The antibody response to different measles vaccine strains given by the aerosol and subcutaneous routes to schoolchildren

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Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Paediatrics and Child Health, Faculty of Medicine, University of Natal

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Author's Declaration

The contents of the research described in this thesis is the original work of the author. This thesis has not been submitted previously to this or any other university. The author completed the writing of the protocol, recruitment of subjects, specimen and data management and analysis with the assistance of a team of fieldworkers and other researchers whose roles have been duly acknowledged further on.

Athmanundh Dilraj

28 AUGUST 2003

Date

Dedication

This thesis is dedicated to my sons Kiran, Yashveer and Nikeal, and my parents Dilraj and Chitraika Duttoo for their patience and support.

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Publications

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2. Dilraj A, Cutts FT, Fernandez de Castro J, Wheeler JG, Brown D, Roth C, Coovadia HM, Bennett JV. Responses to different measles vaccine strains given by aerosol and subcutaneous routes to schoolchildren: a randomised trial. Lancet 2000;355:798-803.

3. Dilraj A, Cutts FT, Bennett JV, Fernandez de Castro J, Cohen B, Coovadia HM. Persistence of measles antibody 2 years after revaccination by aerosol or subcutaneous routes. (*Pediatr Inf Dis J 2000;19:1211-3)*

Conferences

International

Dilraj A, Cutts FT, Fernandez de Castro J, Wheeler JG, Brown D, Roth C, Coovadia HM, Bennett JV. Booster doses of measles vaccine in schoolchildren: responses to different vaccine strains and the aerosol route. The Second Annual Conference on Vaccine Research. Bethesda, Maryland, USA. March 1999.

Local

1. Dilraj A, Cutts FT, Abdool Karim SS. Measles vaccination coverage and immunity status among schoolchildren. 18th African Health Sciences Congress. Cape Town, April 1997.

2. Dilraj A, Cutts FT, Fernandez de Castro J, Wheeler JG, Brown D, Roth C, Coovadia HM, Bennett JV. Greater serological response to measles vaccination following the aerosol route of administration. 16th Epidemiological Society of Southern Africa (ESSA) Conference. Midrand, October 1998. (Won the Abdool Karim prize for best paper on Infectious Disease Epidemiology)

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2. Measles vaccines and the aerosol route of administration. London School of Hygiene and Tropical Medicine. May 1999.

3. Measles vaccination by the aerosol route: a randomised trial in South African school children. Hawaii Immunization Programme, State Health Department, Honolulu, Hawaii. 25 October 1999

4. Measles vaccine administered by the aerosol route: a randomised trial in South African school children. School of Public Health, University of Hawaii at Manoa, Honolulu, Hawaii. 28 October 1999

5. Immunogenicity of measles vaccine administered by aerosol or subcutaneous routes in South Africa. WHO, Geneva, March 2000

Local

1. Aerosol measles vaccination trial in school children: Preliminary findings. Medical Research Council, South Africa, June 1998.

2. Aerosol measles vaccination trial in school children: Preliminary findings 2 years post-vaccination. Medical Research Council, South Africa, May 1999.

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List of abbreviations

CDC	Centres for Disease Control
ELISA	Enzyme Linked Immunosorbent Assay
EPI	Expanded Programme on Immunisation
EPISA	Expanded Programme on Immunisation in South Africa
EZ	Edmonston-Zagreb
EZae	Edmonston-Zagreb aerosol
EZsc	Edmonston-Zagreb subcutaneous
GMT	Geometric mean titre
HI	Haemagglutination Inhibition Assay
IgG	Immunoglobulin G
IgM	Immunoglobulin M
mIU	milli Intenational units
MMR	Measles-mumps-rubella
MRC	Medical Research Council
pfu	Plaque forming units
PHLS	Public Health Laboratory Services
PN	Plaque Neutralisation Assay
RIA	Radioimmuno assay
SKB	SmithKline Beecham
SW	Schwarz
SWae	Schwarz aerosol
SWsc	Schwarz subcutaneous
WHO	World Health Organisation

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Preface

In 1994, the Department of Health conducted a review of the Expanded Programme on Immunisation in South Africa (EPISA). With respect to measles, one of the major problems that emerged was the high proportion of cases amongst children who had been previously vaccinated, particularly school-aged children. Within the ambit of her role as the international advisor to the Department of Health, Dr Felicity Cutts proposed several research questions aimed to address a range of problems experienced with each of the six preventable diseases under the routine immunisation programme in this country. Thus this trial was proposed to test small studies that suggested that measles vaccination by aerosol may be more immunogenic than subcutaneous injection.

Executive Summary

Background

The epidemiology of measles in many countries, including South Africa, has changed from being a disease that primarily occurred in pre-school children up till 1991 to that which now occurs frequently in schoolchildren and older individuals. In the presence of high measles vaccination coverage, the role of waning immunity has been suggested for the loss of protection against measles in these schoolchildren and older individuals. Thus the administration of more than one dose of measles vaccine is necessary for the sustained control of measles. This gives the opportunity to study the immune responses to measles virus when antibodies are already present. Immune responses could also be dependent on the presentation of the antigen (mucosal versus subcutaneous). Information is lacking on vaccine strain effects, particularly when given as an aerosol. Although historical data suggest that the aerosol route might be more immunogenic for booster doses than traditional subcutaneous injections, there have been no randomised comparative trials. In mass measles campaigns, the aerosol route of vaccination would be easier and quick to administer to a large number of children. The aerosol route is attractive over the subcutaneous route as it is painless and avoids the risks of transmission of blood borne infections.

Objectives

1. To randomise schoolchildren, most of whom that have been previously vaccinated against measles, into 4 groups to receive two different measles vaccine strains (Schwarz or Edmonston-Zagreb (EZ)) given by two different routes of administration (subcutaneous injection or aerosol).

2. To compare the serological responses in the different strain-route groups at 1 month, 1 year and 2 years after vaccination in all children with low or absent antibody levels and in a sample of children with high antibody levels at baseline.
3. To investigate the effect of previous and concurrent upper respiratory tract infections on serological responses.
4. To investigate the frequency of adverse events after vaccination in the different groups and in the aerosol vaccinators.

Methods

We assigned 4987 primary schoolchildren (5-14 year old) by block randomisation of classrooms to receive standard titre doses of either Schwarz or Edmonston-Zagreb measles vaccines subcutaneously or by aerosol. Blood samples for antibody assay were collected on the day of vaccination from all children present. All children who were seronegative, as well as a 9% random sample of seropositives, were subsequently followed up at 1 month, 1 year and 2 years post-vaccination. The fieldwork was conducted between July 1996 and September 1998. Antibody titres were determined using the haemagglutination inhibition assay, with a cut-off of 1:4 which corresponded approximately to 300 milli-international units per ml (mIU/ml). The main endpoints (antibody titres at 1 month, 1year and 2 years after vaccination) were compared between groups.

Results

82.4% of children had histories of previous vaccination and only 5% of the children mounted an IgM response, suggesting that nearly all the children had been previously exposed to measles antigens. Of those that were initially seronegative at baseline or were

part of the random sample of seropositives, 992 children had antibody titre data available for each of the 3 sampling time-points that were used for initial analyses (pre-vaccination, 1 month and 1 year). The initial results based on these children showed that Edmonston-Zagreb vaccine by aerosol was significantly better at boosting titres at 1 month and 1 year than any of the other vaccine groups. Seroconversions (proportion with at least four-fold titre increases from baseline) in the Edmonston-Zagreb aerosol group at 1 month and 1 year were 85% and 60%, compared to Edmonston-Zagreb vaccine given subcutaneously (79% and 34%) and Schwarz vaccine given subcutaneously (63% and 25%). The Schwarz aerosol group performed poorly, and its reconstituted vaccine was found to lose potency quickly in the nebuliser.

Measles antibody persistence assessed up to 2 years after revaccination for 851 of the 992 children showed that at 2 years, seroconversions from baseline continued to be significantly more frequent for Edmonston-Zagreb vaccine by aerosol (55%) than for Edmonston-Zagreb vaccine subcutaneously (23%) and Schwarz vaccine subcutaneously (21%). The Edmonston-Zagreb aerosol group had the lowest proportion of children who became seronegative at 2 years (6%) compared to Edmonston-Zagreb vaccine subcutaneously (12%) and Schwarz vaccine subcutaneously (19%). For all groups, geometric mean titres declined substantially from 1 month to 1 year but the rate of decline flattened out in the second year post-vaccination. The geometric mean titres remained highest for Edmonston-Zagreb vaccine by aerosol (1:13), with Edmonston-Zagreb vaccine subcutaneously and Schwarz vaccine subcutaneously both being 1:7.

Seroconversion was significantly reduced in children who reported illness in the month before vaccination compared to those without any illnesses. There was a borderline reduction in effect of Edmonston Zagreb vaccine by aerosol in presence of cough but not with rhinitis (p=0.1). However, the average antibody titre increases in

those with these symptoms in the aerosol group still exceeded the overall responses in the subcutaneous groups

Adverse events in the 2 weeks post-vaccination were lower in the aerosol groups compared to the subcutaneous groups. Fewer children in the aerosol groups were absent, took medications or consulted a doctor compared to the subcutaneous groups. Except for a higher frequency of fever in the Edmonston-Zagreb subcutaneous group in year 1, there were no significant differences in illnesses between groups in the first and second year following vaccination. There was no significant boosting in antibody titres in the vaccinators nor were there any overt clinical symptoms experienced by them. Thus, the aerosol vaccination was safe to both the vaccinees and vaccinators.

Conclusions

The trial afforded an opportunity to study important immunological responses in measles. The immunological responses to different vaccine strains administered by different routes have been clearly demonstrated. EZ vaccine administered by the aerosol route produced significantly higher and more persistent antibody responses after re-vaccination than any of the other strain/route combinations tested. We believe the aerosol route using presently available devices is safe (to both vaccinees and vaccinators), painless, effective and readily adaptable to mass campaigns, and that this approach could significantly abet global measles elimination/eradication efforts.

CHAPTER 1

Purpose and objectives

1.1 Purpose

The purpose of this study was to get a greater understanding of the antibody responses to different measles vaccine strains when administered by different routes. Such studies are required to facilitate programmes aimed at further global control and elimination of measles. This trial contributed towards achieving this purpose by comparing the serological responses at 1 month, 1 year and 2 years after vaccination to Schwarz or Edmonston Zagreb measles vaccines administered by the subcutaneous or aerosol route in schoolchildren.

1.2 Objectives

1.2.1 To randomise schoolchildren, most of whom have been previously vaccinated for measles, to receive one of four combinations of two different measles vaccine strains (Schwarz or Edmonston-Zagreb) given by two different routes of administration (subcutaneous injection or aerosol)

1.2.2 To measure the baseline measles antibody levels in these children and identify those with high, low or absent measles antibody levels.

1.2.3 To compare the serological responses in the different strain-route groups at 1 month, 1 year and 2 years after vaccination in all children with low or absent antibody levels and in a sample of children with high antibody levels.

1.2.4 To investigate the effect of previous and concurrent upper respiratory tract infections on serological responses

1.2.5 To investigate the frequency of adverse events after vaccination in the different groups and in the aerosol vaccinators.

CHAPTER 2

Literature review

2.1 Introduction

Measles is a highly contagious acute viral disease characterised by fever, cough, coryza, conjunctivitis, and a pathognomonic enanthem (Koplik's spots), followed by a generalised maculopapular eruption (Norrby and Oxman, 1990). The virus is transmitted primarily from person to person by large respiratory droplets or aerosols when an individual in the catarrhal stage of the disease talks, coughs or sneezes. Once accepted as an inevitable part of childhood, isolation of the measles virus in 1954 by Enders and Peebles and subsequent development of vaccines against this virus has changed the epidemiological pattern of this disease wherever it has been extensively used (Black, 1984). Immunisation against measles has proved to be the safest and most effective way of reducing the morbidity and mortality from this disease. However, measles remains one of the major causes of childhood mortality in developing countries. In Africa, it was estimated in 1997 that there were about 11 million cases of and about 550 000 deaths from measles.

2.2 Measles virus

Measles virus is a member of the genus Morbillivirus in the family Paramyxoviridae (Kingsbury *et al* 1988). Measles viruses are spherical, enveloped single stranded RNA viruses. There are six identified structural proteins; three proteins complexed with viral RNA, and three proteins in the virus envelope (Norrby and Oxman, 1990). The envelope components comprise the M protein in the inner surface and the H and F proteins on the outer surface. The H protein attaches the virus to cell surfaces. The

F protein fuses virus and cell membranes, allowing viral penetration of the cell and cell destruction.

2.3 Mode of infection and clinical aspects of the disease

Measles is a ubiquitous, highly infectious disease affecting nearly every person in a given population by adolescence in the absence of immunisation programmes (Black 1984). Measles is transmitted primarily from person-to-person by large respiratory droplets or by the airborne route as an aerosol. Measles is most infectious during the prodrome. First there is localised infection of the respiratory epithelium of the nasopharynx and possibly the conjunctivae, with spread to regional lymphatics. Primary viremia occurs 2 to 3 days following exposure, and an intense secondary viremia occurs 3 to 4 days later. The secondary viremia leads to infection of and further replication in the skin, conjunctivae, respiratory tract and other distant organs. The amount of virus in blood and infected tissues peaks 11 to 14 days after exposure and then falls off rapidly over the next 2 to 3 days (Cutts 1993).

These events correspond with an incubation period between exposure and the onset of symptoms of 10 to 12 days. The prodomal period then begins, with fever, malaise, conjunctivitis, coryza, and tracheobronchitis. The appearance of enanthema and erythema mark the end of the prodrome. Koplik spots appear on the buccal musoca 1 to 2 days before rash onset and may be noted for an additional 1 to 2 days after rash onset. The rash is an erythematous maculopapular eruption that usually appears 14 days after exposure and spreads from the head to the extremities over a 3 to 4 days period. Over the next 3 to 4 days, the rash fades; in severe cases desquamation may occur. Other constitutional signs and symptoms, such as anorexia, diarrhoea and generalised lymphadenopathy may also be present (Preblud and Katz 1988).

2.4 The immunological response to natural infection

The body's mucosal surfaces, tissues and blood all need to be protected against infections and antibodies of different isotypes are adapted to function in different compartments, namely systemic and mucosal.

Systemic compartment:

In primary acute infection, there is a B cell (Norrby and Gollman 1972) and T cell (Graziano *et al* 1975) response to most of the six measles virus proteins. Both IgM and IgG antibodies are initially produced, however IgM antibodies peak at 7-10 days after rash onset and fall rapidly, rarely being detectable more than 4 weeks after rash onset. Serum IgA is also produced but is usually transient (Pederson *et al* 1986). IgG antibodies become detectable in the serum within the first days of rash onset, peak within about four weeks and subsequently decline somewhat, but persist for life (Stokes *et al* 1961). The IgG antibodies to the H protein appear to be most important in determining immunity (Black 1989, Norrby and Oxman 1990).

Immunity after natural infection is usually lifelong (Panum 1940, Black and Rosen 1962). The antibody induced by immunization with attenuated measles virus reaches lower peak titres than those induced by wild virus (Krugman *et al* 1965, Hilleman *et al* 1968, Krugman 1983). Antibody persists longer when there is boosting from exposure to circulating wild virus (Krugman 1977, Krugman 1983, Zhang and Su 1983). When measles antibody titres fall to low levels, re-exposure to measles virus (wild or vaccine virus) stimulates memory cells, which are B cell clones primed to produce specific IgG which remain dormant after the initial infection. An anamnestic (secondary) immune response occurs, in which titres of IgG rise rapidly and peak

approximately 12 days after reinfection (Krugman *et al* 1965, Schleuderberg 1965). If antibody titres are high prior to exposure, reinfection is prevented and a boost in titre is rarely seen (Krugman 1977, 1983, Zhang and Su 1983, Zhuji measles vaccine study group 1987). Since viruses replicate inside cells where they cannot be detected by antibodies, destruction of these viruses infected cells is by T lymphocytes (Janeway *et al* 1999). Cell-mediated immunity plays an important role in recovery from, and possibly prevention of measles, and it has been postulated that sufficient stimulation of cell-mediated immunity may be a prerequisite for the development of lifelong protection (Gallagher *et al* 1981). However, tests for cell-mediated immunity are less readily available than those for humoral immunity and the role of cell-mediated immunity in providing long-term immunity after immunization has not been as clearly defined. An emerging theme is that measles virus immunity is conferred by appropriately polarised anti-viral CD4+ and CD8+ cell populations (van Els and Nanan 2002). Recent technological advances have permitted the analysis of the composition and dynamics of these CD4+ and CD8+ T cell responses at single cell level (Nanan *et al* 2000). They have shown that measles virus-specific CD4+ and CD8+ T cells are readily detectable long after acute infection and are thus contributing to long-term immunity. Others have also shown the role of CD4+ and CD8+ T cells in memory, control and elimination of measles infection (van Binnendijk *et al* 1990, Nanan *et al* 1995, Jaye *et al* 1998).

Mucosal compartment:

Most vaccines are given by injection. However, in addition to the practical drawbacks, the immunological drawback is that the injection is not the usual route of entry of the majority of pathogens against which the vaccination is directed. Many

respiratory and enteric microorganisms infect mucosal surfaces or enter the body through mucosal surfaces (Janeway *et al* 1999). Examples of these include respiratory microorganisms such as measles, pertussis, rhinoviruses and influenza viruses, and enteric microorganisms such as polio, rotaviruses and cholera. Exposing mucosal membranes to vaccines is a strategy that can produce an immune response in a less stressful and better targeted manner. The oral polio vaccine, in use since 1950s, is an early example of the effectiveness of this strategy. This shows that the route of entry of a disease-causing organism is also an effective vaccine route as well.

Mucosal membranes are located throughout the body but are the most accessible to microorganisms in the lungs, nose, mouth, throat, gastrointestinal tract, rectum and vagina. The mucosal-associated lymphoid tissue (MALT) comprises all lymphoid cells in epithelia and in the lamina propria lying below the body's mucosal surfaces. The main sites of MALT are the gut-associated lymphoid tissues (GALT) and bronchial-associated lymphoid tissues (BALT) (Janeway *et al* 1999). IgA is the predominant immunoglobulin in secretions of the gastrointestinal and respiratory tracts (Galazka 1993). IgA in mucosal secretions is produced by plasma cells in the submucosal tissue which lie in close proximity to the epithelium (Cohen and Heine 1992). In the mucosal epithelial tissue, plasma cells lie in close proximity to microfold (M) cells, T lymphocytes and macrophages (Brandtzaeg 1989). M cells have a thinner overlay of mucus and may be more accessible to luminal antigens than other epithelial cells. Antibody produced at mucosal sites differs dramatically from serum IgA produced in bone marrow. Secretory IgA (SIgA) contains a J chain which leads to the formation of an IgA polymer (Breitfeld *et al* 1988). The polymeric IgA binds to a secretory component produced by the endoplasmic reticulum before it is excreted across the luminal surface of these cells. The functions of SIgA involve neutralization of viruses, toxins, enzymes and elimination of

bacterial plasmids. SIgA can block the attachment of antigenic particles to epithelial cells, stimulate the alternative pathway of complement, and act in concert with other non-specific host defenses (Janeway *et al* 1999, Russell and Mestecky 1988, Lachmann 1988).

2.5 Definitions of a serological response to infection

The response to primary infection with measles virus (either measles disease or measles immunisation) is usually measured by documenting a significant increase in titre of IgG antibodies, or by documenting the presence of IgM antibody. At present, no serological tests can distinguish between antibodies, whether IgG or IgM, produced by measles infection and that produced by immunisation.

To document a significant increase in antibody titre, paired sera are required: pre and post-immunisation in the case of response to vaccine, and, in the case of disease, as early as possible in the acute phase and 10-14 days later. The paired specimens should be analysed in the same laboratory in the same test procedure, so that variations in test conditions do not reduce the comparability of the two results. A significant titre rise is generally taken to be a rise in antibody titre by a factor of at least two twofold dilutions (fourfold increase) between the first and second specimens (Gershon and Krugman 1979, Centers for Disease Control 1982), or a change from undetectable antibody (seronegativity) to seropositivity. For example, a change in titre from 16 to 64 or higher would indicate seroconversion; a change in titre from 16 to 32 would not be a significant increase. While this definition of seroconversion is in common use, some plaque neutralisation (PN) tests are so sensitive that persons may demonstrate a fourfold rise in antibody titre after immunisation, but have a low post-immunisation titre which may not provide long-term protection.

The development of very sensitive serological assays such as PN tests has raised questions as to the clinical significance of low antibody titres. Although low titres of antibody measured by PN assay have been shown to indicate previous exposure to wild or vaccine measles virus, there are now data which show that titres below 200 milli-International Units (mIU) may not be protective. In an outbreak in the USA in 1985, all measles cases occurred among college students with pre-exposure antibody titres less than 200 mIU. No cases occurred among 71 persons with pre-exposure titres greater than 200 mIU (Chen *et al* 1990).

2.6 Brief history of the development of measles vaccines

The development of live attenuated measles virus vaccines began soon after the isolation of the virus by Enders and Peebles in 1954 (Enders and Peebles 1954). By the end of the 1950s, Enders and colleagues had developed the Edmonston B strain of live attenuated measles vaccine by subjecting the virus to 24 passages in primary human renal cell cultures, 28 passages in primary human amnion cell cultures, and six serial chick embryo passages before adapting the virus to chick embryo fibroblasts (Enders 1962). Because the Edmonston B vaccine was associated with fever greater than 39.5°C in 20-40% and rash in approximately 50% of recipients (Krugman *et al* 1962, 1965), gamma globulin was often administered simultaneously to reduce clinical reactions.

By the middle-late 1960's, new strains of measles vaccine were developed in the USA, Japan, Yugoslavia, the USSR and China, by further attenuation of Edmonston (AIK-C), Edmonston A (Schwarz), Edmonston B (Moraten, Edmonston Zagreb (EZ)) or separate isolates (Leningrad 16, CAM-70). Further attenuation was first achieved by Schwarz by 85 additional passages of Edmonston A virus in chick embryo fibroblast cultures at 32°C instead of 36-37°C (Schwarz 1964). Although antibody titres attained

after further attenuated vaccines such as Schwarz vaccine were lower than those after Edmonston B vaccine or natural infection, further attenuated vaccines were associated with lower rates of clinical reactions and were suitable for widespread use without the need for concurrent administration of gamma globulin.

One of the first measles vaccines was a formalin-inactivated vaccine derived from the Edmonston strain. Usually, three doses of inactivated vaccine or two doses of inactivated and one dose of live vaccine were administered at monthly intervals, with few side effects (Krugman *et al* 1965). Use of inactivated vaccine was stopped in 1967, when it was realised that immunity was short-lived (Karelitz *et al* 1963), and that recipients were at risk of atypical measles, a presumed hypersensitivity reaction, on exposure to live measles virus (Centers for Disease Control 1967).

2.7 Measles vaccine potencies

In an attempt to standardise measles vaccine potencies, the Expanded Programme on Immunisation established that the minimum quantity of the vaccine virus that should be contained in one human dose is generally considered to be 1000 (3.0 $\log_{10}$) viral infective units (WHO 1988). However, no maximum potency was specified. Studies have shown that the EZ vaccine in potencies of >4.0 $\log_{10}$ infective units per dose was as immunogenic in 4-6 month old infants as Schwarz vaccine at 9 months (Whittle *et al* 1988, Tidjani *et al* 1989, Markowitz *et al* 1990, Job *et al* 1991). This lead to use of terminology such as "medium" and "high" titre measles vaccines. Vaccine of potency between 3.0 and 4.0 $\log_{10}$ was generally considered to be "standard" titre. "High" titre was initially defined by WHO as >5.0 $\log_{10}$ and subsequently as >4.7 $\log_{10}$ infectious units per dose (Expanded Programme on Immunisation 1990). Intermediate titres were described as "medium" titre, although that nomenclature was unofficial (Cutts *et al*

1995a). Currently, the official definitions of measles vaccine potencies are "standard" titre measles vaccine (those with a potency between 3.0 and 4.6 $\log_{10}$ infectious units) and "high" titre measles vaccine ($\geq$4.7 $\log_{10}$ infectious units) (Bolotovski *et al* 1994).

2.8 Brief epidemiology of measles and history of measles vaccination

The changes in measles epidemiology that occur as immunisation programmes mature and their potential implications for measles immunisation policy have been reviewed in detail (Cutts 1990). The major changes which occur as high immunisation coverage is achieved are an overall reduction in measles incidence rates, a shift in the age distribution to older persons, and an increase in the interepidemic interval which may lead to the occurrence of outbreaks after a long disease-free period. As high coverage of young children is reached and sustained, measles transmission decreases. Cohorts of unimmunised children from previous years can then reach older ages without contracting measles. The number of susceptible older children gradually builds up so that the potential for outbreaks among these children exists. With the introduction and maturation of a measles immunisation programme in South Africa, this country has experienced these epidemiological changes as well (*Epidemiological Comments 1995b).*

2.8.1 Measles epidemiology and history of measles vaccination in developed nations

Following the introduction of measles immunisation in the USA in 1963, measles incidence fell markedly. However, measles incidence fluctuated over the first 10 years as monies available for immunisation varied. During the late 1970's, only about 67% of children aged 10-13 years were immunised. In 1976, almost double the number of measles cases than that of the preceding 3 years were reported (Mitchell and Balfour 1985). The age distribution of cases in the USA changed markedly after the introduction

of immunisation. Before the introduction of immunisation, only 10% of the cases were in persons over 10 years old. In 1976, more than 60% of cases were in individuals greater than 10 years of age, with 20 % over 15 years old.

