MEASLES: FACTORS INFLUENCING THE MORBIDITY AND MORTALITY IN THE UNDER FIVE YEAR OLD CHILDREN AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA

BY

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DEDICATION

Dedicated to my lovely wife Edith Nkumba Mpabalwani who was very understanding during the preparation of this dissertation. To my children Mutimba, Chilufya, Shula, and Mulenga who have continuously taught me growth and developmental paediatrics, and to them I say thank you ever so much. They however, resented my long hours on the computer during the weekends and my daughter Chilufya once remarked "Dad when are you going to take us down town, always on the computer, are you crazy!"

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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 in the University of Zambia or any other University.

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ABSTRACT

Reported here are factors that influence the morbidity and mortality in hospitalised Zambian children with measles. A prospective study on 2174 children was undertaken to analyse the clinical presentations and complications in children hospitalised for measles between 1993 to 1996 and between 1998 and 1999 at the University Teaching Hospital (UTH), Lusaka, Zambia. The age distribution was from 1 month to 15 years. Hospitalisation for measles infection was most common in children below the age of one year (36.9%, 803/2174). Diarrhoea, pneumonia and oral candidiasis were the most common complications of measles (67.6%, 53.3% and 38.6% respectively). The overall measles case fatality rate (CFR) was 13.7%, and was higher in children less than 3 years of age (16.6%). The CFR was significantly associated with otitis media (45.0%), oral candidiasis (20.1%), pneumonia (18.6%), and diarrhoea (15.4%).

When the above were stratified with HIV-1 sero-status, the CFR was significantly higher in the HIV-1 sero-positive than in the sero-negative (27.3% Vs 7.7%, $p<0.001$). The CFR decreased by age in the HIV-1 sero-negative children. On the contrary, CFR was also high in older children in sero-positive children. In HIV sero-positive group more children had been

previously vaccinated, and the CFR was high even in vaccinated children, suggesting low vaccine efficacy in HIV-1 sero-positive children. Complications such as pneumonia, oral candidiasis and otitis media were more common in HIV sero-positive children, but diarrhoea was equally seen in both HIV sero-positive and sero-negative groups.

Molecular analysis of the currently circulating measles virus showed that they belonged to group D2, and this was consistently demonstrated across a period of 7 years. Only silent minor mutations were detected, documenting the point that measles virus is a very stable virus antigenically. Therefore, the current measles vaccine available in Zambia, the Schwarz vaccine is still immunogenic in inducing protective antibodies against the wild measles virus circulating in Zambia.

The study demonstrates that children with severe measles admitted to UTH had a high CFR, and that measles infection was more severe in HIV-1 sero-positive children. Measles is increasingly becoming a major problem in young infants below the immunisation age of 9 months. Therefore, new strategies should be developed to protect young infants below 9 months.

Finally, it is recommended that there is urgent need to develop new immunisation strategies to protect young infants from measles. These could take the form of immunisation of adolescent girls as they enter the reproductive age group and a two-dose measles schedule in infants. Prevention of mother to child transmission of HIV could subsequently prevent severe measles infection in HIV positive children. More importantly, there is urgent to increase routine measles immunisation coverage to over 95%. Life threatening complications like hypoglycaemia and dehydration should be identified and treated promptly and adequately in children especially those with oral candidiasis.

ACRONYMS

CD 46 Cluster of Differention number 46

EPI Expanded Programme on Immunisation

MV Measles virus

RPMI Rosewell Park Memorial Institute (Enriched Nutrient Medium)

UTH University Teaching Hospital

PCR Polymerase Chain Reaction

VTM Viral Transport Medium

WHO World Health Organisation

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I wish to record my sincere appreciation to my supervisors Prof. G.J. Bhat, and Dr. G.M. Shakankale for the encouragement in the preparation of this dissertation. Dr. C. Kankasa (MMed) and Dr. T. Msoka gladly accepted to read the manuscript and I found their comments and suggestions very valuable, and to them I say thank you.

I also wish to thank the doctors in the Department of Paediatrics and Child Health who made the diagnosis of Measles and transferred these children to the measles isolation ward, without them this work would have not been possible. The assistance of the nurses on the isolation ward was valuable in the follow up of patients on the ward. Sincere thanks goes to the parents and guardians who agreed to participate in this study, as the success of this study depended on their co-operation.

My unique interest in both laboratory and clinical medicine was first kindled to me during my final undergraduate days by my Professor of Paediatrics, Prof. Chifumbe Chintu in one of his onco-haematology clinics when he once said "I will do a bone marrow aspirate on this patient, stain it in the laboratory, read it and then come back to the patient and treat him

accordingly". In this dissertation I have attempted the same – examined the patient, took the clinical specimens, processed them in laboratory and came back to the patient. I have found this to be very satisfying. Therefore, in this dissertation I have retained a combined interest in clinical and laboratory investigation of a childhood viral infection – Measles.

CHAPTER 1

1.0 INTRODUCTION

Measles is a highly contagious, acute viral disease causing an estimated 65 million cases world-wide annually (1,2). The vast majority of measles cases occur in the developing world, whereas elsewhere the widespread use of an effective vaccine has drastically reduced the incidence of measles (1,2). In sub-Saharan Africa measles remains a significant cause of morbidity and mortality among children and infants (1,2,3).

Measles claims 1.5-2 million deaths world-wide every year making it the biggest killer among the six Expanded Programme on Immunisation (EPI) target diseases (3,4). Although an effective and safe vaccine against measles is widely available in most developing countries, the control of measles is a difficult challenge in sub-Saharan Africa (5). Since 1976, the World Health Organisation (WHO) EPI has recommended that a single dose of measles vaccine be administered to all children at the age of nine months in the developing countries (5,6). There has been significant and notable control of measles in the developed countries over the last 10 to 15 years using the vaccine, but despite the availability of an effective measles vaccine, control of measles infection in sub-Saharan Africa particularly in

large cities remains elusive. However, in recent years there has been high measles vaccine coverage rates documented although measles epidemics still occur frequently in this region (3,4).

Measles and the nutritional status of a child form a complex inter-ralationship. The painful mouth with stomatitis or oral candidiasis, fever, diarrhoea and purulent foci, in addition to loss of appetite, severely impair the nutritional state of a child who has a diet poor in proteins, calories and other essential micronutrients like Vitamin A, iron and folate among many others. Measles in these children tends to be severe and accompanied by many complications like diarrhoea, pneumonia, and reactivation of pulmonary tuberculosis. Both tuberculosis and persistent diarrhoea leads to malnutrition in children who have had measles in the recent past. Malnutrition also makes the child to be susceptible to infections like septicaemia, pneumonia, and reactivation of tuberculosis (1,4).

Diagnosis of measles remains clinical and based on the characteristic findings. However, in certain cases like very young infants and those presenting without a typical rash, clinical diagnosis can be very difficult and laboratory tests can be of value in arriving at a definitive diagnosis.

CHAPTER 2

2.0 STATEMENT OF THE PROBLEM

Measles infection in children still remains as a major public health problem in Zambia. In 1993 there were 8,749 reported cases of measles and in 1994, the number had increased to 14,548 (Annex 2,3). During the same period the reported national measles immunisation coverage was 63% and 89% respectively (2,4). Since 1995, there had been a steady decrease in the reported measles cases. However in 1999 there was a sudden increase in the number of reported cases to 23,518 (Annex 2). Between 1995 and 1998, measles immunisation coverage ranged between 73% and 93% (Annex 5).

In spite of the ongoing national immunisation programme, Lusaka district has been experiencing measles outbreaks. Earlier, these outbreaks used to be every two years, but they seem to occur yearly (7,8). During the years 1993 and 1994, measles immunisation coverage for urban Lusaka was 58% and 64% respectively (Annex 5). Surprisingly, in the year 1994, Lusaka urban alone had 8,454 measles cases accounting for 58.1% (8,454/14,548) of the total national reported cases (Annex 2). In subsequent years, 1995 – 1997, measles immunisation coverage for urban Lusaka had increased markedly (Annex 5), but Lusaka continued to have more measles cases.

Of the total reported measles cases to the National EPI Secretariat in 1995 and 1996, Lusaka alone contributed 20.2% and 24.8% respectively (Annex 2).

In Lusaka, measles patients are seen throughout the year, with a peak from September to December. There is a cool dry season between April and August followed by a hot dry season between September and November. The hot rainy season starts in December and lasts until March. The peak incidence of measles between September and December is the seasonal period of measles epidemics in Lusaka, Zambia (7). It is therefore possible to predict an outbreak of measles in Lusaka and appropriate action taken well in advance, during the cool dry season (April to August). With this epidemiological data available, measles mass vaccination campaigns can be best done at the beginning of the cool dry season. Sadly, however, in 1998, measles vaccination campaigns were done in August, and the outbreak still occurred (9).

There is limited data on the clinical presentation and outcome of measles infection in hospitalised children. In addition the effect of HIV infection on measles morbidity and mortality has not been studied in this setting. In view of the above, the present study was undertaken.

CHAPTER 3

3.0 LITERATURE REVIEW

3.1 THE MEASLES VIRUS

3.1.1 Historical Aspect

Measles appears to be a relatively new disease of humans. The writings of Hippocrates (460-377 BC) which record the presence in ancient Greece of such diseases as herpes labialis and malaria, include no account of Measles (10). The explanation can be found in the epidemiology of measles. Since measles is a highly contagious acute infectious disease that results in lifelong immunity and has no animal reservoir, a human population of several hundred thousand is required in order to provide a sufficient presence of the virus (11,12,13). Populations of this size did not exist until the development of ancient Egyptian and Sumerian cities late in the third millennium BC, and such diseases as measles and smallpox must have arisen since that time. By the beginning of the Christian era there were large population centres in Europe, China, India, and the Middle East, which were insulated from each other by thinly populated areas. The development of trade between these civilisations provided a means for transmission of infectious agents and led to massive epidemics that were

recorded in China and the Roman Empire during the third and fourth centuries AD (10). These epidemics represented virgin-soil outbreaks of measles and smallpox.

The first written description of measles is attributed to Abu Berc, a 10th century Persian physician also known as Rhazes (14,15,16). Rhazes cited earlier authors as far back as the seventh century physician, El Yehudi, to whom he attributed the initial clinical description of the disease (14.15,16). Rhazes referred to measles as *hasbah,* which means "eruption" in Arabic; he described measles as an affliction of children and distinguished it from smallpox, which he considered a less severe disease.

