few files traced. Another challenge is the unavailability of objective hearing assessment in infant and small children at the four hospitals, (OAE and ABR). Hearing assessment in children relies on parental report and

distraction testing. When asked, an ENT specialist cited the load of patients which made it impossible to thoroughly document other systemic manifestations.

Storage of files was another limitation as we could not trace certain files. Clerical staff cited storage space, lack of stationery and cabinets as some of the reasons why some rooms just had files thrown in a haphazard manner. Referral of children was not adhered to despite being planned. Because of the multidisciplinary nature of the management of these patients, parents would shun the whole process. They complained of the tedious and involving nature of consultations with little improvement in the child's functionality despite repeated counselling. At KCH, particularly with the ORBIS supported eye programme, when drop out of care rate was noted to be high, a budget line for transport refund was established which has greatly improved stay in care.

Because of limitations in documentation, filing and IgM confirmation in our setting, the methodology used in Costa Rica cannot be replicated in a setting like ours i.e. identification of participants originating from an IgM search.

We would have loved to trace and re-evaluate the children in the retrospective group for thorough documentation of their clinical features but their poor documentation and logistic limitations prevented this.

The inability to perform IgG as well as other advanced testing affects the ability to confirm whether clinically confirmed cases had undergone the IgM to IgG switch or whether we were dealing with alternate diagnoses.

Another limitation was the inability to specifically measure the disease burden of CRS due to the complexity of certain indicators like Daily Adjusted Life Years (DALY's) in CRS. This is particularly difficult because of the many clinical manifestations in CRS patients and their varied occurrence. The measurement of DALY's in our patients is further complicated by our health system challenges.

6.3. RECOMMENDATIONS

Increased multidisciplinary collaboration among specialties: Efforts were already started to strengthen collaboration between Ophthalmology and Paediatrics at KCH and ADH.

Improved documentation cannot be overemphasised as it forms the basis of data documentation and analysis for performance as well as a base for future research in many fields. Instead of generating new forms, solutions are easier when based on existing structures. A suggestion we left at KCH was to utilise the ophthalmology form for children with cataracts which has a systemic part. The paediatrician can fill in that portion of the form which stays on the ophthalmology file. We should actually be moving towards computerising all of our patient documents at least at the referral hospitals as this makes monitoring, evaluating and research much easier. In Costa Rica for example, their retrospective   disease burden assessment involved health record search of positive rubella IgM results and then analysing corresponding patient records (Jimenez G et al, 2007).

Integration of IgM testing with quantitative IgG to demonstrate rising titres at least at the highest referral Hospital UTH. In the meantime, with continued WHO support, strengthening of the transportation chain for samples would ensure they reach the virology laboratory at UTH. WHO has tremendously supported the CRS surveillance by technical assistance, training and mentorship. Acknowledging the great input and goodwill by the Zambian government, there is however more room for more ownership of the programme by the Ministry of Health by more budget allocation.

Isolation of all suspected/clinically confirmed CRS cases below the age of 1 year as these infants continue to shed virus from the nasopharynx during this period.

Increased sensitisation of health workers on Rubella and CRS as this strengthens surveillance. This can be via trainings or mentorship visits.

CHAPTER SEVEN

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7.2 APPENDIX A: CRS CASE INVESTIGATION FORM

Congenital rubella syndrome suspected case investigation form. Sentinel CRS surveillance site. WHO AFR

Name of health facility:

Infant's identification

Name of child:

Date of birth (dd/mm/yy)   Age in months:

Sex: M   F

Name of mother:

Notification and Investigation

Date of notification (dd/mm/yy):

Date of investigation (dd/mm/yy):

Clinical signs and symptoms

Group A

			Group B		
Congenital heart disease	Y	N	Purpura:	Y	N
If Yes, describe:			Microcephaly:	Y	N
			Meningiencephalitis:	Y	N
			Splenomegaly:	Y	N
Cataract(s)	Y	N	Mental retardation:	Y	N
Pigmantary retinopathy	Y	N	Jaundice:	Y	N
Glaucoma:	Y	N	Radiolucent bone disease:	Y	N
Hearing impairment:	Y	N	Other abnormalities:	Y	N

If Yes, describe:

Date of examination (dd/mm/yy):

Nem of physician who examined the infant:

If died, date of death (dd/mm/yy):

Maternal History

Mother's age in years

Maculopapular rash and fever during pregnancy?	Y	N
If yes, give month of gestation at illness.		
If yes, was rubella confirmed by lab in the mother?	Y	N

Lab tests on infant

Date blood specimen collected (dd/mm/yy)