In 1978 a measles elimination programme was announced, with 3 major strategies: maintenance of high coverage; mandatory immunisation prior to school entry; careful surveillance and aggressive outbreak control. These strategies resulted in a further drop in cases. After being close to elimination, with a low of 1497 in 1983, reported measles incidence increased since then. This happened despite over 97% of children entering school having proof of prior immunisation. Of 152 outbreaks reported in 1985 and 1986, 101 occurred mainly among school-aged children. Transmission was documented among appropriately immunised children i.e. those immunised at 12 months of age or above (Davis *et al* 1987).

In response to these school-age outbreaks, the Advisory Committee on Immunisation Practices (ACIP) in 1989 recommended more aggressive outbreak control measures. During school or college-based outbreaks, all students whose most recent dose of vaccine was prior to 1980 were to be reimmunised. In pre-school outbreaks, lowering the age at immunisation to 6 months with reimmunisation at 15 months was not effective in preventing a rise in measles incidence in 1988 and the first half of 1989. Consequently, a recommendation for a routine two-dose schedule, the first at 15 months of age and the second at school age, was made in addition to universal immunisation during outbreaks (CDC 1989).

In the United Kingdom, measles vaccination began in 1968 for the vaccination of children between 1 and 2 years of age. The highest incidence of measles continued to be in children <5 years old while coverage with the single antigen measles vaccine was only 50-70% until the mid-1980s (Miller 1983). There were no special strategies for the first

20 years until the introduction of MMR (measles, mumps, rubella) vaccine in 1988 with a series of public health measures to increase coverage (Cutts and Markowitz 1994). There was a sharp decline in the circulation of measles after the introduction of MMR vaccine (Ramsay *et al* 1994).

In other European countries, such as Sweden, measles vaccination was introduced in the early 1970s. Following the experiences in the United States and other countries of inadequate protection from measles infection throughout the school period or for life with one dose of vaccine, Sweden introduced a two-dose programme of vaccination with MMR vaccine in 1982. The vaccination was offered free of charge to all children at the age of 18 months and 12 years (Christenson *et al* 1983). The two-dose programme with MMR was later also adopted in Norway, Denmark and Finland. In the two and half years since the launch of this programme, the incidence of measles fell by 93% compared with a normal year before this programme (1982) (Peltola *et al* 1986).

2.8.2 Epidemiology of measles and history of measles vaccination in South Africa

The official measles vaccination programme in South Africa was launched in September 1975 with the introduction of free measles vaccine for all children. Prior to 1975, certain local authorities had, through their own initiative and resources, obtained supplies of measles vaccine for inclusion in their immunisation services. However, this policy had been implemented only in the larger cities where the prohibitive costs of the vaccine were more easily borne (Dick 1975). To further enhance attempts to control measles, it was felt that surveillance was necessary. To this end, measles became a notifiable condition on 24 August 1979.

As measles only became a notifiable condition 4 years after the introduction of measles vaccination, there is little national data in this country on measles incidence pre-

immunisation or in the early years of immunisation. In the Municipality of Cape Town, with an estimated population of 770780 in 1973, there were 377 admissions to the 2 large measles referral hospitals (City Hospital for Infectious Diseases, and Red Cross War Memorial Children's Hospital) for that year. Thirty three of those admitted died (Dick 1975). In South Africa in 1975, there was an estimated 3148 deaths due to measles (Epidemiological Comments 1979).

While notifications generally underestimate the true incidence of a condition, including measles, it is nonetheless regarded as a useful tool in outlining the basic epidemiology of a condition. Since measles became a notifiable condition, the number of notified cases of measles decreased from 19200 in 1980 to 1058 in 1998 (Epidemiological Comments 1995, 1996, 1998 and 1999) (Table 2.1). The number of notified deaths attributed to measles in this period reduced from 267 to 1.

While there was an overall decline, this country experienced one of its worst recorded epidemics during this period. Although a comparatively low number of cases was reported for 1991 (4777 cases) following the launch of the Measles Strategy (a period of accelerated immunisation) in 1990 (see later), the number of measles cases notified increased dramatically in 1992 to an all-time high of 22745 cases (Epidemiological Comments 1995a). The mechanism of such a "post honeymoon" epidemic is well described (McLean and Anderson 1988, Cutts *et al* 1991). Rapid achievement of high coverage induces a period of low incidence ("honeymoon period"), during which older persons are immune from exposure to the natural disease and younger persons are immune after immunisation. As the cohorts grow older, while the disease incidence is low, most persons acquire immunity from exposure to the vaccine rather than to the disease. If coverage is less than 100% and the vaccine is less than

100% effective, susceptible persons gradually accumulate until the epidemic threshold is reached, when outbreaks occur ("post-honeymoon period").

In the past, measles occurred predominantly in young children (< 5 years old). The < 1-year age showed the highest age-specific incidence at 405 cases/100000. The rate declined rapidly with advancing age (Gibson 1982). However, since 1992, there has been an upward shift in the ages of those infected with the measles virus (Figure 2.1). In many of the later outbreaks, school-aged children accounted for a large proportion of the cases (Coetzee *et al* 1994). These were largely also in previously highly vaccinated communities. Nationally, the < 5 year olds accounted for 71% of cases when immunisations commenced in 1980 and by 1994 it reduced to 45% (Epidemiological Comments 1995a). The changing age distribution of measles i.e. the massive increase in incidence of older age-groups compared to younger age groups, as a result of high, sustained coverage in many areas, is clearly seen in Table 2.2 and illustrated in Figure 2.2

Table 2.1: Number of notified measles cases and measles deaths in South Africa since the introduction of measles vaccination

Year	Cases	Deaths
1980	19193	267
1981	15809	251
1982	12906	265
1983	16735	504
1984	16160	240
1985	17884	331
1986	13459	317
1987	22559	449
1988	15258	302
1989	18268	322
1990	10624	55
1991	4777	29
1992	22745	53
1993	12870	19
1994	3820	13
1995	6826	3
1996	10604	24
1997	1422	5
1998	1058	1

Adapted from *Epidemiological Comments* 1995a, 1996, 1998 and 1999

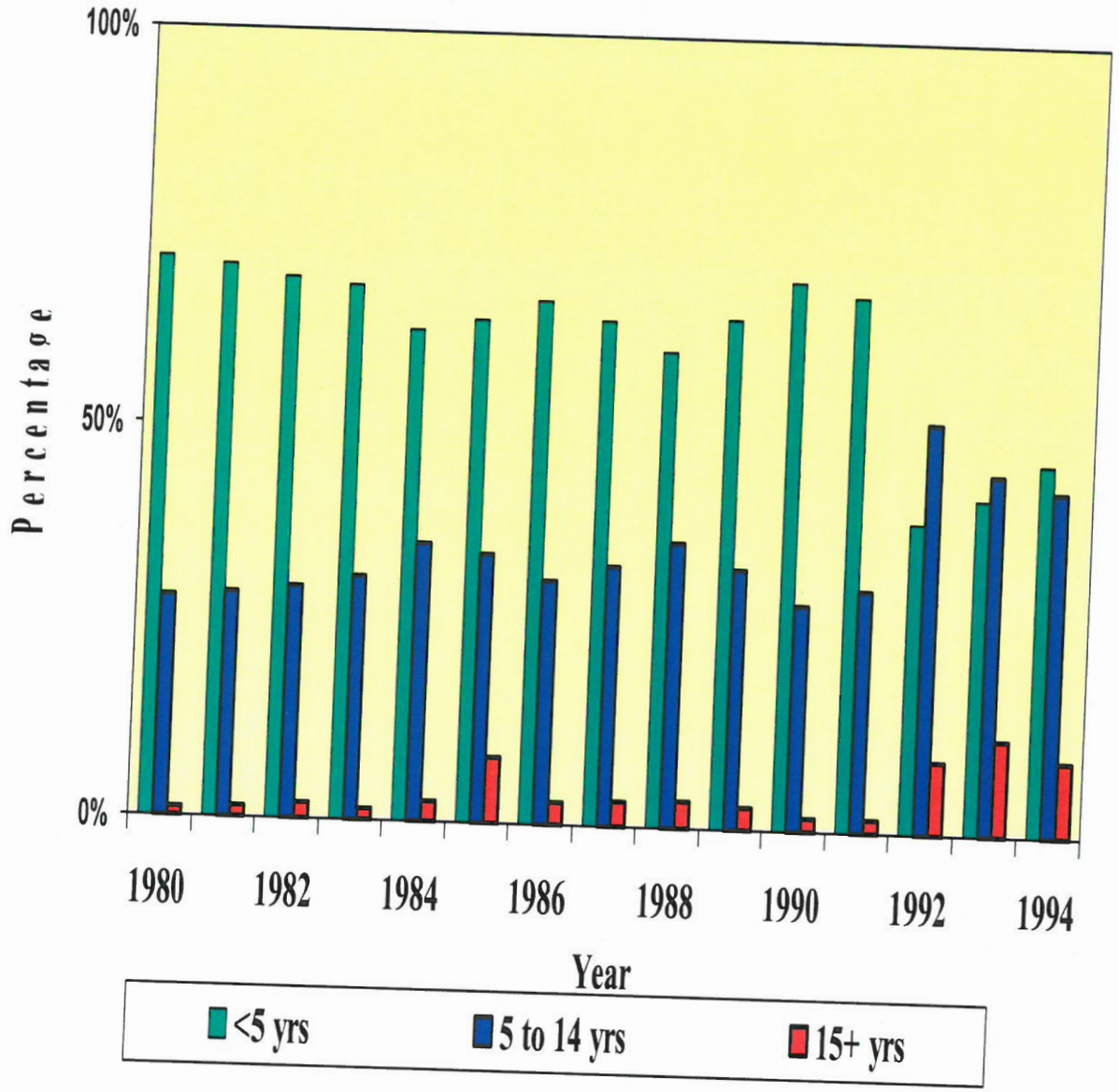

Figure 2.1: Proportional age distribution of measles cases from 1980 to 1994

(Adapted from *Epidemiological Comments* 1995a)

Table 2.2: Percent change in 3-year average measles incidence by age-group, South Africa, 1980-82 to 1992-94

Age groups (years)	3-year average measles IR (per 100 000) 1980-1982 (a)	1992-1994 (b)	Percentage Change (b-a)/a X 100
<1	405,4	147,0	-63,7
1 4	270,1	96,5	-64,3
5-9	108,8	89,0	-18,2
10-14	22,3	51,0	+128,7
15+	1,2	5,0	316,7

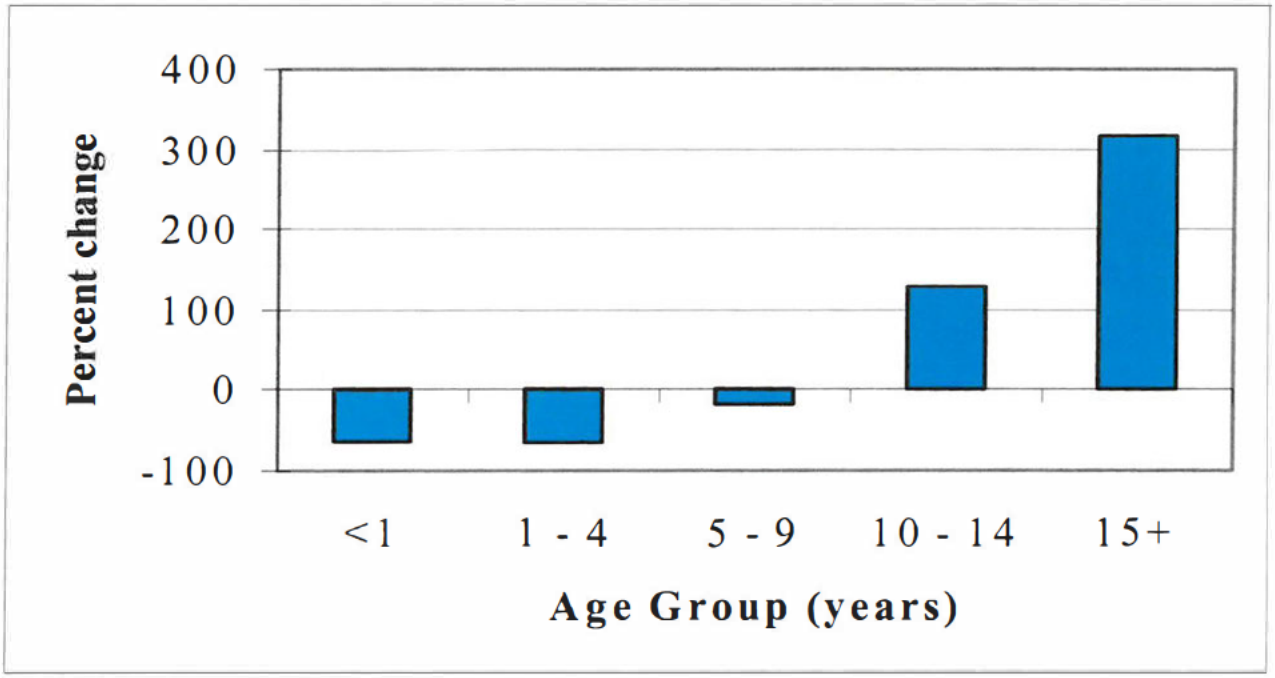

Figure 2.2: Percent change in 3-year average measles incidence in South Africa, 1980-1982 to 1992-1994

(Adapted from *Epidemiological Comments 1995b)*

Unlike developed nations which vaccinated for measles at 15 months or greater and could obtain optimal seroconversion due to absence of maternal antibodies, this age was too late for developing countries like South Africa. Due to the high incidence of measles in children less than one year old, it was recommended in 1975 that measles vaccination be given at 9 months of age. Measles vaccination with Schwarz strain was unsatisfactory at less than 8 months due to interference by circulating maternal antibodies. However, the advent of high-titre EZ vaccine made it possible to vaccinate at less than 8 months as it could overcome the effect of the maternal antibodies as it was highly immunogenic at 4-6 months (Whittle *et al* 1988) and had a high protective efficacy in children followed up for 2 years (Aaby *et al* 1988).

In a study of 20 black South African infants, it was found that the mean measles antibody level at 4 months was 192 mIU, 34 mIU at 6 months, and 13 mIU at 9 months of age (Kiepiela *et al* 1991). Thus at 6 months, 88% of infants were susceptible to measles. Their data supported the WHO recommendation to immunise children in developing countries at 6 months with the high-titre EZ measles vaccine, since most infants had lost passive immunity against measles by this age.

From March 1991, children in high-risk areas (low measles immunisation coverage (<70%) and reported cases of measles under 9 months of age) received 0.5ml/dose high titre ($5\log_{10}$ plaque-forming units [pfu]/dose) Edmonston-Zagreb (EZ) vaccine at 6 months of age, and 0.5ml/dose standard titre ($4.0\log_{10}$ pfu/dose) Schwarz vaccine at 9 and 18 months. Children in low risk areas (high measles immunisation coverage (>70%) and few or no reported cases of measles under 9 months of age) received standard titre Schwarz vaccine at 9 and 18 months. In addition, measles immunisation status of all children 2-5 years visiting a health-care centre was to be

assessed. Those without a definite history of measles vaccination or those who did not have 2 doses were to receive measles vaccine (Epidemiological Comments 1993).

Although EZ vaccine overcame passively transferred maternal antibody better than SW strain in comparable doses (Cutts *et al* 1995a), the WHO recommended that the use of high-titre EZ vaccine be stopped following concerns of excess mortality among female children who received high-titre EZ vaccine (EPI 1992). Although both EZ and SW vaccines showed excess mortality in high-titre vaccine trials, only the high-titre EZ vaccine became the target for safety concerns as the high-titre SW vaccine was never recommended for routine use by the WHO in the first place (Bennett *et al* 1999). In line with the WHO recommendation, South Africa stopped using high-titre EZ vaccine in the above dose in September 1992. The stocks of 0.5 ml high-titre EZ single dose vials were returned to the Department of Health stores, re-issued as 5 doses of 0.1 ml/dose and used until stocks lasted. Despite epidemiologic, clinical and laboratory studies, a plausible and satisfactory biological explanation for the gender differences in mortality due to high-titre EZ vaccine has yet to surface and its use thus remains halted.

The current schedule, which is in operation from April 1995, indicates standard titre Schwarz (SW) vaccine at 9 and 18 months (Epidemiological Comments 1995).

2.9 Strategies to control measles

Many European countries and the United States have instituted a two-dose measles-mumps-rubella (MMR) vaccine schedule with the aim of eliminating measles and rubella (Peltola *et al* 1986, Bottiger *et al* 1987, CDC 1989). The major reason for the second dose is to reduce the number of persons susceptible to measles because of a lack of response to the first vaccine dose.

Countries have adopted the policy of vaccinating children of schoolgoing age, either through a routine dose at school entry (Peltola *et al* 1994, Bottiger *et al* 1987) or through campaigns (EPI 1994, EPI 1995, de Quadros *et al* 1996). The main reasons for this policy are to immunise children who missed vaccination at the age for the first dose (failure to vaccinate), and to immunise children who did not respond to the first dose (vaccine failures). An additional reason for giving another dose is to boost antibody levels in children who responded to the first dose but in whom antibody levels have subsequently waned (Markowitz *et al* 1992).

Unlike lifelong immunity after natural measles, vaccine-induced immunity does not afford a similar duration of protection from exposure to measles virus. In a follow-up study by Krugman (1983), 9 of 70 (13%) children vaccinated with Schwarz vaccine had undetectable haemagglutination inhibition (HI) antibody levels at 16 years, compared with none of 47 children with a history of natural measles. Other clinical and serological studies of measles in vaccinated persons suggest that measles antibody levels do not persist at protective levels for very long in some individuals. Cases of clinical measles have occurred in children known to have seroconverted after vaccination (Mathias *et al* 1989). Others have documented a rise in measles antibody titre following clinical illness in children who had been vaccinated a few years earlier and had seroconverted (Reyes *et al* 1987, Zhuji Measles Vaccine Study Group 1987). Thus, strategies to control measles include reimmunisation which seeks to ensure the persistence of antibody levels at or above protective levels and avoiding measles infection.

There have been conflicting results with reimmunisation studies, however. Some studies in children with undetectable or low antibody levels after the first immunisation have shown no or little antibody responses after reimmunisation (Poland *et al* 1997, Wilkins and Wehrle 1979, Linnemann *et al* 1982). Other studies, however, have shown

that subcutaneous standard titre measles vaccines boost antibody levels among children who are seronegative or whose pre-vaccination antibody level is low (e.g. <200 mIU/ml) (Wittler *et al* 1991, Bottiger 1993, Calvert *et al* 1996, Watson *et al* 1996). However, after an initial antibody response following revaccination of schoolchildren (Deseda-Tous *et al* 1978, Markowitz *et al* 1992) or young adults (Cohn *et al* 1994), antibody levels drop again in approximately 40% of children within 1-6 years.

More than a decade ago, Albert Sabin proposed a radical strategy to prevent outbreaks in older children: mass national immunisation campaigns of a broad age range of children, irrespective of prior vaccination or disease history (Sabin 1986). In recent years, the World Health Organisation has recommended mass campaigns for improving measles control in high-risk areas (including refugee camps, urban and peri-urban slums and in remote regions with difficult access) (Anon 1994) or on a national scale in countries with a measles elimination goal, as in the Americas (de Quadros *et al* 1996). However, scepticism has been expressed about mass immunisation campaigns in this country (Barron *et al* 1987). An extensive mass immunisation campaign, which is not followed up by further mass campaigns or strengthening of routine immunisation services, can produce an equally extensive epidemic. The reason for this is that a large immunisation coverage only temporarily suppresses the transmission of the disease. Newborns can then accumulate as susceptible children until disease transmission again becomes easy, at which point an epidemic may occur (WHO/UNICEF 1985). Another negative aspect of mass campaigns was the diversion of health personnel from normal jobs which were consequently neglected. Nonetheless, it has been viewed as being useful as the first step along the road towards the acceleration of the EPI, in mobilising people, in building community awareness of health and introducing the community to the health care system in general and primary health care in particular. Furthermore, surveys of

immunisation coverage over time in Mpumalanga Province suggest that current immunisation coverage in routine services makes elimination highly improbable by this route alone (Durrheim and Ogunbanjo 2000). The high immunisation coverage achieved during the mass campaign was not sustained at routine immunisation service level, providing support for the complementary approach of combining routine and supplementary immunisation for measles elimination.

All countries in Latin America, and most recently, the United Kingdom and 7 countries in southern African, have conducted mass vaccination campaigns of children under 15 years of age. South Africa has conducted mass campaigns in children <5 years old in 1990, 1995 and 2000, while a mass campaign in children 5-15 years old was conducted in 1997. In all these campaigns, vaccine had been administered subcutaneously. Following earlier studies which showed that measles vaccination with aerosols produced good seroconversion (Kress *et al* 1961, McCrumb 1961, Okuno 1962, Ueda *et al* 1966, Terskikh *et al* 1971, Danilov 1973), Sabin together with Jorge Fernandez de Castro in Mexico, developed simple, inexpensive devices for aerosol administration and evaluated the aerosol route in infants (Sabin *et al* 1983, Sabin *et al* 1984) and several years later in schoolchildren (Fernandez de Castro and Kumate 1990) and found it to be effective. Sabin became convinced of the great potential of the aerosol route of mass measles immunisation to increase cost-effectiveness and avoid potential hazards of subcutaneous vaccination (Sabin 1991, Sabin 1992).

2.10 Elimination goals

Based on the Declaration of Alma Ata in 1978, the World Health Organization (WHO) launched the Expanded Programme on Immunisation (EPI), the targets of which included "…. a 90% reduction in measles incidence by 1995"). In February 1989 the

South African Health Policy Council, under resolutions 8/89 and 9/89, endorsed the objectives of the WHO and a "Measles Strategy" was decided upon (Epidemiological Comments 1994). This strategy was launched in January 1990. The objective was to raise the immunisation levels of all susceptible children against measles (and the other 5 target diseases) and to maintain these high levels in subsequent cohorts of susceptibles. The South African measles goal was set as "reduction of notified cases by 90% and deaths from measles by 95% (compared with pre-campaign levels)". Following the review of the EPI in South Africa in 1994, the elimination goal was set more specifically as: "the reported cases of measles should be reduced to less than 4000 per year for 5 consecutive years starting in 1996". With the recommendation by the WHO to use mass campaigns as a major strategy for measles control and elimination (Expanded Programme on Immunisation 1994), it became more important to evaluate the potential use of other routes for revaccination, such as aerosol, to make the goal of elimination more feasible.

2.11 Alternative routes of measles immunisation

While the percutaneous (subcutaneous or intra-muscular) route has been the preferred way to administer measles vaccine in routine programmes, other routes may be more appropriate for mass vaccination.

Mass campaigns of percutaneously administered vaccines are much more difficult logistically. There is great concern about the potential transmission of infections with vaccination by injection, whether in routine programmes or campaigns (Aylward *et al* 1995, Simonsen *et al* 1999). In many developing countries, re-use of syringes is still common. This is of great concern in areas of high HIV prevalence, such as many parts of Africa. Single-use disposable auto-destruct syringes are recommended for mass

campaigns rather than re-sterilisable syringes (Anon 1997). The use of non-reusable syringes implies not only an increase in cost, but also a difficulty in ensuring their safe disposal, which is particularly evident during campaigns. Administering vaccine by the respiratory route avoids these difficulties as well as having the advantages of being non-invasive, avoids the risk of parenteral transmission of infections, and potentially being administered by non-medical staff.

Vaccination by other routes also needs investigation because of the problem of interference by maternal antibodies with percutaneous vaccination at young ages. The younger the ages, the poorer the antibody response (Reilly *et al* 1961). In a study of 20 South African infants, the mean measles antibody levels measured at 4 months was 192 mIU, 34 mIU at 6 months, and 13 mIU at 9 months of age (Kiepiela *et al* 1991). Vaccination is hampered because these circulating maternal antibodies neutralise conventional Schwarz vaccine. Percutaneous vaccination with Schwarz vaccine at 6 months has shown seroconversion of about 60%. Because of this problem, most countries vaccinate at ages 9 months and greater. However, in developing countries where vaccine coverage is still low or only moderately high, the attack rates are still high for children aged less than 9 months. In specific areas of South Africa, 20-45% of cases occur in black infants under the age of 8 months (Loening 1983). Mucosal vaccination has been suggested to be effective in 4-6 month old infants (Sabin *et al*, 1982, 1983 and 1984, Smerdel 1985, Beck *et al* 1986). It is therefore possible that immunising via the mucosa might enable replication away from the interfering effects of maternal antibodies which are confined to the blood. Thus infants can be protected effectively before they are at risk of disease (Amler *et al* 1983).

