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IMMUNOGENICITY OF QUADRIVALENT MENINGOCOCCAL
POLYSACCHARIDE VACCINE IN CHILDREN,
DURING A MASS IMMUNIZATION CAMPAIGN.

W. JAMES KING

1994



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POLYSACCHARIDE VACCINE IN CHILDREN
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W. James King

Department of Epidemiology and Community Medicine

University of Ottawa

A thesis presented to the Faculty of Graduate Studies
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degree of Master of Science.

August, 1994

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ABSTRACT

Objective: To determine, in healthy children, the immune response induced at one month and one year by the serogroup C antigen of a quadrivalent meningococcal polysaccharide vaccine (*Menomune*TM A/C/Y/W-135), during a mass vaccination campaign.

Design: Prospective before - after intervention study.

Participants: 6 month - 19 year old volunteers recruited during a Public Health Department mass immunization campaign.

Methods: Serum was obtained pre, one month, and one year post immunization and measured by enzyme-linked immunosorbant assay (ELISA) and bactericidal assay (BA) for the immune response to serogroup C *N. meningitidis* polysaccharide antigen. Throat cultures were analyzed for oropharyngeal colonization of *N. meningitidis* and *N. lactamica*.

Analysis: Log transformed mean values, minimum threshold response (ELISA: GMC ≥ 2 $\mu\text{g/ml}$; BA: serial dilution $\geq 1/8$), and fold increase of serologic testing are reported. Geometric mean increases were compared with repeated measures analysis of variance. The percentage of subjects achieving a minimum threshold concentration/serial dilution and the effect of vaccination on oropharyngeal carriage of *N. meningitidis* and *N. lactamica* were explored with McNemar Chi squared analysis. The association of total anticapsular polysaccharide (ACPS) antibody with serum bactericidal antibody (SBA) was examined with correlation analysis.

Results: Pre-vaccination, almost all children had a negligible amount of ACPS

antibody against group C *N. meningitidis*, regardless of age. At one month post-vaccination, a significant response was demonstrated in children older than 6 months of age with regard to total ACPS antibody concentration and in children older than 18 months of age with respect to SBA titre. In children less than 18 months old, despite a significant rise in total ACPS antibody concentration, this measure was not associated with the SBA titre. At one year post-vaccination, children less than 5 years of age had a substantial decline in their total ACPS antibody response, while children greater than 18 months old maintained their SBA levels.

Implications: During an outbreak of invasive meningococcal disease, quadrivalent meningococcal polysaccharide vaccine can induce a 'protective' immune response in children older than 18 months and may be beneficial for children as young as 6 months of age. Further work is required to define the protective antibody concentration/titre, standardize present immune measures, and explore the discrepancy between the ELISA and bactericidal responses in older as compared to younger children. Though ethically and politically sensitive, an efficacy trial should be considered if another mass immunization campaign is to be undertaken. This would be invaluable to determine the vaccine failure rate, define a protective antibody concentration or titre, and as a guide for future immunoprophylaxis. An efficacy trial would also be beneficial to establish the best immune measure (ELISA, bactericidal assay, or other) to assess candidate meningococcal vaccines. The rapid decline of antibody titres in children less than 18 months of age at one year post-vaccination, suggests that if disease activity is still elevated these children require re-vaccination.

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XI. ABBREVIATIONS

ACE:	Advisory Committee on Epidemiology
ACIP:	American Committee on Immunization Practices
ACPS:	Anti-Capsular Polysaccharide
ANOVA:	Analysis of Variance
BA:	Bactericidal Assay
CCDR:	Canadian Communicable Disease Report
CDC:	Centers for Disease Control and Prevention (Atlanta, Georgia)
CHEO:	Children's Hospital of Eastern Ontario
CI:	Confidence Interval
CPS:	Capsular Polysaccharide
ELISA:	Enzyme Linked Immunosorbant Assay
GBSS:	Gey's Balanced Salt Solution
GMC:	Geometric Mean Concentration
GMT:	Geometric Mean Titre
Ig:	Immunoglobulin
IMD:	Invasive Meningococcal Disease
LCDC:	Laboratory Centre for Disease Control (Ottawa, Canada)
MEE:	Multilocus Enzyme Electrophoresis
MHSA:	Mueller-Hinton Horse Serum Agar
OMP:	Outer Membrane Protein
PBS:	Phosphate Buffered Saline
RIA:	Radioimmunoassay
SBA:	Serum Bactericidal Antibody
S-C:	Serum-Conjugate
To:	Time Zero
VAAE:	Vaccine Associated Adverse Events

Units of Measure:

mM:	Millimoles
ml:	Millilitres
μ g:	Micrograms

I. INTRODUCTION

I.1 Statement of the Problem

"Few infections can cause the civil, medical, and social stress that occurs when serious meningococcal disease enters a community"¹.

On December 09, 1991, a 15 year old male died at the Children's Hospital of Eastern Ontario (CHEO) shortly after developing fulminant meningococcal septicemia. Within 24 hours, this illness had also claimed the life of a 16 year old contact. These two cases were the first indication of increased invasive Group C meningococcal disease activity in the Ottawa-Carleton/Outaouais region. Ultimately, eleven cases of invasive meningococcal disease, with a high case-fatality rate in adolescents (55%), led to the mass vaccination of over 225,000 children.

Invasive meningococcal disease (IMD), primarily a childhood infection, is an important cause of morbidity and mortality in Canada. Recently, due to advances in conjugate vaccines, *Neisseria meningitidis* has supplanted *Hemophilus influenzae* type b as the number one bacterial cause of meningitis in children and young adults. An endemic infection, the clinical manifestations range from asymptomatic oropharyngeal carriage to

bacteraemia with or without meningitis. Once established, IMD may follow a rapidly fulminating course, with an overall 10% case-fatality rate that may rise to 50% in an outbreak. The recent trend to clusters of Group C disease in Canada has resulted in mass immunization campaigns in Ontario, Quebec, British Columbia, Nova Scotia, and Prince Edward Island in 1992 and Saskatchewan and Manitoba in 1993.

The increased disease activity in Canada raised questions regarding the role of serogroup C meningococcal immunoprophylaxis, particularly for young children. Specifically, the ability of meningococcal vaccine containing group C polysaccharide antigen to evoke a protective immune response in children less than 2 years of age, was not clear. Also, the minimum age for successful immunization, the duration of immune response, and a clear definition of what constituted a protective immune response were required. This uncertainty has led to a discrepancy in the recommendations of different provincial health departments during the regional immunization campaigns with regards to the minimum age for immunoprophylaxis. In both Ontario and Quebec 6 month olds were selected as the lower age limit for vaccination while other provinces have restricted their programs to children greater than 2 years of age.

This thesis examines the immunogenicity of meningococcal vaccine, containing serogroup C polysaccharide antigen, in children. Specifically, the immune response as

measured by total anticapsular polysaccharide (ACPS) and functional (bactericidal) antibody, the association of these measures, and the duration of response was determined. The effect of immunization on the oropharyngeal carriage of *Neisseria meningitidis* and *Neisseria lactamica* was also examined.

1.2 Thesis Contribution

There are three major areas where this thesis provides a valuable contribution to our understanding of the immune response to vaccination against meningococcal disease:

1. Characterization of the immune response in young children to the group C component of meningococcal polysaccharide vaccine will enhance our knowledge of the mechanisms involved in the development of protection against meningococcal disease. At present there is an abundance of data on the vaccine response in young adults, especially military recruits, but the measures of immunologic response associated with adult protection have not been adequately studied in young children. Due to development and maturation of immune function in the first years of life, a young child may respond differently to a polysaccharide vaccine than an adult. Previous studies of the immune response to serogroup C meningococcal vaccination in young children have not clarified

whether the perceived immunologic differences represent a true lack of vaccine response, and hence lack of protection, or if this simply reflects inadequate laboratory testing due to our lack of understanding of how an evolving, immature immune system develops functional antibodies against this organism. Clarification of the immune response will facilitate the development of appropriate recommendations for the use of meningococcal polysaccharide vaccine in young children.

2. The laboratory 'gold standard' to determine the immune response to meningococcal vaccine is the bactericidal assay, a measure of functional antibody. This assay is considered the method of choice for assessing the immune response for candidate meningococcal vaccines, even though it is still being standardized in a large number of children. This is a time-consuming, expensive test that may have significant inter- and intra-laboratory variability. It is dependent upon a complement driven reaction and requires the use of 'live' meningococcal bacteria. A second method that has been advocated for immunologic testing is the Enzyme Linked Immunosorbant Assay (ELISA), which measures the total meningococcal ACPS antibody response. This is a sensitive, simple test to perform and has acceptable intra-laboratory variability. ELISA has been correlated with the bactericidal assay as a marker of vaccine immune response in adults but similar

studies have not been performed to determine the association of these measures in children. This thesis has examined the immune response as measured by the above techniques and explored the association between them. These data will clarify the usefulness of ELISA as a laboratory measure of vaccine response and immunologic status against *N. meningitidis* in different age groups of children.

3. The effect of quadrivalent meningococcal vaccine on the oropharyngeal carriage of *N. meningitidis* and *N. lactamica* has not been adequately explored in previous studies. As the preceding event for the development of meningococcal disease is felt to be the oropharyngeal acquisition of an invasive form of *N. meningitidis*, knowledge of the vaccine's effect on colonization is extremely important. The ability to influence carriage of this organism with vaccination has numerous public health implications. If vaccination decreases *N. meningitidis* colonization then further benefit of immunization may be seen; analogous to the induction of herd immunity. Conversely, if carriage with *N. lactamica*, important in the development of natural cross-protective antibodies, is decreased, this may interfere with the development of natural immunity.

In summary, the information obtained during this thesis will provide the scientific basis for clinical and laboratory recommendations for the immunization of young children

against *N. meningitidis* during outbreaks of meningococcal disease.

1.3 Organization of the Study

This thesis began during a public health meningococcal mass immunization campaign in the Ottawa-Carleton/Outaouais region. On January 13, 1992 the Health Department announced *Operation Meningo*, a community-wide vaccination of all individuals aged 6 months to 19 years against meningococcal disease. Due to the uncertainty regarding the vaccine response in young children, a plan to study their immunologic response was conceptualized.

Within 48 hours after the announcement of the vaccination campaign and without guaranteed funding, the following was accomplished:

- study protocol developed and a study coordinator recruited.
- strategy for systematic literature review was developed.
- approval was obtained from the Research Ethics Committee.
- a vaccine supply was procured from the Health Department.
- CHEO administration was consulted and a commitment to provide resources and support was obtained.
- CHEO Research Institute approval for 'bridge financing', until hard funding was

available, was obtained.

- funding was solicited from the Ontario Ministry of Health and Connaught Laboratories Inc..
- a commitment from the Laboratory Centre for Disease Control (LCDC; Ottawa, Canada) to provide laboratory assistance for immunologic testing was obtained.
- a commitment from the Bacteriology department at CHEO to process and test all throat swabs and to process and store sera was obtained.
- questionnaire for demographic information was developed.
- medical supplies for vaccination, blood letting, and throat swabs were obtained.
- personnel for vaccination, blood testing, and throat swab testing were identified, hired, and trained
- community pediatricians and family physicians were notified of the study.
- CHEO 'hotline' to handle questions from the community and to assist in the identification of study subjects was organized.
- support services for all children considered to be at increased risk for an adverse reaction to vaccination by the Health Department were provided.
- an after-hours clinic was set up at CHEO for the study site
- a mechanism for flow of study participants including registration and documentation of the CHEO visit was developed.
- information sessions were held with the local media.

The above was accomplished within 48 hours and the first subjects were entered into the study on January 15, 1992. Between 100 - 150 subjects were seen daily over the next 18 days. This was followed by a 10 day interval after which the first subjects returned for their one month follow-up visit.

I have been involved with the conceptualization and implementation of the study with responsibility for the study design, logistics, data collection, and data analysis. Dr. Noni MacDonald, principal co-investigator, and my thesis supervisor, Dr. George Wells, have provided expertise and guidance with the management of the study, data collection and analysis. The thesis is part of a larger two phase study in which 2240 participants aged 6 months to 19 years were immunized at the Children's Hospital of Eastern Ontario (CHEO) and assessed for their immunologic and psychologic response to the IMD outbreak and meningococcal polysaccharide vaccine.

This study has been financed by Connaught Laboratories, the Ontario Ministry of Health, and the CHEO Research Institute. The laboratory investigations have been performed in collaboration with the LCDC, under the direction of Dr. Fraser Ashton, and the Bacteriology Laboratory at the CHEO.

II. REVIEW OF THE LITERATURE

2.1 Meningococcal Disease

2.1.1 Bacteriology

2.1.1a *Neisseria meningitidis*

N. meningitidis, an aerobic gram-negative diplococcus belonging to the genus *Neisseriaceae*², is capable of causing endemic or epidemic invasive disease³. A fastidious organism, the clinical manifestations range from asymptomatic oropharyngeal carriage to systemic disease characterized by meningitis and/or meningococemia. Classification of *N. meningitidis* into serogroups is based on an agglutination reaction to the capsular polysaccharide located in the bacterial cell wall⁴. Thirteen serogroups associated with systemic disease have been identified by this technique of which the five most common are designated A, B, C, Y, and W-135^{5,6}. Further differentiation into serotypes and subtypes is based on the major outer membrane protein (OMP) antigens and the antigenicity of the lipopolysaccharide^{4,7}. Serogroups B, C, Y, and W-135 share common OMP serotypes. Finally, multilocus enzyme electrophoresis (MEE), a technique whereby the genetic variation among meningococci can be identified by the electrophoretic

mobility of cytoplasmic enzymes, is used to distinguish specific clones among different serotypes and subtypes of the organism⁸.

This form of classification provides invaluable epidemiologic information on the specific strain of *N. meningitidis* associated with disease occurrence. Also, it has enhanced our understanding of the immunologic mechanisms involved in both the development of and protection from systemic disease. Using this form of nomenclature the organism responsible for the outbreak of Group C meningococcal disease in the Ottawa-Carleton/Outaouais region was determined to be: *N. meningitidis* serogroup C; serotype 2a; subtype P1.2; electrophoretic pattern 15 (*N. meningitidis* C:2a:P1.2:ET15).

2.1.1b *Neisseria lactamica*

Often found in the nasopharynx of young children, *N. lactamica* is a low virulence organism with little potential to cause systemic illness². Also a member of the *Neisseriaceae* family, it is closely related to *N. meningitidis*. Differentiation of the two organisms is based on their ability to degrade carbohydrate; *N. lactamica* is able to ferment lactose whereas *N. meningitidis* cannot. Though unlikely to cause systemic illness, this organism is felt to play an important role in the development of natural cross-protective immunity to *N. meningitidis*.

2.1.2 Epidemiology

2.1.2a Disease Patterns

Invasive Meningococcal disease (IMD) has been recognized as a worldwide public health problem since the early 1800's⁹ and remains a significant cause of morbidity and mortality¹⁰. *N. meningitidis* is able to cause endemic, local clusters, or epidemic illness dependent upon the specific organism and region of the world involved. The rates of endemic IMD vary from 1-3/100,000 in North America to 10-25/100,000 in developing countries, while rates during periodic epidemics may exceed 500/100,000¹¹.

The experience of the US Army during World Wars I and II illustrates the ability of this organism to cause epidemics¹². During World War I there were 5,839 cases of meningococcal disease with a case-fatality rate of 31.4%. IMD killed more soldiers than any other infectious illness during the Second World War¹³, resulting in an average of 19 hospitalizations/day during all of 1943¹². While there was a total of 13,922 cases, the case-fatality rate declined to 4.0% due to the introduction of sulfonamide treatment.

Invasive disease is primarily a childhood infection. The majority of cases occur in children less than 5 years of age, with the greatest incidence seen between 6 - 12 months

of age. A child less than 1 year of age has an eleven times greater risk than the general population of acquiring meningococcal infection³. In recent years there has been a shift in the age of onset, particularly during clusters of illness. Adolescents have accounted for a greater proportion of cases and IMD in this age group may represent a marker of an outbreak in the community¹⁴.

Temporal patterns and seasonal changes appear to influence the occurrence of meningococcal disease. In industrialized countries disease rates start to increase in the Fall to the Spring with a distinct peak between February and March¹⁵. Developing countries in South America and Africa are influenced primarily by climactic changes, especially during transition from rainy to dry seasons¹⁰. Susceptibility to disease is also increased for those with terminal complement deficiency¹⁶ or close contact with an infected individual - especially in a closed community (household, daycare, military barracks)¹⁷. Preceding upper respiratory tract infections (Mycoplasma, Influenza A and Adenovirus) may also play a role in the acquisition of invasive disease, though the mechanism for this is unknown^{18,19}.

2.1.2b Global Perspective

N. meningitidis serogroups A, B, and C are the strains most commonly responsible for

invasive disease. Each serogroup has a particular pattern of disease association, however this appears to undergo evolution as new strains appear and spread. Since World War II most large epidemics have occurred in developing countries and are caused by serogroup A and to a lesser extent serogroup C. Group A is associated with periodic epidemics in a hyper-endemic region in Africa known as the 'meningitis belt'. This area, bounded by the Ethiopia in the east to Gambia in the west (sub-Saharan Africa), experienced 340,000 cases and 53,000 deaths in an approximate population of 35 million people in a ten year period from 1951-1960¹⁰. Other developing countries such as Brazil, Nepal, and China have experienced large group A meningococcal epidemics with attack rates approaching 1% of the population. Major meningococcal epidemics have been infrequent in developed countries since World War II and their occurrence has been associated with an overall low incidence rate²⁰. The disease patterns in industrialized nations tend to involve sporadic cases and occasional disease clusters caused by serogroups B and C.

International travel provides the potential for global dissemination of a particular virulent strain. The entrance of a new strain of *N. meningitidis* into a community with a low rate of natural occurring protective immunity may be devastating. A distinct form of serogroup B (ET-5 complex) has been associated with localized outbreaks of disease in numerous countries. It has been shown that this strain arose in Europe and then

spread internationally with resultant epidemics in North, Central, and South America as well as Africa²¹. Also, in 1987 during the pilgrimage to Mecca, Group A meningococcus was responsible for an outbreak of invasive disease that subsequently spread throughout the Persian Gulf states and Africa as the pilgrims returned home²².

