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EVALUATION OF RISK FACTORS
ASSOCIATED WITH DIMINISHED IMMUNE RESPONSE
TO *HAEMOPHILUS INFLUENZAE* TYPE b PRP-D VACCINE
AMONG INUIT INFANTS
OF THE
NORTHWEST TERRITORIES

by

NANCY JEAN WILLIAMSON

Thesis submitted to
the School of Graduate Studies and Research
in partial fulfilment of the requirements for the
M.Sc. degree in Epidemiology

UNIVERSITY OF OTTAWA

August 1993



Nancy Jean Williamson, Ottawa, Canada, 1994



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ABSTRACT

Haemophilus influenzae type b (Hib) is the most important cause of serious invasive bacterial disease in young children in many countries, particularly industrialized countries. The intensive search for an effective vaccine against this organism has continued for nearly two decades. Immunogenicity and efficacy trials among North American Aboriginal infants, who are considered to be among the most severely affected, with disease rates of up to ten times the national level, have been disappointing. A 1986 study¹ using PRP-D vaccine among Aboriginal infants in the Northwest Territories (N.W.T.) reported that all ethnic groups responded poorly, but the proportion of Inuit (44%) who responded with protective anti-PRP antibody levels of $\geq 0.15 \mu\text{g/mL}$ was smaller than that of the Dene (60%). This study was undertaken to explore possible reasons for the poorer results of the Inuit infants.

Design. To explore differences between the Inuit and Dene infants who had received the Hib vaccine, a secondary analysis of the data from the 1986 N.W.T. safety and immunogenicity trial was conducted. A design which combined cohort and cross-sectional elements was employed. Stratified analysis using Mantel-Haenszel Summary Odds Ratios was used to explore whether ethnicity, or any of the other available variables, was associated with the reported lower immune response.

Results. The power of the study to detect differences between the ethnic groups was limited, and statistical significance was generally not achieved in the analyses due to inadequate sample size. The results suggested that the difference between two vaccine lots and sex, and possibly age and region, were implicated in the differences between the groups.

Conclusions. The research did not entirely achieve a resolution of the part which ethnicity and other factors played in Inuit response to PRP-D vaccine. The importance of the study, however, was that, by examining the data in more detail, factors other than ethnicity were identified as potentially having an effect on the poor immune response of the Inuit infants in the 1986 N.W.T. study.

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To my dear friends and family, I give heartfelt thanks for their patience, love and advice during this part of my life challenge.

I would also like to remember the nurses of Northwest Territories who have provided excellent service to the people of the North for many decades.

ABBREVIATIONS

HbOC	Conjugate vaccine of Hib oligosaccharide with non-toxic, mutant diphtheria toxin, CRM ₁₉₇
Hib	<i>Haemophilus influenzae</i> type b
N.W.T.	Northwest Territories
PRP	Hib polyribosylribitol phosphate vaccine
PRP-D	Conjugate vaccine of Hib polyribosylribitol phosphate with diphtheria toxoid
PRP-OMP	Conjugate vaccine of Hib polyribosylribitol phosphate with outer membrane protein complex of <i>Neisseria meningitidis</i> group B
PRP-T	Conjugate vaccine of Hib polyribosylribitol phosphate with tetanus toxoid
≥	Equal to or greater than ...
μ/mL	Microgram (one-millionth part of a gram, one-thousandth of a milligram) per millilitre

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1. INTRODUCTION

1.1 Importance of *Haemophilus influenzae* type b (Hib)

In the Northwest Territories, *Haemophilus influenzae* type b (Hib) meningitis is the most frequently reported cause of meningitis, and the most important manifestation of Hib invasive disease. It occurs at much higher rates, and at a younger age, than elsewhere in Canada. The high rates of disease among Inuit infants is mainly responsible for these differences.¹

1.2 The Search for an Effective Vaccine

A *Haemophilus influenzae* type b capsular polysaccharide vaccine against Hib disease, an anti-polyribosylribitol phosphate (PRP) vaccine, became available in 1985 for older children and adults. Anti-Hib antibody response to this vaccine was less than expected, especially in children less than 18 months of age. These results prompted the development of conjugate vaccines which could provide better immune response.

An immunization program using the PRP-D conjugate vaccine (PRP conjugated with diphtheria toxoid) was commenced in the Northwest Territories for children over 2 years of age and

later, as licensure requirements changed, at 18 months of age. This program was not effective in decreasing the morbidity of Hib meningitis since most Hib disease (65%) occurred in infants.¹ Disease rates, therefore, remained very high.