Thomas Sydenham's remarkable description of an outbreak of measles in London in 1670 provides an accurate clinical picture of the disease, called attention to its increased severity in adults, and recorded the danger of pulmonary complications (14,15,16).

Measles appears to have been carried by Europeans to the New World. The American colonies were repeatedly swept by measles during the 17th and 18th centuries; adults were affected as well as children, a pattern

characteristic of thinly populated areas and there was considerable morbidity and mortality (10).

Our understanding of measles was greatly enhanced by the remarkable observations of a young Danish physician, Peter Panum, who was sent to assist with an epidemic of measles in the Faroe Islands in 1846. Panum confirmed that measles was contagious and was transmitted directly from person to person. He defined the 14-day incubation period between exposure and the appearance of the rash, and he demonstrated that patients were most infectious at the end of the prodrome when the rash was just breaking out. He also observed an attack rate of almost 100%, documented increased mortality in children under one year and in adults over 50, demonstrated the efficacy of quarantine, and showed that infection conferred lifelong immunity (13).

In 1883, Hirsch (12) described the devastating impact of measles on virgin populations in the Fiji Islands and the Amazon Basin, recording mortalities in excess of 20% and the extinction of entire tribes. He also extended Panum's observations and concluded that an epidemic persisted as long as susceptible individuals remained to support it and died out when the pool of susceptibles was exhausted.

3.1.2 THE INFECTIOUS AGENT

Measles virus (MV) is a member of the *Morbillivirus* genus of the *Paramyxoviridae* family. Measles is a uniquely human disease, although primates can contract it. However, neither a primate nor any other animal reservoir has been identified that is capable of maintaining the disease and permitting its reintroduction in or spread into human populations (16,17).

Since measles virus is very specific to the human host, this suggests a more recent emergence in the human populations. Measles is believed to have emerged as a sustained threat to human populations only when large communities came into existence. Such a large population living in close proximity is believed necessary to generate sufficient numbers of susceptible individuals and sufficient contact frequency to maintain the virus in the population (11,17).

Measles virus was first isolated in 1954 by Enders and Peebles (18) from the blood of a patient with acute measles, David Edmonston, using primary human kidney and rhesus monkey kidney cells. Recently, Kobune et al. (19) reported that an Epstein-Barr virus-transformed marmoset lymphocyte line, B95-8, is superior for primary isolation of measles from nasopharyngeal aspirates. Marmosets have been shown to be extremely

sensitive to measles virus infection, and the outcome is often fatal. Thus, the marmoset appears to be an attractive model for the pathogenesis and virus attenuation studies (20).

3.1.2.1 In Vitro Propagation

While humans and primates are the only natural hosts, MV can be propagated in vitro in an impressive number of cell cultures and lines (21,22,23,24). Of these cell lines the most commonly used include B95a and vero cell lines (Fig 1). The virus can be isolated with great success from respiratory secretions, blood and urine during the prodromal phase up to about the second or third day of rash.

3.1.2.2 Cytopathic Effects

MV replication on cell culture monolayers of vero and B95a cell lines generally results in cytopathic changes of essentially two varieties. The first cytopathic effect consists of the formation of multinucleated giant cells resulting from cell-cell fusion as more and more cells are involved in syncytium formation (Fig 2). It is not unusual to observe more than 50 nuclei bounded by a single cytoplasmic membrane during the course of infection (24,25). The second type of cytopathic effect observed is the alteration of the infected polygonal shape to a stellate or dendritic

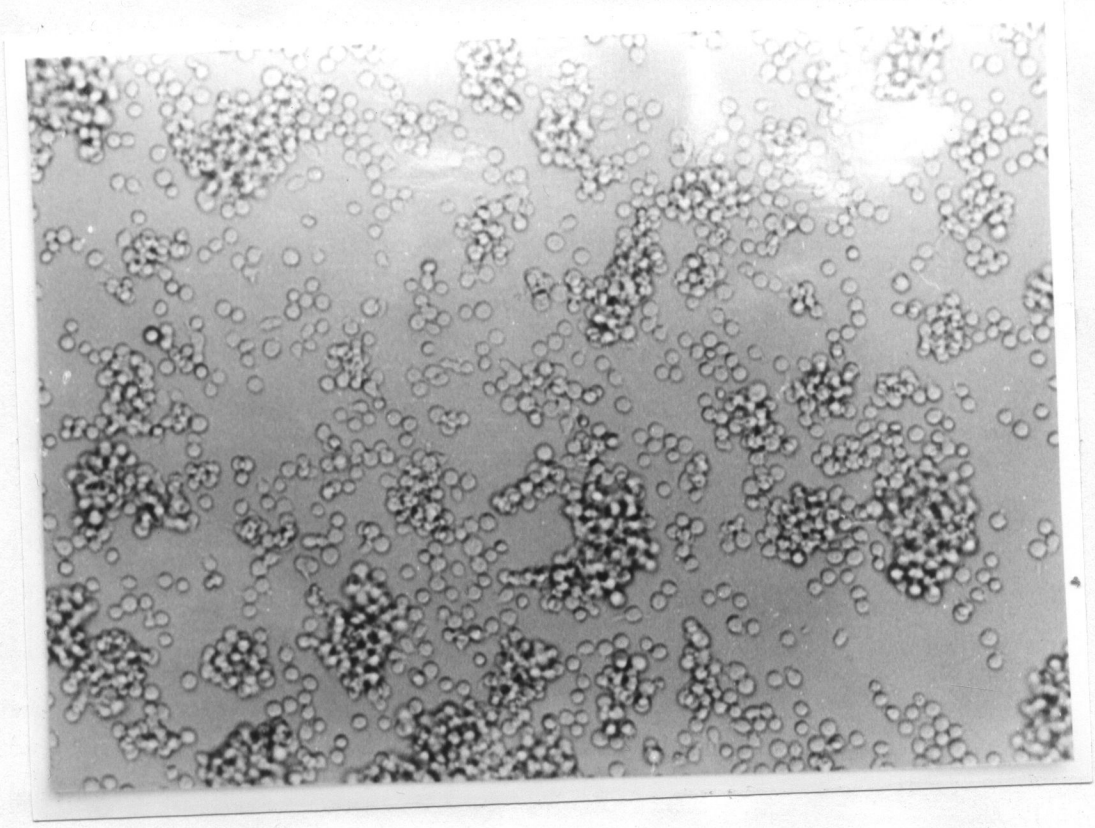

Fig 1: Picture showing uninfected B95a cells

Magnification X 10; Picture taken by the author

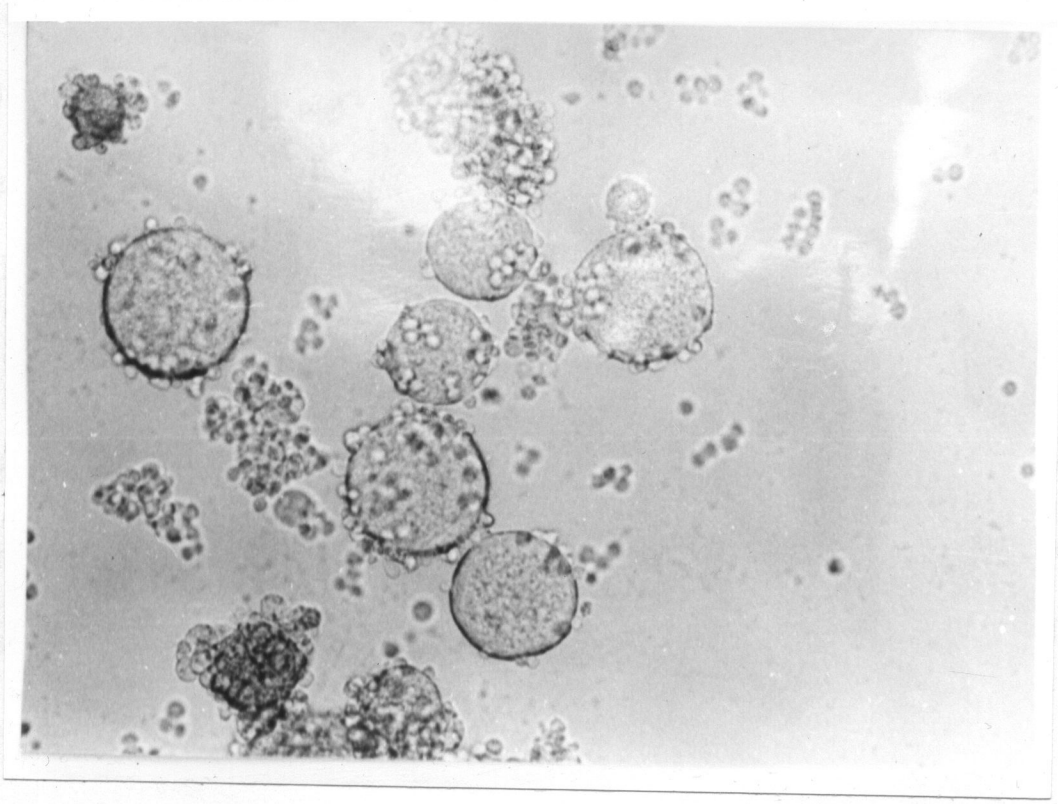

Fig 2: Picture showing measles infected B95a cells – Syncytial formation (giant cells). Typical measles cytopathic effect on B95a cells

Magnification X 20; Picture taken by the author

appearance. These cells do not fuse but are distinctive in their increase in refractility to light (16,24).

3.1.2.3 Morphology

Measles virions are pleomorphic, generally spherical, enveloped particles measuring 100-250 nm in diameter. The lipid envelope surrounds a helical nucleocapsid composed of RNA as the repository of genetic material.

The transmembrane glycoproteins of the virus are present on the envelope surface and appear as projections from it and include the F-glycoprotein (fusion factor) and the H-glycoprotein (haemagglutinin). The M-glycoprotein (matrix) is inside the virion envelope (16,24).

3.1.2.4 Chemistry and Genetics

MV is composed of a single-stranded nonsegmented RNA genome of negative polarity. The entire genome of the Edmonston strain of MV has been sequenced (24,25,26,27,28). The genome encodes six major structural proteins. The most important clinically are the haemagglutinin – protein (H-protein) and the fusion – protein (F-protein).

The H – protein is a major immunogen of the MV, and antibodies directed against this polypeptide have both haemagglutination-inhibiting (HI) and virus neutralising (NT) activities. This is presumably accomplished by blocking the attachment of virus to target cells (29).