Though serogroup C frequently causes sporadic infection, and infrequently large outbreaks²³, it has recently demonstrated the ability to cause clusters of meningococcal disease in the United Kingdom²⁴, United States²⁵, and Canada. Distinct, though closely related serogroup C strains, sharing identical enzymes identified by multilocus enzyme electrophoresis, were responsible for the majority of these disease clusters. The clustering of cases, particularly in adolescents, has prompted growing concern that the epidemiologic pattern of Group C meningococcal disease may be changing.

2.1.2c Canada

In Canada there are approximately 200-400 (mean: 278) cases per year of invasive meningococcal disease, with an annual reported incidence rate of 1.40 cases/100,000 in 1987 and 1988²⁶. There appears to be a mild increase in disease incidence every 12-16 years though no consistent pattern or epidemic cycle has been identified¹⁵. The occurrence of localized clusters of disease in three provinces in late 1991 has not been

associated with an overall increase in either the disease incidence or the case-fatality rate²⁷. What has been intriguing is a shift in the age and serogroup accounting for the majority of cases of IMD.

In the 1970's most infections were caused by serogroups A and C but in the early 1980's serogroup B became the predominate organism²⁶. Since 1986, serogroup C has played an increasingly important role¹⁵ and in 1990 accounted for greater than 60% of invasive disease²⁷. Ashton et al²⁸ have demonstrated that 96% of the Group C strains were serotype 2a belonging to a single clonal complex. Using MEE, they further demonstrated that a specific clone (ET 15), with an allelic variation for the enzyme fumerase, infrequent prior to 1986, accounted for 65% of the Group C isolates in Canada in 1990. This particularly virulent group C strain - 2a:P1.2: ET15 accounted for 81% of 1992 group C isolates identified in Canada (personal communication Dr. Fraser Ashton; Acting Director Bureau of Microbiology, LCDC) and was also responsible for the 1989 localized cluster of meningococcal disease in Victoria county, Ontario²⁹ and the outbreak in the Ottawa-Carleton/Outaouais region.

2.1.2d Ottawa-Carleton/Outaouais Region

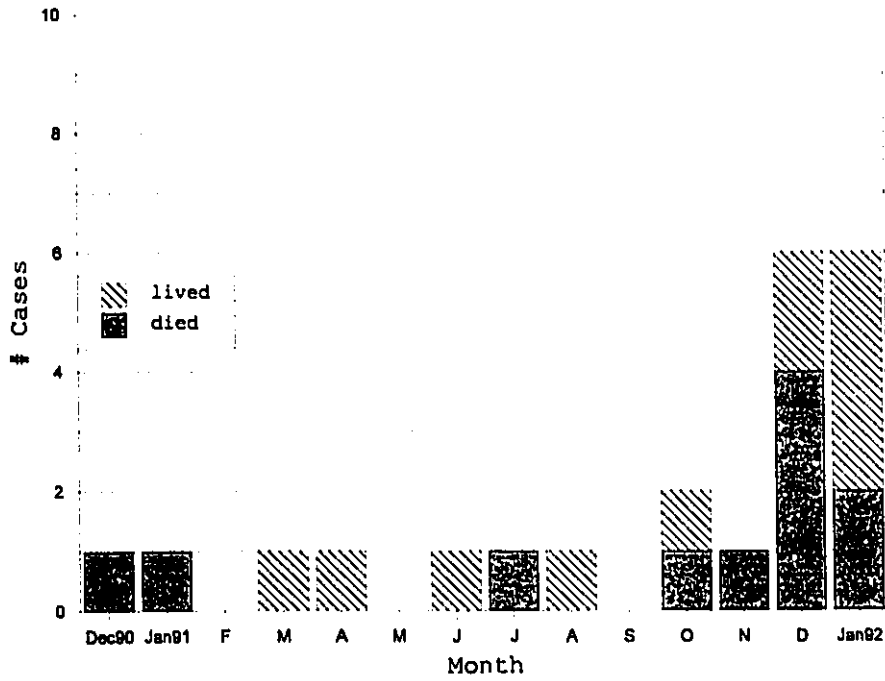
The Ottawa-Carleton/Outaouais region is represented by two separate health boards -

the Ottawa-Carleton Health Board in Ontario and the Département de santé communautaire (DSC) de l'Outaouais in Quebec. From an epidemiologic perspective the geographic boundaries of the regions are closely intertwined and are reviewed together. On average, Ottawa-Carleton experiences 5 - 6 sporadic cases of invasive meningococcal disease each year with a range of 0 - 14 cases³⁰ while approximately 4 cases per year are normally seen in the Outaouais (press release Françoise Bouchard, director, DSC de l'Outaouais). The only previously recorded disease outbreak occurred in the late 1940s during the global increase in the rate of meningococcal disease associated with World War II³¹.

The number of cases associated with the increased disease activity in the Ottawa-Carleton/Outaouais region varies dependent upon the time period and case definition. During the one-month period from December 09, 1991 to January 10, 1992 there were 11 cases of confirmed or clinically suspected serogroup C meningococcal disease. All but one of these cases were between the ages of 14 - 19 years and 6 died as a result of fulminant meningococemia. All cases of meningococcal disease in the region for the year preceding the outbreak are shown in Appendix 1, while Figure 1 shows the specific trend and for Group C disease.

Insert Figure 1 Near Here

Figure 1: Serogroup C Invasive Meningococcal Disease in the Ottawa-Carleton/Outaouais Region Dec. 1990 to Jan. 1992.



From a public health perspective, the increased disease activity in the Ottawa-Carleton/Outaouais region was concerning. Careful investigation had failed to establish strong epidemiologic links between all the cases; the only distinct exposure identified was for the initial four cases who had attended the same high school dance. The emergence of a new Group C clone, in this well-defined older age group, raised the possibility that the level of natural immunity to the organism in the community was low. These new cases presented atypically with fulminant meningococemia, a high case-fatality rate (55)%, and the temporal pattern revealed that the cases were occurring prior to the usual 'seasonal risk' period for IMD (February - March). This was also compounded by the concern that the risk of IMD acquisition was further increased by a preceding Influenza A outbreak in the community.

2.1.3 Carrier State/Transmission

Oropharyngeal carriage plays a crucial role in the life-cycles of both *N. meningitidis* and *N. lactamica*. In humans, the only known natural host for these organisms, asymptomatic carriage represents the mechanism by which both group-specific natural immunity and transmission occur. Transmission requires person to person contact and the spread of aerosolized respiratory droplets. Oropharyngeal colonization with *N. meningitidis* is highest among 15-20 year olds and varies from 5 - 30% depending upon

the population, season, age, and living conditions^{2,32,33}. There has been difficulty comparing carriage rates between studies due to problems ranging from technical difficulty with bacteriologic identification to changes in organism nomenclature.

Our present knowledge is also inadequate regarding factors that influence acquisition of virulent organisms. Exposure is affected by crowded living conditions with household contacts of infected individuals having rates of carriage from 17 - 50%^{17,34} while military recruits may have rates exceeding 50%¹². Carriage may also be influenced by the season, viral infections, and the presence or absence of meningococcal pili³³. After acquisition of a virulent organism the time to onset of disease is brief, generally within one week³⁵. During outbreaks, increased carriage rates have been noted with serogroup A IMD and in closed communities such as the military, but not in other populations¹⁰. High carriage rates of group C during increased IMD activity have not been demonstrated²⁹.

Gold et al found that the rates of carriage for *N. meningitidis* and *N. lactamica* are very different³⁶. Despite having the highest rate of invasive disease, oropharyngeal carriage of *N. meningitidis* was extremely uncommon in children less than four years of age. By 14-17 years of age the carriage rate had increased to 5.4%. In contrast *N. lactamica* was very common in young children with a carriage rate of 3.8% in three month olds increasing to 21% by 18 months of age. With its ability to ferment lactose, the primary

sugar of milk products, *N. lactamica* has an ideal place of residence in the oropharynx of young children. The commensal benefit of this organism may be its ability to stimulate the formation of antibodies that cross-react with *N. meningitidis*. These antibodies assist in the development of natural immunity that helps prevent meningococcal disease.

2.1.4 Clinical Features of *N. meningitidis*

2.1.4a Clinical Manifestations

The spectrum of illness for *N. meningitidis* ranges from asymptomatic oropharyngeal carriage to invasive fulminant disease. Major clinical manifestations are seen in susceptible individuals following an incubation period of 1-10 days. The initial presentation is often nonspecific with symptoms of an upper respiratory viral illness. The illness may then progress rapidly with abrupt onset of fever, chills, emesis, myalgia, and malaise. In the case of meningococcal meningitis this is accompanied by frontal headache, neck stiffness and cervical rigidity. A rash which initially may be urticarial, maculopapular or petechial accompanies this stage of the disease in approximately 50% of cases. Fulminating cases progress rapidly with purpura, metabolic acidosis, disseminated intravascular coagulation, shock, and coma. Death may occur within hours, even with aggressive treatment.

Complications of IMD include adreno-cortical insufficiency (Waterhouse-Friderichsen's syndrome), arthritis, osteomyelitis, endophthalmitis, pneumonia, myocarditis, and pericarditis³⁷. Less common manifestations of meningococcal diseases include occult febrile bacteremia, conjunctivitis, pharyngitis, anogenital infection, and primary pneumonia. A less common presentation, chronic meningococcemia, is associated with low grade fever, rash, and joint swelling.

2.1.4b Diagnosis

IMD is first suspected on the basis of clinical presentation. While presumptive diagnosis may be made by the presence of typical signs and symptoms of septicemia and a characteristic purpuric rash, initial presentation may be nonspecific with features characteristic of many common viral illnesses. Clinical differentiation of infection from that due to other encapsulated organisms, such as *Streptococcus pneumoniae* or *Hemophilus influenzae b*, may be difficult.

Confirmation of the clinical diagnosis relies on isolation of the organism from previously sterile body sites and phenotypic identification by standard bacteriologic techniques. The ability to detect *N. meningitidis* is influenced by its fastidious nature and is affected by prior antimicrobial therapy, specimen transport and handling, and isolation

techniques. Gram stain and culture of the organism from blood, cerebrospinal fluid (CSF), and/or skin lesions, or detection of the organism by latex agglutination of serum, CSF, or urine results in laboratory confirmation of 77 - 94% of clinically suspected meningococcal disease^{37,38}. This is consistent with the laboratory confirmation for 8 of the 11 cases (73%) during the IMD outbreak in the Ottawa-Carleton/Outaouais region.

2.1.4c Treatment

Therapy is dependent upon early recognition of infection. Aggressive supportive care may be necessary for severe meningococemia with concomitant shock and/or disseminated intravascular coagulation. Intravenous (I/V) antibiotics are the mainstay of therapy and include penicillin and/or a third generation cephalosporin for at least 5-7 days after defervescence³⁷. All cases require respiratory isolation for at least 24 hours after I/V therapy has been started. Patients receiving penicillin require rifampin to eradicate *N. meningitidis* oropharyngeal carriage prior to discharge.

2.1.4d Prognosis

Despite improvements in the recognition of illness and supportive care, the overall case-fatality rate has remained between 10-20%^{15,39}. In general, a worse prognosis has been

associated with meningococemia compared with meningitis, epidemic disease compared with sporadic, and infection by caused by serogroup C compared with serogroup A¹⁰. In 1992, during the increased rate of group C IMD in Canada, the overall mortality rate was 11.4%³⁹.

The diversity of clinical measures used in the various outcome studies has made the comparison of prognostic criteria difficult. Gold³⁷ has reviewed these various factors and found that the greatest risk for a poor outcome was associated with shock, seizures, coma, and disseminated intravascular coagulation. Other studies have found that patients with purpuric or petechial skin rash have a poor prognosis⁴⁰.

If the initial infection is not fatal, a number of potential complications may occur. Neurologic sequelae, the most common of which is sensorineural hearing loss, is seen in approximately 5% of survivors while other permanent neurologic deficits occur in less than 1% of survivors³⁷. In a Finnish study, 13.3% had evidence of myocarditis, 7.6% had joint involvement, and 3.2% eighth nerve damage⁴¹. Metastatic spread of infection to distal organs, may also occur. An uncommon, but devastating complication is the occurrence of peripheral symmetric gangrene. This occurs when the infectious process leads to occlusion of small vessels and subsequent loss of circulation to the extremities⁴⁰, the result of which may be gangrene requiring amputation of the affected limb.

2.1.5 Natural Immunity

Our current understanding of the development of natural immunity to *N. meningitidis* is that it is mediated by the humoral immune system and dependent upon the organism's capsular polysaccharide (CPS), and to a lesser extent the major OMP (which respectively form the basis for the identification of specific serogroups and serotypes¹²). The principal work performed in this area occurred in the late 1960's by Goldschneider et al^{13,14} and Gotschlich et al¹⁵. In a series of eloquently executed studies they demonstrated that:

1. Complement-mediated immune lysis by bactericidal antibodies directed against the CPS are essential to prevent the development of invasive group C disease.
2. The response consisted of antibodies from the three major immunoglobulin classes: IgG, IgM, and IgA.
3. Serum bactericidal activity is inversely proportional to the age-specific incidence of meningococcal disease.
4. US Army recruits without serum bactericidal antibody against the homologous serogroup had a significantly increased incidence of invasive disease compared to matched controls with adequate bactericidal antibodies.
5. The development of natural antibody is age-dependent. Approximately 50% of newborns acquire maternal antibody secondary to passive transplacental passage

of immunoglobulin G (IgG). This reaches a nadir over the next 6 - 24 months then starts to increase in a linear fashion through early childhood and into adolescence. By adulthood over 80% of the population has naturally occurring bactericidal antibody against *N. meningitidis*.

6. Oropharyngeal carriage of *N. meningitidis* plays a significant role in the development of natural immunity. This has been confirmed by others who have also demonstrated that meningococcal species and bacteria with cross-reactive antigens (such as *N. lactamica* and certain strains of *Escherichia coli*) play a significant role in the development of immunity³⁶.

In summary, natural immunity to group C meningococcal disease is age-dependent, serogroup specific, and determined by complement-dependent bactericidal antibodies directed against the CPS. Antibody directed towards the CPS (serogroup immunity) takes precedence over the antibody directed towards subcapsular components (serotype immunity), regardless of the antigenic specificity of the latter⁴⁶.

The human complement system is activated by *N. meningitidis* through either the classical or the alternate pathways. After activation both pathways utilize the same route to achieve immune lysis of the bacteria by causing irreversible damage of the bacterial cell wall. Three major circulating immunoglobulins (Ig) IgA, IgG, and IgM are

involved in the humoral immune response against *N. meningitidis*⁴⁶. Of these, IgM is invariably bactericidal and the best activator of complement. IgG, which has four subclasses (IgG₁₋₄), also activates complement, but less than IgM. Of the subclasses, IgG₂ and IgG₄ have almost no ability to activate complement. IgA does not activate, but may actually block complement activity by both the classical and alternate pathways.

Antibody development occurs in stages throughout childhood. The newborn infant acquires passive immunity from maternal IgG antibody against *N. meningitidis* that is transferred across the placenta. Initially colonization with *N. lactamica*, which lacks CPS, leads to serotypic immunity. Later, intermittent colonization with nonpathogenic forms of *N. meningitidis* and other cross-reacting organisms further reinforce the serotypic immunity. Subsequent oropharyngeal colonization with pathogenic forms of *N. meningitidis* allows the formation of serogroup specific antibody.

Unfortunately, the preceding is an oversimplification of how our immune systems resists an extraordinarily complex organism. While the lack of bactericidal antibodies increase the risk for disease, the presence of specific bactericidal antibodies is not exclusively protective. During epidemics in both Finland and the Gambia it has been shown that 16% of the population that develops invasive meningococcal disease had protective bactericidal antibody titres prior to disease onset^{47,48}. Of note, a large proportion of the

antibodies that were measured in the Finnish study were IgA, which is postulated to prevent other immunoglobulins from binding to complement (see below Section 2.6.1a). Other functional properties of the immunologic system, such as opsonic and phagocytic activity, may play an important role, especially in younger children.

2.1.6 Virulence Factors

N. meningitidis is a normal inhabitant of oropharyngeal mucosa and its pathogenic potential is inconsistent and low. Disease acquisition is dependent upon a variety of factors, including exposure to the organism, transmission, establishment of oropharyngeal carriage, and finally disease development. The contribution of many of these factors to disease establishment is not completely understood. Knowledge of the complex interaction between host, organism, and environment is crucial to our understanding of the processes by which IMD develops.

2.1.6a Host

As humans are the only known natural host for *N.meningitidis*, the development of meningococcal disease is directly linked to person-person spread. The first step in the process involves exposure to *N. meningitidis*, followed by organism acquisition which may

lead to a carrier state or invasive disease. Several factors influence exposure and colonization, including: i) age - young adults and adolescents have the highest rates of *N. meningitidis* carriage while children < 4 years have the highest rates of *N. lactamica* carriage³⁶; ii) socioeconomic status (SES)⁴⁹ - a lower SES is associated with increased risk of colonization, though this may be a marker of other risk factors such as crowding; and iii) closed communities - especially military barracks and college dormitories¹². Because the disease is spread through respiratory droplets, factors which increase the degree or duration of contact will increase exposure (eg. sharing foods/drinks, kissing, and symptomatic upper respiratory infection) and the risk of organism acquisition.

Though oropharyngeal carriage is an important step in of disease development it may be neither a necessary nor sufficient cause of IMD. Edwards et al³⁵, found that 11% of military recruits that developed IMD had negative oropharyngeal cultures the day prior to disease onset. In a further 86% of these recruits, carriage occurred within the 2 weeks prior to disease development. Also, the relationship between the extent of carriage in a community and the appearance of meningococcal disease is unclear¹⁰. It would appear that factors that increase either the susceptibility to or the rate of acquisition of disease are more important than the overall rate of oropharyngeal carriage.