Serological responses to measles vaccine after intradermal, conjunctival, oral, intranasal and aerosol administration showed that the aerosol route was the most promising of these non-percutaneous routes of vaccination (Cutts *et al* 1997). The oral and

conjunctival routes generally gave poor results, and intradermal administration of vaccine using a needle and syringe is more difficult than subcutaneous vaccination. The intranasal administration of vaccine gave more variable results than the aerosol route, which gave consistently good results in seronegative children. The interference by maternal antibody would only apply to primary vaccination and not to booster doses.

2.12 Aerosol techniques

Early aerosol techniques utilized a hand-held nebuliser which directed the aerosolised vaccine into the open mouth or in front of the mouth and nose (Kress *et al*, 1961; McCrumb, 1961 and 1962; Okuno, 1962). Subsequently, the aerosol was generated using a compressor with a nebuliser which was held in front of the nose and mouth for 30 seconds (Okuno *et al*, 1965; Ueda *et al*; 1966). A different technique was employed by Terskikh *et al* (1971) and Danilov (1973) who vaccinated groups of children rather than individuals. An ultrasonic generator delivered aerosol in a 12 m^3 room for 20 minutes or a 24 m^3 room for 30 minutes where children were allowed to play.

The nebuliser plus compressor technique was later modified with the inclusion of a paediatric mask which fitted over the nose and mouth of the child (Sabin *et al*, 1982, 1983, 1984 and 1985; Fernandez-de-Castro *et al*, 1986, 1990). This allowed most of the aerosol generated to be inhaled during the 30 seconds of administration.

The above devices generated particles which were predominantly less than 7 microns in diameter such that it was more likely to reach the lower respiratory tract.

2.13 Effect of measles vaccine strain and aerosol route of administration

The magnitude and duration of the boosting effect of a second dose of measles vaccine in children with low antibody levels may depend on the strain of vaccine and route of administration used, in a similar way in which infants respond differently if vaccinated with different vaccines when they still have maternal antibody. A review by Cutts *et* al (1995a) of 30 studies conducted on the serological responses to measles vaccine in infants less than 9 months showed that seroresponses are higher after EZ and AIK-C vaccines than after Schwarz vaccine. Several studies have compared the effect of EZ and Schwarz vaccines by subcutaneous injection at the same dose and age, and found that EZ vaccine gave superior seroconversion rates to Schwarz vaccine (Khanum *et al* 1987, Whittle *et al* 1988, Tidjani *et al* 1989, Markowitz *et al* 1990a, Job *et al* 1991). In aerosol trials of EZ and Schwarz vaccines in infants under 9 months, the seroresponses were substantially better with EZ vaccine than with Schwarz vaccine (Sabin *et al*, 1982, 1983 and 1984). The estimated retained dose of EZ vaccine in these trials ranged from 375 to 5000 infectious units per dose. The EZ vaccine is superior to Schwarz vaccine even in children who are seropositive. Aerosol administration of EZ vaccine boosted antibody levels in a substantial proportion of seropositive children (Sabin *et al*, 1983; Fernandez-de-Castro, 1997), whereas no such boosting was seen with Schwarz vaccination in such children (Sabin *et al*, 1983).

Most of the early comparative studies of aerosol and subcutaneous routes were done in children who had no antibody prior to vaccination. In the studies of Terskikh *et al* (1971) and Danilov (1973), the seroresponses in children receiving aerosol vaccine was better (82%-85%) than those vaccinated subcutaneously (72%-76%) for all 3 strains (SW, L-16 and USSR-58). Other studies of seronegative children also showed as good as or better seroresponses to aerosol than percutaneous vaccination (Kress *et al*, 1961;

Fernandez-de-Castro *et al*, 1986). The study by Torigoe *et al* (1986), however, showed a poorer response by aerosol due to the children being too young to co-operate in inhaling the aerosol. While children in most studies of aerosols were vaccinated for at least 30 seconds, aerosol vaccinations in the study by Khanum *et al* (1987) was done for only 10 seconds. Antibody responses by aerosol in this study were poorer than that by subcutaneous vaccination.

While many of the earlier studies done in seronegative children showed better seroresponses following aerosol vaccination compared to subcutaneous vaccination, it is also likely that administration of vaccine by the aerosol route could be more effective in boosting antibody levels in children who had been previously vaccinated. Two small studies of children who had previously received inactivated measles vaccine showed better boosting after aerosol of live vaccine than subcutaneous vaccination (Okuno *et al*, 1965; Ueda *et al*, 1966). Other studies have found similar responses between both routes. A study in China found comparable responses and duration of immunity after revaccination using a local strain of vaccine by either the aerosol or subcutaneous route (Dai *et al* 1991). Similarly, administration of EZ vaccine by aerosol has given equivalent seroconversion rates to the subcutaneous route in another study (Whittle *et al* 1984).

There are theoretical and practical advantages to administering vaccines by the aerosol route (Cutts *et al* 1997). This mimics the natural route of measles infection, and could allow vaccine virus to replicate locally on the respiratory epithelium without interference from maternal antibody. The aerosol may also be more efficient in stimulating the formation of secretory IgA to provide local immunity against re-infection (Ogra *et al* 1980). The aerosol route is particularly suitable for mass vaccination due to the ease and rapidity of administration, it's attractiveness to health professionals and parents because it is painless, it is non-invasive and avoids the risk of transmission of

blood-borne infections such as hepatitis B and HIV associated with vaccination by needles, and the cost saving in terms of the saving on expensive single-use needles and syringes and the potential for non-medically trained personnel to give the vaccine. While especially suited for older children who can actively cooperate, it has also been successfully given to infants during a large campaign in Monterrey, Mexico (Sabin *et al* 1982). In Mexico, several million children have been immunised by the aerosol route, which was found to be simple, practical, free of unusual side effects, and with a possible superior protective effectiveness as well (Fernandez de Castro *et al* 1990 and 1997).

2.14 Effect of acute illnesses on seroresponse

While measles immunisation of children with acute illnesses in developing countries has been shown to be safe and effective (Halsey *et al* 1985, Ndikuyeze *et al* 1988), there are differing results on the effect of acute illnesses on seroresponse. A small study in the United States found lower seroconversion rates after measles-mumps-rubella (MMR) vaccine in 15-18 months old afebrile children with rhinorrhoea compared to controls without rhinorrhoea (Krober *et al* 1991). Thirty seven of 47 children with rhinorrhoea and 50 of 51 children without rhinorrhoea seroconverted at 6-8 weeks post-immunisation. This is in contrast to large studies conducted in Haiti (Halsey *et al* 1985), where 81% of infants with rhinorrhoea and 78% of infants without rhinorrhoea seroconverted, and in Rwanda (Ndikuyeze *et al* 1988) where 81% of ill and 80% of well infants seroconverted.

In a study of 102 Thai infants who were vaccinated at 9 months of age during the season when respiratory illnesses were prevalent, symptoms densities of illnesses at or following vaccination were significantly lower among seroconvertors (Migasena *et al* 1998). In particular, titres were lower in those who had rhinorrhoea when vaccinated and

during the first 2 weeks post-vaccination, and diarrhoea in either of the 2 weeks of follow-up, compared to those without these symptoms. In contrast, in a randomised trial of AIK-C, high-titre or medium-titre EZ measles vaccines in 3.5 and 6 month old infants in Zaire, in which the occurrence of rhinorrhoea, cough, diarrhoea, fever, conjunctivitis or rash were monitored for 15 days post-vaccination, the seroresponse tended to be higher in children with mild illness after vaccination than those without (Scott *et al* 1999). In the medium-titre EZ group, the proportion of children attaining at least the median post-vaccination antibody level was significantly higher in children with rhinorrhoea in the first week after vaccination than those without. Significantly higher antibody levels in the first week were also seen among children with at least one symptom compared with children with no symptoms. Fever on the day of vaccination or in the 2 weeks following vaccination did not affect seroconversions or GMTs. Antibody levels at 6 months after vaccination showed no consistent differences according to presence or absence of symptoms, providing support that mild illness is not a reason to delay measles vaccination. Epidemiologic support for this comes from a case series and case controlled study by Edmonson *et al* (1996) which did not find any increased risk of measles vaccine failure in the respiratory season compared to the summer months.

In another Thai study where 6 month-old infants were vaccinated with standard titre EZ vaccine either subcutaneously or intranasally, most children given vaccine subcutaneously seroconverted while few children given vaccine intranasally seroconverted. Since upper respiratory infections following vaccination were equally common to both groups, upper respiratory infections may have adversely affected response to intranasal vaccine (Simasathien *et al* 1997).

2.15 Adverse events following vaccination

Percutaneous measles vaccination of schoolchildren as a primary or secondary dose of vaccine has been conducted for years in many European countries and the United States and has been shown to be safe and associated with only minor side effects such as fever and occasionally rash. An evaluation of vaccination of schoolchildren in the United Kingdom (Roberts 1995) showed that in boys, fever, headache, and rash were more common in vaccinees than non-vaccinees (relative risk 2.3, 2.0 and 1.3) respectively); no significant increases in symptoms were recorded in girls. Local redness or swelling was reported by 5% of children. Immunised children were significantly less likely to visit an outpatient department than non-immunised children, and there were no differences in general practitioner consultations or hospital admissions.

In a study in India, about 12500 children who were 9 to 15 month were immunized with measles vaccine of EZ strain and it was found to be safe (Bhargava *et al* 1996). Mild side effects were documented in 31% of the children. Of these, 90% were seen in the first 2 weeks. The commonest side effects were coryza (10%), fever (9.8%), cough (3.2%) and diarrhoea (3.2%). Convulsions, with no later sequelae, were documented in 2 cases only. This indigenously produced vaccine derived from EZ strain, had a level of reactogenicity which was lower than that reported in India with the Schwarz strain.

In a comparison of standard and medium titre AIK-C, EZ, Leningrad-16 and Schwarz strains, the rate of mild adverse events (fever, rash, conjunctivitis, or pharyngitis) was low (6-14%) for all strain groups (Bolotovski *et al* 1994). There were no significant differences in adverse events by age, sex, or vaccine titre.

In the studies conducted by Fernandez de Castro in Mexico, fewer side-effects were reported after aerosol vaccination than those usually seen after subcutaneous vaccination (Fernandez de Castro *et al* 1990 and 1997).

2.16 **Concluding remarks**

Earlier studies involving aerosol measles vaccine have been small and examined a few aspects. These studies sometimes gave conflicting results.

This trial is a large and comprehensive study which aims to expand on the body of knowledge in this area by examining the following:

Effect of route on antibody responses

Effect of strain on antibody responses

Antibody responses in the presence of varying levels of pre-existing antibody

Persistence of antibody over time

Effect of previous and concurrent illness on seroresponse

Adverse events following vaccination in vaccines

Adverse events in vaccinators following aerosol vaccination

CHAPTER 3

Pilot studies

Pre-testing of parents' responses, salivary assays and aerosol equipment

Two pilot studies were done before the main trial at one of the primary schools in Verulam (Trenance Park Primary). The first pilot was done to test parents' responses, a salivary assay, and logistics. The main purpose of the second pilot was to test the aerosol equipment.

3.1 PRE-TESTING OF PARENTS' RESPONSES AND THE SALIVARY ASSAY

The first pilot study was done in February 1996 by a team led by Mr Dilraj and Prof Felicity Cutts.

3.1.1 Objectives

The objectives of the pilot study were:

1. To determine the response rate of parents and estimate the proportion of parents that would consent for the main trial, and to decide whether or not to include children in grade 5.
2. To estimate the proportion of children with low or with undetectable measles antibody levels
3. To determine the degree of correlation of antibody levels between blood and saliva (since it was envisaged that a salivary assay would be used to screen children for low or undetectable antibody levels).
4. To work out the logistics of the main trial

3.1.2 Methods

Prior to determining the response rate of parents at the pilot school, the consent form was first tested with about 15 parents at another school (Jhugroo Primary School). Concerns raised by some parents related largely to the possibility of transmission of HIV, pain to their child and the volume and frequency of blood samples. The consent form was subsequently revised to address these concerns in greater detail.

The revised consent forms explaining the purpose of the pilot study were sent to parents/guardians of 480 children in grades 1 to 5 via the class teachers. The proportion of children for whom consents were received was determined. Both blood and saliva samples were taken from each child for whom consent was received.

The saliva was collected using an OraSure^{R} collection device (a small, chemically treated absorbent cotton pad with a plastic handle). Actually, oral fluid rather than whole saliva was collected. The pad trapped the mucosal transudate that is produced in the gingival crevices. Transudate collected from the general oral mucosa has been shown to contain immunoglobulin at concentrations significantly lower than those in serum but still well above levels in whole saliva (Gronblad-Saksela 1986). The pad was placed between the gum and cheek at the rear of the oral cavity for about 2 minutes. The pad was then inserted in the tube containing a preservative solution. It was sealed and placed in a cooler box with ice bricks.

A blood specimen was taken up in a plain vacutainer tube an hour after a local anaesthetic cream, EMLA ^{R} was applied to the site of venepuncture. The blood samples were taken to the MRC in cooler boxes, where it was centrifuged and the sera separated and dispensed into duplicate serum storage tubes.

Both the sera and saliva samples were airfreighted in cooler boxes with icebricks to the Public Health Laboratory Services, London, where the assays were conducted. The

serum specimens were assayed for measles-specific IgG by using a commercial ELISA (Gull Laboratories, Salt Lake City, Utah). Serum specimens found to be negative or equivocal by ELISA were subsequently subjected to the plaque-reduction neutralisation (PN) assay. The neutralisation test is based on the appearance of plaques in sensitive cell culture monolayers due to the growth of measles virus (the "cytopathic effect"). The PN assay measures the inhibition of virus activity i.e. reduction in plaque formation caused by the neutralisation of measles virus by antibody in the test serum. The PN assay in this study was performed using established methods (Brugha *et al* 1996). In this assay, dilutions of sera from each subject were reacted with a standard inoculum of measles (challenge) virus. The mixture was added to Vero cells and incubated for 7 days. Thereafter, the number of plaques was counted. The dilution of serum reducing the number of plaques by 50% was taken as the end point titre. The mIU/ml was calculated by direct comparison with the titre of the international reference serum (Forsey *et al* 1991). Those with antibody levels <200 mIU/ml were considered as seronegative.

The saliva specimens were assayed for measles specific-IgG using an antibody capture radioimmunoassay (RIA) (Perry *et al* 1993). The radioimmunoassay is a competitive binding assay that employs radiolabelled antibody. Polystyrene beads were coated with rabbit antibody to human IgG (anti-human IgG) which became irreversibly adsorbed to the polystyrene surfaces. The beads were washed and then placed in wells and "blocked" by a massive dose of protein (bovine serum albumin) so that all subsequent binding events were specific ones due to the antibody rather than to the non-specific ones due to the bead surface. Saliva samples were added to the wells and the antibodies became bound to the anti-human IgG. After incubation and washing, anti-measles monoclonal antibodies were added, which bound to the antibodies on the bead surface. After further incubation and washing, a fixed amount of radiolabelled antibody

(^{125}I labelled anti mouse IgG) was added to the wells. These were then washed to separate the bound and unbound radioactive material. The bound fraction was then counted in a gamma counter to determine the amount of radioactivity. A T:N ratio (ratio of bound radioactivity of test sample : bound radioactivity of negative control) of ≤2.1 was regarded as negative and 2.2-2.7 as equivocal.

3.1.3 Results

3.1.3.1 Response rate

Consent for specimens to be taken was received for 366 of the 480 children, a response rate of 76%.

3.1.3.2 Serum and salivary assays

Serum samples were obtained from 168 children. By ELISA, 41/168 (24.4%) were either negative or in the equivocal range (i.e. 25/168 (14.9%) were negative and 16/168 (9.5%) were equivocal). The sera of these 41 children tested by PN showed that only 13 of the 41 specimens were negative (<200 mIU/ml) (Table 1).

Table 3.1.1: Measles antibody results in 41 children tested by ELISA and PN assays

ELISA	PN (mIU/ml)			
	<200	200 500	500 999	≥1000
Negative	13	6	6	0
Equivocal	0	2	11	3

Saliva samples were obtained from 164 children, of which 93 (56.7%) were negative or equivocal (i.e. 67/164 (40.9%) of the specimens were negative and 26/164 (15.9%) were equivocal).

Thirty eight children had all 3 tests (ELISA, PN and saliva assay (RIA)) done. Of 12 children negative by PN, all 12 (100%) were also negative by ELISA but only 9 (75%) of children were negative by RIA (Table 2). The saliva assay yielded twice as many (12) negative results than the ELISA (6) for specimens that were highly positive by PN (>500 mIU/ml).

Table 3.1.2: Comparative results of ELISA and saliva RIA in children with PN results

PN (mIU/ml)	**Results by ELISA and saliva RIA**				
	Negative		**Equivocal**		**Positive**
(n=38)	ELISA	RIA	ELISA	RIA	RIA
<200 (n=12)	12	9	0	1	2
200-500 (n=8)	6	6	2	1	1
>500 (n=18)	6	12	12	3	3

3.1.4 Discussion and Conclusions

The correlation of antibody levels between serum and saliva specimens was poor. Of 12 specimens that were negative by PN, 9 were negative, 1 was equivocal and 2 were

positive by saliva RIA. Of 26 specimens that were positive by PN, 18 were negative by saliva RIA.

It is well known that the PN is the most sensitive and specific measles antibody assay. Although the ELISA is a less sensitive technique than the PN, it correlated better than did the RIA. The RIA produced fewer true negatives and overestimated the proportion of negatives to a greater extent than did the ELISA. Because of the poor correlation in antibody status between the RIA and PN, the screening phase using a salivary assay was dropped from the main trial.

Originally, we wanted to study children with detectable antibody by PN assay and a PN antibody level <500 mIU/ml. However, a study in Mexico showed that 44% of children with antibody levels of 500-999 mIU/ml demonstrated a fourfold increase in titre after aerosol EZ vaccine. Thus we could include children with antibody levels up to 999 mIU/ml. In that case, about 23% of children would be included in the follow-up after vaccination (all the ELISA negatives and equivocals (i.e. 38/168 from Table 3.1.1)); about 8% would be seronegative pre-vaccination, and 15% would have levels of 200-1000 mIU/ml. Therefore, if we had a total sample size of about 5000 children, we would expect about 400 antibody-negative children pre-vaccination and about 750 children with detectable antibody levels between 200-1000 mIU/ml.

The estimated number of children in grades 1-5 in the target study areas was about 8000. As the proportion that consented in this pilot school was about 76%, including grade 5, this projection would give a sample size of just over 6000 children. However, it could not be predicted that the rest of the proposed schools would also have such a high response rate. A more conservative response rate of 60% would give a sample size of 5000 children. This sample size would give an adequate number of children to follow up, as calculated

above, and also appeared more easily achievable. Thus it was decided to include the grade 5 children in the main trial.

The overall conduct of the pilot, the strengths, weaknesses and gaps were noted and used in planning the logistics of the main trial. In particular, the role of class teachers proved to be very useful. Not only did the teachers assist in distributing the consent forms, but also constantly reminded the children to ask their parents to complete and return the consent forms. This no doubt played an important part in the high response rate from parents. In most classes, the teacher collected the returned forms and had it ready to be picked up by the study team. As most teachers could incorporate the above functions into their daily tasks easily, these functions were assigned to the teachers in the main trial.

3.2 PRE-TESTING OF AEROSOL EQUIPMENT

The second pilot study was conducted in May 1996 by Drs Fernandez de Castro, Mr Dilraj and Prof Cutts, primarily to test the aerosol equipment and logistics.

3.2.1 Objectives

The objectives of this pilot study were:

1. To test the overall operation of the aerosol equipment for the local conditions
2. To conduct potency tests of the reconstituted vaccine under local conditions
3. To determine the volume of vaccine administered per dose
4. To work out the logistics of conducting the aerosol vaccination in the main trial
5. To test the understanding of parents/guardians in completing the diary of side-effects

3.2.2 Methods

The initial operation of the equipment, potency tests and determination of volume of vaccine administered per dose were done at the Medical Research Council (MRC). A detailed description and illustration and operation of the aerosol equipment appears in Chapter 4.11.3 (Methods)

As vaccines that were to be used in the main trial had not been sent by the manufacturers (SmithKline Beecham, Belgium) at that stage, Edmonston-Zagreb (EZ) vaccine produced in Mexico that was brought by Dr de Castro was used in the pilot. For potency testing, the lyophilised vaccine was reconstituted in 5 ml diluent and placed in the nebuliser on crushed ice. Using a syringe, about 0.5 ml of vaccine was withdrawn from the nebuliser before operation of the equipment and after 10 and 20 simulated vaccinations (a simulated vaccination entailed operation of the aerosol equipment for 30 seconds). The withdrawn vaccines were placed in brown, rubber-stoppered vials, wrapped in foil and frozen immediately on dry ice. It was stored at -70^{0}C and later sent on dry ice to the Public Health Laboratory Services in London for potency testing. Unfortunately, the samples did not remain frozen due to customs delay during shipment and a separate simulation of trial conditions was done later (see section 5.3.2).

Inactivated EZ vaccine was used in the determination of volume of vaccine administered per dose. The initial volume of reconstituted vaccine in the nebuliser was measured, the equipment operated for 10 minutes, the final/residual volume measured, and the volume in a 30 second exposure calculated.

The logistics of conducting the aerosol vaccination in the main trial was tested at the pilot school. This was achieved by doing a dummy run using distilled water in the nebuliser. Children from two classrooms were used for this purpose.

We also took this opportunity to test how well parents/guardians understood the covering letter regarding monitoring of side-effects after vaccination and how well they completed the diary of symptoms. Parents were requested to fill the diary on a daily basis and return the diary 3 weeks after "vaccination". A sample of parents for whom telephone numbers were available was also contacted and problems regarding understanding the letter and completing the diary were solicited.

3.2.3 Results

3.2.3.1 Volume of vaccine administered

Initial weight of empty nebuliser: 43.01 g

Weight with 5 ml reconstituted vaccine: 48.26 g

Therefore, 5 ml of reconstituted vaccine weighed 5.25 g

1.05 g/ml

Weight of nebuliser with vaccine after 10 minutes operation: 45.46 g

Weight difference in 10 minutes: 2.8 g

Weight "used" per 30 second: 0.14 g

Volume administered per 30 sec 0.14/1.05

0.133 ml per dose.

3.2.3.2 Dose of vaccine administered

The titre in 0.5 ml of vaccine was $4.62\log_{10}$ (or $4.92\log_{10}$ per 1.0 ml)

($4.62\log_{10}$ per 0.5ml = 41686 pfu/0.5ml)

= 11088 pfu/0.133 ml

Approximating the volume to 0.135 ml, the administered dose was thus 11255pfu/0.135 ml ($4.05\log_{10}$ per 0.135ml).

3.2.3.3 Diary of symptoms

Telephonic contact with several parents/guardians revealed that most parents understood how to complete the diary. However, quite a few parents were not filling the dairies on a daily basis. Most parents were of the working class and cited competing tasks in a short period of time in the evenings as the reason for being unable to or forgetting to make an entry on a daily basis.

3.2.4 Discussion and Conclusions

The aerosol equipment with the transformer worked well. The volume of vaccine administered per dose was 0.133 ml (rounded off to 0.135 ml), which is similar to that administered by Sabin *et al* (1983) (0.145 ml). Thus the volume of 0.135 ml delivered a dose of 11255 pfu/dose. It is estimated that approximately 25% of the administered dose is retained in the lungs (Sabin *et al* 1983). Thus a 30 second administration of vaccine would result in a retained dose of approximately 2814 pfu/dose. This conforms to the WHO requirements of a minimum of 1000 pfu/dose stipulated for injected measles vaccine.

The completion of the diary of symptoms by parents/guardians was not optimal. For the main trial, it was noted that children would be asked to remind their parents/guardians on a regular basis to improve completion of dairies.

As most children spent the major part of their day at school, observation of the children by their class teachers seemed useful in supplementing the information from

parents. Thus it was decided that for the main trial, each class teacher would have a list of participants where he/she could note what, if any, symptom any of the children experienced.

The overall conduct of the pilot, the strengths, weaknesses and gaps were noted and used in planning the logistics of the main trial.