The F – protein promotes fusion of an infected cell with adjacent normal cells and this is important in the pathogenesis of measles infection. Antibodies directed against the F – protein are required for effective containment of virus infection, because local infection can be maintained by cell to cell fusion (30).

3.1.2.5 Measles virus receptor

Both the H and F glycoproteins are required for virus-host cell membrane fusion, which permit virus entry and release of nucleocapsid of RNA-Protein complex into the cytoplasm. The H-glycoprotein of MV is believed to interact with a specific receptor(s) in the attachment phase of infection. The MV receptor has been considered to be a cell surface protein.

Recently, the human CD46 molecule, or membrane cofactor protein has been identified as a major MV receptor (31,32). The CD46 molecule has a

wide distribution in human tissues but appears absent on human erythrocytes.

3.1.2.6 Genetic and Antigenic Variability

MV has long been considered to be an antigenically stable monotypic virus, and recovery from a natural MV infection confers lifelong immunity. Serum specimens from persons infected decades ago retain the ability to neutralise more current wild type MVs, and the serum from recently infected persons can neutralise vaccine strains and early wild type measles viruses.

MV has a single-stranded RNA genome and replicates via an RNA-dependent RNA polymerase, a process with an inherent error rate and no proof reading capacity. Variability in the N and H gene products has been described using monoclonal antibodies but until now has been considered minor and unimportant.

The resurgence of measles infection in North America over the last decade provided an opportunity to obtain MV isolates from clinical specimens and determine the extent of genetic and antigenic variability of these circulating

viruses relative to older isolates and vaccine strains. Initially, major interest was in the two glycoprotein genes, since the protein products play major roles in host cell binding and virus-cell membrane fusion and cell entry. Antibodies to H and F glycoproteins appear to be required for protection against infection; thus, major changes in the predicted protein sequence of these gene products relative to the vaccine strains might be important considerations for formulation of improved immunisation strategies (24,33,34,35).

There is no evidence that changes in the H-protein present in some wild-type viruses have altered vaccine efficacy because the vaccine – induced immune response is effective (35).

Eight clades of measles virus have been identified namely A – H, with 15 genotypes. This classification is based on the nucleocapside gene sequence (N-gene).

3.1.2.7 Analysis of Vaccine Strains

The live attenuated measles vaccine currently used in the United States, Moraten, was derived from the prototype Edmonston strain originally

isolated in 1954 (18). Edmonston strain has been the progenitor strain for many measles vaccines currently used world-wide (36), including Schwarz vaccine used in Zambia. Other measles vaccines were developed using locally isolated strains of measles in Russia, China, and Japan. The methods used for attenuation of the original viruses involve multiple passages of virus, generally through foreign host cell culture systems, and different incubation temperatures (figures 3 and 4).

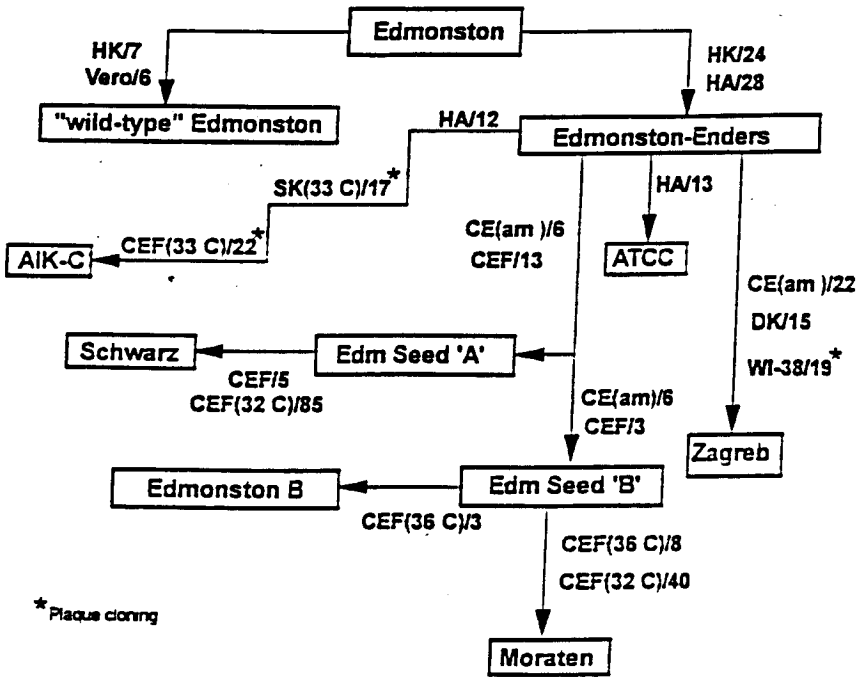

Fig 3: Passage history of Measles vaccine

Passage histories of live attenuated measles virus vaccines derived from John Eders's isolate of Edminstons virus. Temperature of passages assumed to be 37 C unless otherwise stated. HK, human kidney cells; HA, human amnion; CE (am), intraamniotic cavity of chick embryo; CEF, chick embryo fibroblast; DK, dog kidney; WI-38, human diploid cells; SK, sheep kidney.

Source: Fields Virology, 1997, Vol 1, 3rd Ed., page 1294

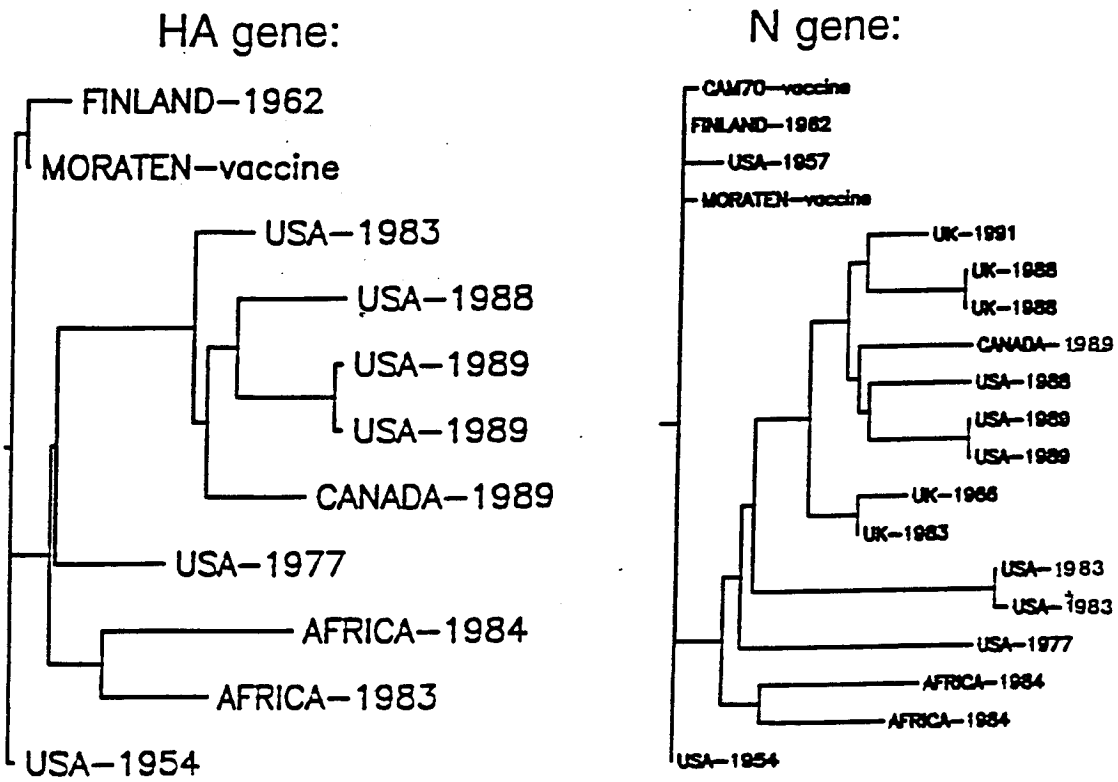

Fig 4: Evolutionary relationships of wild-type and vaccine measles viruses.

The phylogenetic trees are based on sequence analysis of the complete protein coding region of the HA gene and the protein coding region of the N gene.

Source: Fields Virology, 1997, vol 1, 3rd Ed., page 1278

3.2 EPIDEMIOLOGY

The spread of measles has been used as a convenient example to illustrate the principles of epidemiology and it has been calculated that any community of less than 500,000 is unlikely to have enough birth rate to supply the number of susceptible children required for the continuous maintenance of the virus in the population. In fact the complete elimination of measles from isolated groups has been documented. Such communities remain free of the disease until it is reintroduced from outside and the susceptible individuals are once more at risk (37).

In developed countries, measles immunisation programmes have reduced the number of cases reported annually to negligible levels (37). However, measles has remained a major health problem in sub-Saharan Africa, especially in urban areas with high population densities (3). In Zambia, measles is endemic with transmission peaks occurring between August and December, despite the high levels of measles immunisation achieved in recent years (7,8).

Full control of measles infection has not been achieved, in part because of the difficulty in reaching the needed coverage of infant populations to achieve the necessary levels of immunity, and because of the problems of

both primary and secondary vaccine failure (37). Current estimates of the immunisation coverage needed for fully protective herd immunity are 94-97% (38). This level is very difficulty to reach with 15-month-old infants even in the most developed countries, but it may be more readily achievable with school-age children through compulsory vaccination (37,38). In addition, there is the problem of vaccine failure, particularly when the vaccine is administered before the optimal time. Primary vaccination failure occurs at rates of 4-8%, and secondary failure at 4% (38,39). Also large numbers of cases occur among apparently appropriately vaccinated persons during epidemics.

3.3 PATHOGENESIS

Infection occurs via the respiratory tract. The chief mode of transmission is via aerosol. Infected droplets are produced by talking, coughing, and sneezing of an individual in the catarrhal stage of the disease (40). The lower respiratory tract is more susceptible than the nasopharynx, which is, in turn more susceptible than the oral mucosa (40).

During the first 2-4 days after infection, MV replicates locally in the upper respiratory mucosa and spreads, perhaps carried intracellulary in pulmonary

macrophages and other mobile cells, to draining lymph nodes where further replication occurs. The virus then enters the bloodstream, carried within leukocytes (40), producing the primary viraemia which disseminates infection to sites throughout the reticuloendothelial system. The selective infection of lymphoid tissues during primary viraemia may reflect changes in the surface of infected lymphocytes which cause them to *"home"* to the vascular endothelial cells of lymphoid organs, and this accounts for leucopaenia during the incubation period.