The immunologic status of the host is another important consideration in the

development of disease. The first line of defense against meningococci is the nasopharyngeal mucosa. Factors that may disrupt this barrier include viral upper respiratory infections³³, lack of humidity⁵⁰, and cigarette smoking⁵¹. The next line of defense is local immunity. Viral illness along with other enteric or respiratory infections may lead to an enhanced production of immunoglobulin A (IgA), which binds poorly to complement. The high levels of IgA may inhibit immunoglobulins G and/or M, the complement dependent bactericidal antibodies that are normally considered protective (see Section 2.1.5). These 'IgA blocking antibodies' have been postulated to play an important role in establishment of invasive disease⁵².

Finally, the underlying host immunity as described in Section 2.1.5 plays the most important role for the protection against IMD. Bactericidal antibodies have clearly been shown to have a major role in the prevention of disease. Immunologic defects such as deficiencies of either the classical or alternate complement system, terminal components of complement, and asplenia are associated with increased disease risk¹⁶.

2.1.6b Organism

N. meningitidis has many features that may enhance its virulence and lead to systemic illness. Certain strains may be more resistant to killing by complement mediated

antibodies or their lipopolysaccharide may be more likely to induce shock. Strain characteristics influence not only the potential for invasive disease but also oropharyngeal carriage¹¹. Clonal analysis, looking at serotypes and MEE, have demonstrated that different *N. meningitidis* clones have both different rates and duration of carriage. The predominant strain in a community, accounting for oropharyngeal carriage, may or may not be associated with invasive disease.

The impact of the introduction of a new virulent strain into a community, was demonstrated during the meningococcal outbreak at the Haj. As Mecca pilgrims returned home, outbreaks with the same strain occurred in their own countries¹¹. This may be explained by fluctuations in the level of protective ACPS antibody over time. During periods between epidemics there is an underlying disease rate due to varying organism strains. The emergence of a virulent clone, in a population without previous exposure to this organism, may lead to a hyperendemic wave or localized clusters of illness⁴⁶. This may explain the cluster of IMD that occurred when *N. meningitidis* C2a;P1.2;ET15 entered the Ottawa-Carleton/Outaouais region.

2.1.6c Environment

The environment allows the conditions upon which the host and organism can interact.

Several environmental factors that are known to increase the spread of *N. meningitidis* include crowding - especially the number of children sleeping in a room¹⁸, household contacts^{17,33}, and climactic conditions¹⁰.

Many of the factors discussed above are also associated with an increased risk for IMD. While it is helpful to discuss the various factors individually and separate them on the basis of host, organism, and environment, this division is arbitrary. Overall, the available knowledge of the interaction of these factors is very limited, and further research is required. The mechanism by which a new clone of *N. meningitidis* can enter a community and trigger multiple clusters of invasive disease remains to be determined.

2.1.7 Public Health Guidelines

When a case of IMD is detected, the type of public health control measures selected are dependent upon whether the case is sporadic or associated with an outbreak. Guidelines regarding the appropriate public health response with respect to meningococcal disease have been developed by the Advisory Committee on Epidemiology (ACE)³⁹ in Canada and the Advisory Committee on Immunization Practices (ACIP)⁵³ in the United States.

2.1.7a Chemoprophylaxis

Contacts of a sporadic case have an increased risk of secondary disease acquisition and should be aggressively traced and appropriate chemoprophylaxis instituted⁵⁴. High risk contacts are those with exposure to oral and/or nasal secretions and include household members; daycare/nursery school contacts; sexual contacts; and those with close sharing of cigarettes, food, beverages, or kissing. The secondary attack rate for untreated high risk contacts has been estimated to be 300 - 400 times the risk for the general population⁵⁴. Currently rifampin and ceftriaxone are the recommended chemoprophylactic agents and should be administered within 24 hours after the diagnosis of the index case.

Increased disease activity in a defined community should raise concern regarding the possibility of an outbreak. Though the ACE and ACIP guidelines attempt to define increased disease activity, the unpredictable nature of the infection requires latitude in interpretation. Active disease surveillance, with the collection of accurate epidemiologic and microbiologic data, is of utmost importance in determining if an outbreak actually exists. If an outbreak or cluster of cases in a delineated population, caused by serogroups preventable by meningococcal vaccine, is confirmed, it is recommended that immunoprophylaxis be instituted³⁹.

2.1.7b Immunoprophylaxis

The Canadian Immunization Guide, prepared by the National Advisory Committee on Immunization reviews current recommendations for meningococcal vaccine in Canada⁶⁵. Routine immunization to protect civilians against meningococcal disease is currently not recommended. The vaccine is recommended for specific populations at increased risk for IMD such as military recruits, travellers to areas with increased rates of meningococcal disease, and individuals ≥ 2 years of age who have functional or anatomic asplenia. For the control of disease outbreaks that meet the ACE and/or ACIP guidelines, caused by susceptible serogroups in delineated populations, vaccination is recommended for those considered to be at high risk.

2.2 Meningococcal Vaccine

2.2.1 Overview

*Menomune*TM - A/C/Y/W-135, is a freeze dried preparation of the group-specific polysaccharide antigens from *N. meningitidis* Group A, Group C, Group Y, and Group W-135. Immunization consists of a single subcutaneous dose of 0.5 ml, which after

reconstitution contains 50 micrograms of the "isolated product" from each of Group A, Group C, Group Y, and Group W-135, and induces the formation of antibody directed against the CPS antigen that is both independent and group specific. While the degree of clinical protection has not been clearly defined, the vaccine induces the formation of functional immunoglobulin with both bactericidal and opsonic activity that has been correlated with clinical protection (*Connaught Laboratories* product monograph).

A vaccine against group B meningococcal disease is presently under development. Progress with vaccine production has been hindered because the group B capsular polysaccharide is antigenically similar to human brain and blood components^{56,57}. This has interfered with its ability to induce an adequate immune response. Also, it has created the concern that if sufficient antibodies were formed, they could be cross-reactive resulting in an adverse auto-immune response.

2.2.2 Measures of Vaccine Response

Various methods have been developed to measure the immune response provoked by meningococcal CPS antigens. The methods that will be described include ELISA and bactericidal assay (which were used in this study), and radioactive immunoassay (RIA) (the latter of historical importance as the measure previously used by researchers). The

reader is referred to more detailed reviews elsewhere if further information regarding testing is required^{46,58}.

The bactericidal assay, a measure of the amount of functional antibody produced, is the test of choice by the Food and Drug Administration in the United States for the evaluation of candidate meningococcal vaccines⁵⁹. As discussed above (Section 2.1.5), the presence of bactericidal antibody was shown in the late 1960s to be associated with protection against IMD. This is a time-consuming, technically difficult and costly assay. The underlying principle for the bactericidal assay is that immune complexes formed during the assay are able to activate complement and kill intact meningococcal organisms. The procedure (described in full in Section 3.7.2b) involves the use of 'live' meningococci. Serial dilutions of sera from the vaccinee containing complement (from baby rabbits) have a fixed amount of meningococci added to them. The mixture is then incubated and the bactericidal titre expressed as the reciprocal of the final serum dilution required to kill 50% of the meningococci.

A number of factors may influence the results of the bactericidal assay. Both the complement source (rabbit vs human), the type of immunoglobulin present (IgM > IgG >> IgA), and the reference meningococcal strain will cause variation in the titres obtained. While this has made the assay difficult to standardize, it remains the gold

standard by which vaccine response is measured.

Because the bactericidal response is composed primarily of ACPS antibodies, simpler methods to measure the serologic response to meningococcal vaccines have been sought. The general principle for these methods, referred to as solid-phase assays, is that an antigen is fixed to an insoluble matrix and the antibody binding to it is measured by a second antibody that has been labelled with a radiolabel or an enzyme. Evolution of laboratory methods, due to the complexity of the RIA method and the hazards of working with radioactive material, has led to the replacement of RIA by ELISA. Since ELISA has supplanted RIA as the technique of choice for serologic measurement, the technique and potential pitfalls will be briefly reviewed.

At present the ELISA method is being standardized by Dr. G. Carlone (Centers for Disease Control and Prevention {CDC}, Atlanta, Georgia) and has been shown in a multicenter trial to be a sensitive and reproducible method of measuring total anti-meningococcal polysaccharide antibody⁶⁰. Its main advantages are that it is a simple, technically easy, and highly sensitive assay. The method (described in full in Section 3.7.2a) involves mixing group C polysaccharide antigen with serum dilutions obtained from the vaccinee. Enzyme labelled antibody to human immunoglobulins A, G, and M are then added to each well on the plate and a positive reaction results in a colour

change. A computer operated automated reader detects the colorimetric reaction and calculates the antibody values. Therefore this method will give the total amount of anti-meningococcal capsular polysaccharide immunoglobulin that is present in the serum.

A number of factors may influence the results of the ELISA technique. The ELISA assay only provides the total amount of antibody produced and does not give any estimate of functional antibody or actual immune performance. The assay requires pure antigen and specific antibody and results are influenced by the ability of the antibody to bind the specific antigen as well as the amount of antigen that is present. Despite attempts to standardize reagents and conditions specific concerns still exist regarding its reliability and variability, many of which are addressed in the paper by Carlone et al⁶⁰. This is a semiquantitative assay and calculation of the exact quantity of immunoglobulin is determined by reference to a standard amount of known antibody bound to antigen. Depending upon the level of antibody present in the test specimen the result may not fall upon the linear portion of the standard curve which will also influence the assay's accuracy. Also, it is felt that IgM antibodies, due to their size, are more efficient binders in this assay than IgG. Therefore, sera containing disproportionate amounts of one immunoglobulin over another may produce different results even if the total antibody concentrations are the same. This may be significant in meningococcal testing since it is not certain what major immunoglobulin class response is most important and common

in different childhood age groups. Finally, the prozone phenomenon, a form of incomplete reaction, is also a concern. This may arise from several factors including low dilution serum, interference or alteration of proteins, or the presence of extraneous factors, to give erroneous result.

2.2.3 Immunization Studies

Meningococcal group A and C polysaccharide vaccines induce the formation of serogroup specific, complement dependent bactericidal antibodies belonging to the three major immunoglobulins, G, M, and A⁴⁵. The effectiveness of the vaccines to prevent meningococcal disease has been shown in large-scale, controlled field trials involving army recruits in the United States^{61,62}. The effectiveness of the vaccine in these trials to prevent group C meningococcal disease was 87 - 89.7%.

Previous studies have shown that serogroup C polysaccharide vaccine induces an immunogenic response in all age groups, with infants as young as 3 months old demonstrating a rise in bactericidal antibody levels⁶³. However, a significant bactericidal antibody in the protective range was not seen until children were > 18 months old. The duration of vaccine response in young children is relatively brief with return to baseline pre-immunization levels within months after vaccination⁶⁴. Older children show

a much better immunologic response to the vaccine and a longer duration of protection. Specific studies exploring the effect of meningococcal vaccines in children are reviewed in Appendix B.

There has only been one efficacy study of serogroup C polysaccharide vaccine in children. This occurred during an outbreak of invasive meningococcal disease in Brazil in the mid-1970's⁶⁵. The serogroups A and C bivalent vaccine used in this study was new and the epidemic was its first large-scale production. The study was a controlled trial involving infants 6-36 months of age receiving either bivalent meningococcal vaccine or Diphtheria-Tetanus-Pertussis vaccine. Their findings showed poor immunologic response and no clinical disease protection in children less than 24 months of age. Subsequent studies in the United States and Brazil, using different vaccines, have raised concerns regarding the immunogenicity of the bivalent meningococcal vaccine used in the Brazilian trial. These studies have demonstrated ACPS antibody levels in children 6-12 months of age that were associated with protection of older children (> 2 years) in the original Brazilian efficacy trial^{63,66}.

Evaluation of the results from the above studies prompted the recommendation that routine immunization for children less than 2 years of age not be performed, but in the case of an outbreak, consideration should be given to vaccinate children as young as 6

months of age.

2.2.4 Vaccine Response Associated with Protection

Definition of a protective antibody level is extremely important to our understanding of the immunologic processes involved with meningococcal clearance. Because the vaccine is less immunogenic in young children, determination of the lower age limits at which the majority achieve protective levels will help to delineate who should receive the vaccine in an outbreak and to assist in the evaluation of candidate meningococcal vaccines. While it is well established that polysaccharide vaccines are clinically efficacious and result in the production of ACPS antibodies, the assignment of specific protective values has proven problematic and controversial. Another complicating factor is that RIA, used to measure quantitative results and establish protective levels in previous efficacy studies, has now been replaced by ELISA. Reference of the ELISA test to these previous studies is difficult, however Dr. Carlone (CDC) is exploring methods of converting the ELISA antibody response to concentrations, consistent with the previous methods of analysis. The method of conversion of ELISA antibody units to a set concentration of antibody protein is given in Section (3.7.2a).

Theoretically, based on studies of the level of antibody attained in the sera of

agammaglobulinemic children following the administration of gammaglobulin, the antibody concentration that may be protective is $0.2 \mu\text{g/ml}^{67}$. This is also the level of antibody naturally attained by one to two years of age when the risk for developing meningococcal infection is decreasing. Field trials however, have found that higher ACPS antibody levels may be necessary for protection.

Peltola et al⁶⁸, found that 87% of well protected adults had antibody levels $\geq 1 \mu\text{g/ml}$ during an epidemic of group A meningococcal disease in Finland. From the results of their study they found that this level of antibody production was sufficient for protection at all ages. In Brazil, during the serogroup C efficacy trial noted above (Section 2.2.3), the majority of children who were protected against meningococcal disease had antibody levels $\geq 1\text{-}2 \mu\text{g/ml}^{65,69}$.

While there is more agreement with the minimum protective bactericidal level this has not been evaluated in field trials. This is a test of functional antibody and ability to kill viable meningococci, and is therefore felt to be a better measure of true immunologic response. The anti-meningococcal bactericidal antibody response that is felt to correlate with protection against meningococcal disease is a serial dilution greater than or equal to 1 in 4 (personal communication Dr. Frasch, US Federal Drug Administration).

2.2.5 Factors that Influence Vaccine Response

Immune response to the vaccine will be influenced by antigen exposure, recognition, and subsequent formation of specific antibody. The demonstration of antibody production does not guarantee protection against infection, as many factors are involved in the complex process of organism recognition, killing, and clearance.

The first factor of importance is the amount and size of the antigen that is presented to the subject. Animal and human experiments have determined that a dose of 50 microgram with a molecular weight of 5.2×10^5 is the optimal amount of antigen to induce an immunologic response^{67,70,71}. The capsular polysaccharide of group C meningococcus contains an O-acetyl group that can be removed by mild alkaline hydrolysis that may influence vaccine response. Studies of the effect of the O-acetyl group have shown varying results, with the nonacetylated form inducing a better immunogenic response in one study⁷² and the acetylated form demonstrating a better effect in another⁷³. Finally, because the meningococcal polysaccharide lacks protein, it elicits a T-lymphocyte independent response without the formation of immunologic memory. Therefore the response tends to be transient with decline in antibody levels with time.

In children, Gold et al⁷⁴ found that the following factors influenced vaccine response: age of infant, quantity of vaccine, number of doses, molecular size of the vaccine polysaccharide, and the degree of preexisting, naturally acquired immunity to *N. meningitidis*. With respect to age, young children tend to have a less immunogenic response to the vaccine, in terms of both absolute level of antibody and duration, than older children and adults. While the reason for the difference is not clear, it may be due to immaturity of key lymphocyte populations in young children.

Other factors that may impair response to the vaccine include immunodeficiency, severe malnutrition, and infection with malaria⁵⁷. In the present study, these factors should not lead to difficulty with vaccine response as children with immunodeficiencies were evaluated separately, severe malnutrition and malaria were not present in our study population, and children with acute or chronic infections were excluded from the study.

2.2.6 Effect of Vaccine on Oropharyngeal Carriage

While the primary use of meningococcal vaccine is to prevent disease, its effect on oropharyngeal carriage of *N. meningitidis* has been explored in a number of studies. The presumption has been that if local oropharyngeal immunity occurs, then the meningococcal carriage rate in the general population will decrease. This would be

analogous to conferring a secondary, 'herd immunity'. Gotschlich et al⁷⁵ showed that group C vaccine decreased the rate of group C meningococcal carriage in vaccinated vs unvaccinated military recruits. However, studies in Nigeria on group A vaccine have been unable to demonstrate an effect on the carrier state^{76,77}. Therefore, the true effect, if any, remains unanswered. To my knowledge, there have been no studies that have explored the effect of meningococcal vaccine on the carriage of *N. lactamica*.

2.2.7 Reactogenicity

The vaccine is safe in children. The majority of the side effects are minor and consist of transient erythema at the site of injection⁷⁸. Though few systemic side effects have been demonstrated there may be an association with an increased risk of benign febrile seizures⁷⁹.

III. STUDY METHODS

3.1 Objectives

3.1.1 Primary

To determine, in healthy children, the immune response induced at one month and one year by the serogroup C antigen of a quadrivalent meningococcal polysaccharide vaccine (Menomune™ A/C/Y/W-135), during a mass vaccination campaign.

3.1.2 Secondary

1. To determine, in children, the association of total ACPS antibody with bactericidal antibody.
2. To assess, in children, the effect of immunization on the oropharyngeal carriage of *N. meningitidis* and *N. lactamica*.
3. To assess, in children, the rate of adverse events associated with quadrivalent meningococcal polysaccharide vaccine.

3.2 Study Design

Prospective, before - after interventional study in which all subjects received a meningococcal polysaccharide vaccine and then had their immune response measured by development of total ACPS and specific bactericidal antibody. A before-after study was performed because of the meningococcal outbreak and subsequent public health decision to vaccinate all children in the Ottawa-Carleton/Outaouais region (see Section 3.6 Ethical Issues). For overall study numbers and flowsheet see Appendices C and D.