Date serum sent to rubella lab (dd/mm/yy)

Rubella IgM result: *positive* | *negative*

Date results received by clinician/ focal point (dd/mm/yy)

Final classification of case:

No lab test, but clinically consistent with CRS	Clinically confirmed CRS	
Positive IgM and clinically confirmed:	Lab confirmed CRS	
Positive IgM but no clinical manifestations:	Congenital Rubella Infection	

Clinician (focal person)

Name:

Signature:

Date form completed (dd/mm/yy):

7.3 APPENDIX B: INFORMATION SHEET

(THE BURDEN OF CONGENITAL RUBELLA SYNDROME AT FOUR REFERRAL HOSPITALS IN ZAMBIA)

- My name is Chalilwe Chungu and I am learning how to become a doctor for children.
- The reason you have been given this form is so that you are given information and allow your baby to take part in this study.
- Rubella is one of the diseases that causes a rash and a fever. In addition the patient can have a common cold, red eyes and swellings in the back of the head and in the neck. Measles, other viruses and an infection called scarlet fever can also cause these symptoms. Rubella infection itself is usually not serious but if a mother gets it when she is pregnant she can pass it to her baby.
- The baby who gets Rubella from the mother while still in the womb can be born with problems with hearing, problems with the eyes and heart problems.
- This disease can be prevented by giving an injection usually to babies but also to older children, men and women. The injection for babies is given together with the one for measles at the clinic.
- The Rubella injection is not given in Zambia now but will be introduced soon. This will help to stop more babies from getting rubella from their mothers in future.

What will be done to your baby in this study?

- You will be asked some questions.
- Your baby will be examined.
- Blood will be taken from your baby for a test in the lab to see if your baby has Rubella. Only 1millilitre of blood will be collected. This is the same amount as one fifth of a common household teaspoon.
- The blood collected will be taken to the virology lab at UTH in Lusaka where it will be analysed and there after stored for a period of 1 month after which it will be discarded.
- You will be told the results of the blood test.
- Your medical records will be confidential handled in the same manner as other patient files at the health institution. For research purposes your baby will be

issued a unique identification code to help identify your baby but your name will not be made public. Information we obtain from this study will be published but your name will be kept confidential.

- The information collected will be kept private locked in a cabinet only to be accessed by the researchers. The data information sheets will be kept for a period of five years for retrieval if needed after which they will be shredded and burnt.

Why should you agree to take part in your study?

- Participation in this study is voluntary and you are free to withdraw at any point. You will not be materially rewarded or forced to take part. You are also free to decline to answer any question you think is too sensitive or too personal.

The benefits of you participating are:

- Increased awareness among parents of children with this illness such as yourself
- Appropriate referral for management of children with CRS such as your baby as most of the complications e.g. heart defects require specialist management.
- The information gotten will help us to see that less babies are getting the disease from their mothers when the injection becomes available to everyone in Zambia.

Research Related Injury

- Blood collection is generally safe.
- Needle prick will cause pain to baby but it is short-lived.
- Problems that can occur during blood collection include bleeding and excessive pain. Excessive bleeding will be prevented by the prick site being compressed until the bleeding is arrested and excessive pain managed by administration of a pain killer to the child.
- If your child suffers research related injury because of taking part in the study please call the principal investigator Chungu Chalilwe or the Chairperson of the Ethics Committee. The addresses are below
- You can also get in touch if you want further information about this study or your rights as a participant.
- Thank you for taking time to read this document.

Chalilwe Chungu University Teaching Hospital, Department of Pediatrics and Child Health. P/Bag 50013X Ridgeway Lusaka. Phone: +260979 527867 Email: chalilwe@yahoo.com	The Chairperson ERES CONVERGE IRB, 33 Joseph Mwila Road, Rhodes Park, LUSAKA. Phone: +260 966765 503/ +260 955 755 634 Email: eresconverge@yahoo.co.uk

7.4 APPENDIX C: CONSENT FORM
(A STUDY TO LOOK AT THE BURDEN OF CONGENITAL RUBELLA SYNDROME AT FOUR REFERRAL HOSPITALS IN ZAMBIA)

Participant

I_____

(participant's parent or guardian's name, signature or thumb-print) have been informed about the study. I volunteer to have my child and I participate in the study. I have signed two forms. One has been given to me and the other has been kept by the researchers.

Signature/Thumb_____

Date (D/M/Y) _____

Interviewer

I_____

(Name of recruiter) have explained this research study to the Participant. I am available to answer any questions now or in the future regarding the study and the Participant's rights.

Signature_____

Date (D/M/Y) _____