Though clinically inapparent, MV replication at these secondary lymphoreticular sites is evidenced by lymphoid hyperplasia and formation of multinucleated giant cells (29,40). It results in a secondary viraemia of increasing magnitude that disseminates the infection to tissues throughout the body (41). During this secondary viraemia, the virus is carried primarily within lymphocytes and monocytes. The further replication of the virus in these tissues, together with the developing host immune response, is responsible for the prodromal symptoms and signs which occur 9-11 days after infection and mark the end of the incubation period (41).

The prodromal symptoms and signs reflect involvement of epithelial surfaces in the respiratory tract, the gastrointestinal tract, and the

conjuctivae. However, infection of the endothelial cells of vessels in the underlying lamina propria and dermis during the secondary viraemia appears to precede infection of the overlying epithelium, and the inflammatory changes in and around these vessels are an integral part of the local pathology (29,40,41).

3.4 CLINICAL PRESENTATION

Measles is a highly contagious acute viral disease characterised by fever, cough, coryza, conjuctivitis, and a specific enanthem (Koplik's spots), followed by a generalised maculopapular eruption (42,43). Diarrhoea is a very common finding in children with measles infection. Immuno-suppression leading to increased susceptibility to secondary infection is a well-recognised complication of measles infection (44). Therefore these children are prone to secondary bacterial pneumonias and reactivation of dormant tuberculous foci. When diarrhoea becomes protracted, the children go on to develop malnutrition which further depresses their immune status and subsequently exacerbating their susceptibility to infections (45). The child is thus trapped in a vicious cycle of infection and malnutrition (46).

3.5 Immunity

Maternal antibodies provide protection during the first 6 months of life and often longer. However, this is a function of maternal immunity and nutritional status of both the mother and the infant. It is persistence of maternal antibodies that frequently interferes with replication of vaccine virus still seen at the age of 12 months (46). Natural measles infection results in life-long immunity against the disease, although there is a decline in antibody titre with time, but gradual stabilisation occurs. Subclinical reinfections, presumably of the respiratory tract, occur despite this immunity if virus is circulating in the community. These reinfections may boost the immune response, but immunity is retained even in absence of such exogenous reexposure (46,47).

Cell-mediated immunity (CMI) is required to clear measles virus infection. However both humoral and CMI appear to be capable of preventing infection in normal individuals exposed to the virus.

3.6 Prevention

Measles can now be prevented by the administration of a live attenuated virus vaccine (48). Most of the vaccine strains used today (Schwarz,

Moraten, Beckenham, Edmonston-Zagreb, EKC, and AIK-C) were developed from the original Edmonston isolation. Heat stability of most strains has been increased through the addition of stabilisers. Because of differences in the duration of protection from passive maternal protection and differences in risk of exposure, selection of the age of immunisation requires a balancing of two factors: "the earliest age at which high rates of seroconversion can be obtained, and the age group with the greatest risk of infection" (49). On the basis of epidemiologic data on age- specific measles incidence and age-specific seroconversion data, WHO has recommended nine months as the optimal age for measles immunisation in most developing countries (50,51). In Zambia the recommended age of immunisation is thus nine months.

Immunisation with potent vaccine administered at the recommended age does not ensure seroconversion or protection. Primary vaccine failures (the lack of a serologic and immunologic response to initial immunisation) do occur. Secondary vaccine failures (the occurrence of disease in previously successfully immunised children) have been reported but are thought to be rare (50,51).

3.7 Cultural Perspective in Zambia

Long accepted as an inevitable part of childhood infection (52), every tribe in Zambia recognises the disease entity of measles and its no surprise that every language spoken in the country has a name for it. Most parents and especially the grandparents are very familiar with the symptomatology of measles infection. All the major seven dialects spoken on the national radio and television have a name for it;

i) ***IciBemba – Kansakala***; covering Northern, Luapula, and parts of Central and Copperbelt Provinces.

ii) ***ChiNyanja – Kansamwe***; covering Eastern, Lusaka, and parts of Central Provinces.

iii) ***ChiTonga – Chihumu (Chimfumu)***; covering Southern, and parts Lusaka, and Central Provinces.

iv) ***SiLozi – Muukwaukwani***; covering Western and parts of Southern Provinces.

v) ***IciKaonde – Kansamwe***; covering Northwestern and parts of Central Provinces.

vi) ***IciLunda – Kansakala***; covering parts of North-Western Province and in (I) above.

vii) ***IciLubale – Kashakala***; covering parts of both North-Western and Western Provinces.

It is with this background that this study was undertaken to determine factors that contribute to morbidity and mortality in hospitalised children with measles.

CHAPTER 4

4.0 AIMS AND OBJECTIVES

4.1 General objective

To analyse the factors that contribute to the morbidity and mortality in children hospitalised for measles at UTH.

4.2 Specific objectives

4.2.1 To compare measles morbidity and mortality during the period 1993-1996 and 1998-1999.

4.2.2 To evaluate the efficacy of the current measles immunisation by inspection of children's card (under-5-card).

4.2.3 To study the impact of HIV infection on the outcome of measles infection.

4.2.4 To verify the clinical measles diagnosis by virus isolation.

4.2.5 To undertake molecular analysis of the measles virus isolates.

CHAPTER 5

5.0 METHODOLOGY

5.1 Study site

The University Teaching Hospital (UTH) is a 2000 bed hospital which is a referral facility for the whole of Zambia. Most of the patients come from within Lusaka, the capital city of Zambia which has a population of over 1.2 million people. The Department of Paediatrics and Child Health has a bed capacity of about 500. The measles isolation ward has a bed capacity of twenty beds and with an additional four beds in the acute bay, where the extremely ill children with measles are admitted.

5.2 Subjects

Clinical measles cases admitted to UTH isolation ward were recruited to the study. Most of the patients were initially seen at the local health centres and the children who could not be managed at these centres were referred to UTH, which is the only public facility in the city for hospitalisation of ill

children. Therefore, it is the severely ill children with clinical diagnosis of measles that were admitted to the measles isolation ward at UTH.

5.3 Methods

This study was undertaken between November, 1998 and November, 1999. A similar survey was done by the author between January, 1993 and December 1996. In both periods, clinical information was collected using a questionnaire format and thorough physical examination by the author. The patients were examined daily during their stay in hospital.

The study was approved by the Research Ethics Committee of the University Teaching Hospital of the University of Zambia.

5.4 Clinical diagnosis

Patients who met the World Health Organisation (WHO) clinical case definition were considered for the analysis in this study. A clinical case of measles was defined as a child with generalised maculo-papular rash of

three or more days duration, fever, of 38 °C or more and with at least one of the following; cough, coryza, or conjuctivitis (53).

Presence of crepitations or bronchial breathing was defined as pneumonia in addition to tachypnoea, chest indrawing and recession of intercostal spaces. Where appropriate, chest radiographs were done, and the presence of focal or diffuse infiltrates or consolidation were also defined as pneumonia. Diarrhoea was defined as increased frequency of loose stool or more than three loose stools per day. Nutritional status was grouped according to the Wellcome classification (54). Altered level of consciousness was defined as the presence of irritability, drowsiness, or coma. Measles household contact was defined as being in contact in a house with a person who had clinical features of a WHO case definition of measles in the preceding two weeks.

5.5 Clinical specimens

Throat swabs were collected in all the children admitted for measles and transported on ice (cold box) in viral transport media (VTM) for MV isolation.

In some patients, 1 - 2 mls of venous blood was collected, and by ficoll method, lymphocytes were separated and MV isolation attempted.

In children whose parents consented to an HIV test after counselling, venous blood was collected, and screened for HIV.

5.6 Detailed Laboratory procedures

5.6.1 Measles virus isolation

Throat swab suspensions in VTM were centrifuged at 10 rpm, and 50 microlitres of the supernetant was innoculated on pre-seeded B95a cell line on a 24 well microplate in nutrient medium (RPMI - 1640). This was then put in a carbon dioxide incubator set at 36 °C. The plates were checked daily for typical cytopathic effect – syncytial formation (Fig 2) up to day 7. Secondary passage on B95a cell line was done on positive wells with syncytial formation and negative wells for syncytial formation were discarded. The MV isolates were stored at –80 °C for further molecular analysis.

5.6.2 Lymphocyte separation: Ficoll method of lymphocyte separation preparation

The principle of the separation procedure of lymphocytes is based on the fact that when blood is mixed with a compound that aggregates erythrocytes it increases the sedimentation rate of erythrocytes, and the leucocytes

remain in the plasma supernatant. The sedimentation of leucocytes is only slightly affected and can thus be collected from the upper part of the tube when the erythrocytes have settled.

5.6.3 HIV serology

HIV serology was done on those children whose parents gave verbal concent, following pretest counselling. Serum samples were collected on recruitment to the study and stored at –20 °C until tested. HIV-I antibody was detected by ELISA (Wellcozyme HIV recombinant, VK 56, Murex diagnostics, Dartford, UK) and particle agglutination test (Serodia HIV, Fujirebio, Tokyo, Japan).

5.6.4 Molecular analysis of MV isolates

Molecular analysis of MV isolates was briefly done in the following steps;

- RNA extraction from the MV isolates
- PCR
- Sequence analysis was done using a Sequence Analyser at John Hopkins University, Baltimore, Maryland, USA.

5.7 Data analysis

Data was analysed using an EP-Info software programme supplied by WHO. P-value was calculated by chi-square test.

CHAPTER 6

RESULTS

Measles cases

The total number of children who fulfilled the WHO case definition of measles during the two study periods 1993 – 1996 and 1998 – 1999 were 1768 and 406 respectively. Therefore the total number of children admitted to the study were 2174 between the two study periods (Table 1).

Age distribution

The age distribution of these children was between 1 month and 14 years (1993 – 1996) and 1month to 15 years for the period 1998 - 1999. One third of these children were below the age of 1 year (36.95%; 803/2174), (Table 2).

Sex distribution

The sex distribution was the same.

Table 1 : Age distribution of hospitalised children with Measles

Period	1993-1996	1998-1999	
Age (years)	No. cases	No. cases	Total
<1	654	150	804
1-2	391	80	471
2-3	301	68	369
4-5	160	41	201
6-10	177	50	227
>11	85	17	107
Total	1768	406	2174

Measles infection in infants

Measles infection was common in infants as over one third of the patients (36.9%; 803/2174) were below the age of 12 months (Table 2). Of these infants, 64.8% (520/803) were below the vaccination age of nine months. Furthermore, 23.9% (520/2174) of all the total admissions were below the vaccination age of nine months (Tables 2).