3.3 Study Participants

Participants were 6 months to 19.9 years of age recruited from the Ottawa-Carleton/Outaouais region. For the purpose of this thesis 'children' will be defined as those participants aged 6 months to 19.9 years. These children were recruited through public advertisement or by referral from their pediatrician or family physician. Subjects were eligible for the study if they met the criteria for either the Ottawa-Carleton Public Health Department or the Department de sante communautaire de l'Outaouais mass vaccination programs. The criteria included: i) residents of Ottawa-Carleton/Outaouais region; ii) age 6 months-19.9 years; iii) Non-resident but within age range and attending an educational institution within the region; iv) completion of Ottawa-Carleton Public

Health Department or the Department de sante communautaire de l'Outaouais consent form. Participants were excluded from the study if they had a major acute or chronic systemic illness, fever, known hypersensitivity to Meningococcal vaccine, allergy to Thimerosal, or previous immunization with Meningococcal vaccine within the last five years.

Participants who met the eligibility criteria were entered into the study and identified as a healthy group who received vaccination in the outpatient clinic at CHEO. Participants meeting exclusion criteria were eligible to enter into the "Special Needs Group" portion of the study. These patients were identified by either the Health Department or CHEO as requiring special medical surveillance due to potential risk associated with vaccination. This included children with acute or chronic medical illness, significant allergies or asthma, potential allergy to the meningococcal vaccine, immunodeficiency, or bleeding diathesis. These children were immunized at the CHEO Medical Day Unit and observed for vaccine reaction.

A final group of participants were recruited from a local high school that is geographically in the greater Ottawa-Carleton region but outside of the boundaries established by public health officials for the vaccination campaign. These boundaries were established within a health district and children who resided outside of these

boundaries, but were bused to schools within the district, were included in the immunization campaign. Therefore, some neighbours of the school members we had chosen to study had received vaccination within the guidelines set by the public health department. These participants were entered into a study to assess intramuscular versus subcutaneous routes of immunization.

The subject group does not constitute a true random sample from the Ottawa-Carleton/Outaouais region. There are few known factors that influence the immune response to immunization with meningococcal polysaccharide vaccine (see Section 2.2.5 Factors that Influence Vaccine Response) and would hence impair the comparability (internal validity) and generalizability (external validity) of the study results. The study participants that entered into the study would not be at an increased risk for the factors that influence vaccine response any more than the general population of such children. Demographic information will be looked at in order to compare our study group with the general population of the Ottawa-Carleton/Outaouais region.

Participants were divided upon study entry into each of 7 study groups based on age. The study groups consisted of children aged 6-11 months, 12 to 17 months, 18 to 23 months, 2-4 years, 5-10 years, 11-14 years, and 15-19.9 years. From each of the groups approximately 50 subjects per group were randomly selected for serologic analysis.

The random selection and detailed immunological testing of a limited number of participants was planned in advance of the study commencement to assess a controlled amount of sera to validate the assay technique and ensure that there were no methodological problems. We also wanted to confirm that the method for the ELISA was reproducible and an acceptable coefficient of variation ($\leq 15\%$; personal communication Dr. Fraser Ashton, LCDC) could be achieved. Finally, we also wanted to ensure that a minimum amount of sera for each test was shipped to LCDC. This sera had to be unfrozen and the samples divided, then re-frozen with potential for loss of measureable antibody.

After testing the initial random group by the ELISA method we had detected a significant immunologic response with respect to the total ACPS antibody in all age groups. It was felt at this point that further ELISA testing was unnecessary and an inappropriate use of resources. The decision was then made to proceed with the bactericidal testing of the sera from the random group.

3.4 Intervention Schedule

Each participant (parent/guardian) registered at the CHEO and completed an informed consent from both the Health Department and the Study Group. At this time *EMLA*TM

Cream 5% (Astra Pharmaceuticals), a topical anesthetic, was applied to the forearm over the site of blood letting. Participants then completed a questionnaire concerning demographic information (Appendix E). Next, an oropharyngeal throat culture and a venipuncture for serology was performed. After testing they received a subcutaneous injection with 0.5 ml of quadrivalent meningococcal vaccine (*MenomuneTM-A/C/Y/W-135*) as per the Health Department protocol. Information regarding the vaccination was recorded on the CHEO hospital record and on the health Department consent form/information sheet. A record of the vaccination was also given to each participant for future reference for their immunization booklet or family physician. Also, a study information/reactogenicity sheet with instructions to return this sheet with the follow-up visit was provided (Appendix F). At this time an appointment for one month follow-up was made. At the one month and at one year follow-up visits participants were registered at CHEO and serologic testing and an oropharyngeal swab were again performed as described above.

3.5 Statistical Analysis

All information on participants was entered into *dBASE IV* as the primary database. *SPSS-PC* was the statistical software package upon which the statistical analysis was performed. Taking into account the difficulties with accurate determination of a

protective immunologic response to the vaccine (see Section 2.2.4), this thesis will consider the following as the minimum threshold (protective) response:

- I. Total ACPS antibody concentration $\geq 2 \mu\text{g/ml}$.
- II. Bactericidal antibody serial dilution ≥ 1 in 8.

These values were chosen as a conservative estimate of vaccine response.

Immunologic response was measured by ELISA and bactericidal assay. For ELISA, data was converted from units/ml to micrograms/ml to provide consistency with the units of measure that are reported in the literature (see page 53; Section 3.7.2a). Transformation to the log of base 10 was then performed to normalize the data and to calculate the geometric mean concentration (GMC). The bactericidal assay reports values in serial dilution starting from a minimal value of 1 in 2 (1/2). Again, to provide consistency with the reported units of measure the reciprocal of the serial dilution was logarithmically transformed to the base 2 to normalize the data and to calculate the geometric mean titre (GMT). For reporting, the antilog (appropriate base) of the GMT and GMC are given. Mean values with 95% confidence interval (CI), minimum threshold response, and fold increase to serologic testing are reported.

Repeated measures analysis of variance was used to calculate statistically significant changes in either the geometric mean concentration or geometric mean titre over time. McNemar Chi squared analysis was used to assess statistically significant changes in the percentage of subjects that achieved the minimum threshold response in either the ELISA ($\text{GMC} \geq 2 \mu\text{g/ml}$)¹ or the bactericidal assay (serial dilution $\geq 1/8$)²

McNemar Chi squared analysis was used to assess statistically significant changes in the effect of vaccination on oropharyngeal carriage of *N.meningitidis* and *N. lactamica*. Correlation analysis was performed to determine the Pearson correlation coefficient (R) and assess the association of total ACPS antibody with specific bactericidal antibody.

3.6 Ethical Issues

This study was approved by the CHEO Research Ethics Committee and all patients participating in the study were required to sign both the health department informed consent and the study group informed consent. As the decision to vaccinate was a Public Health Department recommendation for **all** children in the region we felt it would have been unethical to perform an analytical trial with a comparison, unvaccinated group.

¹ See above and discussion page ... 38

² See above and discussion page ... 38

3.7 Laboratory Techniques

3.7.1 Microbiology

Throat swab specimens (deep sweep of tonsillar region and posterior pharyngeal wall), were taken by research nurses trained in this procedure, using *Starswab*[®] rayon tip swabs and transported in modified Stuart's media (*Starplex Scientific*). Specimens were kept at room temperature to optimize recovery of *N. meningitidis* and transported to the microbiology laboratory at the Children's Hospital of Eastern Ontario within 12 hours of collection.

The following culture media were inoculated and incubated at 35°C: a modified Thayer-Martin agar plate (*PML Microbiologicals*) was incubated in 5% CO₂; a 5% Columbia sheep blood agar plate was incubated in 5% CO₂; and a 5% Columbia sheep blood agar plate was incubated anaerobically. The modified Thayer-Martin plates were examined after two and three days incubation for the presence of *N. meningitidis* and *N. lactamica*. Using standard microbiological procedures⁸⁰, *N. meningitidis* and *N. lactamica* were identified by oxidase carbohydrate utilization and O-nitrophenyl β-D galactopyranoside tests. All *N. meningitidis* isolates were also identified by the Vitek System (*BioMérieux USA*) and

forwarded to the National Neisseria Reference Centre (LCDC) for confirmation, which included serotyping, subtyping, and multilocus enzyme electrophoresis.

3.7.2 Serologic Response

3.7.2a Enzyme-Linked Immunosorbant Assay

The ELISA was carried out as previously described for the detection of antibodies to groups A and C polysaccharides^{60,81}. Equal volumes of methylated human serum albumin and groups A or C polysaccharides (*Connaught Laboratories*) dissolved in coating buffer consisting of 10 mM phosphate-buffered saline (PBS), pH 7.4, were mixed dropwise to give a final concentration of 5 $\mu\text{g/ml}$ each. The mixtures were used to coat (100 μL per well) Immulon 1 microtiter plates (*Dynatech Laboratories*). The plates were sealed and stored overnight at 4°C. Plates were washed 3 times with wash buffer composed of 10 mM PBS containing 0.1% Brij 35. Then 200 μL of serum-conjugate (S-C) buffer (5% newborn bovine serum, 0.1% Brij 35, and 0.05% sodium azide) were added to each well. Plates were sealed and kept at room temperature for 1 hour and contents emptied. Serum dilutions (total volume 150 μL) were first made by well-to-well transfer in U-bottom plates and mixed 7-10 times in each well. Then 100 μL of each serum dilution were transferred to Immulon 1 microtiter plates which were sealed and incubated

overnight at 4°C. Plates were washed 3 times in wash buffer, then 100 μ L of alkaline phosphatase-labelled affinity-purified goat antibody to human immunoglobulins G, A and M (TAGO Lot #2501) diluted 1:2000 in S-C buffer, were added to each well. The plates were incubated for 2 hours at room temperature and washed 3 times in wash buffer: 100 μ L of substrate (1 mg of p-nitrophenyl phosphate [Sigma] per ml of 1 M Tris, pH 9.8, containing 0.3 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) were added to each well. The plates were incubated for about 20-30 minutes at room temperature and the enzyme reaction was allowed to proceed to an absorbance value of about 2.5. The reaction was stopped by adding 100 μ L of 0.4 M NaOH to each well and storing the plates in the dark for 10 minutes. The absorbance values were read at 405 nm on a computer operated *BioRad Microplate reader (3550)*. Antibody values were calculated by extrapolation from a standard serum dilution curve using a logit-log plot. The standard serum was obtained from Dr. G. Carlone (CDC) and contained anti-groups A and C polysaccharide antibody of 3754 units/ml and 21,769 units/ml respectively. Each serum dilution was assayed in duplicate and the total group A or C antibody (units/ml) was determined by averaging all values (4-6) that fell within the linear region of the standard dilution curve (absorbance values between approximately 0.05 and 2). Values were within a coefficient of variation of $\leq 15\%$.

To convert the total group C ACPS antibody response (Antibody Units/ml) to the

concentration of antibody measured (micrograms/ml) the following transformation was used: 3400 Antibody units/ml = 32 micrograms/ml (personal communication Dr. G. Carlone, CDC). The minimum level of detection reported for total ACPS antibody was 0.10 $\mu\text{g/ml}$.

3.7.2b Bactericidal Assay

The microbactericidal assay⁸² was modified according to CDC protocol (Dr. G. Carlone, personal communication). Sera were heat inactivated at 56°C for 30 minutes and then diluted two-fold serially in Gey's balanced salt solution (GBSS-Gibco) plus 0.2% gelatin (GBSS-B) so that 50 μL of serum dilution were present in wells of a microtiter plate. Complement (25 μL of 3-4 week baby rabbit serum, *Pel Freeze Biologicals Lot 15912*) was added to each well. *N. meningitidis* strain 60E (NT:P1.1) was grown overnight at 36°C in 5% CO₂ on Mueller-Hinton agar containing 5% normal horse serum (MHSA), then subcultured on MHSA and grown for 4 hours as above. Bacteria were suspended in GBSS-G (O.D.₆₀₀ = 0.23) and 25 μL of a 1:2000 dilution of cell suspension were added to wells so that about 100 colony forming units were present in each well. For each experiment a positive control consisted of anti-group C rabbit serum and two negative controls which included GBSS-G, complement and bacteria (used as the time zero (To) count), and GBSS-G, human serum, heat inactivated complement and bacteria. All sera

were assayed in duplicate. After plating for To colonies, the plates were sealed and incubated at 36°C for 60 minutes on a rotary shaker (100-150 rpm). The "tilt" method was used to enumerate surviving bacteria. Briefly, an 8 channel multipipette was used to remove 10 μ L from wells. The contents were inoculated onto and allowed to run down the surface of MHSA. Plates were incubated at 36°C in 5% CO₂ overnight and colonies enumerated. The bactericidal titre was expressed as the reciprocal of the final serum dilution giving \geq 50% killing of the To inoculum.

IV. RESULTS

The results section will be presented in the following format: Demographic information, looking at the specific study and the general Ottawa-Carleton/Outaouais population will be presented first (Section 4.1). As results for oropharyngeal carriage of *N. meningitidis* and *N. lactamica* and the vaccine associated adverse events include all 2240 participants, these data are presented next (Section 4.2 and 4.3). This is followed by the results for the immune response to the serogroup C antigen of the meningococcal vaccine and the association of total ACPS with bactericidal antibody (Section 4.4).

4.1 Demographics

2240 children participated in this study with a median age of 4.7 years (range 6 months to 19.9 years). The gender ratio was 1.1:1 male to female. Mean total family size was 4.0 and the number of children per household 2.1. The median family income, derived from postal code data, was \$49,600.00 (Special Family Income Tabulations 1990. Small Area and Administrative Data Division, Statistics Canada.). Comparable data for the Ottawa-Hull region revealed that the family size was 3.8 and the number of children per household 1.2⁸³ with a median income of \$50,800.00 (Special Family Income Tabulations 1990. Small Area and Administrative Data Division, Statistics Canada.).

The percentage of children in the study group reporting allergies was 20% and reactive airway disease was 12%. The number of participants with smoking in the household was 35%. At follow-up, 86% (1922) of the participants returned at the 1 month visit and 72% (1612) returned at 1 year.

4.2 Oropharyngeal Carriage

Results for all participants are given for the oropharyngeal carriage of *N. meningitidis* and *N. lactamica* and this is displayed in and Table 1 and Figure 2.

Insert Table 1 / Figure 2 Near Here

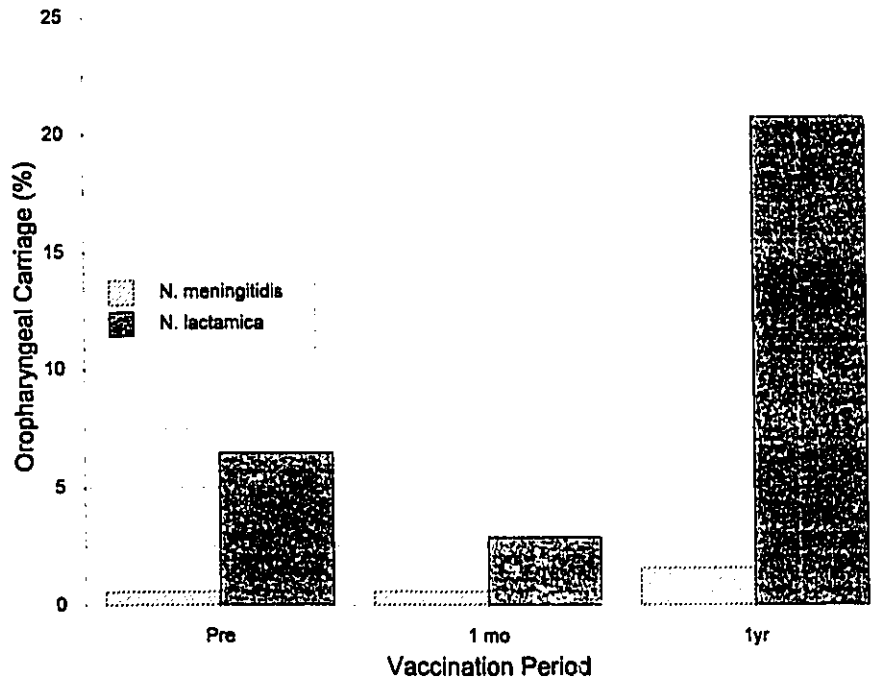
Table 1: Oropharyngeal Carriage Rate of *Neisseria meningitidis* and *Neisseria lactamica* Pre-, One Month Post-, and One Year Post-Vaccination.

	Pre	One Month Post	One Year Post	p value*
N	2240	1922	1612	
<i>N.meningitidis</i>	0.6	0.6	1.6	ns
<i>N.lactamica</i>	6.5	2.9	20.8	<0.001**

* McNemar Chi square analysis

** p value for comparison of the pre, one month post, and one year post vaccination *N. lactamica* carriage rates

Figure 2: *N.meningitidis* and *N. lactamica* Oropharyngeal Carriage: Pre, One month Post, and One Year Post Vaccination.



Pre-vaccination, 13 (0.58%) subjects were colonized with *N. meningitides*. One month post-vaccination 11 (0.57%) had oropharyngeal carriage and this increased to 26 (1.6%) at 1 year post-vaccination. Greater than 30% of the initial isolates were serogroup Y and no serogroup C isolates were identified at any time in the study. With respect to age, children older than 15 years of age accounted for 80% of the *N. meningitides* isolates. While we did not demonstrate an effect with regard to immunization on *N. meningitides* colonization, the overall carriage rates were too low to make any significant conclusions.

With respect to *N. lactamica*, 146 (6.5%) children were colonized pre-vaccination. One month post-vaccination there was a significant drop in the percentage to 2.9% (McNemar Chi Square analysis $p < 0.001$). However, one year post-vaccination, there was a statistically significant increase in the percentage of *N. lactamica* oropharyngeal carriage (20.8%) with respect to both pre-vaccination and one month post-vaccination (McNemar Chi Square analysis $p < 0.001$). As expected, 89% of the *N. lactamica* isolates occurred in children < 4 years of age.

4.3 Vaccine Associated Adverse Events

Due to differences in either observation or presumed risk for a vaccine associated adverse event (VAAE), the total study population has been broken down into three distinct

groups for reporting. An overview of each group with sample size and percent follow-up is given in Table 2. Table 3 shows the rate of VAAE for each group.