CHAPTER 4

Materials, Subjects and Methods

4.1 MATERIALS

4.1.1 Office supplies

Consent forms

Guidelines for teachers

Lists of children in each class in each school

Questionnaires for vaccination and follow-up

Diaries with guidelines for parents

Diaries for teachers

Envelopes for diaries to be stored in on return to schools

Pre-printed study identity labels: for parent and teacher diaries, questionnaires, blood collection tubes, serum storage tubes

4.1.2 Blood taking and processing

EMLA^{R} local anaesthetic cream (Astra Pharmaceuticals, Sweden)

Tegaderm^{R} patches (3M)/ micropore tape (Millipore)

Surgical gloves

Vacutainers tubes and needles

Cooler box with rack inside

Sharps disposal box

Cotton swabs

Pipettes in laboratory

Serum storage tubes (Sarstedt Inc Microtubes, Product no. 72.694.006) and boxes

Sweets for children

4.1.3 Vaccination

4.1.3.1 Subcutaneous vaccination

1500 doses each of SW and EZ vaccine strain with 0.5 ml vials of diluent

Clean sheet to put over tables where vaccinating

Towels to clean tables before and after use

Needles and syringes

Cooler box for vaccine and diluent

Ice pack for vaccine during session

Sharps disposal box

Anaphylactic kit (particularly adrenaline and 1 ml syringe)

4.1.3.2 Aerosol vaccination

70 vials each of SW and EZ vaccine with 5ml vials of diluent

Earplugs (optional for study team)

Cooler box for crushed ice

Cooler box for vaccine and diluent

Stopwatch

Compressor (Black and Decker Airstation^{R})

Stepdown transformer

Nebulisers and tubing (Aeromist Treatment Set, Cat. No. 4107; Inhalation Plastics Inc., Illinois, USA)

Paper masks

Filter paper

Sheet to put over table

Alcohol wipes to clean table

Towels to clean table after use

Anaphylactic kit (particularly adrenaline and 1 ml syringe)

4.1.4 Examination of child

Thermometer for each nurse (plus for nurses doing side effect monitoring)

Tongue depressors: 1 box each school (plus for nurses doing side effect monitoring)

Torch for each nurse (plus for nurse doing side effect monitoring, in case of complaint of sore throat)

Auroscope: 1 for the team (in case of complaint of otitis)

4.2 SUBJECTS AND METHODS

4.2.1 Study design

The study was a block randomised controlled trial in primary schoolchildren.

4.2.2 Study population

To study the effect of a booster dose of measles vaccine, it was ideal for several years to have elapsed since the last vaccination in order to allow measles antibody levels to wane. Thus, local authorities in the province of KwaZulu-Natal that did not have a policy of vaccinating children for measles at schools were targeted. For logistical purposes, the Durban and surrounding districts were favoured. Two local authorities, Verulam and

Shallcross, which are on the outskirts of Durban, were chosen on this basis. These two areas had a total of 22 primary schools between them with an adequate population size for the study.

Schoolchildren from Class 1 to Standard 2 (grades 1 to 5), corresponding to ages between 5 and 14 years, were studied. This age group was selected to minimise loss to follow up as it was expected that the majority of the participants would still be in the same school at the end of the study period when some children would have entered grade 7. Most primary schools do not cater for grades beyond grade 7. Thus, children in the study would have to go to various secondary schools, some of which are out of the study area, making follow-up difficult. Furthermore, the present primary schools are not usually informed of which secondary school the child has moved to.

4.2.3 Sampling

All primary schools in the local authorities of Verulam (N=13) and Shallcross (N=9) were considered for entry into the study. With the inclusion of children in grade 5, preliminary estimates indicated that there would be approximately 8000 children in the target classes who would be eligible for initial screening. A conservative estimation from the pilot study indicated that consent would be obtained for about 60% of children. Thus, approximately 5000 children were expected to be in the study.

The proportion of children with low or absent antibody levels was not known as there were no local data on this. This proportion, however, depended on the age at vaccination. According to past vaccination schedules, most of the younger schoolchildren would have been vaccinated at 9 months. At this age, around 15% of vaccine failures (seronegative children) were expected (Diaz-Ortega *et al* 1994). Many of the older children were vaccinated at 15 months and the proportion of vaccine failures would have been less

(Stetler *et al*, 1986). A study in urban Bolivia found that 30-40% of 2-14 year old children had serum antibody levels below 200 mIU/ml, of which around 7% were seronegative (Cutts *et al*, 1995).

4.2.3.1 Sample size:

At least 192 children were required to be followed up in each group to detect a 20% difference in seroconversion at 2 years after vaccination between any two groups at 90% power and 95% significance, assuming 50% seroconversion at 1 month and allowing for 20% losses and 20% increases for clustering within schools. Therefore, if we had a total sample size of about 5000 children, we would expect about 400 antibody-negative children pre-vaccination and about 750 children with detectable antibody levels between 200-1000 mIU/ml.

4.2.4 Ethical considerations

Ethical approval to conduct the study was obtained from the Ethics Committee of the University of Natal and the London School of Hygiene and Tropical Medicine. A letter of support from the KwaZulu-Natal and National Department of Health (Directorate of Communicable Diseases) was also obtained. Permission from the Department of Education (KwaZulu-Natal) to conduct the study in this province was sought before principals of schools in the selected areas were approached to discuss participation of their schools.

Informed consent was obtained from all parents/guardians. Letters were sent via the schools to all parents/guardians of children in the chosen grades. The letter explained the purpose of the study, field procedures and possible side-effects, and sought their approval

for their child to participate. Contact details of Mr Dilraj and Prof Coovadia were provided in this letter for those seeking clarification on any issue.

As promised to parents/guardians at the time of seeking their consent, the pre-vaccination antibody status of children was made known to them via a letter. An arrangement with the Department of Health was an undertaking by the trial co-ordinators to revaccinate children who became seronegative during the course of the study. Letters were sent to parents/guardians of those children who had measles antibody below that which was protective after one year, asking for consent to revaccinate them. Those for whom consents were received were vaccinated immediately after the second year follow-up blood sample was taken. Following the accepted policy of the Department of Health, this vaccination was done using Schwarz vaccine given by the standard injection method with a sterile disposable needle and syringe.

Blood was drawn by experienced venepuncturists. A local anaesthetic cream (EMLA^{R}) was applied to the skin before taking the blood to reduce discomfort, and sterile disposable needles and vacutainers were used for each child. Parents were requested to take their child to their local doctor or clinic if any side effects occurred after vaccination, and Prof Coovadia was available for consultation.

4.2.5 Exclusion criteria

At the discretion of the nurse, those children judged too ill on the day of vaccination were excluded. As the vaccines are prepared on egg albumin, any child with a definite history of allergy to egg or egg products were also excluded. The large number of parents who responded in the affirmative regarding allergy to egg raised doubts whether all parents understood the question clearly. An additional form (in English and Zulu) was sent to these parents asking more detailed questions. Those who were initially said to have an

allergy to egg but for whom the detailed information did not suggest a true allergy were retained in the study but were observed more closely after vaccination. Children who previously reacted adversely to measles vaccine were also excluded.

4.2.6 Withdrawals

Parents /guardians were free to withdraw their child from the study at any time in the study without having to furnish any reasons.

4.2.7 Effect on vaccinators

Although no effects were seen on people administering the aerosol vaccine in the Mexican study, those conducting the aerosol vaccination in this study were asked to note any possible side effects as well. Blood samples from all vaccinators were taken before the aerosol vaccination phase commenced and one month later to measure any boosting in antibody levels.

4.2.8 Recruitment of schools

A list of primary schools was obtained from the KwaZulu-Natal education authority and contact was made with the principals of all primary schools in the target area (13 schools, including the pilot school in Verulam and 9 schools in Shallcross). Mr Dilraj visited each school and explained the project to the Principal, the Secretary and teachers, and sought their approval and support. Principals were obliged to inform their school governing bodies before granting permission. Where requested, school governing bodies and parents were addressed to discuss their concerns regarding participation of their schools and children. Only one school in Verulam did not participate following refusal by the school governing body. Thus 21 schools (including the pilot school) were recruited into the study.

4.2.9 Recruitment of subjects

Information on number of classes from grades 1-5, the internal system of designating divisions within each grade, and the enrolment of children in each class were obtained from the secretary of each of the participating schools. The corresponding number of consent forms was given to each class teacher who distributed it to the pupils. Each teacher was also given a set of guidelines to assist the trial team in conducting the study. The pupils were asked to hand the forms to their parents/guardians and return the form within a week, even if it was unsigned. Parents were asked to send the child's road to health card (RTHC) with the completed form. Initially, the entries for measles vaccination dates on the consent form were checked by MRC personnel against the Road to Health card which was attached, then sent back to children. This created problems when returning it to the schools as the child's class was not recorded on it and official names on the RTHC sometimes varied from that used at the school. Teachers in each class were subsequently asked to check the vaccination dates against cards and give the RTHC back to the child immediately, before sending the consent forms to the MRC.

The schools were revisited one week after consent forms were distributed, to make sure the teachers received them and handed them out to the children, and that there were no questions. Teachers forwarded the returned forms to the school secretary from whom it was picked up by the trial team. The teachers were asked to give the children regular reminders to return outstanding forms. Non-responders were also followed up at intervals by the trial team. The number of forms that were distributed initially and those returned (consented, refused or unsigned) were noted for each class at all schools.

When it was apparent that no further consent forms were forthcoming, a database was constructed from the information on the returned forms. A list of the names and study

numbers of each child, by class in each school, was produced (see below section 4.2.10). These lists were sent to the schools for verification.

4.2.10 Coding and assignment of study numbers

All children for whom consent was received were assigned study numbers. The study number was envisioned as a key variable to link the various databases and to glean certain information at a glance. The study number was a 5-digit alpha-numeric variable (eg. A0101) (Appendix 1).

The first digit, an alphabet, identified the school. Alphabets were assigned to schools in each of the two areas randomly. Verulam schools were assigned alphabets A to L and Z, and Shallcross schools were M to U.

The second and third digits stood for specific classes at each school. Classes were arranged in ascending grades and the divisions within each grade were further arranged by ascending alphabetic order. Each class was then assigned a sequential number in ascending order. The number of classes at the different schools ranged between 6 and 20.

The last two digits represented the serial number of children within each class. Surnames of children were arranged in an alphabetically ascending order and children were assigned to an ascending sequential number. The maximum number of children in any one class was 35.

Pre-printed labels with the study numbers were computer-generated in sets for each child and used on all questionnaires, blood collection tubes and serum storage tubes. Study numbers were suffixed with "a", "b" or "c" for the one month, first and second year follow-up, respectively, to differentiate samples from that collected at vaccination (baseline).

4.2.11 Randomisation

There were a total of 236 classes at the 20 of the 21 participating schools. The pilot school was assigned vaccination differently (see below). Classes at the other 20 schools were randomly assigned to one of four combinations of route and strain below using block randomisation:

1. Aerosolised Schwarz measles vaccine (SWae)
2. Aerosolised Edmonston-Zagreb measles vaccine (EZae)
3. Subcutaneous Schwarz measles vaccine (SWsc)
4. Subcutaneous Edmonston-Zagreb measles vaccine (EZsc)

The randomisation was done by Mr Dilraj and Prof Cutts.

Steps performed in block randomisation:

1. All schools were listed in ascending alphabetical order with the classes within each school arranged in an ascending number as assigned (Appendix 1).
2. The above combination of vaccine strain and route of administration was assigned a number from 1-4 (1=SWae; 2=EZae; 3=SWsc; 4=EZsc)
3. All possible combinations of numbers of 1,2,3,4 (Appendix 2) were written down (a total of 24 combinations). With eyes closed, one investigator twirled a pencil over these numbers while the other investigator was moving the paper, and the pen-point was dropped at random on a combination. The other investigator noted the combination and assigned the sequence to the first 4 classes (as arranged in step 1). The following 4 classes were assigned the sequence as determined by the combination of numbers obtained next. This process was repeated until all classes in each school were assigned a number (i.e. a vaccine/route combination). A new sequence was always started for a new

school. Thus, children received the vaccine/route combination according to the class they were in (Appendix 1).

At the pilot school, the antibody status of children had already been determined previously in the pilot phase. Only the seronegative children were approached to enter the main trial. As relatively few (21/37) seronegative children consented for the main trial, they were randomised individually to either EZsc or EZae group.

4.2.12 Pre-vaccination preparations (April-July 1996):

The vaccination phase was scheduled to be conducted in the third school term between the winter and spring vacations (August to September 1996). Most of the preparations for this phase had to be done in the second term (April to June 1996) before schools closed for the winter vacation in July. Schools were contacted to check if any activities were being held during the third term that might interfere with the vaccinations. These activities included tests or examinations, sports, excursions, school photography, and parents' day. Some schools had a history of poor attendance on a Monday and Friday, and on the day before or after a public holiday. A draft timetable of visits to the schools was drawn up, avoiding the above days at those particular schools. At schools where the enrolment was low, two schools were scheduled for vaccinations on the same day. The timetable was finalised after several rounds of consultation.

Visits were also made to schools to identify a room or rooms for the vaccination phase. The rooms had to be spacious enough to accommodate the vaccination team(s) as well as serve as a holding area for the children who have been bled and vaccinated. For the subcutaneous vaccination phase, one room was adequate, whereas two rooms were needed for the aerosol vaccination phase. This was necessary as to avoid any

contamination of children in the holding area who have been bled and vaccinated with one strain to inhale vaccine of the other strain done just previously that may have been present in the air.

In this preparatory period, a doctor, a team of nurses and several general assistants were recruited for the study. The doctor was responsible for supervising the nurses, act appropriately in case of an adverse reaction to the vaccine, and to assist nurses in venepunture. The main tasks of the nurses were to conduct clinical examinations, collect blood specimens, do subcutaneous vaccination and complete the questionnaires. A week before commencement of vaccinations, a training session was held at one of the study schools to familiarise the team with the procedures of the trial. This included a discussion of the study organisation, questionnaires, venepuncture procedures, vaccination procedures, action in case of an adverse reaction to the vaccine and checks to be done to minimise errors. Nurses were also trained how to do the follow-up of side-effects.

4.2.13 Vaccines

The vaccines for subcutaneous and aerosol vaccination was kindly donated by the manufacturer, SmithKline Beecham Biologicals (Belgium). Lyophilised SW and EZ vaccines were provided in sealed ampoules with the stabilizers used in routine preparations. The vaccine used in both phases were of standard titre, i.e. less than 4.7 $\log_{10}$ (<50120 plaque-forming units [pfu]/dose). The following quantities and information on batches was sent by the supplier:

2000 vials standard titre SW vaccine 1 dose – Lot M174H44

2000 vials standard titre EZ vaccine 1 dose – Lot MZ13D44

70 vials of SW vaccine 10 doses (100000 infectious units per ml) Lot M174D13

70 vials of EZ vaccine 10 doses (100000 infectious units per ml) Lot MZ05AA12

the viraemic phase 7-14 days after vaccination and cause cross-contamination when they sit together in a classroom, or mingle during breaks, and at home where more than one child from a family had participated. Thus, the subcutaneous and aerosol vaccination phases were conducted approximately two weeks apart. About half the number of classes at each school was visited in August and the other half in September, according to the randomisation. The subcutaneous phase was coordinated by Mr Dilraj, Dr Cutts and Dr Ahmed, while the aerosol phase was coordinated by Mr Dilraj, Dr de Castro and Dr Bennett.

4.2.14.1 Daily preparations and procedures followed at schools

Preparations for each school visit were done the previous day. This included packing all equipment and supplies for venepuncture and vaccination, anaphylactic kits, labels, questionnaires, diaries and chocolates for children. At the beginning of each day, the required number of vials of subcutaneous vaccines and diluent would be taken from cold room and transported to the school in a cooler box with ice bricks to maintain the cold chain. For the aerosol phase, only a few vials of vaccine and diluent were required each day. It was thus feasible to keep these in a home fridge overnight. It was also logistically simpler to go directly to the school from home instead of diverting to the MRC to pick up the vaccines.

At the school, a few members of the team prepared the vaccination room while the others went to the classrooms. Starting with the lowest grade, in each classroom the team was introduced by the team leader and the procedure explained to the class. The names of the participants were called out and the questionnaires with their study number already affixed were handed to them. In most classes, the teacher assisted in confirming that the correct children were identified. A note of absenteeism was made against the relevant name on the class list. Information was obtained from teachers (and older children in the higher

grades) on a history of illnesses during the previous 4 weeks to assess the effect of recent illness on seroresponse to vaccination. Days of absenteeism due to illnesses during this period were noted from the class register. A small amount of local anaesthetic cream, EMLA^{R} (Astra Pharmaceuticals, Sweden) was applied to the venepuncture site on the forearm. Initially about 1g was applied. This was reduced to about 0.2g after several children experienced serious side-effects which was thought to be associated with use of the cream (see Section 7.4: Adverse events associated with use of EMLA cream and Discussion in Section 7.6). The cream was kept in place with a Tegaderm^{R} patch or a strip of Millipore tape. The cream took about an hour for the anaesthesia to become effective. During this hour, the above procedure was repeated in each class from grades 1-5. If the cream had not been applied to all children after the first hour, some nurses continued with this task while the other nurses returned to the vaccination room to commence with collecting blood specimens and vaccinations.

An hour after applying the cream to the first class, sufficient local anaesthesia would have been induced in these children for the commencement of blood specimen collection and vaccination.

Children from one classroom at a time were brought to the blood specimen collection room by an assistant. The assistant would guide each child to a nurse who would first double-check that the child had the correct form by asking their name and checking it against that on the form. The nurse noted whether the child was well or had any current illness. Children were examined for current upper tract infection (rhinitis, cough, conjunctivitis) to assess potential interference with the response to vaccination (Krober *et al* 1991; J. Bennett unpublished data 1995). An oral temperature reading was also taken. About 3ml of baseline blood sample was taken up into a plain blood collecting tube using a sterile vacutainer needle set. A pre-printed label with the child's study number

was fixed to the tube. The nurses recorded on the questionnaire whether or not sufficient blood was collected and reasons for failure, when that occurred.

Up until this point, the above procedures were common to both the subcutaneous and aerosol phases. The procedure between the two phases diverged at the next step, the administration of the vaccine. For the subcutaneous phase, children remained in the same room and received the vaccine after having the blood sample taken. In the aerosol phase, children were sent to another room where the vaccination was being conducted, or to their classroom if there were any delays with the aerosol vaccination and called later when the vaccinators were ready.

After the blood collection and vaccination for each class was completed, the coordinator checked that all questionnaires were collected and that the study number on the blood tube matched that on the questionnaire. In the event that mislabelling occurred, blood was redrawn from those involved and the first samples were discarded. If the children involved could not be located timeously or the attempt at rebleeding was unsuccessful, then these children were excluded from the study. During the course of the study, the introduction of writing the child's name on the label as well helped to overcome this problem. The coordinator recorded all blood specimens collected, unsuccessful bleeds, errors if any, refusals, withdrawals and absentees against a master list for each class and school. We did not go back to the school on another day to vaccinate absentees.

4.2.14.2 Subcutaneous vaccination

Vaccine for subcutaneous injection was provided in single dose vials (0.5ml per dose). Each vial of lyophilised vaccine was reconstituted with 0.5ml cold sterile water for injection immediately prior to vaccination. After a blood specimen was taken, the child then received either the Schwarz or EZ vaccine (depending on which class was being done)

subcutaneously on the upper arm. The subcutaneous injection delivered standard titre doses of 12000 pfu/dose for Schwarz vaccine and 10000 pfu/dose for EZ vaccine. Each vaccination was done using a sterile disposable needle and syringe. These were disposed into sharps containers which were later sent to the incinerators at the King Edward VIII Hospital. The vaccine given was also noted on the questionnaire. The vaccinated children were given a chocolate to eat and remained for about 15 minutes in a post-vaccination observation area in the vaccination room to check that no one experienced any adverse reactions to the vaccine.

4.2.14.3 Aerosol vaccination

Once children from each class had their blood specimens taken, they were sent to another room where the aerosol equipment was set up. Outside the vaccination room, the procedure was explained to each group of children and steady breathing demonstrated to them. Several children at a time were then sent into the vaccination room.

The aerosol method and equipment used in this trial has been previously used extensively in campaigns in Mexico (Fernandez-de-Castro *et al* 1990 and 1997). Aerosol was generated with a plastic nebuliser (Aeromist treatment set) by passage of pressurised air from an electric powered compressor (Black and Decker AirStation^{R}) operating at 30-40 psi (200-275 kPa) (Figure 4.1). Both items were kindly supplied by Dr Fernandez de Castro. The compressor was designed to operate at 110 volts and a stepdown transformer was used to convert the local outlet voltage of 220 volts to 110 volts. The nebuliser was kept in a container of crushed ice. A plastic tube, 3 metres long and 5mm in diameter, conducted air from the compressor to the nebuliser. A plastic tube, 14 cm long and 2cm in diameter, joined the nebuliser to a plastic cone of 8,5 cm maximum diameter, in which single-use conical paper cups with the pointed end cut off were inserted as disposable

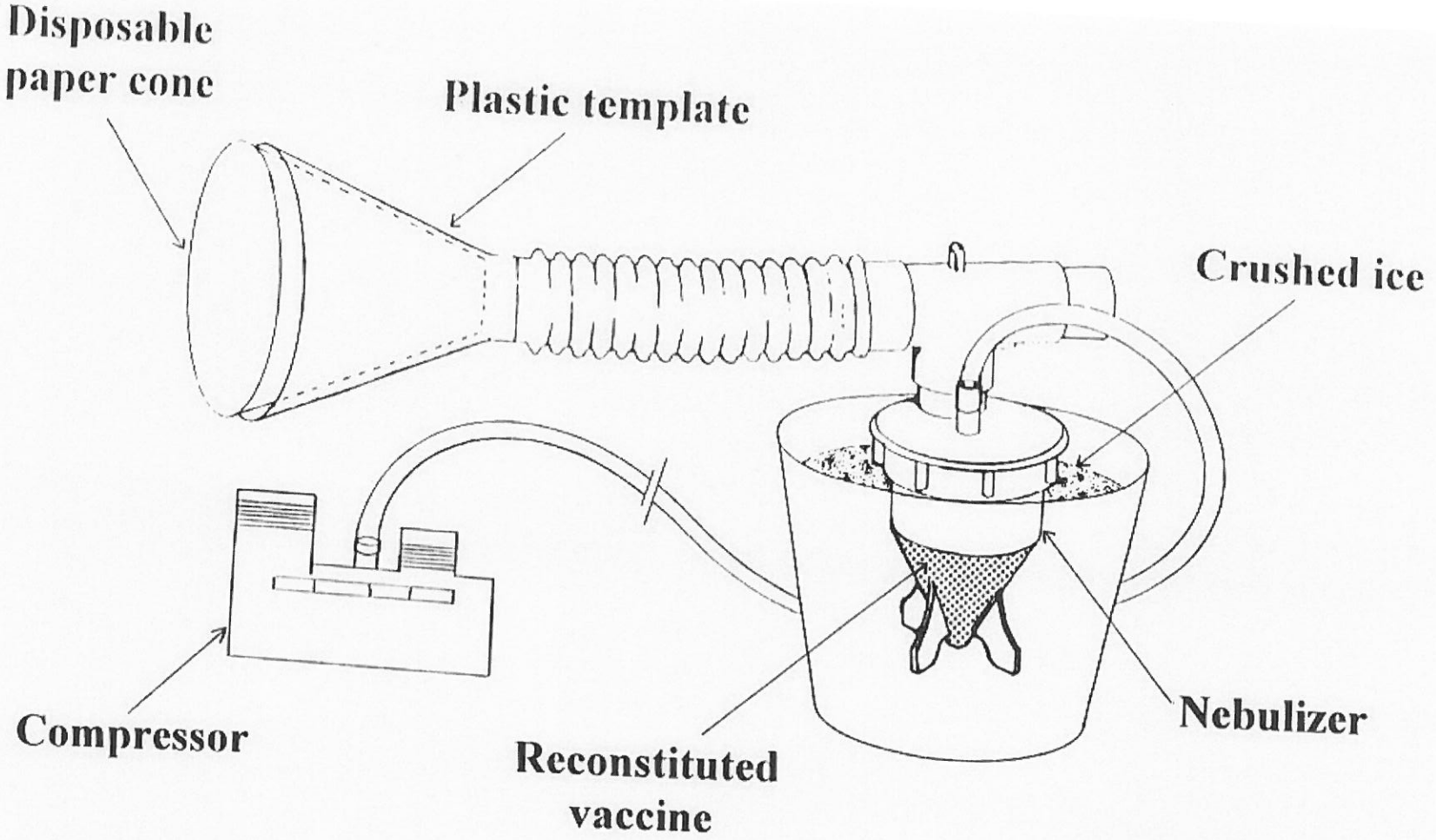

Figure 4.1: Diagram of aerosol equipment

masks. The dispersed particles were predominantly <5 microns in diameter (manufacturers' data). Being true aerosols rather than a spray, the size of the emitted particles mainly targeted the lower respiratory tract. The volume of vaccine delivered was estimated in the pilot study with EZ vaccine to be approximately 0.135 ml in the 30 second burst. The administered doses were thus 14,000 pfu/dose for SWae and 5000 pfu/dose for EZae.