Two cases of measles infection in young infants are highlighted here to demonstrate the burden of measles in young infants in Zambia.

Table 2: Measles infection in infants (children 11 months and below)

Period	1993 – 1996		1998 – 1999			
Age (months)	No.	Percent	No.	Percent	Total	Percent
1	5	0.7%	15	10.0%	20	2.5%
2	4	0.6%	18	12.0%	22	2.7%
3-5	98	15.0%	43	28.7%	141	17.6%
6-8	308	47.1%	29	19.3%	337	42.0%
9-11	238	36.0%	45	30.0%	283	35.2%
TOTAL	653	100%	150	100%	803	100%

Case 1

A 2 month old female infant was referred to the UTH for increasing respiratory distress and fever for six days. The infant was noted to be febrile (38.5 C), tachypnoeic, intercostal recession with chest indrawing and had bilateral crepitations. The infant was admitted with a diagnosis of severe bronchopneumonia and was treated with intravenous ampicillin and gentamicin and intravenous fluids (half strength darrows in 10% dextrose) with Oxygen by nasal catheter.

On day 2 of admission, the patient remained pyrexic and was noted to have injected conjunctivae with a maculo-papular rash. A diagnosis of measles was made. On further inquiry, the mother reported that two other siblings had measles infection which was introduced in the household two weeks previously. Both siblings had been previously vaccinated against measles. IgM specific antibody for measles was positive. Further investigations showed that the patient had a low CD4+ lymphocyte count (419) and a high HIV-I viral load (12,576).

The baby remained pyrexic with worsening respiratory distress. On day 5 of hospitalisation, intravenous cefotaxime and cloxacillin were introduced and the initial antibiotics stopped. The temperature started to stabilise and on day 15 the patient developed a swelling in the neck which on examination showed a subcutaneous emphysema which finally extended from below the ear lobes down to the breast line anteriorly and posteriorly from the neck to mid way down the scapular.

Over the next six days the infant recovered and was discharged home on day 21 on oral ampiclox. On review a week later, the baby was well and there was no evidence of subcutaneous emphysema.

Case 2

A three week old baby presented to UTH with a two day history of fever, cough and fast breathing. A diagnosis of severe peumonia was made and was commenced on crystalline penicillin and gentamicin. On day 2, it was noted that the neonate was being looked after by the grandmother and further inquiry reviewed that the 23 year old mother was admitted to the adult wards for measles infection and had been vaccinated in childhood.

Re-examination of the neonate showed that the baby had conjunctivitis with a poorly defined "generalised papular rash". A provisional diagnosis of Measles was made and an IgM specific measles antibody test on the blood was positive. The baby made an uneventful recovery and was discharged home on day 7.

Symptoms and signs

Fever was present in all the children with measles. Cough was noted in 98.1% (2132/2174) as a presenting symptom and nasal discharge was documented in 65 % (1413/2174), (Table 3) .

On clinical examination, conjunctivitis was the commonest finding accounting for 90.2% (1961/2174) with the least finding being otitis media 12.2% (265/2174), (Table 3).

Table 3: Clinical presentation of children hospitalised for measles

Clinical Presentation	Number	Percent
Symptomatology		
Fever	2174	100%
Cough	2132	98.1%
Nasal Discharge	1413	65.0%
Clinical findings		
Conjunctivitis	1961	90.2%
Diarrhoea	1470	67.6%
Pneumonia	1159	53.3%
Oral Candidiasis	839	38.6%
Pneumonia and Diarrhoea	817	37.6%
Koplik's spot	370	17.0%
Otitis media	265	12.2%

Clinical complications

Diarrhoea was the most common complication followed by pneumonia in 67.6% and 53.3% respectively (Table 3). Pneumonia and diarrhoea occurred together in 37.6% of the children.

Age and Measles complications

A high proportion of measles cases were below the age of 2 years and most of the complications were seen in children in this age group (Table 4).

Table 4: Age and measles complications in hospitalised children

Age (yrs)	Diarrhoea (%)	Pneumonia (%)	Pneumonia and Diarrhoea (%)	Total
<1	517 (64.3)	496 (61.7)	335 (41.7)	804
1-2	342 (72.6)	279 (59.2)	213 (45.2)	471
2-3	224 (60.6)	173 (47.0)	123 (33.3)	369
4-5	103 (51.0)	87 (43.1)	51 (25.5)	201
6-10	146 (64.1)	96 (42.3)	76 (33.3)	227
11-15	59 (54.9)	35 (43.2)	21 (21.1)	102
Total	1391 (64.1)	1166 (53.3)	818 (37.6)	2174

Age and Case Fatality Rates (CFR)

Two hundred and ninety seven deaths were documented in the 2174 cases, giving an overall case fatality rate of 13.7%. On average, the CFR in children 0-3 years was 16.6%, which was significantly higher than in children aged 4-14 years (5.5%), (Table 5).

Table 5: Age distribution and Case Fatality Rate in children hospitalised for measles

Age (yrs)	No. Cases	No. Died	CFR
<1	749	106	14.2%
1-2	488	84	17.2%
2-3	417	77	18.5%
4-5	158	10	6.3%
6-10	242	13	5.3%
11-15	120	6	5.0%
Total	2174	297	13.7%

Complications and CFR

All complications of measles like pneumonia, diarrhoea, and oral candidiasis were significantly associated with CFR (Table 6). The CFR was significantly higher in the children who had pneumonia (18.4%) than those without 8.3%, p-value 0.00014). Oral candidiasis was even associated with the highest CFR (20.1%, p-value 0.000013), (Table 6).

Table 6: Measles complications and CFR in hospitalised children

Complications Pneumonia	No. Cases	No. Died	CFR	p-value
Yes	1159	213	18.4%	0.00014
No	1015	84	8.3%	
Diarrhoea				
Yes	1470	226	15.4%	0.072
No	704	70	10.5%	
Pneumonia & Diarrhoea				
Yes	817	148	18.1%	0.009
No	1357	149	11.0%	
Oral Candidiasis				
Yes	839	167	20.1%	0.000013
No	1335	129	9.7%	
Otitis media				
Yes	354	90	25.3%	0.000010
No	1820	146	8.0%	

Measles skin rash duration before presentation to hospital and CFR

Measles CFR was significantly higher in children who were brought to hospital seven days after the onset of the rash, (29.5%, p-value 0.0047),(Table 7). The onset of rash was defined as point time that the patient developed clinical measles infection recognised by the parent / guardian.

Measles 7: Measles skin rash duration before presentation to hospital and Case Fatality Rate

Skin rash eruption (days)	No. of patients	No. Died	CFR
1	276	33	12.0%
2	447	44	10.1%
3	654	63	9.6%
4	288	50	17.3%
5	165	36	21.8%
6	33	6	18.2%
>= 7	132	39	29.5%

Overall Case Fatality Rate = 13.6%

Other factors contributing to high CFR

Other clinical findings like nutritional status, hydration status and level of consciousness as correlated to CFR are summarised in Table 8. These findings were also significantly associated with a high CFR ($p<0.0001$). One child aged 5 years had encephalitis. Measles children with altered level of consciousness had a very high CFR (52.4%). Household contact in this hospital based study was not significantly associated with a high CFR ($p>0.78$).

Table 8: Other factors contributing to high CFR in children hospitalised for measles

Nutrition	Died	CFR	Discharged	Total	p-value
Malnutrition	146	23.3%	484	630	<0.0001
Normal	145	9.4%	1399	1544	
Nutrition					
Total	291	13.4%	1883	2174	
Dehydration					
Yes	61	31.4%	133	194	<0.0001
No	230	11.7%	1750	1980	
Total	291	13.7%	1883	2174	
Level of consciousness					
Altered conscious	71	52.4%	65	136	< 0.0001
Alert	224	11.0%	1811	2038	
Total	295	13.7%	1876	2174	
Measles household contact					
Yes	68	14.3%	407	475	> 0.78
No	228	13.4%	1471	1699	
Total	296	13.7%	1878	2174	

Measles vaccination status as seen by under-5-card

Immunisation cards were available in 59.5% (1263/2174) of all children admitted for measles infection (Table 9).

Table 9: Availability of Immunisation card (under 5 cards seen)

Availability of card	Number	Percent
Seen	1293	59.5%
Not seen	881	40.5%
Total	2174	100%

Measles vaccination status Vs CFR

Of the total under-5-cards seen, 30.9% of the children showed that they had received the measles vaccine, while 69.1% were not vaccinated. The case fatality rate was more in the unvaccinated children (19.7%) than the vaccinated children (9.3%), (Table 10).

Table 10: Measles vaccination status Vs CFR in children hospitalised for measles

Vaccination Status	Alive	Died	Total (%)	CFR
Vaccinated	365	34	399 (30.9%)	9.3%
Unvaccinated	747	147	894 (69.1%)	19.7%
Total	1112	181	1293 (100%)	14.5%

HIV-I sero-prevalence in children hospitalised for measles

The overall sero-prevalence of HIV in children hospitalised for measles was 20.1% during the period 1993-1996 and the prevalence had increased to 31.1% during the period 1998-1999 (Table 11).

Table 11: HIV sero-status in children hospitalised for measles

Period	1993 – 1996		1998 - 1999	
HIV status	No.	Percent	No.	Percent
Positive	88	20.1%	103	31.1%
Negative	349	79.9%	228	68.9%
Total	437	100%	331	100%

Prevalence of HIV-I sero-positivity in children admitted for Measles

A total of 768 children were tested for HIV-I antibodies and the overall prevalence of HIV-I in children hospitalised for measles was 24.9%, (Table 12). The antibody test (IgG-based tests) does not indicate infection in young children below the age of 18 months.

Table 12: Sero-prevalence of HIV-I in children hospitalised for measles

HIV status	Number	Percent
Positive	191	24.9%
Negative	577	75.1%
Total	768	100%

Measles Case Fatality Rate in the HIV-I sero-positive children

The prevalence of HIV sero-positivity was highest in children in the age group 9 – 17 months (35%, 66/191) than the children below 9 months (25%) and those aged above 18 months (28%), (Table 13).

Measles CFR in HIV-I sero-positive children was highest in the 9 – 17 months age group (36.0%) and lowest in those below the age of 9 months. The average measles CFR in the HIV-I sero-positive children was 27.3% (Table 13).