The first group consisted of healthy children vaccinated as the main study group at CHEO. This group had passive surveillance and participants were given a questionnaire (Appendix F) for completion and return at the 1 month follow-up visit. Overall, 792 (57%) of CHEO healthy participants returned the questionnaire at the one month follow-up. Within three days of the vaccination, 482 (34.7%) participants had a self-reported reaction to the vaccine. As indicated in Table 3, 97 (7.0%) reported having a fever, either $\geq 39^{\circ}\text{C}$ or subjectively 'felt warm'. 316 (22.7%) reported having a local reaction consisting of either redness, swelling, or pain while 121 (8.7%) reported redness ≥ 2.5 cm.. 31 (2.2%) reported having a rash and 26 (1.9%) reported an allergic reaction consisting of either hives, swelling, wheezing, or respiratory difficulty. Other events noted at rates $< 1\%$ included emesis, diarrhea, or headache. Ten percent sought medical help for symptomatology that they felt secondary to the vaccine. There were no reported episodes of anaphylaxis. Results for the CHEO healthy group by specific age group are given in Appendix G.

Insert Table 2 and 3 Near Here

Table 2: Vaccine Associated Adverse Events: Group Division and Follow-up.

	CHEO Healthy	Special Needs	High School	Total
N	1621	272	347	2240
Follow-up	1390	216	316	1922
Questionnaire Complete (%)	792 (57.0)	109 (50.5)	306 (96.8)	1207 (62.8)

Table 3: Vaccine Associated Adverse Events: Rate of Reported Adverse Events for the Healthy, Special Needs, and Highschool Groups.

	CHEO Healthy N = 1390		Special Needs N = 216		Highschool N = 316	
	N	%	N	%	N	%
Fever	97	7.0	20	9.3	2	0.6
Any Local Reaction	316	22.7	63	29.2	20	6.3
Local Reaction >2.5cm	121	8.7	43	19.9	16	5.1
Rash	31	2.2	11	5.1	5	1.6
Allergic Reaction	26	1.9	28	13.0	0	0
Any Reaction (Self-report)	482	34.7	180	83.3	98	31.0

The next group consisted of the children designated as 'Special Needs'. This was a high risk group of children and included those with severe environmental and drug allergies, children who had a potential allergy to the meningococcal vaccine, reactive airway disease, and immunodeficiency. Because these children have an underlying increased risk for VAAE they are considered separately. They were immunized in the Medical Day Unit at the Children's Hospital of Eastern Ontario with close medical surveillance. The results are presented in Table 3.

Overall, 216 (50.5%) of the 'Special Needs' participants returned the questionnaire at the one month follow-up. Within three days of the vaccination, 180 (83.3%) participants had a self-reported reaction to the vaccine. There was a significantly greater risk (Mantel-Hanzel Chi Square analysis $p < 0.0001$) of having a reaction in this group compared to the healthy group, though this was not unanticipated. 20 (9.3%) reported having a fever, either $\geq 39^{\circ}\text{C}$ or subjectively 'felt warm'. 63 (29.2%) reported having a local reaction consisting of either redness, swelling, or pain, while 43 (19.9%) reported redness ≥ 2.5 cm.. 11 (5.1%) reported having a rash and 28 (13.0%) reported an allergic reaction consisting of either hives, swelling, wheezing, or respiratory difficulty. There were no major adverse events or anaphylaxis reported in this group.

The final group consisted of highschool students that had evaluation of an intramuscular

(I/M) versus a subcutaneous (S/C) route of injection for both reactogenicity and immunogenicity. These subjects had detailed assessment of VAAE and were assessed at 15 minutes and 24 hours by a study nurse blinded to injection route, and were also contacted at 72 hours by telephone. There were 343 participants in this group who were randomized 2:1 with regards to S/C versus I/M injection.

Overall, the group of highschool students showed a low rate of reactogenicity to the vaccine when assessed by the study nurse. Self-reporting of adverse events demonstrates that 98 (31.0%) of the highschool participants perceived themselves as having a reaction, however this was not documented during the assessment by the study nurse. As indicated in Table 3, 2 (0.6%) reported having a fever, either $\geq 39^{\circ}\text{C}$, 20 (6.3%) reported having a local reaction consisting of either, redness, swelling, or pain, while 16 (5.1%) had redness ≥ 2.5 cm.. 5 (1.6%) reported having a rash and there were no reported allergic reactions. There was a trend towards the S/C group having a lower rate of reactogenicity but this was not statistically significant. There were no major adverse events or anaphylaxis reported in this group.

4.4 Immune Response to the Serogroup C Antigen of Quadrivalent Meningococcal Polysaccharide Vaccine

From the total study population (2240 participants), a random sample of 345 children were selected from each of these 7 age groups. The number of subjects initially tested with the bactericidal assay or ELISA and the one month and one year follow-up are given in Table 4. Participants were selected at the 1 month follow-up to ensure an adequate amount of pre- and one month-sera.

Insert Table 4 Near Here

Testing was completed on all specimens pre- and 1 month post-vaccination, except for the following: there was an inadequate amount of serum to perform the bactericidal assay for one specimen pre-vaccination in the 5-10 year age group and at one month post vaccination for one specimen in the 11-14 year age group. Despite intensive encouragement, numerous telephone and mail reminders, and the opportunity for the testing to be performed at the participant's home, 255 (72%) subjects returned at 1 year. The oldest age group (15-19.9 years) and the 12-17 month age group had the lowest percentage participation at one year.

Table 4: Immunogenicity Testing Group C Polysaccharide Antigen.
 Initial Sample and Follow-up for Bactericidal Assay and ELISA:
 Numbers of Subjects Tested per Group.

Age (Years)	Pre Vaccine		1 Month Post Vaccine		1 Year Post Vaccine	
	BA	ELISA	BA	ELISA	BA	ELISA
0.5-0.9	47	47	47	47	39	39
1.0-1.4	49	49	49	49	25	25
1.5-1.9	47	47	47	47	34	36
2.0-4.9	50	50	50	50	37	38
5.0-10.9	49	50	49	50	43	44
11.0-14.9	52	52	51	52	44	44
15.0-19.9	51	51	51	51	29	29
Total	345	346	344	346	251	255

4.4.1 Immune Response to the Serogroup C Antigen of Quadrivalent Meningococcal Polysaccharide Vaccine: Total ACPS Antibody

The results for concentration of total ACPS antibody as determined by ELISA are presented as follows: geometric mean concentration (GMC), percentage of subjects with ACPS antibody $\geq 2 \mu\text{g/ml}$, and fold increase. The results are displayed in Tables 5 and 6, and Figures 3 and 4.

Insert Tables 5 and 6 / Figures 3 and 4 Near Here

Pre-vaccination the GMC for group C total ACPS antibody was $\leq 0.26 \mu\text{g/ml}$ in all age groups. The mean GMC was $0.16 \mu\text{g/ml}$ (95% CI: 0.14 to 0.18). Overall, only 3.5% of children had a GMC $\geq 2 \mu\text{g/ml}$.

Table 5: Geometric Mean Concentration ($\mu\text{g/ml}$) of the Total Anticapsular Antibody Level (paired sera) to Serogroup C Polysaccharide Antigen: Pre, One month Post, and One Year Post Vaccination.

Age (Years)	Pre Vaccine	1Mo Post Vaccine	1Yr Post Vaccine
	GMC ($\mu\text{g/ml}$) 95%CI	GMC ($\mu\text{g/ml}$) 95%CI	GMC ($\mu\text{g/ml}$) 95%CI
0.5-0.9	0.07 0.05-1.0	3.05 2.19-4.24	1.11 0.90-1.37
1.0-1.4	0.21 0.17-0.27	5.57 4.40-7.04	0.88 0.66-1.17
1.5-1.9	0.14 0.10-0.21	5.89 4.83-7.18	1.34 1.00-1.79
2.0-4.0	0.09 0.07-0.12	6.78 5.39-8.54	2.05 1.51-2.77
5.0-10	0.21 0.15-0.29	9.38 6.62-13.27	4.46 3.09-6.43
11-14	0.26 0.19-0.37	19.22 14.08-26.24	10.81 7.54-15.51
15-19	0.19 0.13-0.28	10.29 6.86-15.45	12.60 7.23-21.95
Total	0.16 0.14-0.18	7.56 6.67-8.58	3.03 2.54-3.61

Figure 3: Geometric Mean Concentration ($\mu\text{g/ml}$) and 95% CI of the Total Anticapsular Antibody Level (paired sera) to Serogroup C Polysaccharide Antigen: Pre, One month Post, and One Year Post Vaccination.

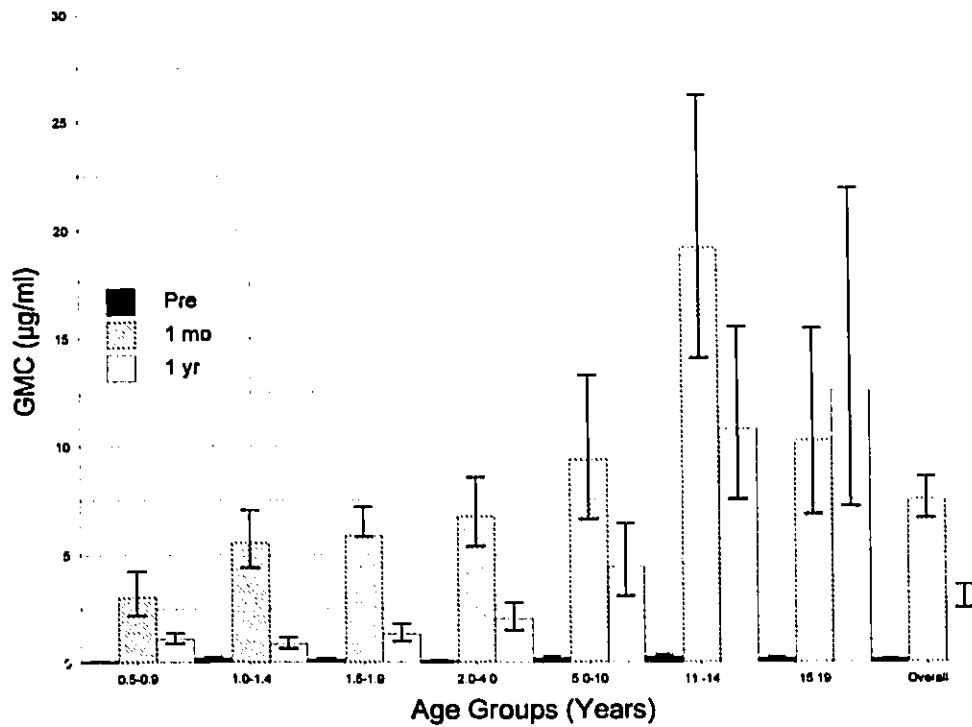


Table 6: Percentage of Participants (with 95% CI) by Age Group with Total Anticapsular Antibody Level to Serogroup C Polysaccharide Antigen ≥ 2 ($\mu\text{g/ml}$): Pre, One month Post, and One Year Post Vaccination.

Age (Years)	Pre Vaccine	1Mo Post Vaccine	1Yr Post Vaccine
	% ≥ 2 ($\mu\text{g/ml}$) 95%CI	% ≥ 2 ($\mu\text{g/ml}$) 95%CI	% ≥ 2 ($\mu\text{g/ml}$) 95%CI
0.5-0.9	0.0 0.0-2.0	68.1 54.8-81.4	23.1 9.9-36.3
1.0-1.4	2.0 0.0-5.9	87.8 78.6-97.0	16.0 1.6-30.4
1.5-1.9	6.4 0.0-13.4	89.4 80.6-98.2	33.3 17.9-48.7
2.0-4.0	0.0 0.0-2.0	96.0 90.6-100	55.3 39.5-71.1
5.0-10	8.0 0.5-15.5	90.0 81.7-98.3	81.8 70.4-93.2
11-14	5.8 0.0-12.1	100 94.0-100	90.9 82.5-99.3
15-19	2.0 0.0-5.8	90.2 82.1-98.3	93.1 83.9-100
Total	3.5 1.9-5.7	89.0 85.7-92.3	59.4 53.4-65.4

Figure 4: Percentage of Participants (with 95% CI) by Age Group with Total Anticapsular Antibody Level to Serogroup C Polysaccharide Antigen ≥ 2 ($\mu\text{g/ml}$): Pre, One month Post, and One Year Post Vaccination.

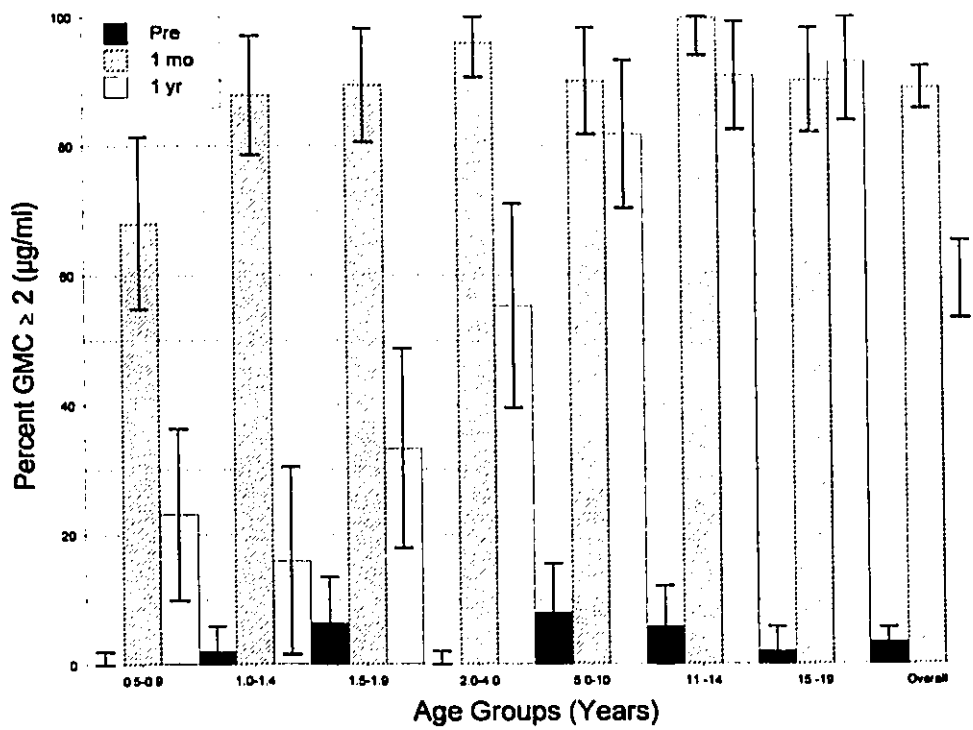


Figure 1

At 1 month post vaccination, 89% of children responded with a GMC $\geq 2 \mu\text{g/ml}$. This included 68.1% of those 6-11 months old and greater than 85% of all the older age groups. The overall mean GMC had increased to 7.56 (95% CI: 6.67 to 8.58), statistically significant at $p < 0.001$ (Repeated measures ANOVA). This represented a mean fold increase of 113 times the pre-vaccination total ACPS antibody level. A significant difference in the fold increase between the different age groups could not be demonstrated (Repeated measures ANOVA $p = 0.25$). For children 6-11 months old the fold increase was 210 times, 12-17 months old - 37 times, and 18-23 months old - 60 times the pre-vaccination level.

By 1 year post-vaccination, the overall mean GMC had declined to 3.03 (95%CI: 2.54 to 3.61). There was a significant fall (Repeated measures ANOVA $p < 0.001$) in the GMC and the percentage of children < 5 years of age who still had ACPS antibody $\geq 2 \mu\text{g/ml}$ when compared to the 1 month post-vaccination results. 33.3% of the 18-23 month olds and 55.3% of the 2-4 year olds maintained ACPS antibody $\geq 2 \mu\text{g/ml}$. Overall, there was still a fold increase of 88 times the pre-vaccination ACPS antibody level. For children 6-11 months old this represented a fold increase of 45 times, 12-17 months 6 times, and 18-23 months 14 times the pre-vaccination level. The GMC of ACPS antibody at one year post-vaccination was still significantly greater (Repeated measures ANOVA $p < 0.001$) than the pre-vaccination level in all age groups.

4.4.2 Immune Response to the Serogroup C Antigen of Quadrivalent Meningococcal Polysaccharide Vaccine: Serum Bactericidal Antibody

Bactericidal assays are presented as follows: geometric mean titres (GMT), percentage with serial dilution $\geq 1/8$, and 4-fold titre increases. Results are presented in Tables 7 and 8 and in Figures 5a,b and 6.

Insert Tables 7 and 8 / Figures 5a,b and 6 Near Here

Pre-vaccination, the GMT for the bactericidal assay was 1.17 (95%CI: 1.09 to 1.24). Overall, only 2.6% had pre-existing bactericidal antibodies with a serial dilution titre $\geq 1/8$ against group C meningococci. This ranged from a low of 0% in the 6-12 month age group, up to 7.7% in the 11-14 year old age group.

Table 7: Geometric Mean Titre (with 95% CI) of the Reciprocal Serial Dilution to Serogroup C Polysaccharide Antigen: Pre, One month Post, and One Year Post Vaccination.

Age (Years)	Pre Vaccine	1Mo Post Vaccine	1Yr Post Vaccine
	GMT 95%CI	GMT 95%CI	GMT 95%CI
0.5-0.9	1.05 1.00-1.10	2.77 2.04-3.76	2.77 2.02-3.14
1.0-1.4	1.01 0.99-1.04	2.81 1.85-4.27	2.23 1.92-2.60
1.5-1.9	1.05 0.96-1.14	7.01 3.72-13.18	11.55 7.43-17.96
2.0-4.0	1.03 0.99-1.07	18.90 10.68-33.43	39.32 27.32-56.59
5-10	1.33 1.13-1.55	145.38 73.01-289.50	54.26 26.27-112.09
11-14	1.35 1.01-1.80	411.94 243.83-695.96	97.92 50.87-188.49
15-19	1.40 1.11-1.77	533.30 281.62-1009.90	89.44 38.57-207.37
Total	1.17 1.09-1.24	33.52 24.69-45.50	22.19 16.80-29.32

Figure 5a: Geometric Mean Titre (with 95% CI) of the Reciprocal Serial Dilution to Serogroup C Polysaccharide Antigen: Pre, One month Post, and One Year Post Vaccination.