The lyophilised vaccine was supplied in 5ml multidose vials. Two vials were reconstituted with 10ml of cool diluent and placed in the nebuliser for each round of vaccination. One operator kept the mask applied over the nose and mouth of each child, and sometimes placed a hand behind the child's head to ensure good contact. A second person timed the 30-second exposure to vaccine, switched the compressor on and off, and recorded vaccine given, date and other relevant data on the questionnaire. Each vaccinated child had a sticker applied to the outer garment to signify that they had been vaccinated and to avoid double exposure to the immunisation procedure. Because of the potential for the nebulisation process to inactivate some of the virus during operation of the nebuliser, the number of children vaccinated by this route was restricted to 35 from each freshly charged nebuliser. A pilot study in Mexico in April 1996 showed little loss of potency with 20-25 children (titre of 4.42 logs at time 0; 4.40 logs at 10 minutes and 4.30 logs at 20 minutes of operation of the nebuliser (personal communication : Dr JF de Castro)). As with the subcutaneous vaccination, the vaccinated children were given a chocolate to eat and remained for about 15 minutes in a post-vaccination observation area in the vaccination room to check that no one experienced any adverse reactions to the vaccine.

All classes randomised to one of the vaccine strains were vaccinated consecutively. To avoid any possible chance of involuntary immunisation by viral particles that might theoretically have remained suspended in the air, the same classroom was never used for aerosols of more than one vaccine strain. After completing aerosol vaccinations of one strain in a classroom, the doors were locked and the teachers were instructed to keep the room free of children until the next day.

Different nebulisers were used for the different vaccine strains. The tubing was rinsed between vaccines with distilled water while operating the nebuliser. Used nebulisers were sterilized daily by submerging the disassembled parts into very hot water (about 70°C). Boiling water was not used as it would have deformed the nebulisers. The various parts were dried and then assembled and kept in clean plastic bags up to the moment of use. The aerosol system was purged with reconstituted vaccine before commencement of vaccination each day. When a new nebuliser was used, the system was purged with inactivated measles vaccine.

During the trial, samples of vaccine were taken from the nebuliser at the beginning, after 20 and 35 vaccinations of some rounds, frozen immediately on dry ice and sent on dry ice for potency analysis at the Public Health Laboratory Services (London). Unfortunately, the samples did not remain frozen due to customs delay during shipment, so a separate simulation of trial conditions had to be done.

4.2.15 Follow-up phase

All children who were seronegative at baseline and a 9% random sample of seropositives (see Section 4.2.17) were followed-up at 1 month after vaccination and blood specimens collected.

For the 1 and 2 year follow-up, the schools were visited a few months before specimen collection to incorporate class or school changes. New master lists for each school had to be drawn up as there was mixing of children from the old grades to the new grades. Where it was possible to locate children who had taken transfer to another school within or near the study districts, they were followed-up there. Permission had to be obtained from the principal if the school that the child was transferred to was not already participating in the trial.

Unlike the vaccination stage, several attempts were made to obtain blood samples from those who were absent on main collection days for all follow-up periods. Questionnaires were completed for all children at each follow-up occasion. Information was collected on illnesses experienced, visits made to doctors/hospitals and length of absenteeism from school during each period. Reasons for loss to follow-up were noted.

4.2.16 Specimen handling and storage

Blood samples were placed in racks in a cooler box and transported to the MRC laboratory. They were centrifuged and sera separated using a sterile pipette. Sera were separated into 2 aliquots and dispensed into 2 ml serum storage tubes. The tubes were labelled with pre-printed labels bearing the study number, placed in a serum storage box with the year and school name on it, then stored in a freezer. One set was airfreighted in cooler boxes with a sufficient number of ice-bricks to the PHLS in London and the duplicate was kept in storage at the MRC. Because of the volume and the need to know the antibody status of the children to decide those to be followed-up at 1 month, the pre-vaccination sera collected during August and September 1996 were sent on a weekly basis. The sera collected for the 1 month, 1 and 2 year follow-up were sent in single batches each of those occasions.

4.2.17 Laboratory assays

All pre-vaccination sera were screened for measles-specific IgG using a commercial ELISA (Gull laboratories, Salt Lake City, Utah) to determine the baseline antibody status. The results were calibrated with the international reference preparation (Forsey *et al* 1991) and expressed in mIU/ml. The international reference preparation contains 5 international units in 1 ml after reconstitution and is run together with the test sera. It is used to eliminate inter-laboratory variations and standardize serological assays. The ELISA was chosen for baseline antibody determination to enable rapid identification of children for the 1 month follow-up. We aimed to follow-up all children with pre-vaccination levels of <200 mIU/ml (seronegatives), but only a 9% random sample of those with higher levels were followed-up, since serological responses to revaccination have previously been reported to be low among children with high antibody levels.

The serological response to vaccination was measured using the Haemagglutination Inhibition (HI) assays (Norrby *et al* 1962). The HI assay is based on the ability of the H protein of the measles virus to bind to erythrocytes, causing a lattice of haemagglutinated erythrocytes in the bottom of a tube or well. When serum containing measles antibody is incubated with measles virus or purified haemagglutinin prior to the addition of red cells, the antibody combines with the haemagglutinin and prevents its attachment to red cells (inhibits haemagglutination). The starting dilution was 1:4 (approximately 300 mIU/ml), which is slightly higher than that considered protective (Samb *et al* 1995). Children with antibody titres <1:4 were regarded as seronegative by this assay even though many probably had low levels of antibody. Sera from the first three time points (baseline, 1 month and 1 year after vaccination) were assayed simultaneously. Plaque-reduction neutralisation (PN) assays were performed on a randomly selected sub-sample of the sera from each group using established methods

(Brugha *et al* 1996). Measles-specific IgM was assayed at one month post-vaccination by radioimmunoassay (Perry *et al* 1993). Detection of IgM antibody was used for the classification of subjects as primary responders following vaccination. The assay for IgM was identical to that described in Section 3.1.2 for IgG antibody capture radioimmunoassay. The exception was that the solid phase beads were coated with anti-human IgM instead of anti-human IgG.

The second year follow-up sera were assayed separately.

The laboratory analyses were done at the Public Health Laboratory Services, London.

4.2.18 Data collection and management

Data from the consent forms and questionnaires (vaccination, follow-ups at 1 month, 1 and 2 year after vaccination, parents' and teachers' diaries, and absentee forms) were double entered using EpiInfo 6.04. The study number was the key variable that was used to link and manipulate the various datasets to aid data cleaning. It was necessary to go back to schools, parents and the laboratory on several occasions in order to clean the datasets sufficiently for analysis.

4.2.19 Data analyses

Seroconversion was defined as a four-fold rise in measles IgG antibody level after vaccination. Seroconversion with detection of measles specific IgM was considered a primary response to vaccination; seroconversion or boosting without measles IgM detection was considered as a secondary response.

Seronegative children were assigned titres of 1:2 ($\pm$ 150 mIU/ml). The outcomes between the different strains of vaccines and different routes of administration of vaccine were compared at 1 month, 1 and 2 year after vaccination by:

1) the proportions with at least four-fold titre increases from baseline ("seroconversion");
2) geometric mean titres (GMT);
3) average fold increase in titres from baseline, analysed on a log transformed scale;
4) proportion of children who were seronegative.

Analyses used EPI-INFO (Dean *et al* 1994), STATA (Statacorp 1997) and ML3 (Dallal *et al* 1988). Results are presented on an intention-to-treat basis, although 14 children randomised to receive EZae actually received SWae.

Chi-square or Fisher's tests were used to assess impact of dichotomous variables on seroconversions and seropositivity, and ANOVA (or Mann Whitney U test if non-parametric) to evaluate effects of variables on geometric mean titres and fold increases. Multiple logistic regression was used to analyse seroconversion adjusting for other variables.

The statistical advisor on the trial, Jerry Wheeler, used multi-level modelling to determine the proportion of variability at child, class and school levels. Since the randomisation to vaccine group was by class, certain analyses were performed using this unit of randomisation. The school was also taken into account as an extra component of variability, to avoid understating standard errors produced from a single level regression model.

CHAPTER 5

Early responses to Schwarz and Edmonston-Zagreb measles vaccine administered by the subcutaneous or aerosol route

5.1. Overview of chapter

The emphasis of this chapter is the comparison of serological responses between the various groups from baseline to 1 month and 1 year after vaccination. The results at 2 years after vaccination are presented separately in the next chapter as fewer subjects were followed up at 2 years. This chapter first deals with the recruitment of participants and follows up their participation to the end of study period. The baseline characteristics of the initial and follow-up participants are described. The chapter touches on the problem of potency of the administered vaccine in one of the study arms and how data analysis was modified to address this problem. The effect of previous and concurrent upper tract infections on seroresponse are also explored.

5.2. Results

5.2.1. Recruitment of children and follow-up

Recruitment of children, numbers followed-up and reasons for loss to follow-up in each group are detailed in the Trial Profile (Fig 5.1). The trial profile includes follow-up at 2 years for completeness.

Figure 5.1: Trial profile

Of the 8134 children who were invited to participate in the trial, parental consent for 4987 children was received, while 417 refused to participate and forms were not returned by the remaining 2730. The outcome of randomisation of the 4987 participants was EZae (1219), SWae (1277), EZsc (1302) and SWsc (1189). On the day of vaccination, adequate blood samples were obtained from 4327 children who were then vaccinated. Of the remaining 660 children, 497 were absent, 15 were transferred to schools out of the study area, 34 parents subsequently withdrew consent, 81 had insufficient blood obtained, 7 refused to have blood taken, 16 were ineligible because of illness and 10 had severe allergy to egg products.

Of the 4327 children who had a pre-vaccination sample taken, 1976 were eligible for follow-up at 1 month (1740 with pre-vaccination ELISA antibody <200 mIU/ml and 236 with $\geq$ 200 mIU/ml). Blood was obtained from 1772 (90%) children. Of the 204 remaining children, 144 were absent at repeated visits, 44 had insufficient blood obtained, 11 were withdrawn by parents and 5 had been transferred.

At 1 year, samples were collected from 1119 of 1261 children from 3 groups only (EZae, EZsc and SWsc – see Section 5.3.2). Of the remaining 142 children, 75 had been transferred, 14 were repeatedly absent, 21 had insufficient blood obtained, 24 were inadvertently vaccinated in the national measles immunisation campaign, 3 were withdrawn by parents, 1 died in an accident and 4 were excluded for miscellaneous reasons.

5.2.2 Potency of vaccine in nebulisers

The 1-month post-vaccination serological results revealed that only 116/511 (22.7%) of children given SWae seroconverted. The GMT at 1 month for the SWae group was 1:11 which was substantially lower than that for the other 3 vaccine groups (see later - section 5.3.4.3). Subsequently, laboratory simulations of field aerosol conditions showed that the SW vaccine had no detectable potency after only a few doses (Fig 5.2), whereas the EZ vaccine remained

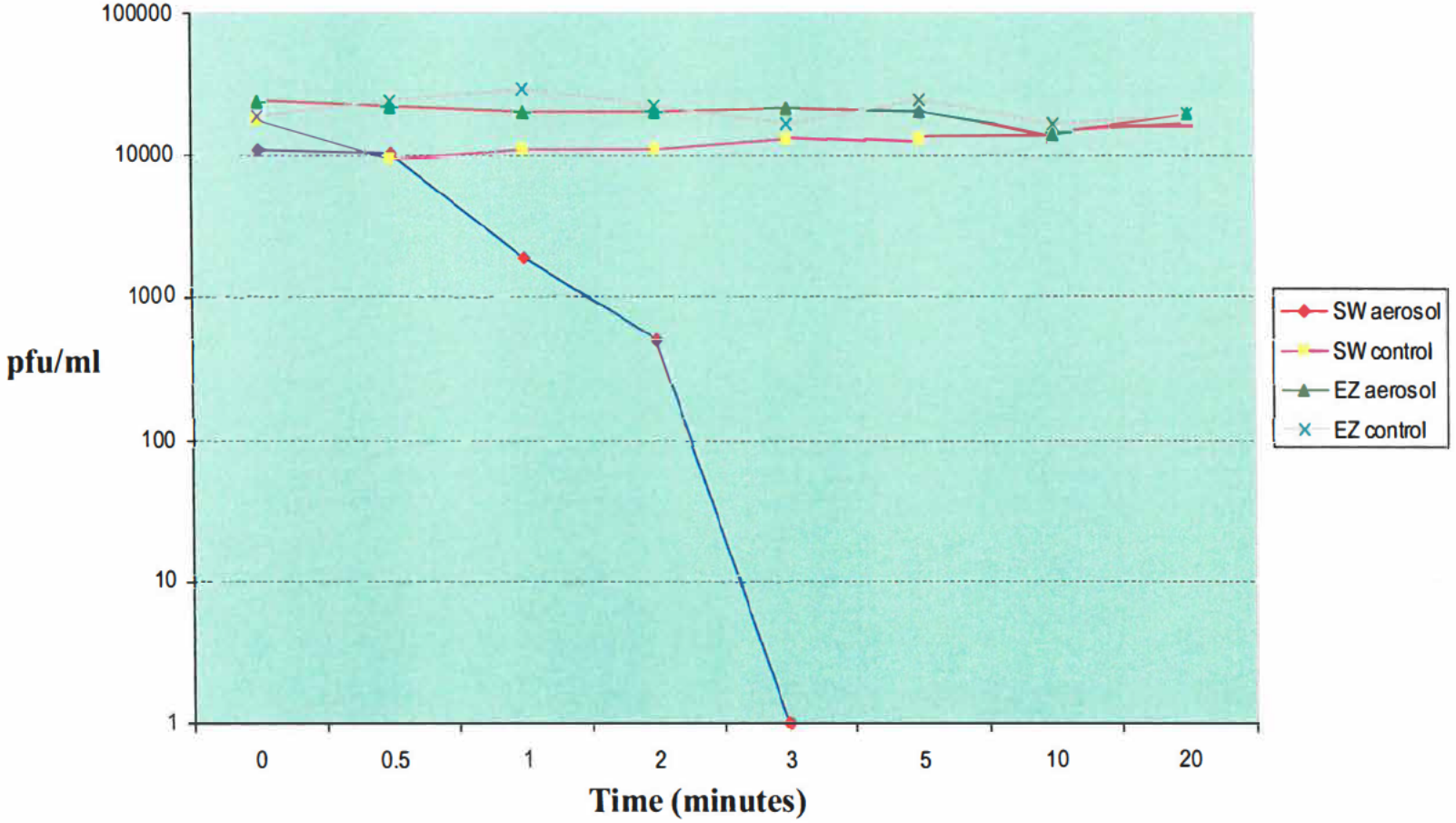

Figure 5.2: Simulation trial of aerosolised Schwarz and EZ vaccines (SKB)

stable for 40 administered doses (the maximum tested over 20 minutes of operation of the compressor). Controls (i.e. reconstituted SW and EZ vaccines kept on ice but not subjected to nebulisation) also remained stable over this period. Thus, we concluded that the SW vaccine had become inactivated during aerosol administration. As the majority of children in the SW group received a vaccine that was deemed largely inactive, any follow-up antibody data at 1 and 2 years would not have yielded true comparisons with other groups. It was therefore considered unethical to collect further blood samples from this group, and further antibody tests and analysis were not done on this group. These children (and seronegatives at 1 year) were later offered revaccination by injection.

5.2.3 Baseline characteristics

Baseline characteristics are presented for all 4987 children who provided consent (Table 5.1) and for the 992 children who had sufficient sera remaining at each timepoint available for analysis (Table 5.2). 817 of 992 (82.4%) children had been previously vaccinated.

Table 5.1 Baseline characteristics of 4987 children who consented and were randomised.

Characteristic	EZae	SWae	EZsc	SWsc	p value
Number of children	1219	1277	1302	1189	
Mean (SD) age (years)	8.16 (1.67)	8.44 (1.70)	8.45 (1.54)	8.17 (1.64)	0.001
Sex: Male	13 (50.3%)	609 (47.7%)	606 (46.5%)	580 (48.8%)	0.28
Race: Indian	855 (70.1%)	857 (67.1%)	892 (68.5%)	845 (71.1%)	0.15
African	364 (29.9%)	420 (32.9%)	410 (31.5%)	344 (28.9%)	
Previous vaccination:					
1 documented	528 (43.3)	514 (40.2)	556 (42.7)	487 (41.0)	0.06
≥ 2 documented	276 (22.7)	276 (21.6)	250 (19.2)	236 (19.8)	
Undocumented history					
Of ≥ 1 dose	166 (13.6)	191 (15.0)	170 (13.1)	164 (13.8)	
No vaccination history	249 (20.4)	296 (23.2)	326 (25.0)	302 (25.4)	
Reported history of measles	388 (31.8%)	384 (30.1%)	422 (32.4%)	363 (30.5%)	0.44
No measles or vaccination	188 (15.4%)	232 (18.2%)	245 (18.8%)	244 (20.5%)	0.01

Table 5.2 Baseline characteristics of 992 children analysed

Characteristic	EZae	EZsc	SWsc	p-value
n	385	326	281	
Mean (SD) age (years)	8.4 (1.7)	8.5 (1.4)	8.3 (1.6)	0.06
Sex - Male (%)	187 (49)	146 (45)	149 (53)	0.13
Race: Indian (%)	287 (75)	249 (76)	214 (76)	0.82
African (%)	98 (25)	77 (24)	67 (24)	
Prior doses (%):				
1 documented	182 (47)	171 (52)	138 (49)	0.59
≥ 2 documented	86 (22)	63 (19)	55 (20)	
Undocumented history of ≥ 1 dose	47 (12)	42 (13)	33 (12)	
No vaccination history	70 (18)	50 (15)	55 (20)	
Reported history of measles (%)	124 (32)	111 (34)	85 (30)	0.78
No measles or vaccination (%)	45 (12)	33 (10)	36 (13)	0.58
Geometric mean HI titre	1:4.4	1:4.3	1:4.2	0.68

Data are missing for some variables

Although there were no significant differences between groups by age, sex, race, previous measles vaccination or history of measles in the 992 children who were analysed (Table 5.2), there were differences in age and those with no measles or vaccination in the 4987 children who initially consented (Table 5.1). There were no significant differences in baseline antibody levels between groups by HI (Table 5.2) or in the subset of sera assayed by PN. Baseline HI titres did not significantly decrease with age, and did not correlate with a history of measles or measles vaccination.

On multi-level analysis, there does appear to be some "clustering" of HI response within schools, such that 7% of the variation in baseline HI titres is accounted for by between-school variation (Table 5.3). This component of variation significantly improves the model (likelihood ratio test X^2= 6,6; p=0.01). In the 3 level analysis, there was no additional clustering of HI response within classes, after allowing for the school level clustering, and the variance estimate for the class component was zero.

Table 5.3: Proportion of variability in the fold difference at baseline and 1 month at child, class and school level, in models of vaccination group including all potential confounding variables

Level	Single level analysis (same as normal linear regression)	Two level analysis: School + child	Three level analysis: School + class + child
Child	100%	93%	93%
Class			0%
School		7%	7%

5.2.4 Effect of route and strain on serological responses by the different outcome measures

In all groups, antibody levels rose sharply at one month then fell rapidly. Responses by the 4 outcome measures are presented below.

5.2.4.1 Proportion with ≥4-fold increase in antibody titre (seroconversion)

At 1 month post vaccination, 85% of EZae had seroconverted, which was higher than both the EZsc (79%, p=0.04) and SWsc (63%, p<0.001) groups (Table 5.4). At 1 year, 60% of the EZae group, but only 34% of EZsc and 25% of SWsc groups still had antibody levels at least 4-fold higher than baseline (p<0.001).

Table 5.4: Percentage seroconversion at 1 month and 1 year post-vaccination by vaccine group

Vaccine Group	N	% seroconversion (95% CI)	
		1 month*	1 year**
EZae	385	85 (81-88)	60 (55 65)
EZsc	326	79 (74 83)	34 (29-39)
SWsc	281	63 (57-68)	25 (20-30)

* 1 month: EZae vs EZsc (p=0.04); EZae vs SWsc (p<0.001); EZsc vs SWsc (p<0.001)

** 1 year: EZae vs EZsc and EZae vs SWsc (p<0.001); EZsc vs SWsc (p=0.01)

5.2.4.2 Average fold increase from baseline

Average fold increases were significantly better in the EZae group at 1 month and 1 year after vaccination (Table 5.5). The difference between EZsc and SWsc at 1 year, though small, was significantly different (p=0.03).

Table 5.5: Average fold increase from baseline titre at 1 month and 1 year post-vaccination

Vaccine Group	N	Average fold increases (95% CI) 1 month*	1 year**
EZae	385	11.2 (9.9-12.7)	3.7 (3.3-4.1)
EZsc	326	6.7 (6.0-7.9)	2.1 (1.9-2.3)
SWsc	281	4.7 (4.2-5.4)	1.9 (1.7-2.1)

* 1 month: EZae vs EZsc and EZae vs SWsc ($p<0.001$); EZsc vs SWsc ($p<0.001$)

** 1 year: EZae vs EZsc and EZae vs SWsc ($p<0.001$); EZsc vs SWsc ($p=0.03$)

5.2.4.3 Geometric mean titres (GMT)

The EZae group had highest GMTs at 1 month and 1 year post-vaccination ($p\leq 0.001$). EZsc had significantly higher titres at 1 month than SWsc ($p<0.001$), but the difference was of borderline significance at one year (Table 5.6).

Table 5.6: Geometric mean titres (GMT) at baseline, 1 month and 1 year post-vaccination by vaccine group

Vaccine Group	n	GMT (95% CI) Baseline	GMT (95% CI) 1 month*	GMT (95% CI) 1 year**
EZae	385	1:4 (4-5)	1:50 (45-55)	1:16 (15-18)
EZsc	326	1:4 (4-5)	1:29 (27-32)	1:9 (8-10)
SWsc	281	1:4 (4-5)	1:20 (18-22)	1:8 (7-9)

* 1 month: EZae vs EZsc and EZae vs SWsc ($p<0.001$); EZsc vs SWsc ($p<0.001$)

** 1 year: EZae vs EZsc and EZae vs SWsc ($p<0.001$); EZsc vs SWsc ($p=0.04$)

In the 64 children who had PN assays, the GMT at 1 year post-vaccination was significantly higher for EZae (3605 mIU/ml) compared to either EZsc (1345 mIU/ml) or SWsc (934 mIU/ml) ($p<0.001$ for both comparisons).

5.2.4.4 Proportion seronegative

Seronegativity was comparable at about 40% in each group at baseline, and virtually no children were seronegative at 1 month (Table 5.7). However, at 1 year far fewer of the EZae group were seronegative (4%) than the EZsc (9%, $p=0.009$) or SWsc (14%, $p=0.001$) groups. Among those initially seronegative, seronegativity at 1 year for EZae (8%) was also substantially less than for the other 2 groups (19% and 27%, $p<0.001$ and 0.004, respectively).

Table 5. 7: Percentage seronegative at baseline, 1 month and 1 year post-vaccination

Vaccine group	n	% seronegative (95% CI) Baseline	% seronegative (95% CI) 1 month	% seronegative (95% CI) 1 year*
EZae	385	42 (37-47)	1 (0.2-3)	4 (2-6)
EZsc	326	39 (34-45)	0 (0-1.0)	9 (6-12)
SWsc	281	46 (40-52)	2 (0.7-5)	14 (10-18)

* 1 year: EZae vs EZsc ($p=0.01$); EZae vs SWsc ($p<0.001$); EZsc vs SWsc ($p=0.05$)

5.2.5 IgM responses

Only 49 (5 %) of the children mounted an IgM response, suggesting that nearly all the children had been previously exposed to measles antigens. IgM responses were significantly more frequent among the EZsc and SWsc groups (5.7% and 7.9%) than the EZae group (2.5%, p=0.007).

5.2.6 Effect of pre-vaccination antibody level

With increasing baseline antibody titres, the magnitude of boosting response at both 1 month and 1 year decreased (Figure 5.3). The EZae group had greater fold increases at baseline titres $\leq$1:8 at both 1 month and 1 year ($p<0.001$ for all comparisons with the other groups). The EZsc group outperformed the SWsc group at 1 month ($p<0.001$), but fold increases at 1 year exceeded those for SWsc only in those with baseline titres of 1:4 ($p=0.05$). No significant differences between groups were seen at baseline titres $\geq$1:16 at either 1 month or 1 year.