A 1986 intervention study in the Northwest Territories (N.W.T.) using PRP-D vaccine in infants concluded that the vaccine was safe, but that it provided only 44% of the Inuit infants with anti-Hib antibody levels greater than $\geq 0.15 \mu\text{mL}$, the minimum protective level. Other groups in the study, despite an overall poor response to the vaccine, achieved higher antibody levels than the Inuit (59.5-60%).¹

1.3 Thesis Objective

The results of the 1986 N.W.T. intervention study suggested that the Inuit may have had different, and diminished, immune response to the PRP-D vaccine based on their ethnicity. This difference was not statistically significant ($P > 0.05$). This thesis explored possible reasons, in terms of risk factors, risk markers, confounders or effect modifiers, which could answer the question "What factors, other than ethnicity, might explain the observed differences in anti-PRP antibody response between the Inuit and Dene infants?".

The sample size for the secondary analysis was small and it is possible that the results of my analyses occurred by chance. The analyses were conducted, however, because of my interest in the information in the literature related to response to vaccines and ethnicity. I felt that it would be useful to examine the Northwest Territories' data set and explore whether factors other than ethnicity might have influenced the 1986 study findings.

1.4 Study Design

The design used in this investigation was similar to the "follow-up prevalence study" design as defined by Kleinbaum, Kupper and Morgenstern², and included cohort and cross-sectional elements.

Factors believed to be associated with poor immune response were explored by conducting a secondary analysis of the data from the 1986 N.W.T. study intervention group. The data were examined by using stratified analyses and Mantel-Haenszel summary odds ratio.

In addition, literature related to North American and Circumpolar *Haemophilus influenzae* type b vaccine studies, particularly those involving Aboriginal populations, was reviewed. The purpose of the review was to identify the

factors reported to be associated with the wide range of immune responses found among the different groups studied, and to determine if ethnicity was considered to be important.

2. REVIEW OF LITERATURE

2.1 Epidemiology of *Haemophilus influenzae* type b Disease in Children Under Five Years of Age

2.1.1 Introduction

Haemophilus influenzae type b (Hib) disease has been, and continues to be, among the most important causes of serious invasive bacterial childhood infections in many countries, particularly in children under the age of five years, where 85 percent of invasive Hib disease occurs.^{3,4,5,6,7,8,9,10,11,12} The problem is greatest in those six to 12 months of age, with less than 15 percent of disease found in those under six months of age.^{3,7,11} Annual rates as high as 1100 per 100,000 have been reported depending on the country and the population which was studied.^{6,8,9,11,13,14,15}

The most frequent manifestation of Hib disease is meningitis, although countries report some variation in rates.^{3,4,6,9,11,12} Hib disease is also associated with epiglottitis, pneumonia, cellulitis, and otitis. Hib is the most important cause of all meningitis in infants.^{4,10,13} Mortality from Hib meningitis has been reported from four to ten percent, and neurological sequelae occur in 20 to 50 percent.^{3,14,16,17}

It has been found that Hib disease in Aborigines differs from non-Aborigines in various ways. Aboriginal infants have experienced as high as 38 percent of Hib disease under the age of six months, rather than the 15 percent among the general population. Furthermore, 70 to 80 percent of disease occurs as meningitis, as compared to only 49 percent among Scandinavian (Caucasian) infants.^{4,6,8,9,12,13,14,15,17,18} On the other hand, epiglottitis is a prominent characteristic of infection in some Scandinavian countries^{5,9}, but is rare or absent among Aboriginal peoples.^{8,13,15}

2.1.2 Country Specific Epidemiology of Hib Disease

In order to focus on both Northern and Aboriginal populations, I shall describe the morbidity and mortality of Hib disease in Scandinavia, specifically Finland, the United States (U.S.), Australia and Canada. Except for Australia, these countries all have extensive circumpolar geographical areas. Furthermore, the epidemiology of Hib disease, especially meningitis, has been well documented in these countries.^{3,4,5,6,9,10,11,12,13,14,15,17,18,19} Australia is also considered because of the Aboriginal population found there.⁸