Age 0.5 - 4.0 years

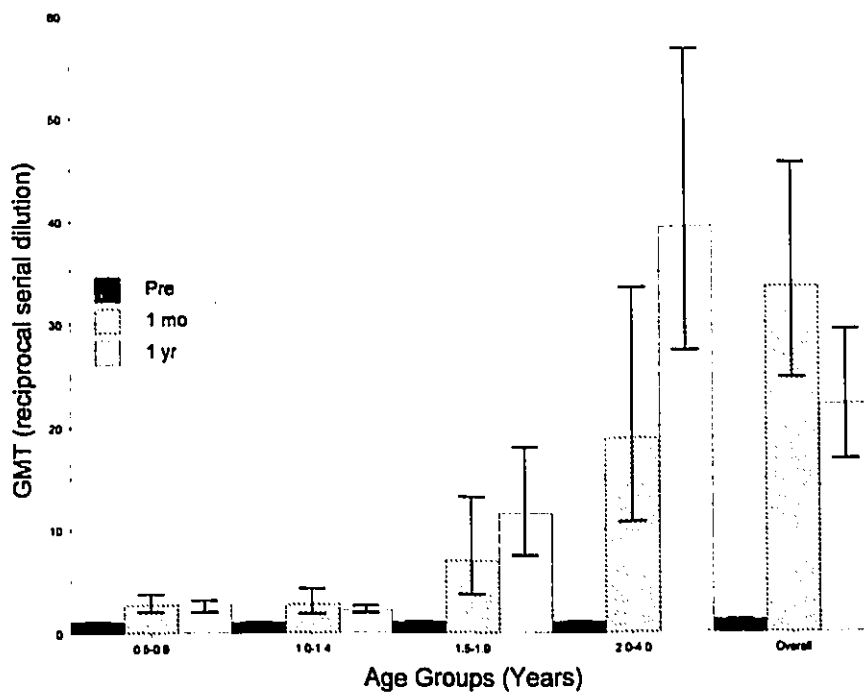


Figure 5b: Geometric Mean Titre (with 95% CI) of the Reciprocal Serial Dilution to Serogroup C Polysaccharide Antigen: Pre, One month Post, and One Year Post Vaccination.

Age 5 - 19.9 years

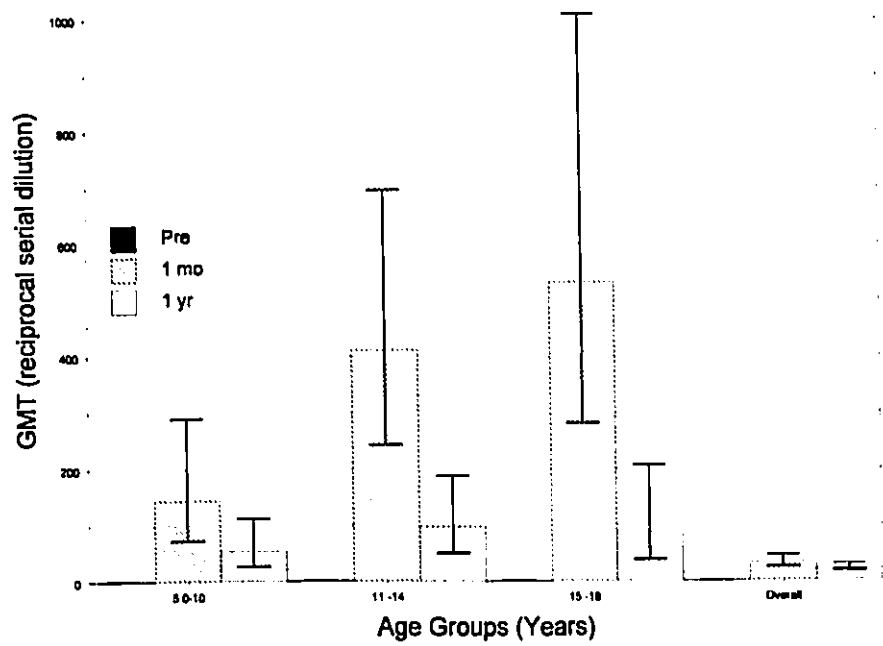
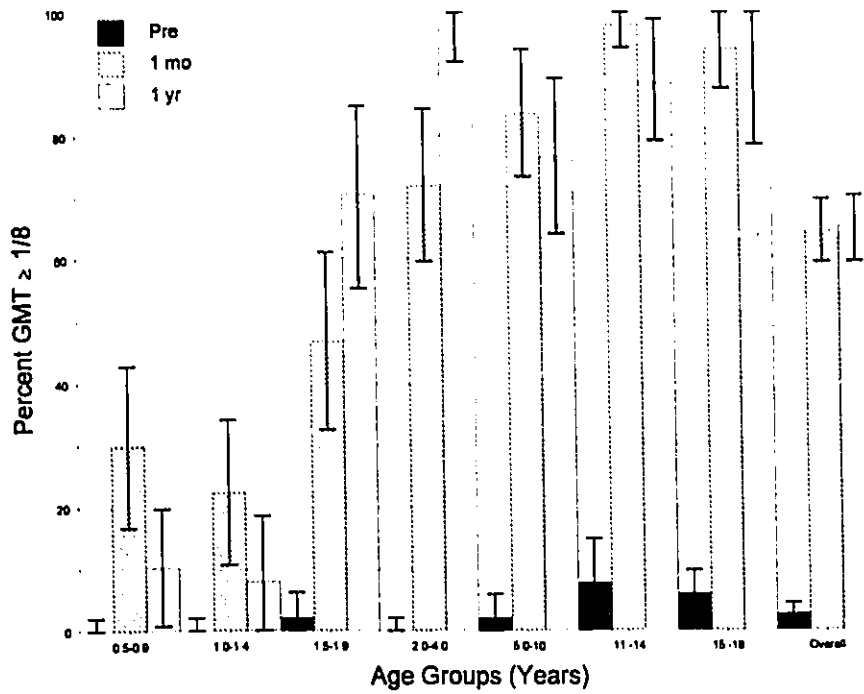


Table 8: Percentage of Participants (with 95% CI) with Bactericidal Antibody to Serogroup C Polysaccharide Antigen $\geq 1/8$: Pre, One month Post, and One Year Post Vaccination.

Age (Years)	Pre Vaccine	1Mo Post Vaccine	1Yr Post Vaccine
	% $\geq 1/8$ 95%CI	% $\geq 1/8$ 95%CI	% $\geq 1/8$ 95%CI
0.5-0.9	0.0 0.0-2.0	29.8 16.7-42.9	10.3 0.8-19.8
1.0-1.4	0.0 0.0-2.0	22.4 10.7-34.1	8.0 0.0-18.6
1.5-1.9	2.1 0.0-6.2	46.8 32.5-61.1	70.6 55.3-85.8
2.0-4.0	0.0 0.0-2.0	72.0 59.6-84.4	97.3 92.0-100
5.0-10	2.0 0.0-5.9	83.7 73.4-94.0	76.7 64.1-89.3
11-14	7.7 0.5-14.9	98.0 94.2-100	88.6 79.2-98.9
15-19	5.9 2.1-9.7	94.1 87.6-100	89.7 78.6-100
Total	2.6 0.9-4.3	64.5 59.4-69.6	65.3 59.4-70.2

Figure 6: Percentage of Participants (with 95% CI) with Bactericidal Antibody to Serogroup C Polysaccharide Antigen $\geq 1/8$: Pre, One month Post, and One Year Post Vaccination.



At 1 month post-vaccination, a statistically significant increase in the GMT was noted for all age groups (Repeated measures ANOVA $p < 0.001$). The overall GMT was 33.52 (95%CI: 24.69 to 45.50), representing a 30 fold increase over the pre-vaccination titre with a range from 2.77 in the 6-12 month old age group to 533 in the 15-19 year old age group. When explored by age group, 30% of children 6-11 months old, 22.4% 12-17 months old, and 46.8% 18-23 months old achieved bactericidal antibody titres $\geq 1/8$. By 5 years of age over 80% of children had achieved this level of bactericidal antibody activity. As shown in Table 9, a 4-fold increases in bactericidal titres was seen in 33.3% of children 6-12 months old, 27.7% 12-18 months old, and 46.8% 18-23 months old. By 5 years of age greater than 85% of children had a 4-fold increase in their bactericidal titre.

Insert Table 9 Near Here

Table 9 : Percentage of Participants with 4-Fold Increase in Bactericidal Antibody to Serogroup C Polysaccharide Antigen: One month and One Year Post Vaccination.

Age (Years)	.5-.9 %	1-1.4 %	1.5-1.9 %	2-4 %	5-10 %	11-14 %	15-19 %	TOTAL %
1mo Post	33.3	27.7	50.0	76.0	85.7	94.4	95.8	67.1
1yr Post	25.4	8.0	77.8	97.3	85.7	93.5	84.6	68.7

By 1 year post-vaccination, the GMT had decreased to 22.19 (95%CI: 16.80 to 29.32) and 65.3% of children had bactericidal antibody titres $\geq 1/8$. In children > 5 years of age, there was a significant fall (Repeated measures ANOVA $p < 0.001$) in GMT compared with the one month antibody level. However, greater than 75% of children still maintained protective bactericidal titres $\geq 1/8$ and the GMTs were still significantly greater than pre-vaccination (Repeated measures ANOVA $p < 0.001$).

In children < 18 months of age, the GMT was maintained while there was significant decrease in the percentage of children having bactericidal titres $\geq 1/8$. 29.9% of children 6-11 months old and 22.4% of children 12-17 months old maintained bactericidal titres $\geq 1/8$. In the 18-23 month and 2-4 year old groups, there was a significant (Repeated measures ANOVA $p < 0.001$) increase in both the 1 year post-vaccination GMT (18-23 month old GMT = 11.55; 2-4 year old GMT = 39.32) and the percentage children with bactericidal titre $\geq 1/8$ (18-23 months old = 70.6%; 2-4 years old = 97.3%). A 4-fold increase in bactericidal titres was maintained in 10.8% of children 6-12 months old, 8.0% 12-18 months old, and 77.8% 18-23 months old. By 5 years of age greater than 85% of children maintained a 4-fold increase in their bactericidal titre.

4.4.3 Association of Anti-Capsular Polysaccharide Antibody with Serum Bactericidal Antibody (SBA)

The association between bactericidal and ELISA testing was assessed through correlation analysis and the results are given in table 10 and figures 7-9.

Insert Table 10, Figures 7-9 Near Here

Pre-vaccination, the overall R-value for the association between SBA and total ACPS antibody was equal to 0.34 ($p < 0.001$). One month post-vaccination, the overall R-value was 0.63 with the R-value for children ≥ 18 months old: 0.67 and < 18 months old: 0.06. There was a statistically significant association between SBA and total ACPS antibody in children ≥ 18 months ($p < 0.001$). At 1 year post-vaccination, the overall association was 0.69 ($p < 0.001$).

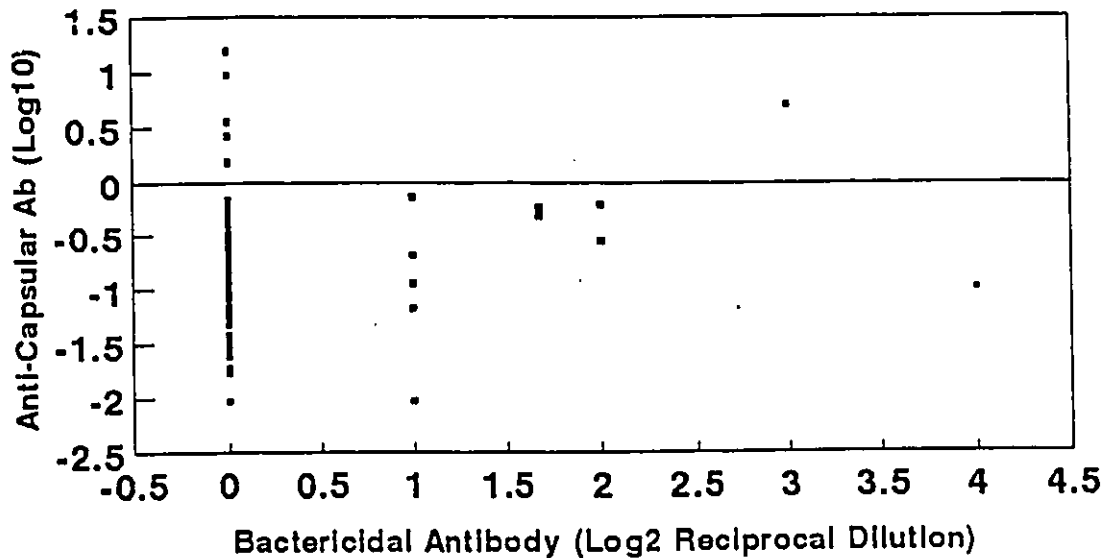
Table 10: Association of Bactericidal Assay and ELISA Response: Pre, 1 Month Post, and 1 Year Post Vaccination.

Pearson Correlation Coefficient: R values.

Age (Years)	Pre Vaccine	1 Month Post Vaccine	1 Year Post Vaccine
	R	R	R
0.5-0.9	-0.27	0.12	0.33
1.0-1.4	0.004	-0.02	0.06
1.5-1.9	0.40	0.53*	0.19
2.0-4.0	0.05	0.63*	0.15
5.0-10	-0.01	0.71*	0.61*
11-14	0.63*	0.52*	0.71*
15-19	0.43	0.81*	0.69*
< 1.5	-0.21	0.06	0.25
≥ 1.5	0.40*	0.67*	0.62*
Overall	0.34*	0.63*	0.69*

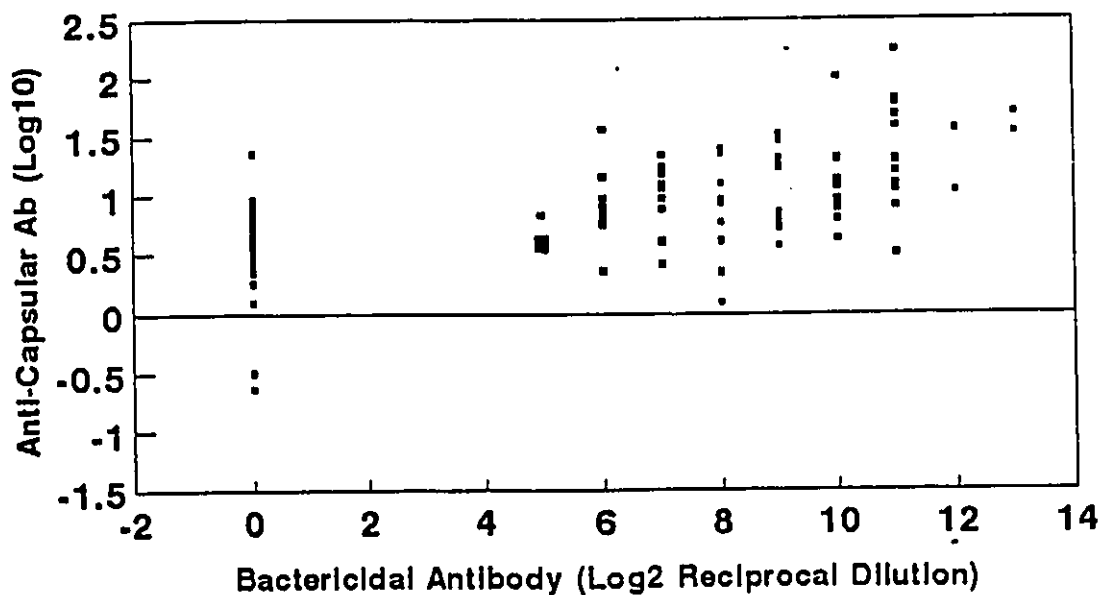
* p < 0.001

Figure 7:
 Association of Total Anti-Capsular Antibody with Bactericidal Antibody: Pre Vaccination



Correlation: Overall R = 0.34 (p<0.001)
 <18 mos R = -0.21 (p=0.04)
 ≥18 mos R = 0.40 (p<0.001)

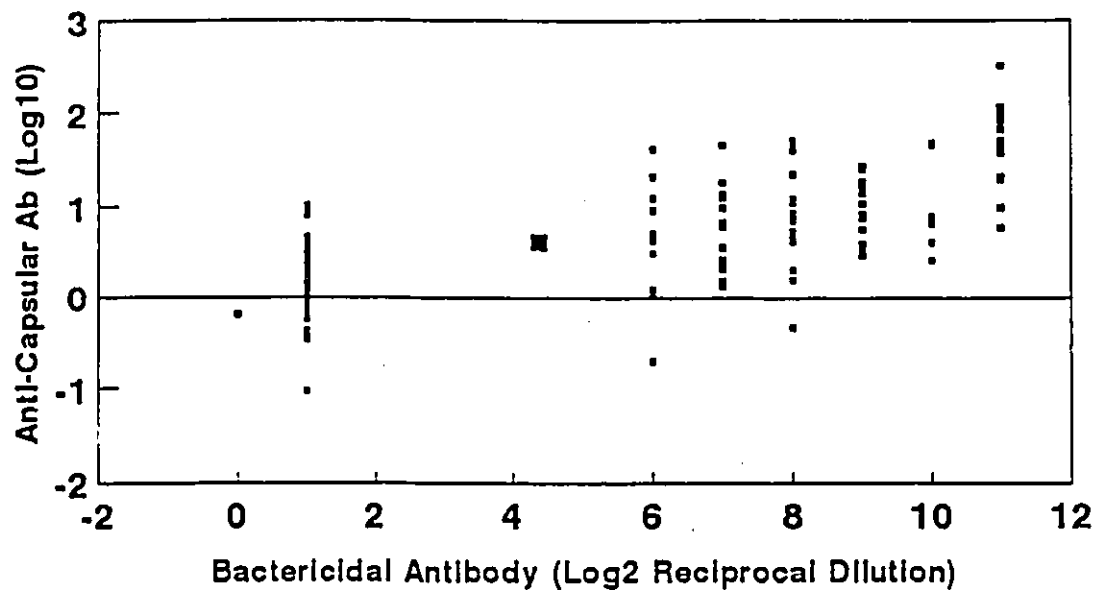
Figure 8:
 Association of Total Anti-Capsular Antibody with Bactericidal Antibody: 1 Month Post Vaccination



Correlation: Overall R = 0.63 (p<0.001)
 <18 mos R = 0.06 (p=0.6)

✓

Figure 9:
Association of Total Anti-Capsular Antibody with
Bactericidal Antibody: 1 Year Post Vaccination



Correlation: Overall R = 0.69 (p < 0.001)
< 18 mos R = 0.25 (p = 0.53)
≥ 18 mos R = 0.62 (p < 0.001)

4.5 Synopsis of Results

The rate of vaccine associated adverse events was low and the majority of events that occurred were minor. Perceived events occurred at a much higher rate than those objectively assessed. There was an effect of the vaccine to decrease oropharyngeal carriage with *N. lactamica* at one month post vaccination, however the rate of carriage actually increased at one year. The rate of *N. meningitidis* carriage was low and as a result a vaccine effect could not be determined.