Table 13: Measles CFR in HIV-I seropositive children hospitalised for measles

Age (months)	Number	Percent	Died	CFR
< 9	47	25.0%	8	18.2%
9-17	66	35.0%	17	25.8%
18-59	54	28.4%	19	36.0%
> 60	26	11.4%	8	30.0%
Total	191	100 %	52	27.3%

Measles Case Fatality Rate in HIV-I sero-negative children

Measles CFR in the HIV-I sero-negative children was highest in children below the age of 9 months (12.3%, 13/108)) and least in children above the age 18 months (7.4%, 10/135).

The average measles CFR in the HIV-I sero-negative children was 7.7 % (Table 14) compared to 27.3% in the HIV-I sero-positive group (Table 13).

Table 14: Measles CFR in HIV-I sero negative children hospitalised for measles

Age (months)	Number	Percent	Died	CFR
< 9	108	18.6%	13	12.3%
9-17	206	35.8%	23	11.2%
18-59	135	23.5%	10	7.3%
60	128	22.1%	0	0.0
Total	577	100%	46	7.7%

Measles complications Vs HIV-I sero-positivity

Diarrhoea and pneumonia were the commonest associated complications (68.2% and 64.8% respectively) of measles in the HIV-I seropositive group. Oral candidiasis was the next common associated complication, 53.4%, and the least was otitis media 25%, (Table 15). However, in the immunocompromised children, Kopliks spots may be generalised and may be mistaken for oral candidiasis.

However, otitis media was significantly associated with a high measles CFR of 45% in the HIV-I sero-positive group followed by oral candidiasis 34.3% (Table 15). Pneumonia and diarrhoea contributed 29.8% and 28.5% respectively to measles CFR in the HIV-I sero-positive children.

Table 15: Measles complications Vs HIV-I sero-positive in children hospitalised for measles

Complication	Number	Percent	Died	CFR
Pneumonia				
Yes	124	64.8%	37	29.8%
No	67	35.2%	15	22.4%
Diarrhoea				
Yes	130	68.2%	37	28.5%
No	61	31.8%	15	24.6%
Oral Candidiasis				
Yes	102	53.4%	35	34.3%
No	89	46.6%	17	19.1%
Otitis Media				
Yes	48	25.0%	22	45.8%
No	143	75.0%	30	21.1%

Measles complications in the HIV-I sero-negative group

In the HIV-I sero-negative group of children hospitalised for measles, diarrhoea was the commonest complication (63.9%) followed by pneumonia (53.0%). Oral candidiasis accounted for 38.4% and otitis media

8.0% (Table 16). However, measles CFR was 15.2% in children with otitis media in the HIV sero-negative group. Diarrhoea contributed least (6.6%) to measles CFR in this group (Table 16).

Table 16: Measles complications Vs HIV-I sero-negative children hospitalised for measles

Complication	Number	Percent	Died	CFR
Pneumonia				
Yes	306	53.0%	33	10. 9%
No	271	47.0%	13	4.9%
Diarrhoea				
Yes	369	63.9%	24	6.6%
No	208	36.1%	21	10.2%
Oral Candidiasis				
Yes	222	38.4%	24	11.0%
No	355	61.5%	21	6.0%
Otitis Media				
Yes	46	8.0%	7	15.2%
No	531	92.0%	40	7.5%

Measles vaccination status and CFR in HIV-I sero-positive children

Measles CFR was higher in measles vaccinated children (37.7%) than the unvaccinated (28.3%) in the HIV-I sero-positive group (Table 17).

Table 17: Measles vaccination status Vs CFR in HIV-I sero-positive

Vaccination status	Number	Percent	Died	CFR
Yes	77	53.7%	29	37.7%
No	67	46.3%	19	28.3%
Total	144	100%	48	33.3%

Measles vaccination status and CFR in HIV-I sero-negative children

In the HIV-I sero-negative children, measles CFR was high in the unvaccinated group (9.2%), than the vaccinated group(4.3%), (Table 18).

Table 18: Measles vaccination status Vs CFR in HIV-I sero-negative

Vaccination status	Number	Percent	Died	CFR
Yes	210	42.6%	9	4.3%
No	283	57.4%	26	9.2%
Total	493	100%	35	7.2%

Measles virus isolates

A total of 333 measles viruses were isolated from the patients. Of these, 11.9% (116/973) were isolated from the throat swabs and 72.6% (217/300) from the separated lymphocytes. The contamination rate was 29.8% with the throat swabs and nil with the lymphocytes (Table 19).

Table 19: Measles virus isolates in children hospitalised for measles

Sample	No. +ve (%)	No. –ve (%)	Contaminated (%)	Total
Throat swabs	116 (11.9%)	567 (58.3%)	290 (29.8%)	973
Lymphocytes	217 (72.4%)	83 (27.6%)	-	300
Total	333	650	-	1273

Molecular sequencing of MV isolates

The MV isolates belong to clade A and B. Only silent and minor mutations were identified. There was no major antigenic drift detected in the measles isolates (Fig 5).

Figure 5: Molecular sequencing of measles virus isolates

Sequence Analysis of current isolates

Sequence of isolate 40 and isolate 8

- only silent mutations detected

```
                10        20        30        40        50        60
40   CCAGACAAGC CCAAGTGTCA TTTCTACACG GTGATCAAAG TGAGAATGAG CTACCAGGAT
8    CCAGACAAGC CCAAGTGTCA TTTCTACACG GTGATCAAAG TGAGAATGAG CTACCAGGAT

                70        80        90        100       110       120
40   TGGGGGGCAA GGAAGATAGG AGGGTCAAAC AGAGTCGGGG AGAAGCCAGG GAGAGCTACA
8    TGGGGGGCAA GGAAGATAGG AGGGTCAAAC AGAGTCGGGG AGAAGCCAGG GAGAGCTACA

                130       140       150       160       170       180
40   GAGAAACCGG GTCCAGCAGA ACAAGCGATG CGAGAGCTGC CCATCTTCCA ACCAACACAC
8    GAGAAACCGG GTCCAGCAGA ACAAGCGATG CGAGAGCTGC CCATCTTCCA ACCAACACAC

                190       200       210       220       230       240
40   CCCTAGACAT TGACACTGCA TCGGAGTCAA GCCAAGATCC GCAGGATAGT CGAAGGTCAG
8    CCCTAGACAT TGACACTGCA TCGGAGTCAA GCCAAGATCC GCAGGATAGT CGAAGGTCAG

                250       260       270       280       290       300
40   CTGACGCCCT ACTCAGGCTG CAAGCTATGG CAGGAATCTC GGAAGAACAA GGCTCAGACA
                    |                 |
8    CTGACGCCCT GCTCAGGCTG CAAGCCATGG CAGGAATCTC GGAAGAACAA GGCTCAGACA

                310       320       330       340       350       360
40   CAGACACCCC TAGAGTGTAC AATGACAGAG ATCTTCTAGA CTAGGTGCGA GAGGCCA
```

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                    190        200        210        220        230        240
CTTCCAACCG GCACACCCCT AGACATTGAC ACTGCATCGG AGTCCAGCCA AGATCCGCAG
CTTCCAACCG GCACACCCCT AGACATTGAC ACTGTATCGG AGTTCAGCCT AGATCCGCAG
---------- ---------- ---------- ----*----- ---*-----* ----------
CTTCCAATCA GCACACCCCT AGACATTGAC ACTGCATCAG AGTCAGGCCA AGATCCGCAG
-------*-* ---------- ---------- --------*- ----**---- ----------
CTTCCAACCG GCACACCCCT AGACATTGAC ACTGTATCGG AGTTCAGCCT AGATCCGCAG
---------- ---------- ---------- ----*----- ---*-----* ----------
CTTCCAACCA ACACACCCCT AGACATTGAC ACTGCATCGG AGTCAAGCCA AGATCCGCAG
---------* *--------- ---------- ---------- ----*----- ----------
CTTCCAATCA GCACACCCCT AGACATTGAC ACTGCATCAG AGTCAGGCCA AGATCCGCAG
-------*-* ---------- ---------- --------*- ----**---- ----------
CTTCCAACCA ACACACCCCT AGACATTGAC ACTGCATCGG AGTCAAGCCA AGATCCGCAG
---------* *--------- ---------- ---------- ----*----- ----------
CTTCCAACCA ACACACCCCT AGACATTGAC ACTGCATCGG AGTCAAGCCA AGATCCGCAG
---------* *--------- ---------- ---------- ----*----- ----------
CTTCCAACCG GCACACCCCT AGACATTGAC ACTGTATCGG AGTCCAGCCT AGATCCGCAG
---------- ---------- ---------- ----*----- ---------* ----------
                    250        260        270        280        290        300
GACAGTCGAA GGTCAGCTGA CGCCCTGCTT AGGCTGCAAG CCATGGCAGG AATCTCGGAA
GACAGTCGAA GGTCAGCTGA CGCCCTGCTT AGGCTGCAAG CCATGGCAGG AATCTCGGAA
---------- ---------- ---------- ---------- ---------- ----------
GACAGTCGAA GGTCAGCTGA CGCCCTGCTC AGGTTGCAGG CCATGGCAGG AATCTTGGAA
---------- ---------- ---------* ---*----*- ---------- -----*----
GACAGTCGAA GGTCAGCTGA CGCCCTGCTT AGGCTGCAAG CCATGGCAGG AATCTCGGAA
---------- ---------- ---------- ---------- ---------- ----------
GATAGTCGAA GGTCAGCTGA CGCCCTGCTC AGGCTGCAAG CCATGGCAGG AATCTCGGAA
--*------- ---------- ---------* ---------- ---------- ----------
GACAGTCGAA GGTCAGCTGA CGCCCTGCTC AGGTTGCAGG CCATGGCAGG AATCTTGGAA
---------- ---------- ---------* ---*----*- ---------- -----*----
GATAGTCGAA GGTCAGCTGA CGCCCTGCTC AGGCTGCAAG CCATGGCAGG AATCTCGGAA
--*------- ---------- ---------* ---------- ---------- ----------
GATAGTCGAA GGTCAGCTGA CGCCCTGCTC AGGCTGCAAG CCATGGCAGG AATCTCGGAA
--*------- ---------- ---------* ---------- ---------- ----------
GACAGTCGAA GGTCAGCTGA CGCCCTGCTT AGGCTGCAAG CCATGGCAGG AATCTCGGAA
---------- ---------- ---------- ---------- ---------- ----------
                    310        320        330        340        350        360
GAACAAGGCT CAGACACGGA CACCCCTATA GTGTACAATG ACAGAAATCT TCTAGACTAG
GAACAAGGCT CAGACACGGA CACCCCTAGA GTGTATAATG ACAGAGATCT TCTAGACTAG
---------- ---------- --------*- -----*---- -----*---- ----------
GAGCAAGGCT CAGATACGGA CATCTCTAGG GTGTACAATG ACAAAGATCT TCTAGACTAG
--*------- ----*----- --*-*---** ---------- ---*-*---- ----------
GAACAAGGCT CAGACACGGA CACCCCTAGA GTGTATAATG ACAGAGATCT TCTAGACTAG
---------- ---------- --------*- -----*---- -----*---- ----------
GAACAAGGCT CAGACACAGA CACCCCCAGA GTGTACAATG ACAGAGATCT TCTAGACTAG
---------- -------*-- ------*-*- ---------- -----*---- ----------
GAACAAGGCT CAGATACGGA CATCTCTAGG GTGTACAATG ACAAAGATCT TCTAGACTAG
---------- ----*----- --*-*---** ---------- ---*-*---- ----------
GAACAAGGCT CAGACACAGA CACCCCTAGA GTGTACAATG ACAGAGATCT TCTAGACTAG
---------- -------*-- --------*- ---------- -----*---- ----------
GAACAAGGCT CAGACACAGA CACCCCTAGA GTGTACAATG ACAGAGATCT TCTAGACTAG
---------- -------*-- --------*- ---------- -----*---- ----------
GAACAAGGCT CAGACACGGA CACCCCTAGA GTGTACAATG ACAGAGATCT TCTAGACTAG
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CHAPTER 7