2.1.2.1 Scandinavia

Scandinavia comprises Denmark, Finland, Iceland, Norway and Sweden and is considered to have "homogeneous socioeconomic conditions". In the period 1960 to 1985, the incidence of Hib disease was 49 cases per 100,000 children (Caucasian) under five years of age.⁹ More specifically, in Finland, a country in which extensive Hib vaccine trials were conducted from 1974 to present, rates of Hib disease of 52 per 100,000 were found, 40 percent of which occurred in children six to 18 months of age. Forty-six percent occurred as meningitis and 29 percent as epiglottitis. Most meningitis (54-61%) occurred between six and 24 months of age, but was mostly concentrated around the age of 12 months.^{5,6,20,21}

2.1.2.2 The United States: Non-Aboriginals

In the U.S., for the period 1976 to 1984, Hib disease occurred somewhat differently than in Scandinavia. It was similar in that it most commonly presented as meningitis (19-69 cases per 100,000), but most cases occurred during the interval of six to 12 months of age. Less than 15 percent of all Hib disease occurred under six months of age. Epiglottitis was more common after two years of age.³

2.1.2.3 The United States: Aborigines

This pattern was not found among the U.S. Aboriginal peoples. Epidemiological studies done among the Navajo and Apaches of the continental U.S., and among the Aborigines of the State of Alaska, have shown that this population has been at very high risk of Hib disease, particularly meningitis.^{13,14,15,17,18,22}

A study among the Navajo Indians, between 1973 and 1980, reported a rate of 214 per 100,000 for all Hib disease in children under 5 years, 70 percent of which was meningitis (rate 152/100,000). Eighty-one percent of the meningitis occurred under 12 months of age, with 64 percent between four and eight months. No epiglottitis was reported.¹⁴ An earlier study of the years 1968 to 1973 had reported a Hib meningitis rate of 550 per 100,000 in infants under 12 months, with few of these under three months of age. This represented 80 percent of all Hib meningitis cases reported.¹⁷

From 1973 to 1983 a study among Apache children reported an annual rate of meningitis of 254 per 100,000 in those under five years of age. All cases were under one year of age, mostly concentrated between the ages of four to seven months. No epiglottitis was reported.¹⁵

Like Scandinavia, Alaska lies for the most part above the 60th parallel. It is, therefore, considered as one of the circumpolar geographical areas. Two reports from Alaska, which covered a ten year period (1971 to 1977, 1980 to 1982), indicated that 90 percent of all Hib disease occurred in children under the age of five.^{13,18} The Eskimo children of Alaska (called Inuit in Canada) experienced a rate of meningitis ten times that of the rest of the U.S., and 68 percent of that was *Haemophilus influenzae*.¹³

In the 1980 to 1982 study, the rate for Hib disease was highest for Eskimo children at 705 per 100,000; for Indian children it was 401 per 100,000 and for non-Aboriginal groups it was the lowest at 129 per 100,000. Most disease was found in those under two years of age, particularly between six and 11 months. Up to 60 percent over the three year period was meningitis. There was no epiglottitis among the Eskimo and Indian children.¹⁸ A 1971 to 1977 study of Eskimos only reported a Hib meningitis rate of 409 per 100,000; 98 percent occurred in those less than 18 months of age, with 23 percent under six months of age. Again no epiglottitis was reported.¹³

2.1.2.4 Australia

Haemophilus influenzae infection also caused excessive rates of Hib disease among the Aboriginal children of Australia. Studies from several areas (Central, Western, Southern, Southeastern, and Northern), and covering the period 1984 to 1990, have shown that, although rates were high for Hib disease among the non-Aboriginal children under five years of age (30/100,000 in the South; 350/100,000 in Central Australia), rates among Aboriginal children under five years were considerably higher (450 - 1100/100,000).^{8,23,24} Most of the disease in Aboriginal children occurred before 12 months of age, and in one area, 40 percent occurred before six months of age.^{8,24}

2.1.2.5 Canada and the Northwest Territories

In Canada, the highest rates of Hib disease are found in the circumpolar region of the Northwest Territories (N.W.T.).^{1,12} Although the Inuit comprised 36 percent of the N.W.T. population, they had, in the period 1983 to 1986, 80 percent of the Hib disease cases. The Dene (Indian) comprised 15 percent of the population and reported 11 percent of the disease; the remaining 48 percent of the population reported nine percent. (1986/1987 N.W.T. population statistics) High disease rates have also been noted for other Canadian

Aboriginal people. For example, Manitoba's Indian people were noted to have rates 1.4 times higher than non-Indians from 1981 to 1984.⁴