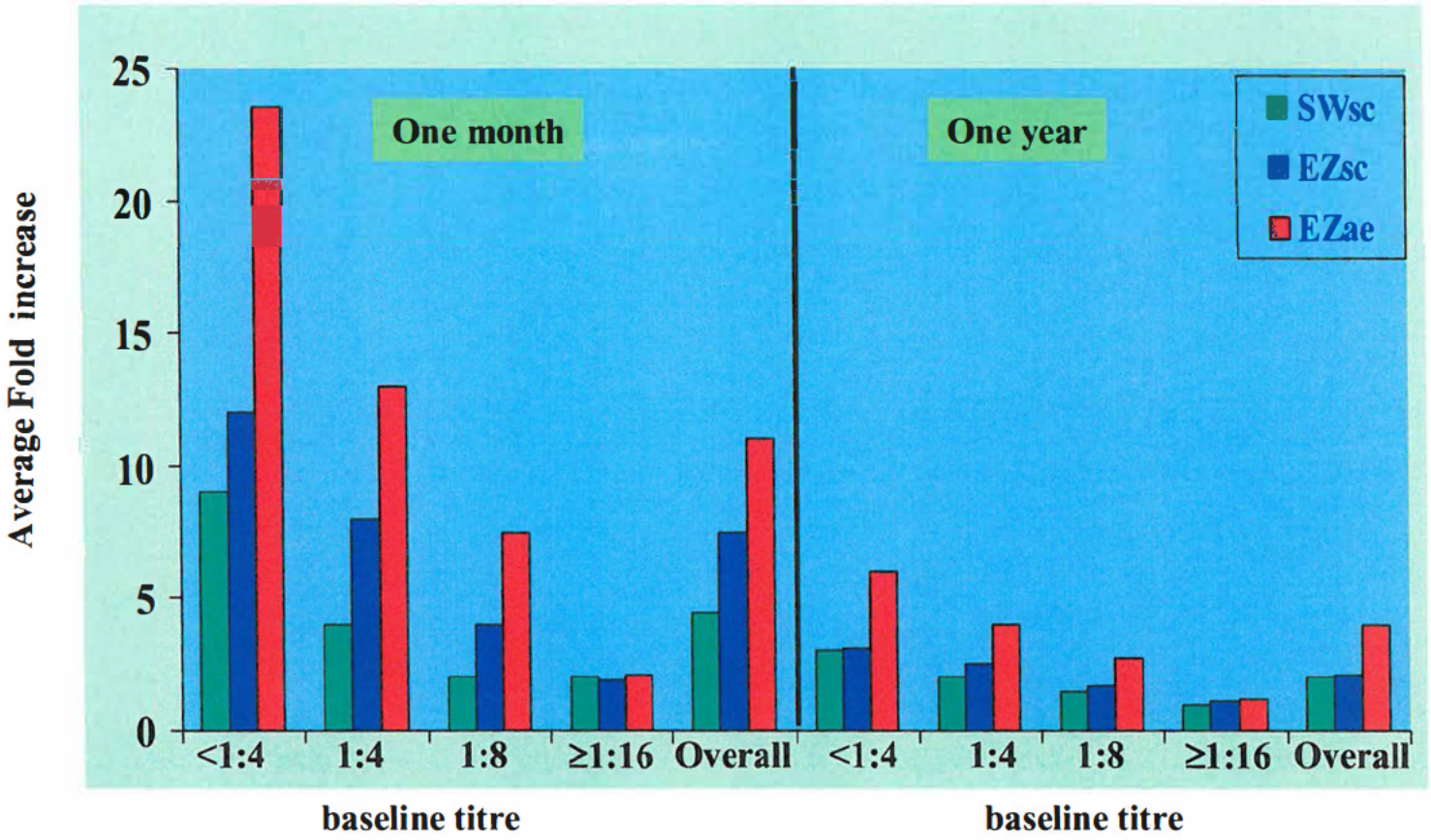

Figure 5.3 : Average fold increases from baseline HI titres at one month and one year post-vaccination by vaccine group

5.2.7 Multivariate analyses

With logistic regression, seroconversions in the EZae group were significantly more frequent than in either of the other groups at 1month and 1 year post-vaccination, after controlling for baseline titre, race, reported illness in the month before vaccination, history of measles or measles vaccination, age and sex ($p \leq 0.002$; Table 5.8).

African children were significantly less likely to seroconvert than Indian children (69% versus 78.9%, p=0.01, Table 5.8). The effect of race remained significant after controlling for pre-vaccination antibody level. Regardless of the initial antibody level, African children had a lower frequency of seroconversion than Indian children at 1 month for all baseline line titres (Table 5.9) with an overall statistical significance of p=0.003. No significant interactions were noted between race and vaccine group. There was no effect on seroresponse of a history of measles, prior vaccine doses, age or sex. The GMT at 1 month was significantly lower (p=0.05) in African children compared to Indian children (Table 5.10).

Table 5.8: Relationship of selected variables to frequency of at least four-fold increases in antibody titres 1 month after booster doses of vaccine

	Number responding/total	Multivariate Adjusted odds ratio (95% CI)	Adjusted p*
Vaccine group			
EZae	326/385 (84.7%)	1.00	
EZsc	257/326 (78.8%)	0.47 (0.29-0.77)	0.002
SWsc	176/281 (62.6%)	0.15 (0.09-0.25)	<0.001
Baseline reciprocal titre			
<4	386/416 (92.8%)	1.00	
4	204/243 (84.0%)	0.33 (0.19-0.58)	<0.001
8	139/207 (67.1%)	0.11 (0.06-0.19)	<0.001
≥16	30/126 (23.8%)	0.02 (0.01-0.03)	<0.001
Ethnic origin			
Indian	592/750 (78.9%)	1.00	
African	167/242 (69.0%)	0.51 (0.32-0.81)	0.01
Illness in month before vaccination			
Absent	594/764 (77.7%)	1.00	
Present	160/222 (72.1%)	0.57 (0.37-0.90)	0.02
Measles history			
Absent	457/612 (74.7%)	1.00	
Present	258/320 (80.6%)	1.45 (0.95-2.20)	0.08
Previous vaccine doses			
None reported	133/175 (76.0%)	1.00	
≥1	626/817 (76.6%)	1.13 (0.66-2.00)	0.67
Age when given booster dose			
5-9 years	551/731 (75.4%)	1.00	
10-14 years	203/254 (79.9%)	1.44 (0.90-2.30)	0.12
Sex			
Female	393/510 (77.1%)	1.00	
Male	366/482 (75.9%)	0.90 (0.62-1.30)	0.59

*Likelihood ratio statistic. Denominators vary due to missing data for some variables.

Table 5.9: Percentage seroconversion at 1 month in Indians and Africans stratified by baseline titre

Group	Race	Baseline titre				Overall P value
		<4	4	8	>16	
All vaccines	Indian	93.7	85.8	70.1	28.6	
	African	91	77.4	58.5	11.4	0.003
Subcutaneous vaccines	Indian	92.2	82.3	56	25	
	African	87.5	68.8	37	4.8	0.002
EZ Aerosol vaccine	Indian	95.9	92.4	90.5	34.3	
	African	94.6	90.5	80.8	21.4	0.2

Table 5.10: GMT at baseline and at 1 month by race

Race	n	Baseline	1 month
Indian	750	1 : 4.3	1 : 33.4
African	242	1 : 4.6	1 : 29.0
p value		0.45	0.05

5.2.8 Effect of recent illnesses on seroresponse

Seroconversion was significantly reduced in children who reported illness in the month before vaccination compared to those without any illnesses (72.1% versus 77.7%, p=0.02, Table 5.8). No individual symptom in the month before vaccination was significantly associated with failure to seroconvert, except fever which resulted in a significantly lower proportion of seroconvertors (53/79 (67.1%) vs 675/874 (77.2%); p=0.04). Looking at all symptoms combined, the presence of any of fever, cough, sore throat, otitis, or diarrhoea in the month before vaccination was associated with reduced seroconversion in the aerosol group (59/78 (75.6%) vs 267/307 (87%); p=0.02) and in the subcutaneous group (43/62 (69.4%) vs 390/545 (71.6%). Rhinitis in the month before vaccination was associated with reduced response in children receiving subcutaneous vaccine (19/28 (67.9%) vs 403/562 (71.7%) but not aerosolised vaccine (31/37 (83.8%) vs 274/325 (84.3%)).

5.2.9 Effect of concurrent upper respiratory tract infections on seroresponse

There was a reduction in effect (seroconversion) of EZae in presence of rhinitis (23/37 (62.2%) vs 196/251 (78.1%); p<0.05) but not with cough (18/28 (64.3%) vs 201/260 (77.3%)). Titre increases were about 1.5 times higher in those without these symptoms. However, the average antibody titre increases in those with these symptoms in the aerosol group still exceeded the overall responses in the subcutaneous groups (8 fold vs 6.6 fold respectively)

5.3 Discussion

We showed that the response to vaccination is better following standard potency EZ vaccine delivered by aerosol than EZ or SW vaccine given by subcutaneous injection. As

previously reported, antibody levels fell sharply between one month and one year post-vaccination, but responses remained significantly better in the EZae group at 1 year, when only 4% of children in this group were seronegative.

The EZae group's response outperformed other groups at pre-vaccination titres ≤1:8 (approximately 600mIU/ml). Most of these children had previously been exposed to measles or measles vaccine. Other studies have shown 93-100% seroresponse to aerosol measles vaccine in seronegative children over 9 months of age (Cutts *et al* 1997). The public health significance of boosting titres to higher levels by aerosol is not yet fully clear, but it could indicate a longer duration of increased protection. As large-scale measles campaigns are increasingly promoted (de Quadros *et al* 1996), the use of a non-invasive method of vaccination is clearly desirable. If antibody titres are high prior to exposure, reinfection is prevented and a boost in titre is rarely seen (Krugman 1977, 1983, Zhang and Su 1983, Zhuji measles vaccine study group 1987).

The lower response seen in children who received measles vaccine subcutaneously could be related to the fact that the injection is not the usual route of entry of measles virus. Some of the injected vaccine could have been neutralised by circulating antibodies, blocking replication of vaccine virus (Barry and O'Callaghan 1997), much like the way measles vaccine is neutralised in infants when circulating maternal measles antibodies are present. This probably results in lower stimulation of antibody producing cells (Okuno *et al* 1965). On the other hand, the superior antibody response in the aerosol group could be related to the fact that vaccination by aerosol in this trial followed the natural route of infection by measles virus. Thus the mucosal surface of the nose, mouth, throat and lungs would be accessible to the vaccine virus. Since the size of the aerosol particles were <5 um, most of the vaccine would deposit in the peripheral airways (O' Callaghan and Barry 1997). Thus, replication of the vaccine

virus would occur over a large surface of the respiratory epithelium. Being removed from the neutralising effect of circulating measles antibodies, greater replication of vaccine virus would presumably be possible in the mucosal cells, with a consequent greater stimulation of antibody producing cells. The initial replication and antibody production at the mucosal cells would be followed by migration of vaccine virus and activated B cells to other tissues via the lymph and blood (Quiding Jarbrink *et al* 1995) where further replication and antibody proliferation would occur.

Standard titre EZ vaccine performed better than SW vaccine when administered subcutaneously, although the differences were much smaller 1 year post-vaccination. In young infants, EZ vaccine overcame passively transferred maternal antibody better than SW vaccine in comparable doses (Cutts *et al* 1995a). Our observations suggest that EZ vaccine also induces a better response in the presence of actively acquired antibodies. Although high titre EZ and SW vaccines were associated with increased mortality (Halsey 1993), these strains of vaccine are safe at standard titre and are widely used (Bennett *et al* 1999). Current global patterns of vaccine use indicate that millions of standard titre doses of both SW and EZ vaccines are used yearly for subcutaneous vaccinations, without any of the safety problems previously recognised with high titre doses of these vaccines. EZ and SW vaccines are distributed interchangeably for routine vaccination programmes in developing countries, according to the tendering procedure each year. The differences between subcutaneous EZ and SW vaccine groups, though much smaller than those between EZae and either subcutaneous group, may nonetheless be important if measles eradication is considered. Further study of strain differences in children with pre-existing antibody is needed.

Antibody profiles probably underestimate protection, since cellular immune responses may be better sustained than antibody titres after revaccination of some

subjects (Samb *et al* 1995, Ward *et al* 1995). Attack rates for children exposed to measles were only 2-3% for those with baseline titres above 125 mIU/ml, versus about 82% for unvaccinated seronegatives. Significant protection was seen for seronegative previously vaccinated children versus unvaccinated seronegatives. Measles vaccination induces humoral and cellular immune responses (Krause *et al* 1980). While evaluation of the cellular immune response to different routes of vaccination is desirable, cell-mediated immunity is difficult to measure and interpret. Thus, antibodies are an accepted, historically useful way to compare responses to measles vaccines and have been the mainstay of evaluation of subcutaneously administered vaccines (Cutts *et al* 1995a). We know what antibody levels correlate with protection against disease, although we cannot be certain that persons with lower levels are susceptible.

Our study is the first to report an effect of race on seroresponse. However, few previous trials have studied this. Different predominant HLA types may limit the selection of peptides presented by antigen processing cells (Jaye *et al* 1998), and affect response to vaccine (Hayney *et al* 1998). The HLA frequencies are different among the Africans, Indians and Caucasians in the province of KwaZulu-Natal (Hammond *et al* 1997). The possibility that this race discrepancy is due to the test having different specificity and sensitivity when applied on sera from individuals of different ethnic origin cannot be ruled out.

Reported illness in the month before vaccination was associated with reduced serologic responses. This is the first report of such an effect with re-vaccination, though one study showed a reduction in seroresponse to primary vaccination (Krober *et al* 1991). The magnitude of the reduction in seroconversions was, however, small (6% overall).

Despite a slight reduction in seroresponse in children with concurrent upper respiratory tract infections at vaccination in the aerosol group, the antibody responses were still superior to that in the subcutaneous group. Studies have shown no or little difference in antibody response to measles vaccination in young children with mild illness compared to those without any illness at the time of vaccination (King *et al* 1996, Scott *et al* 1999). Our findings suggest that aerosol vaccination can be conducted in any part of the year and that it is not necessary to avoid mass campaigns in the seasons when respiratory illnesses may occur more frequently.

The aerosol route is painless, rapid, non-invasive, practicable for non-medical personnel, appears to evoke better humoral immunity, and may also induce superior mucosal immunity. In Mexico, several million children (including infants and preschoolers) have been immunized by the aerosol route without unusual side effects (Fernandez de Castro *et al* 1990 and 1997). However, measles vaccines can only be recommended for aerosol vaccination when shown to be stable in nebulisers under field conditions. The Schwarz vaccine used in this trial lost potency very rapidly in the nebuliser, whereas the other vaccine preparations all retained their potency. The reasons for the instability of the Schwarz vaccine during nebulisation are being investigated in ongoing studies. Concerns about increased hypothetical risks of aerosol vaccine-related adverse events (Cutts *et al* 1997) must be balanced against the real and frequent hazards of unsafe injections in developing countries (WHO 1996, Simonsen *et al* 1999). Although improved aerosol delivery technologies, including the development of inhalers that might use the stable, lyophilised powder vaccine directly (Li Calsi *et al* 1999), are worth devising, there is favourable experience in Mexico with the equipment and approaches used in the South African trial.

CHAPTER 6

Persistence of measles antibody 2 years after revaccination by aerosol or subcutaneous routes

6.1 Overview of chapter

In the previous chapter, we have demonstrated that the response of schoolchildren at 1 month and 1 year after vaccination was significantly higher after EZ vaccine by aerosol than after EZ or SW vaccine by subcutaneous injection. Study of the duration of the increase in antibody after revaccination is important, since antibody levels have been shown to wane rapidly after revaccination by injection (Watson *et al* 1996, Bartoloni *et al* 1977). In this chapter, we present data on the persistence of antibody at 2 years post-vaccination and discuss the implications for the use of aerosol vaccination in sustaining humoral immunity to measles.

Sera were collected at 2 years post-vaccination from the EZae, EZsc and SWsc groups only. Serum samples were not collected from the SWae because of inactivation of the vaccine in the nebuliser (discussed earlier in Section 5.3.2).

6.2 Results

Of the 992 children for whom antibody testing was done up to one year, 851 (86%) were successfully rebled at 2 years post-vaccination. Of the remaining 141 children, blood was unobtainable from 21 children, 12 were absent at repeat visits, 90 were transferred to other schools, and 18 withdrew consent. The follow-up history up to 2 years within each group is detailed in the Trial Profile (see previous chapter - Fig 5.1).

Among children with pre-vaccination antibody levels below ≤1:8, most children showed a significant (≥fourfold) increase in titre at one month post-vaccination, but titres then fell quite rapidly to 1 year. For all groups, the rate of loss of antibody was greatest in the first year after vaccination, with geometric mean titres declining substantially more slowly in the second year (Fig.6.1).

The initial humoral response to revaccination was of greatest magnitude in the EZ aerosol group, and the differences between groups were sustained over time. By 2 years after revaccination, only 6% of children in the EZae group had reverted to a titre below our cut-off of 1:4. The EZsc group was twice as likely (13%, $p<0.01$), and the SWsc group was three times as likely (19%, $p<0.001$), to have titres below the threshold at this time (Table 6.1). Seronegativity in the EZsc group was significantly less than that in the SWsc group at 2 years ($p=0.037$).

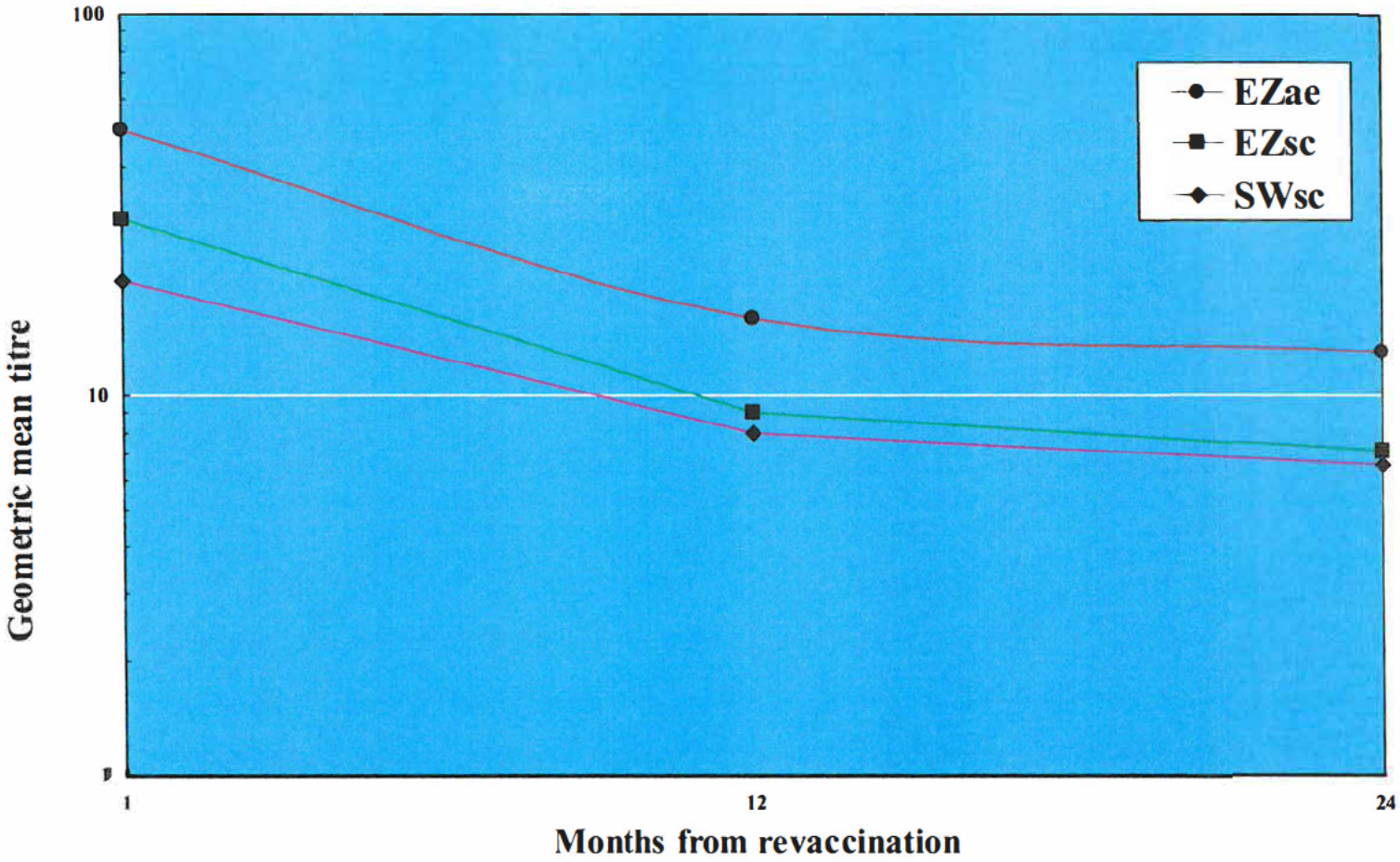

Figure 6.1: Changes in GMT from 1 month to 2 years

Table 6.1 Comparative serological findings at 2 year after vaccination

Outcome Measure	Vaccine group		
	EZae (n = 323)	EZsc (n = 281)	SWsc (n = 247)
% Seroconversion	55 (49-60)	23 (18-28)	21 (16-26)
Average fold increase from baseline	3.0 (2.7-3.4)	1.6 (1.5-1.8)	1.5 (1.4-1.7)
GMT	1:13 (12-15)	1:7.1 (6-8)	1:6.6 (6-7)
% Seronegative	5.9 (4-9)	12.5 (9-17)	19 (14-25)

Amongst those who were seronegative at baseline, seronegativity at 2 years in the EZae group (14/134; 10.4%) was substantially lower ($p<0.001$) than that in the SWsc group (34/110; 30.9%). While a statistically significant difference between the EZae and EZsc groups was seen at 1 year, this was no longer the case at 2 years (EZsc = 21/112; 19%). However, the difference between the EZsc and SWsc groups remained statistically significant at 2 years ($p=0.05$).

The percentage of children who seroconverted was highest in the EZae group at all time points. In the EZae group, 55% (176/323) had seroconverted at 2 years compared with only 23% (64/281) for EZsc and 21% (51/247) for SWsc ($p<0.001$ for both comparisons). At 2 years, the percentage of children with titres >1:8 was significantly higher in the EZae group (193/323; 59.8%) compared with EZsc (82/281; 29.2%) and SWsc (64/247; 25.9%), ($p<0.001$ for both comparisons).

African children continued to have a lower response than Indian children at 2 years after revaccination after controlling for baseline antibody level (Table 6.2). However,

African children getting aerosol had significantly better seroconversion rates after revaccination than the African children getting subcutaneous vaccine (at 1 month: 80% vs 62%, $p=0.005$; at 1 year: 52% vs 26%, $p<0.001$; at 2 year: 38% vs 15%, $p<0.001$). Furthermore, African children vaccinated by aerosol had as good as or significantly better seroconversion rates than Indian children vaccinated by injection (at 1 month: 80% vs 74%, $p=0.33$; at 1 year: 52%vs 31%, $p<0.001$; at 2 year: 38% vs 24%, $p=0.01$).

At one year after revaccination we had found significant differences in serological response by several criteria when comparing EZ vaccine with SW vaccine by injection. At 2 years follow-up, however, the differences in seroresponse were much smaller and only significant for the proportion of children who became seronegative. In addition, children vaccinated at age 10-14 years were more likely than younger children to retain a titre at least fourfold higher than baseline at 2 years post-vaccination.

Table 6.2: Relationship of selected variables to frequency of ≥4 - fold increases in antibody titres at one month and 2 year after booster doses of vaccine.

		At 1 month			At 2 year		
		Percentage seroconverting	Adjusted odds ratio	Adjusted p value*	Percentage seroconverting	Adjusted odds ratio	Adjusted p value*
Vaccine group	EZae	85	1.00	----	55	1.00	----
	EZsc	79	0.47	0.002	23	0.16	<0.001
	SWsc	63	0.15	<0.001	21	0.13	<0.001
Baseline titre (HI)	<1:4	93	1.00	----	54	1.00	----
	1:4	84	0.33	<0.001	32	0.34	<0.001
	1:8	67	0.11	<0.001	17	0.09	<0.001
	≥1:16	24	0.02	<0.001	3	0.01	<0.001
Race	Indian	79	1.00	----	38	1.00	----
	African	69	0.51	0.01	24	0.34	<0.001
Illness in month before vaccination	absent	78	1.00	----	41	1.00	----
	present	72	0.57	0.02	32	0.67	0.06
Measles history	absent	75	1.00	----	33	1.00	----
	present	81	1.45	0.08	36	1.03	0.86
Prior vaccine doses	none reported	76	1.00	----	32	1.00	----
	1 or more	77	1.13	0.67	35	1.10	0.71
Age booster dose given	5-9 years	75	1.00	----	32	1.00	----
	10-14 years	80	1.44	0.12	43	1.84	0.004
Sex	female	77	1.00	----	35	1.00	----
	male	76	0.90	0.59	33	0.90	0.58

* Likelihood ratio statistic.