7.0 DISCUSSION

The presentation of measles was very typical in this study population, with fever being documented in all children, cough, rash, and conjunctivitis in most cases. Koplik's spots were noted in about 17 % our patients partly because of the relatively high rate of oral candidiasis. The classical clinical presentation of measles infection in these patients was largely because of the highly selective population who were referred to hospital from the local clinics. The high rates of complications in these patients were probably due to bias towards referring children with complicated or severe measles.

Diarrhoea was the most common complication in children with measles and was associated with a high CFR (15.4%). Pneumonia was the next commonest complication and was associated with a higher CFR (18.4%). When diarrhoea and pneumonia appeared together, the CFR was not increased significantly (18.1%). Oral candidiasis was a complication in a significant number of children with measles and was associated with a significantly higher CFR (20.1%), suggesting that these children were immunocompromised. It is hypothesised that poor feeding in these

children with oral candidiasis could have led to hypoglycaemia, and this may have contributed to the higher CFR. Otitis media was associated with an even higher CFR of 25.3% in children hospitalised for measles infection. Otitis media in these children was associated with immunosuppression. Therefore, otitis media was a risk factor for high CFR.

The overall CFR of 13.7% reported in this study is much higher than that reported from neighbouring Zimbabwe (1.43%), in hospitalised children with measles (3). In Harare, Zimbabwe where the vaccination coverage rate is high (86%), the age of measles infection has shifted to the older age group and the CFR is lower during measles outbreaks (3). The CFR was higher in children less than 3 years (16.7%). This high CFR is probably due to the low measles vaccine coverage rate in Lusaka (58%, 1993) and the young age of exposure to measles (4,7).

Analysis of other factors which contributed to the high CFR showed that children with malnutrition had a significantly high CFR. Malnutrition has consistently been shown to be a bad prognostic factor in children hospitalised for measles (12,13,14). Dehydration due mainly to diarrhoea was also noted to have a significantly high CFR while fever did contribute. Altered level of consciousness in children was associated with a very high

CFR (52.4%). Probably some of these patients with altered level of consciousness may have had acute measles encephalitis, although this complication was documented in only one patient. Household contact of measles did not seem to significantly contribute to high CFR (14.3%), ($p>o.78$) in these hospital based study. However, community based studies have shown that close contact is associated with high CFR (13,15) probably because of the high measles virus infective load in the households.

Measles infection before the vaccination age of 9 months is becoming a major health problem. There is a significant increase in young infants less than 6 months of age presenting with measles in the period 1998 – 1999, than the period 1993 – 1996. There are two possible reasons for this. The first is related to the increased incidence of HIV in pregnant mothers in the community which currently is estimated to be 30% in urban Lusaka. The second is that HIV-I infected mothers may have defective transfer of specific measles IgG antibodies across the placenta, which results in lower titres of protective antibodies in the infant and enlarges the window of susceptibility to infection before the infant receives routine immunisation (55). HIV-infected children may not respond adequately to measles immunisation and thus remain susceptible to measles infection (55). Therefore, infants born to HIV-I sero-positive women are more likely to be

infected with measles very early in life. This is illustrated by case 1 documented above. The two month old baby had severe measles complicated by severe pneumonia and subcutaneous emphysema. This infant was born to a mother who was HIV sero-positive and by two months of age the baby was already susceptible to measles infection with severe immunosuppression (low CD4+ count) and a high HIV-1 viral load (56). HIV-1 Viral load and CD4+ count were only done in this particular patient mainly because of the costs of these tests.

The other contributing factor could be due to the fact that young mothers who are in the reproductive age group now had received measles vaccination in infancy. Their measles protective antibodies have waned off, thus leaving their infants without protective measles antibodies and therefore at increased risk of developing measles infection. Probably these young infants could be protected by increasing the herd immunity which can be achieved by increasing the national vaccination coverage rate to over 95%. Therefore, as long as the national vaccination coverage rate remains lower than 70% in Zambia, more young infants will be at increased risk of being infected with measles. The other alternative is to introduce a two dose measles vaccination strategy in infants, but the obvious implication would be cost and indeed the other counter argument would be that in the

first place the national vaccination coverage should be increased to higher levels. However, there is urgent need to devise new strategies to protect the young infants against infection.

This aspect is demonstrated by case 2 documented above. The three week old baby was born to a 23 year old mother who was previously vaccinated in infancy according to the mother. The neonate had no protective antibodies and was therefore infected by the mother who had measles infection shortly after delivery. HIV was not a factor in this mother. The neonate had a measles specific IgM antibody test positive. Measles specific IgM antibody is a variable tool in making definitive diagnosis of measles in very young infants (57).

The case fatality rate was higher in young infants (Table 5). Measles infection was more severe in young infants especially if they are HIV-I sero-positive, and were more likely to develop severe complications. Like the first case cited above had severe pneumonia with subcutaneous emphysema.

Young infants may also be protected by vaccinating adolescent girls. This would be a booster dose to the adolescent girls and as they enter the reproductive age group they would transfer more protective maternal

antibodies to the babies (58,59). Therefore, the young infants would be protected in the first few months of life.

A high CFR in the hospital, which tends to see more severe cases, usually means a much higher rate in the community (17). There is urgent need to look at measles morbidity and mortality in the community. The high CFR was associated with complications which included otitis media, oral candidiasis, pneumonia and diarrhoea. It is recommended that nasogastric feeds be given to children with severe oral candidiasis and adequate hydration maintained in children with measles. Measles infection in hospitalised children has therefore still remained a major cause of morbidity and mortality in Zambia. With the current measles vaccination coverage rates particularly in urban Lusaka, measles infection will remain a serial killer in children for a long time to come.

In this study population, about 60% of the children hospitalised for measles had evidence of being vaccinated against measles. This could be attributed to primary vaccination failure or secondary vaccine failure but more importantly, these were break through infections in vaccinated children (60,61). This suggests that there is high measles transmission rates in the community. The CFR was however lower in the vaccinated group

(9.3%) than the unvaccinated group (19.7%). Therefore, the current measles vaccine cannot prevent infection but can modify the outcome with reduced morbidity and mortality.

It was noted that there was a 10% increase in HIV-1 sero-prevalence in the hospitalised children between the two study periods, (1993 – 1996; 20.1% and 1998 – 1999; 30.1%. This increase reflects the increase in HIV-1 sero-prevalence in the community. The average sero-prevalence was 24.9% between the two periods.

In the HIV-1 sero-positive children hospitalised for measles, the CFR was very high (27.3%). The CFR was lower in children below 9 months (18.2%) and highest in children aged 18-59 months (36.0%). This could be attributed to advanced immunosuppression in the children aged 18-59 months. The CFR was also high in children over 60 months. On the other hand, in the HIV-1 sero-negative group, the CFR was highest in infants below 9 months and least in the age group 18-59 months. No fatality was noted in children over 60 months in the HIV-1 sero-negative group. The overall CFR in this group was lower (7.7%) compared to the high CFR in the HIV-1 sero-positive children (27.3%). It is therefore apparent from

these findings that the HIV epidemic has fuelled the morbidity and mortality in children.

Otitis media was highly associated with increased CFR in the HIV-1 sero-positive than the sero-negative group, 45.8% and 15.2% respectively. Oral candidiasis was also associated with a high CFR in the HIV-1 sero-positive than the sero-negative children, 34.3% and 11.0% respectively. Pneumonia and diarrhoea contributed equally but significantly to the CFR in the HIV-1 sero-positive and less significantly in the sero-negative compared to otitis media and oral candidiasis. Both otitis media and oral candidiasis were more frequently present in HIV sero-positive children and the high CFR was attributed to immunosuppression..

The CFR was higher in vaccinated children who were HIV-1 sero-positive (37.7%) than the unvaccinated (28.3%). This suggests that there is decreased efficacy of measles vaccine in the HIV-1 sero-positive children. However, in the HIV-1 sero-negative group, the CFR was higher in the unvaccinated group than the vaccinated group, 9.2% and 4.3% respectively. Therefore, the current measles vaccine in use in Zambia appears to be more efficacious and protective in the HIV-1 sero-negative than the sero-positive children.

Measles virus isolation on the B95a cell line was higher from the separated lymphocytes than from throat swabs. Fungal contamination on the throat swabs contributed to the lower rate of measles virus isolation as almost a third of the children had oral candidiasis. Measles virus recovery from the separated lymphocytes was better than from throat swabs. Therefore, in doubtful cases of measles infection particularly in young infants, measles virus isolation from separated lymphocytes by the ficoll method should be attempted to arrive at a definitive diagnosis.