In a review of the cases for the period 1983 to 1987, the general disease characteristics of the N.W.T. population were found to be similar to other European and North American populations, with some notable differences. The differences were the ages most affected and the type and proportion of presentation manifested.^{4,5,6,7,9,10,11,12,25} As in Alaska, Hib disease affected the age group six to 11 months rather than those concentrated at aged 12 months, as seen in Finland. Like the U.S. Aboriginal experience, the proportion of meningitis was higher (81%), and no cases of epiglottitis were reported.^{1,3,12,13,14,15,17,18}

It was also found that 65 percent of Hib disease occurred in those under the age of 12 months; 29 percent were under the age of five months. The Dene reported 71 percent of Hib disease under 12 months and 14 percent under five months, while the Inuit had 69 percent under 12 months and 36 percent under five months. The non-Dene, non-Inuit had no Hib disease under the age of 18 months.^{1,12}

Meningitis was reported in 81 percent of the cases of Hib disease in the N.W.T. and 88 percent of the cases were

Inuit.^{1,12} This proportion was similar to data from the N.W.T. region of Keewatin, where, for the period April 1981 to May 1984, all cases less than two years of age were among the Inuit, with 89 percent before 12 months. The rate for Inuit children under five years of age was 530 per 100,000.⁴ Two studies of infant mortality and morbidity among Inuit of the Keewatin and Baffin Regions, 1973-1978 and 1978-1983, reported rates of meningitis for children less than five years to be 410 and 530 per 100,000, respectively. A peak incidence at four to six months was noted during both periods.⁴

2.1.2.6 Summary of Hib Disease Epidemiology

Hib disease characteristics were strikingly similar among the Aboriginal children of the N.W.T. and the U.S., particularly between the Alaskan Eskimo and the Inuit of the N.W.T.. Circumpolar North American Aboriginal infants had rates of disease higher than Aboriginal infants of the continental U.S. All had rates of Hib disease and proportions of meningitis that were considerably higher than the non-Aboriginal citizens.

In order to control Hib disease it has been imperative to develop a preventive intervention which would protect infants at the ages during which they were most susceptible. It has been noted that in predominantly Caucasian populations Hib

disease was mainly a disease of older infants, while among the Aboriginal populations it affected much younger infants. Thus, the picture has been one of a very high risk among Aboriginal people, suggesting the possibility of an association between susceptibility and ethnicity. Susceptibility to Hib disease is explored in further review of the literature.

2.1.3 Factors Influencing Susceptibility to Disease

Numerous studies have suggested a variety of factors that influence a particular group's susceptibility to invasive Hib disease. These include altered immunoglobulins, genetic factors, race/ethnicity, socioeconomic status, and other host factors.

2.1.3.1 Immunoglobulins

One way in which the body protects itself from infectious diseases is through the development of immunity against them. Particular subclasses of immunoglobulins (Ig), secreted by the B-cells of the humoral immune system, are able to bind with the antigens and produce antibodies against them. If there is an inability of the immune system to carry out its function, the individual will be more susceptible to that disease for which the immunoglobulin was responsible.

Immunoglobulin IgG subclass deficiency^{26,27,28}, and the presence or absence of certain allotypes^{22,29,30,31,32}, have been implicated as factors responsible for increased susceptibility of some populations to Hib disease.

Studies done as early as 1974 showed that IgG subclass deficiency played a part in chronic susceptibility to disease, particularly that of IgG2 deficiency in relation to polysaccharide antigens.²⁶ This finding was supported by several later studies.³¹ This IgG2 deficiency interfered with antibody production upon exposure to polysaccharide antigens, regardless of whether antigen exposure was through natural means or through vaccination. Some of the studies took note of the effect of age on the normal occurrence of IgG2, as it develops only as a child matures. Maternal antibodies usually protect the infant until its own production begins. It was also noted that sex and race had an effect on the concentrations of IgG2 antibody.³¹

Ferrante (1990) reviewed the state of knowledge of IgG subclasses.²⁷ He found that particular subclasses were predominant with a particular type of antigen. For example, although IgG1 and IgG2 were predominant when viral antigens were involved, IgG1 was the most prominent IgG subclass antibody to Hib polysaccharide antigen, and not IgG2 as previously reported.

The verdict is still out on the role of IgG subclass deficiency in increased susceptibility to infection.²⁸ However, in spite of the difficulties associated with interpretation of results, there were indications that IgG subclass deficiency is an important factor.