6.3 Discussion

We have found that EZ vaccine administered by aerosol gives better short-term responses and better antibody persistence over 2 years post-vaccination compared to EZ or SW vaccine by subcutaneous injection. The rate of decline in antibody level flattened in all vaccine groups one year after revaccination, suggesting that the differences in initial response might have a long-term effect on offering increased protection to children who received EZ vaccine by aerosol. Children receiving vaccine by subcutaneous injection were 2-3 times more likely to have antibody levels below 300 mIU/ml at 2 years post-vaccination and also 2-3 times less likely to have HI titres >1:8. While the majority of children may not develop clinical measles on exposure to wild virus, clinical measles has been demonstrated repeatedly among children with antibody levels below 125-200 mIU/ml (Chen *et al* 1990, Samb *et al* 1995). It has also been consistently demonstrated that individuals with antibody titres over approximately 500 mIU/ml are protected from subclinical infection on re-vaccination (Watson *et al* 1996, Bartoloni *et al* 1997). The lack of a response to vaccine at baseline HI titres of >1:8 seen in this study suggest that there is little or no replication of the vaccine virus, and this level of antibody would provide protection against clinical/subclinical infection on exposure to wild measles virus in a similar way. Hence it is reasonable to predict that the higher the antibody titre, the greater the protection from clinical disease and the lower the chance that a child would be involved in measles transmission after exposure to wild virus.

The aerosol route may enhance mechanisms for preventing infection, including stimulation of mucosal immunity. Aerosol particles generated in our study are of a size that probably deposit in both the upper and lower respiratory tract. It is therefore likely that this route of immunization would elicit a more vigorous mucosal response than

would vaccination by injection. Natural infection by measles virus generally results in lifelong immunity. Okuno *et al* (1965) suggested that the immunity obtained from aerosol vaccination was akin to infection immunity and would thus probably be solid and durable. The aerosol vaccine given in our trial followed the natural route of infection and we have confirmed the suggestion of Okuno by showing that the antibody response was better sustained than the injected route.

The higher antibody titres induced by aerosol may have implications for subsequent generations. Antibody titres are lower among persons with vaccine-induced compared to natural measles immunity; thus infants born to mothers with vaccine-induced immunity lose maternal antibody at an earlier age than those born to mothers who had measles disease (Lennon and Black 1986, Jenks *et al* 1988). The level of antibody in maternal serum explains almost all the variation in cord antibody titre (Gonclaves *et al* 1999). The administration of vaccine by aerosol to young adults, such as in mass catch-up vaccination campaigns, could therefore boost the levels of immunity of women in their early childbearing years and provide longer protection to their infants. Since countries aiming for measles elimination are changing to a schedule involving primary vaccination in the second year of life (de Quadros *et al* 1998, WHO 1998), this would reduce the chance that a large window of susceptibility would occur in infants born to mothers who were vaccinated in childhood.

Despite ethnic differences, children receiving aerosolised vaccine responded better than children receiving vaccine by injection. Thus, the aerosol is more immunogenic than injection, irrespective of race.

Age-related differences in response to revaccination usually reflect differences in antibody level before revaccination. Since our antibody assay was of low sensitivity, we may have missed differences in pre-existing antibody among children who were

classified as "seronegative" before revaccination. Younger children had shorter intervals since their previous dose of vaccine and thus were likely to have had higher baseline antibody titres (though below our threshold).

Our previous findings of strain differences in the response to re-vaccination by subcutaneous injection were much less marked at 2 years follow-up, although sero-susceptibility remained significantly more frequent in the SWsc group. Persistence of antibodies at titres that afford protection against clinical/subclinical infection makes the aerosol route of administering the measles vaccine very attractive. Thus, the main public health implication of our study is the potential to use the aerosol route for administration of vaccine in mass campaigns with associated increased protection from measles and less viral replication upon exposure to measles virus in aerosol vaccinees as well as in infants of mothers vaccinated by this route.

CHAPTER 7

Adverse events

7.1 Overview of chapter

The aerosolised EZ vaccine has been shown to evoke a stronger serological response after vaccination which has been sustained over a period of 2 years better than either of the subcutaneous vaccines. However, immunogenicity on its own is insufficient to base recommendations for widespread use of the aerosol route of measles vaccination. Acceptability of vaccination by the aerosol route would be further enhanced if the side effects are within acceptable limits using subcutaneous vaccination as the standard.

One of the difficulties with this aspect of the study is that we did not know if the symptoms represented a response to the vaccine (which would then be a side effect or adverse event) or symptoms of another virus (which would then be a distinct illness). We had collected information on measles-like symptoms/illnesses in the acute phase (up to 2 weeks) and over a longer term (up to 2 years). Symptoms/illnesses experienced in the first 2 weeks after vaccination are more likely to be due to the measles vaccination than to other viruses and are referred to here as adverse events; symptoms/illnesses experienced in the first or second year after vaccination are likely to be caused by a range of viruses and are therefore referred to here as reported illnesses.

There has also been concern about the safety of health workers to repeated exposures of aerosolised measles vaccine. We have attempted to investigate this issue in those involved in conducting the aerosol vaccination.

7.2 Methods

Information on adverse events was measured by various means for the period up to 2 weeks after vaccination for all those vaccinated in the study, and at 1 month, 1 and 2 year in those followed-up.

On the day of vaccination, all those that were vaccinated were given a diary to be completed by their parents/guardians on a daily basis for 2 weeks after vaccination. Parents/guardians were asked to tick the column that indicated that the child was well, or had one or more specified adverse events (fever, rash, rhinitis, cough, conjunctivitis, diarrhoea, other) and to specify the medication given.

Each class teacher was also given a diary with all the participants' names and study numbers. Every schooling day for 2 weeks after vaccination, each teacher was asked to record the code of the above-mentioned conditions if he/she noticed whether any child experienced one or more of these conditions.

An objective measure of fever was done by nurses who visited the schools and took oral temperature measurements from all children present. While these visits were scheduled for day 7 and 10 after vaccination, this was difficult due to weekends and holidays falling on these days. This was sometimes complicated by poor attendance due to teachers' strikes. Thus, temperature readings were done at least once in this period.

The secretary of each school was contacted daily to check on absentees. Contact details of those suspected of being absent due to an illness were obtained from the secretary. These children were followed-up by telephone or a home visit by a nurse. A questionnaire was completed, noting the reason for absenteeism, symptoms, and whether visits to a doctor or hospital were made.

Longer-term information on illnesses was obtained from the children who were followed up at 1 month, 1 and 2 year after vaccination. These children were asked

whether they had been absent from school due to an illness and whether they had measles. Those who reported having measles were asked to describe the symptoms. The 2-year questionnaires included information on hospitalisations.

For some of the analyses of adverse events in the first 2 weeks after vaccination, data from the various sources (parents' diaries, teachers' diaries and nurses' follow-up on absentees) were combined. The information from the various sources was not checked against each other as it would have been impossible to identify which source was the correct one in the event of discrepancies between sources. Since the correctness of information from any of the above sources could not be verified, it was assumed that an entry for any symptom on any one of the forms from each source was correct.

For the analysis of adverse events, the SWae group has been included as well. Unlike the ethical dilemma that we faced with the collection of blood beyond 1 month from this group, we could and did continue collecting data on adverse events on this group for the full duration of the study. Furthermore, information on illnesses was collected from children who refused to give blood or from whom blood collection attempts were unsuccessful at follow-up. These records have been included in the analysis here.

To investigate any boosting of antibody titres in the vaccinators, a sample of blood was taken before the commencement of the aerosol vaccination phase and approximately 1 month later (by which time the aerosol phase was completed). The measles antibody levels were measured in these pre- and post- aerosol vaccination specimens.

In order to attribute any increase in illness rates to vaccination, we needed to get some idea of background occurrence of illnesses in the community before, during and in the immediate post-vaccination period. We lost an opportunity to utilise the school that did not participate in the vaccinations as a control school where background rates of

illnesses in the community could be gathered. Thus data on selected conditions were gathered retrospectively from a local doctor that served one of the communities where the vaccinations were conducted. As the participating doctor served a large section of the community and saw a large number of patients, collecting information on illnesses retrospectively from one doctor was deemed adequate to determine trends in background illnesses. Information on a range of respiratory and related illnesses was collected from the records of all patients visiting the doctor between July and October 1996. Actual ages were not recorded but patients were classified broadly as adult or child (if of school-going age).

7.3 Results

7.3.1 Adverse events in the 2 weeks post-vaccination

Information on adverse events in the first 2 weeks after vaccination was obtained from 1338 parents' diaries, 2530 records in teachers' diaries and 613 nurses' absentee records. One hundred and six children had information from all three sources, 1144 had information from two sources and 1763 had information from one source only. Combining the information from all of the above 3 sources, a total of 3013 children had some information about illnesses from one or more sources.

Approximately 5% of children had a rash in each group. There were statistically significant differences by overall Chi square (tested at the 0.05 level) among groups in the proportion of reported fever, rhinitis, cough, headache, sore throat, vomiting and otitis (Table 7.1), though the number of reports of vomiting and otitis media were small. In almost all of these adverse events with significant differences, the lowest proportion was in the SWae group, followed by the EZae group. The number of reported measles was low with no significant differences between vaccine groups. Only about one-third

(8/22) who reported measles met the CDC criteria for measles (rash and fever and one of cough, rhinitis or conjunctivitis). Pre- and 1 month post-vaccination HI titres were only available for 2 of these 8 children and both had a 2-fold or greater increase in antibody titre.

Table 7.1: Adverse events reported in the first 2 weeks after vaccination (from combined sources)

Adverse event	Vaccine group				P
	EZae (n=766)	SWae (n=637)	EZsc (n=808)	SWsc (n=802)	
	No. ill (%)	No. ill (%)	No. ill (%)	No. ill (%)	
Measles	7 (0.9)	7 (1.1)	6 (0.7)	2 (0.2)	0.25
Rash	35 (4.6)	32 (5.0)	40 (5.0)	33 (4.1)	0.83
Fever	147 (19.2)	96 (15.1)	180 (22.3)	160 (20)	0.007
Rhinitis	265 (34.6)	181 (28.4)	335 (41.5)	334 (41.6)	<0.001
Cough	224 (29.2)	157 (24.6)	255 (31.6)	226 (28.2)	0.036
Conjunctivitis	45 (5.9)	43 (6.8)	61 (7.5)	51 (6.4)	0.59
Diarrhoea	37 (4.8)	36 (5.7)	25 (3.1)	34 (4.2)	0.11
Headache	39 (5.1)	22 (3.5)	61 (7.5)	50 (6.2)	0.007
Sore throat	29 (3.8)	23 (3.6)	61 (7.5)	31 (3.9)	<0.001
Vomiting	1 (0.1)	3 (0.5)	17 (2.1)	6 (0.7)	<0.001
Wheezing	3 (0.4)	2 (0.3)	6 (0.7)	6 (0.7)	0.56
Otitis	0	1 (0.2)	3 (0.4)	0	<0.001

Other information from 1338 parent diaries showed that 462 children took medications in the 2 weeks after vaccination, with statistically significant differences between groups. Fewer children in the aerosol groups took medications, whether in the first week or second week or over both weeks compared to the subcutaneous groups (Table 7.2).

Table 7.2: Number (%) of children who took medications in the 2 weeks after vaccination

Medications taken	**Vaccine group**				**p**
	EZae	**SWae**	**EZsc**	**SWsc**	
	(n=352)	(n=301)	(n=331)	(n=354)	
In the first week	92 (26.1)	70 (23.3)	119 (36)	99 (28)	0.003
In the second week	41 (11.6)	39 (13)	62 (18.7)	57 (16.1)	0.044
Over both weeks	109 (31.0)	87 (28.9)	140 (42.3)	126 (35.6)	0.002

Information from the absentee records indicated that 344 did not attend school due to illness. There was a significant difference ($p<0.001$) between groups, with both aerosol groups having fewer absentees (Table 7.3). A significant difference ($p<0.001$) between groups was also seen amongst those who consulted a doctor (Table 7.3), a substantially higher proportion being those in the EZsc group.

Table 7. 3: Children absent due to an illness or consulted a doctor in the first 2 weeks after vaccination

	Vaccine group			
	EZae	**SWae**	**EZsc**	**SWsc**
	(n=1219)	(n=1277)	(n=1302)	(n=1189)
Number (%) absent	61 (5.0%)	48 (3.8%)	137 (10.5%)	98 (8.2%)
Number (%) consulted a doctor	5 (0.4%)	4 (0.3%)	41 (3.1%)	5 (0.4%)

Information from the parent diaries and absentee records showed that there were 4 hospitalisations/visits. All were from the subcutaneous groups with a borderline significance between aerosol and subcutaneous routes (0/2497 vs 4/2491, p=0.06).

An analysis of frequency of adverse events between those who responded to the vaccine (responders) and those that did not (non-responders), showed that there were no statistically significant differences in the frequency of symptoms between responders and non-responders within each of the EZae, EZsc and SWsc groups. In the SWae group however, significant differences were seen for conjunctivitis (12/77 vs 8/163; p=0.01), headache (4/77 vs 1/163; p=0.04) and rash (10/77 vs 6/163; p=0.015), which occurred more frequently in responders (Table 7.4).

Table 7.4: Adverse events (%) in responders (R) and non-responders (NR) within each vaccine group

Adverse	EZae		SWae		EZsc		SWsc	
Event	R	NR	R	NR	R	NR	R	NR
Conjunctivitis	7.9	2.8	15.6	4.9*	7.6	6.9	7.0	8.6
Headache	6.3	5.6	5.2	0.6*	10.4	6.9	7.9	1.7
Sore throat	4.0	5.6	3.9	3.7	9.5	10.3	3.7	1.7
Otitis	0	0	0	0	4.8	0	0	0
Diarrhoea	5.2	0	5.2	1.8	3.8	0	4.7	6.9
Vomiting	0.4	0	1.3	1.2	1.9	3.4	0.9	0
Wheezing	0	0	0	0	1.4	0	0.5	0
Measles	0.8	0	1.3	0.6	0.9	0	0.5	0
Fever	25.8	22.2	22.1	14.7	23.7	20.7	24.3	25.9
Rash	6.0	6.3	13.0	3.7*	5.7	5.2	6.5	8.6
Rhinitis	36.9	33.3	33.8	27.0	44.1	34.5	52.8	56.9
Cough	32.9	27.8	29.9	23.9	31.3	32.8	36.0	36.2

* Statistically significant differences

7.3.2 Reported illness in the first year

Five hundred and sixty three of 1647 (34.2%) children reported being absent from school due to an illness in the first year after vaccination. Of these, 453 (80.5%) were absent for less than a week and 108 (19.2%) for longer than a week but less than a month. However, there were no statistically differences between groups for either duration. Only 2 children were absent due to an illness for longer than a month. Both children were from the SWae group, though information on the illnesses was not available.

One hundred and thirteen (6.9%) children reported having measles in the first year after vaccination. Reported measles and symptoms of measles in the 4 groups during the course of the first year are presented in Table 7. 5

Table 7.5: Reported measles and symptoms of measles in the first year after vaccination

	Vaccine group				
	EZae	**SWae**	**EZsc**	**SWsc**	**Total**
	n=439	n=466	n=392	n=350	N=1647
Measles	26 (5.9%)	37 (7.9%)	28 (7.1%)	22 (6.3%)	113 (6.9%)
Rash	20 (4.6%)	25 (5.4%)	26 (6.6%)	17 (4.9%)	88 (5.3%)
Fever	10 (2.3%)	14 (3.0%)	23 (5.9%)	10 (2.9%)	57 (3.5%)
Cough	3 (0.7%)	10 (2.1%)	8 (2.0%)	6 (1.7%)	27 (1.6%)
Rhinitis	2 (0.5%)	4 (0.9%)	2 (0.6%)	4 (1.1%)	12 (0.7%)
Conjunctivitis	3 (0.7%)	0 (0%)	6 (1.5)	3 (0.9%)	12 (0.7%)

There were no significant differences between groups in the proportion of reported measles. Only about half of the children (53/113) reported both rash and fever together, though only 24/113 (21%) of reports of measles met the CDC criteria. Of 61/113 who had HI done at 1 year, there was a 2-fold or greater increase from baseline in 41 children. However, comparing antibody titres from the last blood draw atl month to 1 year, it was boosted in only 2 children. Of the 24 children who met the CDC criteria for measles, 11/15 (73%) who had HI done at 1 year had a 2-fold or greater increase in antibody titre from baseline but the antibody level was boosted in only 1 child since the last blood draw at 1 month.

Forty four of the 113 who reported they had measles said that it was diagnosed by a doctor, while 84 children said that it was diagnosed by the parent. For measles diagnosed by a doctor, 18/26 (69%) who had HI done at 1 year had a 2-fold or greater increase from baseline, which was similar to that for parents (32/47; 68%). However, only 1 child diagnosed for measles by either a doctor or parent showed any boosting in antibody titres from 1month to 1 year. Statistically significant differences between groups in the symptoms of measles were only seen for fever ($p = 0.03$), the highest proportion being reported in the EZsc group. Generally, the EZae group had the lowest proportion of reported measles or symptoms of measles.

7.3.3 Reported illness in the second year

Five hundred and forty three of 1441 (37.7%) children reported being absent from school due to an illness in the second year after vaccination. Of the 543, 438 (80.7%) were absent for less than a week and 101 (18.6%) for longer than a week but less than a month. However, there were no statistically differences between groups for either duration. Only 4 children were absent due to an illness for longer than a month, the

children being from the EZae, EZsc and SWsc groups. Information was only available for the one child from the SWsc group who underwent a circumcision.

Forty six children visited a hospital in the second year after vaccination for illness-related conditions. Of 36 children who were able to provide a diagnosis, the most common conditions were wheezing (5), tonsillitis/tonsillectomy (5), bronchitis (3) and meningitis (2). There were no statistically significant differences between groups. The 2 reports of meningitis occurred in the SWae and EZsc groups.

Sixty four children reported having measles in the second year after vaccination. Reported measles and measles symptoms in the 4 groups during the course of the second year are presented in Table 7. 6

Table 7. 6: Reported measles and symptoms of measles in the second year after vaccination

	Vaccine group				
	EZae	**SWae**	**EZsc**	**SWsc**	**Total**
	n=388	n=403	n=351	n=299	n=1441
Measles	14 (3.6%)	17 (4.2%)	13 (3.7%)	20 (6.7%)	64 (4.4%)
Rash	11 (2.8%)	14 (3.5%)	9 (2.6%)	13 (4.3%)	47 (3.3%)
Fever	8 (2.1%)	13 (3.2%)	5 (1.4%)	9 (3.0%)	35 (2.4%)
Cough	6 (1.5%)	7 (1.7%)	5 (1.4%)	7 (2.3%)	25 (1.7%)
Rhinitis	2 (0.5%)	8 (2.0%)	2 (0.6%)	6 (2.0%)	18 (1.2%)
Conjunctivitis	3 (0.8%)	4 (1.0%)	0 (0.0%)	5 (1.7%)	12 (0.8%)

There were no significant differences between groups of measles reported, though a lower proportion of measles reports were received from the EZ groups. Twenty six of the 64 children reported having both rash and fever but only 17 (27%) met the CDC criteria. Of 36 children with HI done at 2 years, 22 had a 2-fold or greater increase from baseline. Nine of the 36 (25%) had a boost in titre from year 1 to year 2. Twenty two of the 64 who reported they had measles said that it was diagnosed by a doctor while 42 said that it was diagnosed by the parent. For those children who had HI done at 2 years, there was a 2-fold or greater increase in antibody titre from baseline in 8/12 (67%) who were diagnosed by a doctor and 16/24 (67%) who were diagnosed by the parent. A boost in titre from year 1 to year 2 was seen in 3/12 (25%) of doctor-diagnosed measles and 7/24 (29%) in parent-diagnosed measles.

There were no statistically significant differences between the proportions of reports of symptoms.

7.4 Adverse reactions linked to use of EMLA cream

During the subcutaneous vaccination phase, a few children developed unusual adverse reactions. These reactions did not appear to be typical of anaphylaxis following measles vaccination. The cases are described below with modifications done to overcome this problem.

As mentioned in Chapter 4, a mild topical anaesthetic - EMLA^{R} 5% (lidocaine 25mg per gram/prilocaine 25mg per gram) - was applied to the skin of the antecubital fossa of each child at least one hour prior to venepuncture. Approximately 1g of this preparation was applied and covered with an occlusive dressing. This dose was half of that recommended for both adults and children on the product package insert for minor

dermatological applications such as needle insertion. This reduced dose was selected to reduce procurement costs.

Venepuncture was conducted approximately 2-5 minutes before vaccine was administered. Three of the 1648 children on whom the above procedures were performed, developed unusual adverse reactions. Two of the adverse reactions occurred in two eight-year-old boys with no known allergies. Approximately 15 minutes after vaccination with subcutaneous Schwarz vaccine, the one child complained of weakness and dizziness, and examination revealed no palpable pulse while recumbent with, clammy skin. No urticaria or wheezing was noted. He recovered after adrenaline was given. The other boy, from another school, displayed similar though less severe symptoms about 10 minutes after receiving EZ vaccine subcutaneously. His pulse was slow but palpable, and he too recovered well after receiving adrenaline. The researchers later contacted the parents of both boys, who reported full recovery from the incidents.

In another instance, a nine year-old girl with a history of poorly controlled asthma but no history of egg allergy, presented similar symptoms about 20 minutes following venepuncture and vaccination with subcutaneous EZ vaccine. Unlike the boys, she did not fully recover after receiving adrenaline. Although her pulse and initial status did improve, about 15 minutes later she began to wheeze markedly and had peripheral cyanosis and marked shivering. An intravenous saline drip with 50mg hydrocortisone was administered. Following this, the child recovered with no obvious wheezing, but rhonchi were heard on auscultation.

After observing these reactions, which were unrelated to vaccine type and were not typical of anaphylactic reactions, we reduced the amount of EMLA by approximately five-fold and applied about 0.2g to the skin of each child for the rest of the study. No subsequent serious adverse events were seen in the remaining 2756 children on whom these

procedures were performed (3/1648 *vs* 0/2756, p=0.05 by Fisher's test) and the topical analgesia remained satisfactory.

Adverse events reporting forms detailing the above events have been completed and sent to Astra Pharmaceuticals, the manufacturers of EMLA.

7.5 Adverse effects on vaccinators

Pre- and post-vaccination samples were only obtained for 4 of the 6 members who were repeatedly exposed to the aerosol. Post-vaccination samples were not obtained for the 2 international collaborators (JVB and JF de C) as they had left South Africa 2 and 3 weeks respectively after commencement of aerosol vaccination. The sensitivity of the assay for this aspect of the study was 1:8. As antibody levels below the level of sensitivity are traditionally assigned half this value, the reciprocal antibody level of <8 was assigned a value of 4. Though S.H had a post-vaccination reciprocal antibody level of 8 (which represented a two-fold increase), there was no significant boosting in antibody titres in the vaccinators. None of the vaccinators reported any side effects during or after completion of the aerosol vaccination phase.

Table 7. 7: Antibody levels in vaccinators at the beginning and after the aerosol vaccination phase

Vaccinator	Age	Sex	Reciprocal antibody titre	
			Pre-vaccination	Post-vaccination
A.D.	39	M	64	64
R.S.	19	M	16	16
S.H.	19	F	<8*	8
N.A.	30	F	16	16
J.F. de C.	65	M	16	Not done
J.V.B.	55	M	32	Not done

* The sensitivity of the assay was 8

7.6 Illnesses in the community

The total number of patients (both adults and children) seen each month by the doctor was 337 (July), 425 (August), 409 (September) and 361 (October). Over this 4-month period, these patients were diagnosed with (amongst other conditions) asthma (10), allergies (20), bronchopneumonia (1), bronchitis (14), conjunctivitis (4), gastroenteritis (34), headache (27), measles (3), otitis media (12), sinusitis (111), tonsillitis (25), upper respiratory tract infection (URTI) (230), wheezing (32). For all these conditions, there were no statistically significant differences in the frequencies between months. A breakdown by month and age-group of the frequency of the most commonly occurring illnesses is given in Table 7.8.

Table 7.8: Frequency of the most commonly occurring illness in adults (A) and children (C) reported by a local doctor in the study area in the month before, during and after vaccination

Month	URTI			Sinusitis			Influenza			Wheezing			Gastroenteritis		
	A	C	All	A	C	All	A	C	All	A	C	All	A	C	All
Jul	13	11	24	27	2	29	13	2	15	6	1	7	3	2	5
Aug	16	27	43	30	7	37	24	1	25	10	3	13	5	1	6
Sept	14	29	43	18	6	24	14	4	18	11	2	13	11	5	16
Oct	13	24	37	16	5	21	12	1	13	3	6	9	3	2	5

7.7 Discussion

In the first 2 weeks after vaccination, significantly fewer illnesses were reported in the aerosol groups. The fewer illness reports in the EZae and SWae groups are supported by the lower proportion of children who were absent, took medications or consulted a doctor compared to the subcutaneous groups. The differences in absenteeism in the first 2 weeks due to illnesses were, however, no longer seen in the first and second year after vaccination. As the majority of children in this group inhaled vaccine that had become inactivated, the SWae group comes closest to being regarded as a control group. It is therefore not surprising that the SWae group experienced the lowest proportion of adverse events. In addition, those who did have an antibody response to the vaccine (responders) in this group had a significantly higher frequency of several adverse events compared to the nonresponders. These observations suggest that the reactions seen at 2 weeks were related to the vaccine.