With respect to antibody development, pre-vaccination the overall ACPS GMC by ELISA was low, with few participants attaining levels $\geq 2 \mu\text{g/ml}$. At 1 month post-vaccination all age groups demonstrated a significant rise in the total geometric mean concentration and in the percentage of children attaining a GMC $\geq 2 \mu\text{g/ml}$. By 1 year post-vaccination, children < 5 years of age had a significant drop in the percentage of children with a GMC $\geq 2 \mu\text{g/ml}$ while the majority of children ≥ 5 years of age maintained this level. For all age groups, the 1 year post-vaccination geometric mean concentration was significantly greater than the pre-vaccination value.

Few children had pre-existing bactericidal antibody pre-vaccination. At 1 month post-vaccination, there was a significant increase in all age groups with respect to GMT, 4-

fold antibody increase, and SBA titres $\geq 1/8$. By 1 year post vaccination, children less than 18 months of age had a significant decrease in their SBA titres $\geq 1/8$, but maintained their overall GMT. There was an actual increase in the GMT and percentage with SBA $\geq 1/8$ in the age groups 18-23 months and 2-4 years. Children older than 5 years, while showing a decline in GMT and percentage with SBA $\geq 1/8$, maintained titres that were significantly greater than pre-vaccination.

The association between the total ACPS antibody and SBA revealed that there was a low but significant association between the antibody levels pre-vaccination. There was a good association demonstrated at 1 month post-vaccination for children ≥ 18 months of age. By 1 year post-vaccination, while maintaining an overall good correlation, an association between the antibody measures could not be demonstrated for children less than 5 years of age.

V. DISCUSSION

Neisseria meningitidis is presently the leading cause of meningitis in children and young adults in the United States and an important cause of morbidity and mortality in a number of Canadian communities^{39,84}. The diverse clinical manifestations of IMD complicate early recognition and its rapid progression may lead to an adverse outcome despite appropriate management. At present immunization with meningococcal vaccine is the only known method to control an outbreak of IMD due to serogroup C.

The substantial variability in the clinical course of serogroup C IMD in Canada and the United States exemplifies our lack of knowledge concerning the epidemiology of *N. meningitidis*. Compounded by uncertainties regarding the immune response to infection, especially in young children, it is difficult to determine appropriate management strategies. In consequence, it is therefore difficult to establish specific public health guidelines to deal with outbreaks, without leaving an excessive degree of individual interpretation. While immunization with a polysaccharide meningococcal vaccine will induce production of functional, protective bactericidal antibody in adults and older children, the response in young children, especially those less than 2 years of age, is less clear. As vaccination campaigns require considerable resources, information regarding the groups that will benefit from immunization is crucial.

The purpose of this thesis was three-fold: first - to assess, in children, the immediate (one month) and longterm (one year) immune response to a quadrivalent meningococcal polysaccharide vaccine, during a mass vaccination campaign; second- to assess the association between ELISA and bactericidal assay, to quantify whether the known functional antibody was associated with the total ACPS antibody response; and third- to explore the effect of vaccination on the oropharyngeal carriage of *N. meningitidis* and *N. lactamica*. Finally, though the vaccine is considered to be very safe, the rate of adverse events was examined.

The vaccine was safe in this study, the rate of vaccine associated adverse events being low. In the total study population no serious adverse events were documented and the overall rate of reactogenicity was 20.8%, which is consistent with previous studies of meningococcal vaccination. Of note, while participants self-reported a relatively high rate of adverse events, this could not be substantiated in the subgroup of students with careful, objective VAAE assessment. This reflects the subjective nature of self-reported assessments, particularly regarding the use of vaccines, where the general public may already have preconceived ideas of potential adverse events and the overall anxiety in the community during the IMD outbreak. In our study population, despite the vaccine providing reassurance to parents that their child may be protected from IMD, parents appeared to be more concerned regarding vaccine side effects in children less than 12

years of age than the chance of their child developing the illness (personal communication Dr. I Manion, submitted for publication).

The overall meningococcal carriage rate in our study was low. Despite the impression that oropharyngeal carriage rates increase during outbreaks, our findings are in keeping with previous reports which have documented low carriage rates, of both the involved clone and of total meningococci, in communities during serogroup C IMD clusters^{10,29}. Also, the majority of children in our study were less than 10 years of age and would be expected to have a low rate of *N. meningitidis* carriage. The low carriage rate observed in our study population precluded the potential to reach any useful conclusions regarding the effect of vaccination on the oropharyngeal carriage of *N. meningitidis*.

With respect to the influence of vaccination on the carriage of *N. lactamica*, the rate of carriage initially decreased at one month, then increased at one year. The implication of this observation is unknown. *N. lactamica*, a nonpathogenic organism, has an important association with the development of natural protective immunity against *N. meningitidis* by means of cross-reacting antigens. If vaccination induces local mucosal immunity that can decrease *N. lactamica* carriage, then it also could conceivably decrease *N. meningitidis* carriage. This may then confer a broader benefit from the vaccine by providing not only systemic immunity but also decreasing the burden of transmittable

organism in the community. The effect of this added protection may be small. Despite looking at over 2200 children the *N. meningitidis* carriage rate was very low (at the time of the outbreak) and the majority of the isolates were of probable low virulence (79% - Serogroup Y or nonagglutinable). Conversely, if the vaccine effect is specific to *N. lactamica*, it may decrease the rate at which natural immunity to IMD is developing. While this issue remains unresolved, the lack of a vaccine effect on *N. lactamica* carriage at one year suggests that the vaccine's influence on oropharyngeal carriage of *N. lactamica* is temporary.

The serologic results of this thesis provide data on the immediate and long-term immunologic response to this vaccine in children. The first finding of note is that children in all age groups demonstrated a significant rise in total meningococcal anticapsular polysaccharide antibody. If an ACPS antibody response $\geq 2\mu\text{g/ml}$ is associated with protection, then regardless of age, vaccination with this quadrivalent meningococcal polysaccharide vaccine would have protected the majority of children in this study against meningococcal disease. One consideration is that the total antibody measured may simply represent nonspecific, nonfunctional antibody. However, to address this we performed a competitive inhibition assay on a random sample of study subjects that demonstrated meningococcal group C polysaccharide was able to absorb 100% of the group C ACPS antibody measured by ELISA in children < 11 years old and

greater than 97% in children > 11 years old (Dr. Fraser Ashton, LCDC). Therefore, the ACPS antibody measured by ELISA is specific to the group C meningococcal polysaccharide.

By one year, the majority of children less than 5 years of age had a significant fall in their ACPS antibody levels and whatever protection that may have been conferred at one month had almost disappeared. However, in all groups less than 5 years of age, the one month and one year geometric mean concentration was significantly greater than both the original subject pre-vaccination level and when compared at one year to the pre-vaccination antibody levels of children in the same aged group.

Results for the bactericidal assay, which measures functional antibody and has a more clearly defined protective level, revealed that there was a significant response in all age groups greater than 18 months old at both one month and one year post vaccination. While the response in the younger age group was not as dramatic, greater than 20% developed protective levels of bactericidal antibody. Once again, in all age groups the one month and one year response were significantly greater than both the original subject pre-vaccination level and when compared at one year to the same age group pre-vaccination.

The increased bactericidal response at one year in both the 18-23 and the 2-4 year old groups is intriguing. To explore this further, results for the all the one year responders were repeated and except for three subjects they were all within the standard error of the measurement for the bactericidal assay (one serial dilution). Also, the validity of the test was measured with each assay using both positive and negative controls. I am presently qualitatively reviewing each of these subjects to assess if they differ from the main study group. One possibility is the influence of cross reacting organisms promoting an enhanced immune response in this group of children, especially in light of the increased *N. lactamica* carriage at one year.

The association between the ACPS antibodies and the bactericidal antibodies in children greater than 18 months old, suggests that this test may be used as a surrogate measure of functional antibody in older children. The lack of correlation in children less than 18 months of age, despite the good and specific ACPS response, suggests that these children do not make adequate amounts of bactericidal antibody. This does not preclude the development of other types of functional antibody in these children, such as opsonic antibodies, which may be beneficial in protection against IMD. Further investigation is required in these young children to define the immunoglobulin class-specific antibodies formed and whether they may be protective. It is possible that these antibodies may also contribute to the 'unexplained variability' in the correlation analysis.

Methodologic concerns regarding this thesis concern the factors that may influence the internal validity (comparability) and the external validity (generalizability) of the results. The methodological issues of concern that I will discuss will be related to factors that have influenced the study design, study participants, and the laboratory measures.

A before-after intervention study was designed after the announcement of the health department's vaccination campaign for the entire Ottawa-Carleton/Outaouais region. Though the vaccination program was not mandatory, it was felt at the time that a randomized clinical trial would be impracticable, in light of the extremely tight time-limit for start-up, and unethical, given the increased activity of IMD and the heightened public and media awareness. Therefore the decision was made to proceed with a cohort of participants that could be closely followed after receiving vaccination.

Specific strengths of the study include the following: precise knowledge of the baseline state of both the participant's immune status and oropharyngeal carriage of *N. meningitidis*; a definite, known and controlled exposure (vaccination); and a hard outcome (seroconversion). Another specific strength is the ability of each participant to serve as an internal control with respect to seroconversion and vaccine response. Finally, we feel that the follow-up rate at both one month and one year post-vaccination was excellent considering the voluntary nature of the study.

Limitations of the study design include the increased risk of analytical bias, especially with regards to the sample selection. For known variables, it is unlikely that confounding bias has a role in this study as there is no increased susceptibility for the study participants to respond to the vaccine compared with any other similar population of children and there are no other factors that would specifically accompany the vaccination and affect the response. Though there is the possibility that these children may have acquired natural immunity in this period of study, we looked specifically at carriage of the organisms, *N.meningitidis* and *N. lactamica*, felt to play a role in antibody development. Also, we were able to compare children post-vaccination to same aged or older children pre-vaccination. Other than the specific intervention accounting for the results that we have attained (see discussion of laboratory methods below), it is extremely unlikely that any other factor may have accounted for the immune response that has been demonstrated in this thesis.

The participants of this study were members of the population considered at risk for IMD by the health department and therefore eligible to receive vaccination. While they do not constitute a random sample taken from the population, they are comparable, by the demographic information, to similar aged individuals from the Ottawa-Hull area. The idea of random subject selection from the population with regards to vaccine response and serologic measurement may not be as important in this study as factors

that may have influenced vaccine response were either actively excluded (eg. immunodeficiency and acute or chronic illness) or very unlikely to occur in a developed country (eg. malaria or severe malnutrition).

With respect to the laboratory analysis, both the ELISA technique and bactericidal assay have potential problems that have been decreased as much as possible by running positive and negative controls with each assay, testing of duplicate sera, and strict adherence to each assay's protocol. Technical considerations regarding the standardization of the ELISA method in children, extrapolating the protective antibody concentration from previous studies, and relating the ACPS antibody measurement to functional antibody complicate the interpretation of the total antibody response. Dr. Carlone (CDC Atlanta, Georgia) is presently verifying the standardization of the ELISA in children and our results are comparable to ACPS antibody response that they have observed (personal communication). Also, the bactericidal assay is technically difficult, in particular related to the method by which the meningococcus is grown for the assay. Specific care was observed with the bactericidal technique to ensure that the methods utilized would provide the most reliable results.

In summary, this thesis has demonstrated that a quadrivalent meningococcal polysaccharide vaccine can induce a good immune response in all individuals, 6 months

to 19 years old, during a mass immunization campaign for increased IMD activity. The immune response is associated with one measure of functional antibody at one month in children greater than 18 months old, while younger children did not demonstrate this association, despite adequate levels of meningococcal specific ACPS antibody.

VI. IMPLICATIONS AND UNRESOLVED ISSUES

This thesis has provided insight, in healthy children, of the immune response induced by the serogroup C antigen, from a quadrivalent meningococcal polysaccharide vaccine (*MenomuneTM A/C/Y/W-135*), during a mass immunization campaign. The overall implication is that an adequate meningococcal specific ACPS antibody response was seen in children greater than 6 months of age and a protective bactericidal response was demonstrated at one month and at one year in children older than 18 months. In children less than 5 years of age the total ACPS antibody response is short lived and the majority of children less than 18 months old would be considered unprotected at one year. If disease activity persists in the community or recurs, and children less than 18 months are considered at risk, they may require re-vaccination at one year. The total ACPS antibody response was not correlated with bactericidal antibodies until the children were greater than 18 months of age. Therefore, in the assessment of candidate meningococcal vaccines, the enzyme-linked immunosorbant assay may be a surrogate measure of functional antibody response in children greater than 18 months of age but not in younger children.

Despite the advances that have been made in the development of vaccines against IMD our lack of knowledge regarding the definition of a protective antibody level hinders

further progress. The existing techniques for assessing immune response are all surrogate measures that still require standardization in children. Because the vaccine is less immunogenic in younger children it is important to define the mechanism of protection, the immunologic factors involved, and accurate levels of protection. While we know that both bactericidal and opsonic antibodies are important to protect against IMD and the vaccine will induce formation of the three major immunoglobulin classes with both antibody properties, the opsonic response has not been adequately investigated. The lack of correlation of total antibody to bactericidal antibody in young children requires additional exploration to define the immunoglobulin class-specific antibodies formed and whether they may be protective. Other areas that also require investigation include characterization of the specific immunoglobulins formed by age group, the antibody affinity in young children, and the possible influence of IgA blocking antibody. At present, we are planning to follow the participants of this study at three and five years to better define the duration of vaccine response and also, to further evaluate the older subjects (>5 years old) in our study, who did not have an adequate immune response to the vaccine .

This thesis has accumulated a considerable amount of knowledge regarding the immune response of healthy children to meningococcal polysaccharide vaccine. However, if we are to make meaningful progress to prevent this infection in children, further work is

required to standardize present immunologic measures, define the protective antibody level, determine the duration of vaccine protection, and explore the discrepancy between the response in older as compared to younger children. The lack of a definitive, controlled efficacy trial has resulted in meningococcal guidelines that are open to interpretation and though ethically and politically sensitive, an efficacy trial should be considered if another mass immunization campaign is to be undertaken. Without knowing the true benefits in young children, the lower age for immunization varied by province during the provincial health department vaccination campaigns. As adolescent clusters may forbear a widespread community outbreak, and children less than 5 years of age normally constitute the major risk group for meningococcal disease, a number of regions elected to vaccinate children as young as 6 months of age. In the event of another cluster of IMD warranting vaccination, the vaccine program could be directed toward the 'high risk' adolescent group and children less than 5 years of age offered the opportunity to participate in a controlled efficacy trial of polysaccharide meningococcal vaccine. This would be invaluable to define the vaccine failure rate, the protective level of antibody response, and to guide future decisions regarding immunoprophylaxis; both routine and during disease outbreaks. Finally, the success of the *H. influenzae* type b conjugate vaccine, provides incentive for the development of a meningococcal conjugate vaccine to assist in the eradication of invasive group C meningococcal disease.

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VIII APPENDICES

Appendix A: Meningococcal Disease in the Ottawa-Carleton/Ontario Region: Dec. 1990 - Jan. 1992.