Molecular analysis of the currently circulating measles virus showed that there was a co-circulation of clades A and B, and this was consistently demonstrated across a period of 7 years. Only silent minor mutations were detected, documenting the point that measles virus is a very stable virus antigenically. Therefore, the measles vaccine currently in use, the Schwarz vaccine, is still immunogenic in inducing protective antibodies against the wild measles virus circulating in Zambia.

CHAPTER 8

8.0 CONCLUSIONS AND RECOMMENDATIONS

8.1 Conclusions

1. Measles infection is a major cause of morbidity and mortality in children hospitalised for measles in Lusaka, Zambia.

2. Measles infection before the immunisation age of 9 months is increasingly becoming a major problem in Lusaka, Zambia.

3. Diarrhoea, Pneumonia and Oral candidiasis were the most common complications of measles whereas altered level of consciousness and otitis media were associated with high CFR in children hospitalised for measles.

4. Measles CFR was higher in HIV-I sero-positive children than the sero-negative, and the latter was associated with higher CFR in older children because of advanced HIV immunosuppression. There is also low measles vaccine efficacy in the HIV-I sero-positive children as the CFR was high in previously vaccinated children.

5. Complications such as Pneumonia, Oral candidiasis and Otitis media were more common in HIV-I sero-positive children than the sero-negative in children hospitalised for measles.

6. There are more severe measles complications in young infants, and infants born to HIV-I sero-positive mothers are more likely to catch measles infection early in life.

7. There is a high CFR in measles unvaccinated children than the vaccinated and the current measles vaccine in use in Zambia is effective against the circulating measles virus, but there is urgent need to increase the immunisation coverage rate to over 95%. However, the

current measles vaccine does not prevent against measles infection, but appears to protect against severe infection.

8. In difficulty cases like neonates and very young infants laboratory diagnosis of measles is necessary to confirm measles infection.

9. Looking at the antigenic stability of the wild MV isolates with silent and minor mutations detected over a 7 year period, the current measles vaccine is effective in controlling and eliminating measles in Zambia.

8.2 Recommendations

1. There is urgent need to develop new immunisation strategies to protect young infants from measles. These could take the form of;
   a) Immunisation of adolescent girls as they enter the reproductive age group.
   b) Two dose measles schedule in infants.
   c) Development of new measles vaccines which are more immunogenic in early infancy.

2. As measles is a serious infection in immunocomprised patients, prevention of mother to child transmission of HIV would be an ideal strategy.

3. To increase the herd immunity to measles, there is urgent need to increase the measles immunisation coverage rate to over 95%.

4. Hypoglycaemia and dehydration should be treated adequately in children hospitalised for measles, especially those with oral candidiasis.

5. Mass measles immunisation campaigns when planned should be done at the beginning of the cool dry season.

6. Further studies should be instituted to look at:

   a) Delayed mortality in measles

   b) Measles infection in the community

   c) Virologic and molecular analysis of the MV isolates

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Annex 1

Questionnaire: Measles study Form

1. Study No. M.....Hospital No.....
2. Date of recruitment to study.....
3. Date of admission to hospital.....
4. Name of patient.....
5. Home address.....
6. Type of population density area: ( ) Low; ( ) Medium; ( )High
7. CLINICAL SYMPTOMS
   a) Skin rash Y / N / U If yes, duration in days.....
   b) Fever Y / N / U If yes, duration in days.....
   c) Cough Y / N / U If yes, duration in days.....
   d) Wheezing Y / N / U If yes, duration in days.....
   e) Nasal discharge Y / N / U If yes, duration in days.....
   f) Conjuctivitis Y / N / U If yes, duration in days.....
8. Vaccination History
   a) Measles vaccine Y / N / U If yes, when.....yrs.....mths.....
   b) Under 5 card seen Y / N
9. Any measles patients in household in past 2 weeks Y / N / U
10. CLINICAL ASSESSMENT

a) Weight.....kg Mid Arm Circumference.....cm

b) Respiratory Rate...../min Intercostal Recession Y / N

Chest indrawing Y / N

c) Temperature.....C

d) Wheezing Y / N

e) Skin Rash Y / N If yes, type of rash.....

f) Pharyngeal lesion Y / N

If yes, type ( ) Redness ( ) Vesicular ( ) Ulcer ( ) Other.....

g) Oral lesion Y / N

If yes, type ( ) Koplick's spot ( ) Candidiasis ( ) Vesicles

( ) Other.....

h) External ear lesion Y / N

If yes, type ( ) Redness ( ) Rapture of Tympanic membrane

( ) Pulurent ear discharge ( ) Other.....

i) Conjunctivitis Y / N

j) Nutritional status ( ) Normal ( ) Under weight ( ) PEM

k) Dehydration status ( ) No ( ) Some ( ) Severe

l) Consciousness level ( )Alert ( )Irritable ( )Low consciousness

( ) Coma

11. COMPLICATIONS

( ) Diarrhoea ( ) Pneumonia ( ) Otitis media

( ) Meningitis ( ) Encephalitis ( ) Other.....

12. Patients outcome ( ) Died ( ) Discharged

13. Laboratory

a) Throat swab taken Y / N

Viral culture result.....

b) Heparinised blood for lymphocyte separation Y / N

Viral culture result.....

c) HIV-1 serology result Positive / Negative (After pretest counselling)

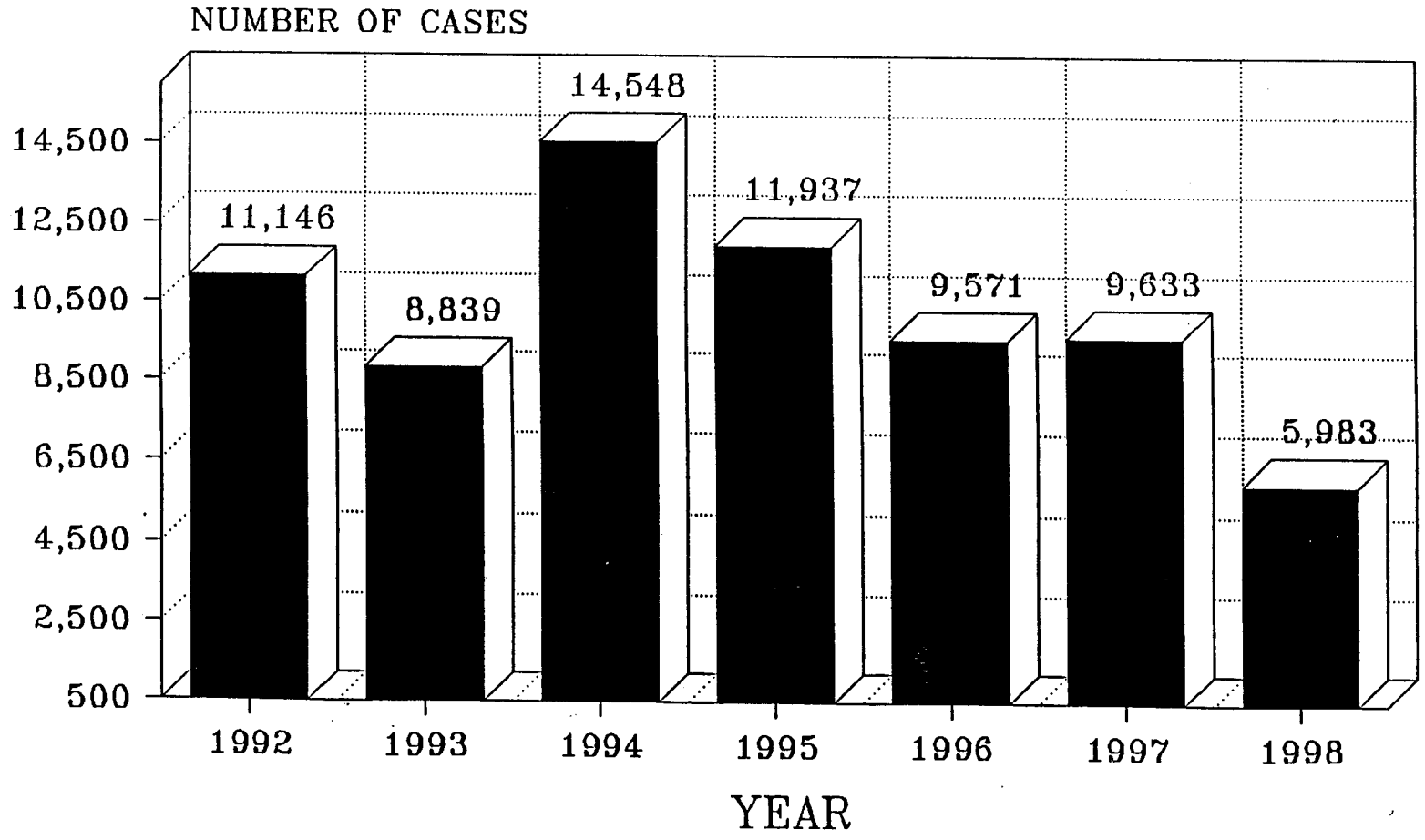
Annex 2: Total number of Measles cases in Zambia

Year	National	Lusaka	Percent
1993	8749	-	-
1994	14548	8454	58.1%
1995	11937	2407	20.2%
1996	9571	2371	24.8%
1997	9633	-	-
1998	5993	-	-
1999	23518	-	-

Source: EPI Secretariat, Lusaka

Annex 3: Trends of Measles cases in Zambia, 1992 – 1998 (All ages)

TRENDS OF MEASLES CASES IN ZAMBIA
1992 - 1998 (ALL AGES)



SOURCE: EPI ROUTINE REPORTING
1997 Based on 95% Routine Reporting
1998 Based on 93% Routine Reporting

Annex 4: Measles immunisation coverage and cases in Zambia, 1992-1998

Year	1992	1993	1994	1995	1996	1997	1998
Measles Coverage	63%	63%	89%	85%	92%	78%	71%
Measles Cases	11,006	8,749	14,548	11,398	9,459	9,633*	5983*

*cases in 1997 and 1998 under reported / many districts missing

Source: EPI Secretariat, Lusaka

Annex 5: Measles immunisation coverage in the three big cities of Lusaka, Kitwe and Ndola in Zambia

Year	Lusaka	Kitwe	Ndola	National average
1993	58%	90%	56%	61%
1994	64%	103%	97%	89%
1995	105%	81%	103%	85%
1996	180%	85%	103%	93%
1997	104%	99%	88%	78%
1998	61%	85%	74%	73%
1999	-	-	-	-

Source: EPI Secretariat, Lusaka