As the subcutaneous and aerosol vaccinations were not conducted concurrently, comparison of adverse events by these 2 routes posed a problem. However, the data from the local doctor on illnesses in the general community showed that there were no statistically significant differences in the frequency of illnesses between the months during and around the months that the vaccination by the 2 routes was conducted. This suggests that the differences in the frequency of illnesses between the 2 routes can be attributed to the route of vaccination.

It is acknowledged that reports of measles obtained in the manner that we did is not totally reliable and that there may have been misdiagnosis of measles. However, it is assumed that this would exist across all groups. It would appear that many of the reports of measles were incorrect as only about half of the children reporting measles had both rash and fever at 1 and 2 year after vaccination. Furthermore, only a fifth to a quarter met the additional CDC criteria of accompanying cough or rhinitis or conjunctivitis. In addition, not more than a quarter of children reporting measles showed any boosting in antibody titre from one time point to the next.

About one-third of children with measles had their condition diagnosed by a doctor while about two-third was diagnosed by parents. The larger proportion of diagnosis made by parents is not surprising as the Indian community still clings to many traditional beliefs with respect to measles (Dilraj, 1995). While not statistically significant, it is interesting to note that the highest proportion of measles was reported by the SWae group in the first year after vaccination. This would seem logical as this group would not have been as well protected as the other 3 groups. By the same token, the EZae group had the most vigorous humoral response to vaccination and thus had the lowest proportion of measles reported. In an investigation of a measles outbreak in an area in Mexico where children had been previously vaccinated with EZ vaccine subcutaneously and by aerosol, the lowest attack

rate was in children receiving aerosol (0.8% of 723), followed by the subcutaneous group (14.6% of 48) and unvaccinated children (26.2% of 61) (Fernandez-de-Castro *et al*, 1997).

It is well known that there are seasonal variations in the number of measles cases. In South Africa, the peak season is in springtime (September-November). The highest number of cases has been reported for September, with a mean proportion of 12.3% of cases for each year from 1980-1994 (Epidemiological Comments 1995a). Even in 1994, the year before our trial, the highest number of cases was recorded in September. Despite this, the number of measles reports in our trial was lower in September (aerosol vaccination period) than in August (subcutaneous vaccination period), suggesting that the lower number of measles reported for the aerosol groups was not due to climatic influences.

With respect to conditions needing a visit to the hospital or hospitalisation, the aerosol proved safe in the short and longer term; there were no hospitalisations/hospital visits in the aerosol groups (as opposed to 4 in the subcutaneous group) in the first 2 weeks after vaccination and the hospitalisations in the second year was not significantly different between groups. Concern was also expressed whether aerosol might provoke more serious or frequent allergic reactions. As with the others conditions, we did not solicit wheezing systematically as a specific symptom, but based on volunteered information, there was no significant difference in occurrence of wheezing between the aerosol and subcutaneous groups in the first 2 weeks nor by 2 years after vaccination.

The theoretical concern of cross-contamination (deposition of organisms in the aerosol equipment by infected children and subsequent transmission to healthy children) that may result in an increased frequency of illness in children being vaccinated by the same aerosol equipment have been raised (Whittle *et al*, 1984). However, since the first

trial of inhaled measles vaccine by Sabin *et al* (1982) in Monterrey, this procedure has been used without any trouble. Almost two decades of experience have elapsed of continuous tests and no problem of cross-contamination has occurred. During the Mexican epidemic of measles in 1989-90 in which 4 million school children were vaccinated by aerosol, a study of side effects in the State of Tabasco demonstrated clearly no more adverse reactions with the use of aerosol than with the classic subcutaneous route (Fernandez Bracho *et al* 1990). More importantly, there was not a single report of severe illness among the children vaccinated by inhalation in 14 Mexican states. Furthermore, Albert Sabin, just a few weeks before his death, approved in a letter, the special nebulisers used in this study with the introduction of a very simple modification - the use of a corrugated filter paper in the pipe which goes from the nebuliser to the nasal-oral pieces of the child, just to avoid the entrance of contaminated particles from an individual to the nebuliser content (Sabin AB documents with JF de Castro which are available on request).

The 3 cases of unusual adverse reactions seen during the subcutaneous vaccination phase, were not typical vaccine reactions, but were nonetheless of concern. All 3 children had previously received measles vaccine and their skin at the application site of the cream was intact and free of lesions. The reported rate of anaphylaxis after measles vaccination is less than 1 case per million (CDC 1998). We hypothesize that these reactions may be unrelated to the vaccines, but to other factors including:

1. Emla 5% contains lignocaine and is known to cause allergic reactions, and in the most severe instances, anaphylactic shock may occur. The dose used per child contained approximately 25mg lignocaine and 25mg prilocaine. While there does not appear to have been prior reports of these specific

reactions (Russell and Doyle 1997), their rarity may have made detection difficult in smaller series of patients.

2. The reactions may have been a response to two traumatic procedures performed in quick succession, blood collection and vaccination, but they occurred many minutes after these events and did not appear to be mere faints nor hysterical reactions.

We suggest that doses substantially lower than those mentioned in the package insert be used when topical analgesia in children is induced with lignocaine and prilocaine.

There have been concerns that aerosol vaccination may be harmful to the vaccinators. In his early study with aerosol vaccine, Sabin *et al* (1983) found that of 3 vaccinators, one showed a four-fold rise in ELISA antibodies of one person, a two-fold rise in another and no change in the third person. We explored the risk to vaccinators from repeated exposure to aerosolised measles vaccine over the 3-week period of aerosol vaccinations and did not find any significant boosting in antibody titres, nor any overt clinical symptoms in any of those conducting the vaccinations. Unbeknown to the one team member who had a 2-fold increase in antibody titre, she was actually about 4 months pregnant at the time of aerosol vaccinations. To this day, her son is a normal, healthy child. This suggests that the aerosol vaccine was safe to the foetus. Health care workers in many developing countries are repeatedly exposed to wild measles virus while caring for sick children, but such exposures have not been recognized as a source of harm in immune persons (Cutts *et al* 1997).

In conclusion, measles vaccination via the aerosol route was not accompanied by any increase in side effects when compared to subcutaneous vaccination. It is therefore

safe to the vaccinees. Whilst the number of vaccinators was small, the data suggest that aerosol vaccination is safe to the vaccinators as well.

CHAPTER 8

Conclusions and recommendations

In this large randomised controlled trial, we have shown that the responses to vaccination with standard potency EZ vaccine administered as an aerosol is superior to EZ or SW vaccines administered by subcutaneous injection. While vaccination by aerosol using current equipment and formulation is not expected to replace subcutaneous vaccination in the standard clinic setting, the findings of this trial has major implications for control and elimination of measles using mass campaigns as a major strategy.

Although Albert Sabin was quite emphatic in the proposal to use aerosols for mass campaigns, this was based on his perceptions of cost and convenience issues. There were no randomised controlled trials of effectiveness, and his own experience was limited to studies in Mexico (with Dr de Castro) and in Brazil in young infants. Although Sabin's thoughts were clearly a factor in our ultimately engaging in the present trial, several other factors were fundamentally important. First, the concept of national campaigns in the control and perhaps ultimate eradication of measles became a reality and not just a vision with Ciro de Quadros and the Pan-American Health Organisation (PAHO) actually implementing such campaigns and demonstrating its effectiveness in controlling and even interrupting measles transmission. Second, Dr de Castro's development of "field friendly" methods and his experience and favourable impressions of the value and safety of the process in giving doses to over 3 million Mexican school children. Third, earlier literature suggested not only that EZ vaccine might perform better than SW vaccine, but that booster doses by aerosol might be more effective than vaccination by the subcutaneous route. Others also believed that aerosol might be highly

effective, in that inducing immunity by the respiratory route might provide superior protection. The large number of small studies of aerosol also gives ample testimony of the persistent interest and belief of many investigators that this route was worth further investigation.

This trial was the first randomised controlled study, and we would have been in a position to endorse the aerosol approach on the basis of obviating HIV/hepatitis B transmission and on the wide-scale and apparently safe use in Mexico, convenience, acceptance, and cost-effectiveness, even if we simply showed comparable responses of aerosol with subcutaneous injection. The superior immunogenicity of the aerosol shown in this trial is a major additional incentive to use this approach.

The factors responsible for instability of nebulised SW vaccine are being investigated in ongoing studies. An unappreciated rapid loss of potency during nebulisation may be a factor underlying the lesser responses seen rather consistently in previous comparisons of SW and EZ aerosols.

The EZ vaccine has been frequently and unfairly maligned with persons frequently believing that EZ vaccine is unsafe, *per se*. Although both high titre EZ and SW vaccines were associated with increased mortality in 5-6 month old infant girls compared with receipt of standard doses at 10 months of age for a few years after vaccine receipt, both vaccines have a long history of safety in standard doses and have been extensively used globally. We have shown in this trial that both the EZ and SW vaccine at standard doses to be safe to the vaccinees.

Concern for the safety of the vaccinators involved in the aerosol vaccination phase has been shown to be unfounded in this trial. None of the vaccinators seroconverted nor showed any side effects from exposure to vaccine virus that may be present around the aerosol equipment.

The rate of antibody decline has been suggested to be faster among persons who attain the highest antibody levels post-immunisation, so that the range narrows with time. In our trial, the EZae attained the highest antibody level 1 month after vaccination and consistently retained a higher level than the other two groups over the 2 year period of the trial. Although the EZsc group attained a higher antibody level than the SWsc group 1 month after vaccination, antibody levels declined faster in the EZsc group such that it was not significantly greater than that of the SWsc group 2 years after vaccination.

Although seroresponse in HIV-infected individuals has been reported to be lower (Palumbo *et al* 1992), there has been no apparent increase in serious adverse events after immunisation at age 9 months in developing countries or at older ages in the USA. The only case report of Giant-cell pneumonia in a severely immunocompromised HIV-infected case happened to a college entrant in the USA who received subcutaneous measles-containing vaccine (CDC 1996), and it is probably the atypical host response rather than the route that may be important in this. The case was also unusual because the patient did not have clinical onset of measles pneumonitis until almost a year after vaccination. The patient died about 2 months later with cytomegalovirus (CMV) encephalitis as the immediate cause of death and pulmonary measles listed as one of the contributing causes. While this case may have been an atypical host response, we still need to be careful in giving any live virus by any route to severely immuno-compromised persons. Studies underway in Malawi and Zambia (high HIV prevalence countries) may provide additional data on serious adverse events. Although our investigation of measles vaccination by the aerosol route was the largest randomised trial, the numbers were still too small to collect information on serious adverse events. Agammaglobulinemia, giant cell pneumonia and encephalitis occur in ratios of 1:1000 measles cases, while subacute sclerosing pan encephalitis (SSPE) occur in ratios of

1:300000 measles cases. Other than the case of the college student, there is no evidence of any relationship between measles vaccination and the above-mentioned serious adverse events. Given the rarity of these conditions following measles illness, concerns that aerosol vaccination may lead to one or more of the above serious adverse events will be difficult to assess even if aerosol vaccination is in large-scale use.

As measles control (by current methods) improves, the circulation of wild virus will decrease. Thus, persistence of antibody may become shorter because of less boosting of antibody titres in immunised persons from exposure to wild virus. In this respect, our trial has shown that vaccination by the aerosol route has an added advantage in that there is the longer persistence of antibodies after revaccination.

Some concern was also expressed that children with upper respiratory tract infections may not respond adequately to vaccination by aerosol. The superior immunogenicity of the aerosolised EZ vaccine despite the presence of upper respiratory tract infections adds to the attractiveness of the vaccination by aerosol. This is an important consideration in a mass campaign, particularly where staff and time constraints may not allow for assessment of upper respiratory tract infections.

Although observations on adverse events in the first 2 weeks after vaccinations were not temporally concurrent by the two routes of immunisation, background illness occurrence in the community were similar in both months of subcutaneous and aerosol vaccination, enabling conclusions about the reactogenicity of the different routes in our trial to be made. It was encouraging to note that significantly fewer side effects occurred in the aerosol groups compared to the subcutaneous groups in the 2 weeks after vaccination. In the longer term, illness patterns were similar by both routes of administration.

Although the experience with the equipment and approaches used in this trial were favourable, aerosol delivery of measles vaccine can only be put to widespread use once it has been licensed for use by this route. The current measles vaccines are only licensed for use by injection. The findings of this trial have been presented at the WHO meeting of Research Related to Measles Control and Elimination held on 27-29 March 2000 in Geneva. Aerosol studies with measles-rubella vaccine done in Mexico have supported our findings and have encouraged the WHO to recognise the potential of using aerosol vaccination in mass campaigns to control and eliminate measles. The WHO has therefore placed further research on vaccination by the aerosol route on a high priority (Appendix 3). The highest priority issues in this area relate largely to safety issues to meet licensing requirements. Subsequent to this meeting, information from existing data from our trial has addressed some of the issues (safety to vaccinators and vaccinees). Urgent studies in macaques have been recommended and are being undertaken to address other safety issues not possible in the trial to fast-track licensing requirements. The WHO has also recommended that a meeting of regulatory authorities, companies and researchers be convened to clarify steps, time frames and hurdles to bring this product to the marketplace.

Aerosol vaccination using EZ was found to be apparently safe in the age group we studied. There is little information on HIV status available for this age group. A recent study estimates HIV prevalence to be 5.6% for children 2-14 years old (Nelson Mandela/HSRC 2002). However, HIV prevalence was expected to be much lower in 1996 when the children in this trial were vaccinated. Children who may have contracted HIV at birth would have probably not survived to this age. Furthermore, the majority of children were not old enough to be sexually active. In this light, the recommendation is that aerosol vaccination be done first in children over 5 years of age. Further research of

aerosol vaccination in immunocompromised children <5 years old needs to be done before aerosol vaccination can be recommended for this age group as well.

If the measles vaccine is approved for aerosol use by the WHO, then vaccinations could be done by a few countries as demonstration projects of the use of aerosol vaccination on a large scale with careful monitoring of impact and safety.

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Appendix 1

Coding for school, class, vaccine/route codes and child study numbers

SCHOOL AND CHILD ID NUMBER CODES

Eg A0101

The child study number is a 5 digit number
1st digit: letter for school
2nd and 3rd digits: numbers for class (01...15)
4th and 5th digits: serial number for child in class (01..40)

CODES

A Dawncrest

A01 Class 1A AE 2
A02 Class 1B SC 3
A03 Class 2A AE 1
A04 Class 2B SC 4
A05 Std 1A SC 4
A06 Std 1B SC 3
A07 Std 2A AE 1
A08 Std 2B AE 2
A09 Std 2C AE 1
A10 Std 3A SC 3
A11 Std 3B AE 2

B Everest Heights

B01 Class 1A AE 2
B02 Class 1R SC 4
B03 Class 1S SC 3
B04 Class 2K AE 1
B05 Class 2P AE 2
B06 Std 1O SC 4
B07 Std 1S AE 1
B08 Std 1M SC 3
B09 Std 2A AE 1
B10 Std 2N AE 2
B11 Std 2T SC 4
B12 Std 3K SC 3
B13 Std 3R AE 1
B14 Std 3Y SC 4

C Mounthaven

C01 Std 1K	AE 2
C02 Std 1B	SC 4
C03 Std 2M	SC 3
C04 Std 2S	AE 1
C05 Std 3T	AE 1
C06 Std 3N	SC 4
C07 Class 1C	SC 3
C08 Class 1H	AE 2
C09 Class 2G	AE 1

D Jhugroo

D01 Class 1	SC 3
D02 Class 2	SC 4
D03 Std 1	AE 1
D04 Std 2A	AE 2
D05 Std 2B	AE 2
D06 Std 3	AE 1

E Lotusville

E01 Class 1A	(No replies)
E02 Class 1B	SC 3
E03 Class 1C	SC 4
E04 Class 2A	(No replies)
E05 Class 2B	AE 1
E06 Std 1A	AE 2
E07 Std 1B	AE 2
E08 Std 1C	SC 4
E09 Std 2A	AE 1
E10 Std 2B	SC 3
E11 Std 2C	SC 4
E12 Std 3A	AE 1
E13 Std 3B	AE 2
E14 Std 3C	SC 3
E15 Std 3D	SC 3

F Redcliff :
School governing body refused

G Umhloti

G01 Class 1A	SC 3
G02 Class 1B	SC 4
G03 Class 2A	AE 1
G04 Class 2B	AE 2
G05 Std 1A	AE 1
G06 Std 1B	SC 3
G07 Std 2A	SC 4
G08 Std 2B	AE 2

H Verulam Madressa

H01 Class 1A	AE 2
H02 Class 1B	AE 1
H03 Class 2A	SC 4
H04 Class 2B	SC 3
H05 Std 1A	AE 2
H06 Std 1B	SC 3
H07 Std 2A	AE 1
H08 Std 2B	SC 4
H09 Std 2C	SC 4
H10 Std 3A	AE 2
H11 Std 3B	AE 1

I Verulam Primary

I01 Class 1A	AE 1
I02 Class 1B	AE 2
I03 Class 2A	SC 4
I04 Std 1A	SC 3
I05 Std 1B	SC 3
I06 Std 2A	AE 1
I07 Std 2B	SC 4

J Acacia

J01 Class 1A	AE 1
J02 Class 1B	SC 4
J03 Class 1C	AE 2
J04 Class 1D	SC 3
J05 Class 2A	SC 4
J06 Class 2B	AE 1
J07 Class 2C	SC 3

J08 Std 1A	AE 2
J09 Std 1B	SC 3
J10 Std 1C	SC 4
J11 Std 1D	AE 1
J12 Std 2A	AE 2
J13 Std 2B	AE 2
J14 Std 2C	SC 4
J15 Std 2D	SC 3
J16 Std 3A	AE 1
J17 Std 3B	AE 1
J18 Std 3C	AE 2
J19 Std 3D	SC 4

K Parkgate primary

Class 1A	AE 2
Class 1S	AE 1
Class 2	SC 4
Std 1A	SC 3
Std 2	SC 3
Std 3R	SC 4
Std 3S	AE 2

L Dianthus

L01 Class 1A	SC 4
L02 Class 1B	SC 3
L03 Class 2A	AE 2
L04 Class 2B	AE 1
L05 Std 1A	AE 2
L06 Std 1B	AE 1
L07 Std 2A	SC 3
L08 Std 2B	SC 4
L09 Std 3A	SC 4
L10 Std 3B	(No reply)
L11 Std 3C	AE 2
L12 Std 3D	AE 1

M Everest (Shallcross)

M01 Class 1A	SC 4
M02 Class 1B	Ae 1
M03 Class 1C	SC 3
M04 Class 2A	AE 2
M05 Class 2B	AE 1
M06 Std 1A	AE 2

M07 Std 1B SC 3
M08 Std 1C SC 4
M09 Std 2A SC 3
M10 Std 2B SC 4
M11 Std 2C AE 1

N Glenridge

N01 Class 1A AE 1
N02 Class 1B AE 2
N03 Class 1C SC 3
N04 Class 2A SC 4
N05 Class 2B SC 4
N06 Std 1A AE 2
N07 Std 1B SC 3
N08 Std 1C AE 1
N09 Std 2A AE 2
N10 Std 2B SC 3
N11 Std 2C AE 1
N12 Std 2D SC 4
N13 Std 3A AE 2
N14 Std 3B AE 1
N15 Std 3C SC 3
N16 Std 3D SC 4

O Simla

O01 Class 1A AE 2
O02 Class 1B AE 1
O03 Class 2A SC 4
O04 Std 1A SC 3
O05 Std 1B SC 4
O06 Std 2A AE 2
O07 Std 2B AE 1
O08 Std 3A SC 3
O09 Std 3B AE 2

P Marianhill primary

P01 Class 1B AE 2
P02 Class 1D SC 3
P03 Class 1J AE 1
P04 Class 2I SC 4
P05 Class 2N AE 2
P06 Class 2V AE 1
P07 Std 1A SC 4

P08 Std 1G SC 3
P09 Std 1N AE 1
P10 Std 2B AE 2
P11 Std 2M SC 3
P12 Std 2N SC 4
P13 Std 2R SC 4
P14 Std 3C AE 2
P15 Std 3G AE 1
P16 Std 3R SC 4
P17 Std 3P SC 3

Q Marianpark
Q01 Class 1A SC 4
Q02 Class 1B AE 2
Q03 Class 1C AE 1
Q04 Class 1D SC 3
Q05 Class 1E AE 1
Q06 Class 1F SC 3
Q07 Class 2A SC 4
Q08 Class 2B AE 2
Q09 Class 2C AE 2
Q10 Std 1A AE 1
Q11 Std 1B SC 3
Q12 Std 1C SC 4
Q13 Std 1D SC 3
Q14 Std 2A AE 1
Q15 Std 2B SC 4
Q16 Std 2C AE 2
Q17 Std 3A AE 1
Q18 Std 3B AE 2
Q19 Std 3C SC 3
Q20 Std 3D SC 4

R Parklands

R01 Class 1A SC 3
R02 Class 1B AE 2
R03 Class 1C AE 1
R04 Class 2A SC 4
R05 Class 2B AE 2
R06 Std 1A SC 3
R07 Std 1B AE 1
R08 Std 1C SC 4
R09 Std 2A AE 1
R10 Std 2B SC 3
R11 Std 2C AE 2

S Shallcross primary

S01 Class 1A	SC 3
S02 Class 1B	AE 2
S03 Class 2A	SC 4
S04 Class 2B	AE 1
S05 Std 1A	SC 3
S06 Std 1B	AE 1
S07 Std 2A	AE 2
S08 Std 2B	SC 4
S09 Std 3A	SC 3
S10 Std 3B	AE 1

T Malvern

T01 Class 1M	SC 3
T02 Class 1P	AE 2
T03 Class 1S	AE 1
T04 Class 2P	SC 4
T05 Class 2M	AE 2
T06 Std 1M	SC 3
T07 Std 1P	AE 1
T08 Std 1S	SC 4
T09 Std 2M	SC 4
T10 Std 2S	SC 3
T11 Std 2P	AE 1
T12 Std 2T	AE 2
T13 Std 3M	AE 1
T14 Std 3P	SC 3
T15 Std 3S	AE 2

U Savannah Park

U01 Class 1A	AE 2
U02 Class 1B	AE 1
U03 Class 2A	SC 4
U04 Std 1A	SC 3
U05 Std 1B	AE 2
U06 Std 2A	SC 3
U07 Std 2B	AE 1
U08 Std 3A	SC 4
U09 Std 3B	SC 4
U010 Std 3C	AE 2

Z Trenance: pilot school

Z01 Class 1A Z0101 : SC EZ / Z0102 AE EZ
Z02 Class 1B Z0201 AE EZ
Z03 Class 1C Z0301 AE EZ
Z04 Class 2A ---
Z05 Class 2B Z0501, 502 : AE EZ
Z06 Std 1A Z0601 :SC EZ / Z0602, 603, 604 :AE EZ
Z07 Std 1B Z 0701,702 : SC EZ / Z0703 : AE EZ
Z08 Std 1C Z0801, 802 : SC EZ
Z09 Std 2A Z0901 : AE EZ
Z10 Std 2B Z1002 SC EZ / Z1001 AE EZ
Z11 Std 2C Z1102 SC EZ
Z12 Std 3A Z1202 SC EZ / Z1201 AE EZ

Appendix 2

Sequences used for block randomisation

1 : aerosol SW vaccine
2 : aerosol EZ vaccine
3 : subcutaneous SW vaccine
4 : subcutaneous EZ vaccine

1	1 2 3 4
2	1 2 4 3
3	1 3 2 4
4	1 3 4 2
5	1 4 2 3
6	1 4 3 2
7	2 1 3 4
8	2 1 4 3
9	2 3 1 4
10	2 3 4 1
11	2 4 1 3
12	2 4 3 1
13	3 1 2 4
14	3 1 4 2
15	3 2 1 4
16	3 2 4 1
17	3 4 2 1
18	3 4 1 2
19	4 1 2 3
20	4 1 3 2
21	4 2 1 3
22	4 2 3 1
23	4 3 1 2
24	4 3 2 1

3. additional studies of safety and immunogenicity in immunologically naive younger children;
4. use of aerosolised MMR as a booster dose to school age children (Phase I and II);
5. further evaluation of methods to deliver the nebulised vaccine to pre-school children;
6. cellular immunity after aerosol vaccination (already planned);
7. mucosal immunity after aerosol vaccination of pre-school children;
8. potential suitability of different vaccine strains; and
9. cost/benefit of aerosol vaccination.

Co-ordination of studies in the area of alternative routes of vaccination

WHO should convene a meeting that includes regulatory agencies and companies and researchers developing the three proposed products/vaccines. This meeting should focus upon clarifying the steps, time frames, and hurdles for bringing these products to the marketplace. At the end of this meeting, WHO and other collaborators should be able to prioritise the different approaches. Before the regulatory meeting, the developers should provide to WHO the projected time frame and costs of developing (and approximate unit costs for use) the three approaches: jet injector system, nebulised formulation, and the powder formulation.