ONSET	SEROGP	REGION	AGE	GENDER**	OUTCOME*
29-12-90	C	OTTAWA	16 years	F	D
29-01-91	C	OTTAWA	18 years		S
10-03-91	C	OUTAOUAIS	6 years	M	S
07-04-91	C	OTTAWA	15 years	M	S
10-04-91	B	OTTAWA	2 months	M	S
02-07-91	C	OTTAWA	14 months	M	D
12-08-91	C	OTTAWA	29 years		S
07-09-91	B	OTTAWA	7 months	F	S
22-10-91	C	OTTAWA	17 years	M	S
23-10-91	C	OUTAOUAIS	38 years	M	D
06-11-91	?	OUTAOUAIS	3 years	M	D
21-11-91	C	OTTAWA	2 years	F	S
26-11-91	?	OTTAWA	16 years	F	S
09-12-91	C	OTTAWA	15 years	M	D
10-12-91	C	OTTAWA	15 years	F	D
11-12-91	C	OTTAWA	17 years	M	S
11-12-91	?	OTTAWA	14 years	F	S
18-12-91	C	OTTAWA	15 years	M	D
26-12-91	C	OUTAOUAIS	16 years	F	D
02-01-92	B	OUTAOUAIS	6 months	F	S
03-01-92	B	OUTAOUAIS	2 years	M	S
05-01-92	C	OUTAOUAIS	37 years	M	D
07-01-92	C	OTTAWA	19 years	M	S
07-01-92	C	OTTAWA	19 years	M	D
08-01-92	C	OUTAOUAIS	17 years	M	S
10-01-92	?	OUTAOUAIS	14 years	F	S
17-01-92	C	OTTAWA	2 months	M	S
20-01-92	Y	OTTAWA	14 years	M	S

* D = Died; S = Survived

** M = Male; F = Female

Appendix B: Group C Meningococcal Vaccine Studies in Children

Author (Year)	Vaccine	N	Age	Test	Outcome
Lepow 1986 Ref#:78	A,C,Y,W-135	73	2-12yr	Bact RIA	<u>SE</u> : Local Rcn 40% <u>Carriage Rate</u> : NM - 1 nontypable <u>Response</u> :Pre vaccine levels low 1mo:Good response A,C > Y,W-135 1yr:Sig decline BA IgG > baseline
Cadoz 1985 Ref#:85	A,C,Y,W-135	26	3-13yr	Bact	<u>SE</u> : 80% - mild <u>Response</u> :Pre vaccine A levels high; C low 1mo:100% seroconverted
Peltola 1985 Ref#:73	A,C,Y,W-135 OAc+ vs OAc-	118	6-23 mo	Bact	<u>SE</u> : 50% - mild <u>Response</u> :Age dependent good booster response OAc- > OAc+ but NS
Picnichero 1985 Ref#:86	C OAc+ vs OAc-	18	6mo	RIA	<u>SE</u> : OAc+ - Nil OAc- - 71%(mild) <u>Response</u> :No difference OAc- vs OAc+
Andre 1983 Ref#:72	A,C,Y,W-135 OAc+ vs OAc-		6- 24mo	Bact	<u>SE</u> : Mild <u>Response</u> :OAc- > OAc+ Y = W-135 = A > C 6-11mo old:short lived
Steinhoff 1981 Ref#:87	C	31	2yr	RIA	<u>SE</u> : 52% - mild <u>Carriage</u> : No NM found <u>Response</u> :Pre level low 8wk: OAc->OAc+ : NS 50wk:OAc=OAc+ ;Both ↓

Appendix B continued:

Author (Year)	Vaccine	N	Age	Test	Outcome
McCormick 1980 Ref#:88	A,C	90	Fetus Baby	RIA	Vaccinated pregnant ♂; looked at response vs unvaccinated: <u>Response:</u> Cord blood 3x> 3mo:Infant level ↓ 80% 6mo:Level =unvaccinated No immune tolerance
Gold 1979 Ref#:66	A,C	176	2yr 5yr	RIA	<u>SE:</u> Nil significant <u>Response:</u> Related to pre- existing Ab levels. Immunized = unimmunized by 2 years. GrC decline > GrA
Lepow 1977 Ref#:89	A C	20 1345	2-11yr 6-8yr	RIA	<u>SE:</u> Nil significant <u>Carriage:</u> 1 GrC NM <u>Response:</u> Similar peak Ab GrA = GrC; but ↓ GrC decline and better GrA Ab persistence
Gold 1977 Ref#:74	A,C A C	Vary	3mo 7mo 12mo 2yr	RIA	<u>SE:</u> Low: 5% irritable 2.5% erythema <u>Response:</u> GrC 3mo old: 90% 7 & 12mo old: 100% ↓ booster response
Gold 1975 Ref#:63	A C	396	2-3mo	RIA	<u>SE:</u> Total reaction 9.8% <u>Response:</u> GrC - good response at 3,7,& 12mo to primary dose. No corr. to preexisting Ab No amnestic response
Taunay 1974 Ref#:69	C	67000	6-35 mo	RIA	Random, unblinded: <u>Response:</u> No protection in children 6-23 mos; protection by 24-35mo

Appendix B continued:

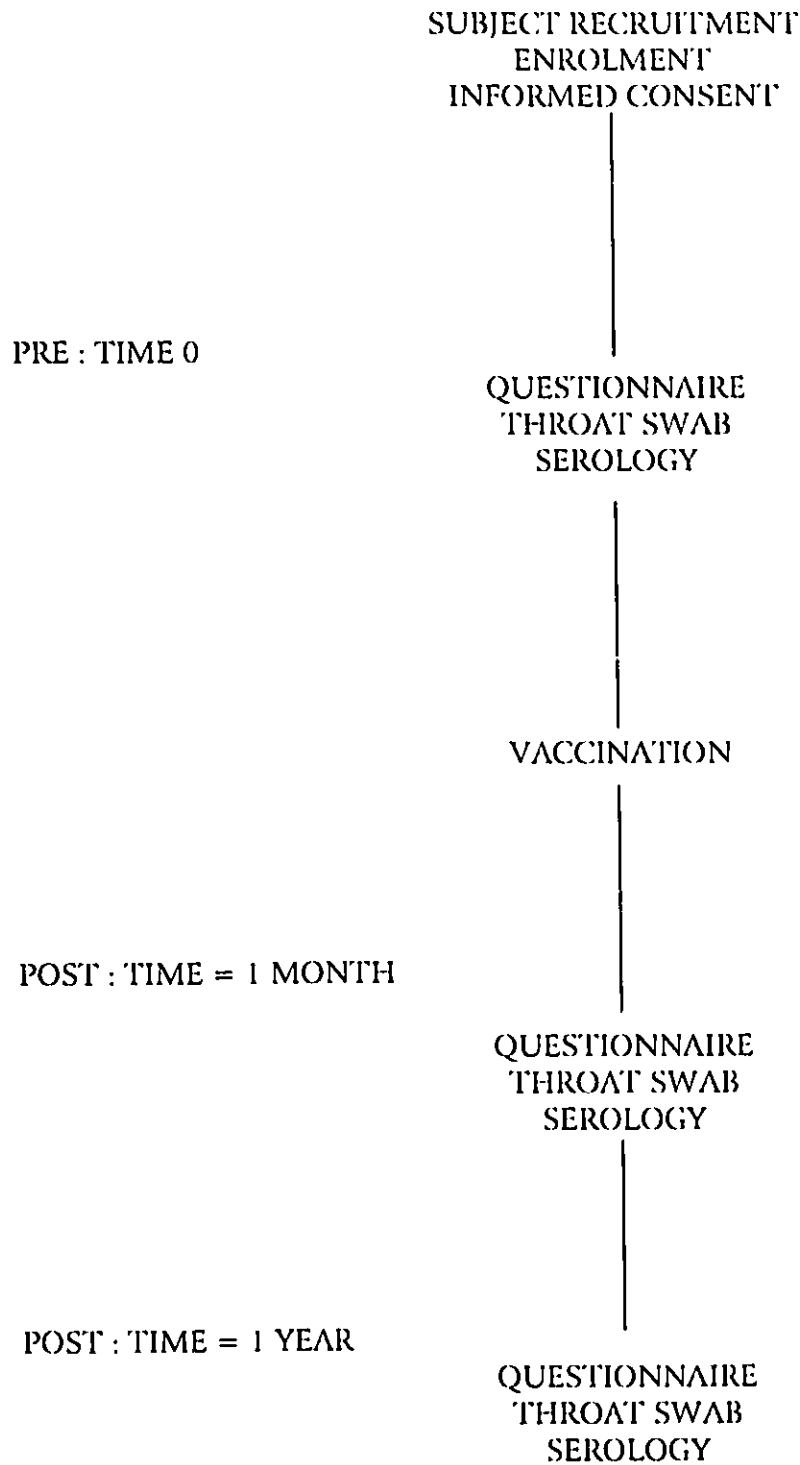
Author (Year)	Vaccine	N	Age	Test	Outcome
Amato Neto 1974 Ref#:65	C	166	6mo- 6yr	RIA	<u>Response</u> : All age groups respond to vaccine with older have better Ab levels
Monto 1973 Ref#:90	A C	102	2-6yr	RABA HA	<u>SE</u> : Fever 7.8% <u>Response</u> : Children < Adult Better with age, ↑ wt, & preexisting Ab Booster response seen
Goldschneider 1973 Ref#:91	A C	34 13 45	3mo 7mo 18mo	RABA	<u>SE</u> : Nil <u>Carriage</u> : 16% NM but only 3 typable (1-Y;2-29E) <u>Response</u> : 3,7,18mo old infants all responded. ↑ Ab at 7,18mo compared with unimmunized. No booster response. No crossreactivity with group A.
Goldschneider 1972 Ref#:64	A C	48	7mo- 9yr	II HA RABA	<u>SE</u> : 11.5% - mild <u>Carriage</u> : 17% - NM (1-C;2-Y) <u>Response</u> : Group specific antibody. All children had immune response but those < 2 years old had significantly lower immunity.

Table Abbreviations:

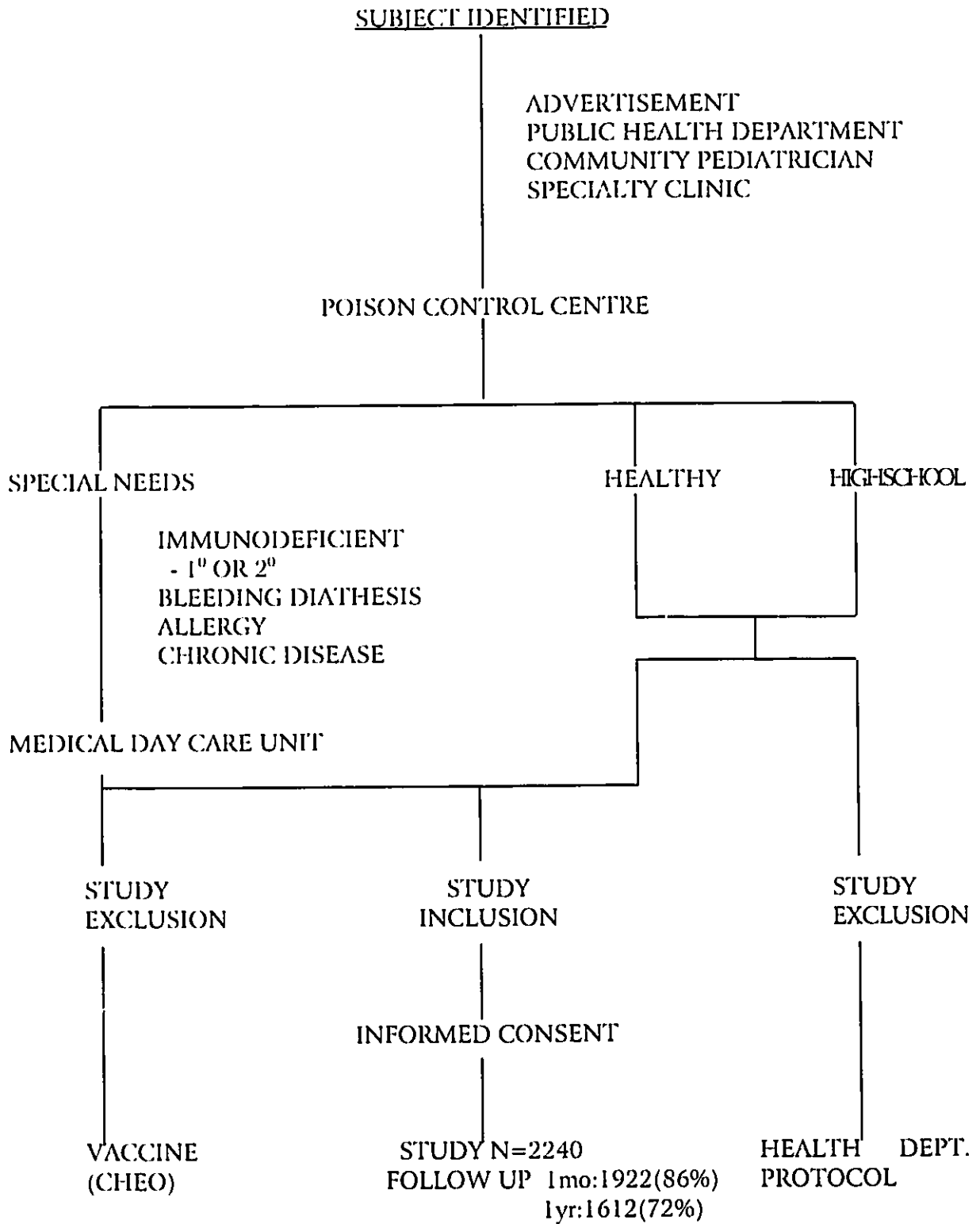
Bact: Bactericidal Assay
 NM: *Neisseria meningitidis*
 II: Indirect immunofluorescence
 RIA: Radioimmunoassay
 BA: Bactericidal Antibody

RABA: Radioactive Antigen Binding Assay
 HA: Passive Hema-agglutination assay
 OAC+: GrC O-Acetyl Group Positive
 OAC-: GrC O-Acetyl Group Negative

Appendix C: Study Outline



Appendix D: Flowsheet



Why _____

18. Previous meningococcal vaccine? _____ 1=Yes; 2=No
If yes, when _____

19. Did your child receive influenza vaccine? _____ 1=Yes;2=No
If yes, when _____
Month Year

Recent History

IN THE PAST MONTH HAS YOUR CHILD HAD

20. "Cold" _____ 1=Yes; 2=No
(eg. cough, runny nose, fever, earache)

21. Vomiting and/or diarrhea _____ 1=Yes; 2=No

22. Rash _____ 1=Yes; 2=No

23. Antibiotics _____ 1=Yes; 2=No

Reason for Antibiotic _____

Type Antibiotic _____

For Children < 12 years old

24. In school _____ 1=Yes; 2=No
Grade _____
Name of School _____
25. In nursery school _____ 1=Yes; 2=No
Half days _____
Full days _____
Name of School _____
26. In day care _____ 1=Yes; 2=No
Type _____ 1= home daycare < 10 children
2= group daycare > 10 children
Name daycare _____
27. Attends play group _____ 1=Yes; 2=No
#1/2days/week _____
Approximate # children in playgroup _____
28. Outside of school, my child plays with 5 or more different children per week _____ 1=Yes; 2=No

Appendix E cont: Questionnaire

MENINGOCOCCAL VACCINE STUDY: 1 month follow-up

- 1a. Did your child (you) have a reaction to the vaccine?
_____ 1= Yes 2=No
- b. If yes, what kind? _____ (may choose more than one)
1= fever within 3 days:
checked by thermometer T > 38 C/100.4F
felt warm, temperature not measured
2= allergic reaction:
hives, wheezing, difficulty breathing
face swelling, mouth swelling
3= reaction at injection site within 3 days
a) redness(> 1 inch (2.5 cms) in diameter)
b) pain at site
4= vomiting:
< 24 hours after injection 24-48 hours
48-72hours
5= other, specify _____
- c. Did your child (you) seek medical treatment for this?
_____ 1= Yes 2= No
If yes, specify _____ 1) visited family doctor/pediatrician
2) visited emergency room/clinic
3) called meningococcal HOT LINE
4) called emergency/hospital
5) called doctor
6) other, specify _____
2. In the month since receiving the meningococcal vaccine, has your child (you) received any antibiotics? _____ 1= Yes 2=No
If yes, what antibiotic? _____
why? _____
how long? _____
3. Has your child (you) had :
1. a rash (not related to vaccine) _____ 1= Yes 2= No
2. diarrhea _____ 1= Yes 2= No
3. sore throat _____ 1= Yes 2= No
4. fever (> 3 days after the vaccine) _____ 1= Yes 2= No

Appendix F: Reactogenicity

PATIENT INFORMATION SHEET
PLEASE RETURN THIS SHEET AT NEXT APPOINTMENT

WHY ARE WE DOING THIS STUDY?

This study is being performed to determine whether the vaccine will help you (your child) develop immunity against meningococcal disease. In order to tell if you (your child) developed immunity we **HAVE** to repeat the blood test in four weeks. The second blood test is very important for us to answer the question of whether the vaccine works or not. We will notify you with the results of the test when they become available. The response to the vaccine is variable and it will take at least two weeks for you to develop immunity. It is also important to know that the vaccine will not provide protection against all forms of meningitis and meningococcal disease.

WHAT ARE THE SIDE EFFECTS OF MENINGOCOCCAL VACCINES?

- You may experience redness, swelling, and tenderness at the site of the vaccine for up to 2 days after vaccination
- Mild fever occurs rarely
- Headache, chills, feeling unwell occurs rarely
- Those who had meningococcal vaccine less than 5 years ago may experience more severe reactions
- **PREGNANT WOMEN** - the safety of the vaccine during pregnancy has not been determined. Please discuss this with one of the nurses at the clinic if you are pregnant.

IF YOU (YOUR CHILD) EXPERIENCE(S) ANY OF THE FOLLOWING IN THE 3 DAYS FOLLOWING VACCINATION, PLEASE RECORD BELOW.

PLEASE CIRCLE YES OR NO

- | | | |
|--|-----|----|
| 1. Fever: | Yes | No |
| If yes: Date _____ | | |
| Temperature _____ | | |
| 2. Reaction at injection site: | Yes | No |
| If yes: Size greater than 1 inch (2.5 cms) | Yes | No |
| 3. Rash: | Yes | No |
| 4. Allergic reactions: | | |
| Hives | Yes | No |
| Wheezing | Yes | No |
| Difficulty breathing | Yes | No |
| Swelling of mouth | Yes | No |
| 5. Other | | |

PLEASE RETURN THIS SHEET AT NEXT APPOINTMENT

Appendix G: Vaccine Associated Adverse Events: Percent of Reported Adverse Events for CHEO Healthy Group by Specific Age Group.

	Age Groups (Years)						
	.5-.9	1-1.4	1.5-1.9	2-4	5-10	11-14	15-19
Fever	16%	13%	14%	16%	6%	5%	0%
Any Local Reaction	39%	48%	32%	35%	37%	57%	32%
Local Reaction >2.5cm	10%	17%	11%	10%	19%	31%	21%
Rash	9%	1%	4%	4%	5%	2%	6%
Allergic Reaction	3%	6%	4%	4%	5%	7%	16%
Any Reaction (Self-report @ 1 month)	34%	37%	31%	30%	36%	36%	0%