Specific allotypes of immunoglobulin in particular races seemed also to represent differences in the availability of immunoglobulin to protect against bacterial polysaccharide antigens, both in children and in adults.³¹

2.1.3.2 Genetic Markers

In addition to genes related to immunoglobulin production, other genes have been reported to provide markers of increased disease susceptibility.³³ These genetic markers were found among particular racial or ethnic groups, such as the uridine monophosphate kinase 3 enzyme (UMPK-3) genetic variant associated with increased Hib disease among Alaskan Eskimos.²² It was acknowledged, however, that other factors, such as age, exposure and presence of antibody, could have contributed to the susceptibility.

A study, between 1971 and 1982, of 35 genetic markers among Alaskan Eskimos again reported that some genetic factors were associated with disease, and also that the G2m(n) and Km(1)

markers previously associated with susceptibility to Hib disease were not associated.²⁹ The study acknowledged the influence of age, ethnicity, breast feeding, household characteristics, geographic area and season. In a Finnish study of an all Caucasian population the G2m(n) allotype was not considered to be associated with Hib disease.³⁰

2.1.3.3. Race/Ethnicity

In the U.S., Hib meningitis has been reported to be from three to four times more prevalent in black people, and 1.6 times higher in Hispanics, than in the white population.³⁴ Three studies on different populations found a relationship between race, age group and susceptibility.³⁴ Black and white infants were reported to have a similar incidence of disease, but black children one to four years of age were 30 to 110 percent more susceptible. Rates of Hib disease among Hispanics were in between rates for whites and blacks.¹⁹

Aboriginal people in the continental U.S., in Alaska, in Canada and Australia have been shown in several studies to be especially susceptible to Hib disease, particularly meningitis.^{12,15,17,18,35,36}

2.1.3.4 Socioeconomic Conditions

Several studies agreed that, although there was potential for genetic and racial/ethnic susceptibility to disease, socioeconomic factors provided the most important means for increased susceptibility to Hib pathogens.^{33,37,38,39} Household crowding, the number of people per room in the house, the number of people sharing a bed, daycare attendance, and the presence of siblings are cited as socioeconomic factors which influence risk of disease.

2.1.3.5 Summary of Factors

There are many factors associated with Hib disease and the patterns of manifestation. These are host factors (such as age, antibody acquisition and genotype presence, race, ethnicity, history of otitis media, respiratory infections), agent factors (such as Hib strains) and environmental factors (such as socioeconomic status, number of siblings, overcrowding, day care attendance, parental smoking, breast feeding, seasonal variations, temperature, humidity).

Disease epidemiology is often stated in terms of race or ethnicity, and certain groups are definitely more susceptible than others. Their susceptibility to disease, however, may or may not be related to their race or ethnicity but to some

other factor found among that particular population, such as household crowding.

2.2 Vaccines for the Prevention of Hib Disease

2.2.1 Immunity and Vaccines

The purpose of administering vaccines is to eliminate or, at least, to lessen the consequences of diseases. This purpose is achieved by providing adequate levels of antibody against the antigen and by conditioning a response of increased antibody production to subsequent exposures.^{40,41}

2.2.2 Measuring Immune Response and Efficacy

The immune response to a vaccine generally can be measured by the ability of the vaccine to stimulate the production of levels of antibody resembling that which occur after exposure to the actual organism. An effective vaccine is one which maintains antibody levels over time.⁴²

There are as many factors which affect the immune system's response to a vaccine as there are factors which influence susceptibility to disease. Development of an adequate immune response is primarily dependent on the ability of the host to respond to the vaccine.⁴² Passively acquired antibody through

the placenta and through breast milk may inhibit production of antibodies, as may an immune system which is unable to function.

The anti-Hib antibody response levels determined to be satisfactory for short term and long term protection against disease are ≥ 0.15 μ/mL and ≥ 1.0 μ/mL respectively.^{16,43,44} When antibodies are naturally acquired in an unvaccinated population, levels of ≥ 0.15 μ/mL have been suggested as being adequate for protection. A higher level of ≥ 1.0 μ/mL is believed to be required for protection, both short and long term, in vaccinated children.^{45,46}

A vaccine is determined to be efficacious if it produces clinically beneficial results, as demonstrated in a randomized controlled trial. That is, it prevents (to an acceptable degree), or lessens the severity of, the disease for which the person is being vaccinated.^{42,47} Adequate antibody response to a vaccine does not equate with efficacy as it is the decrease in disease which indicates efficacy, not the ability to produce antibodies.^{42,48}

2.2.3 Hib Vaccines

The efficacy of the early Hib polysaccharide vaccine varied from -58 percent to +88 percent in different populations. The

negative efficacy occurred in one study which found that those who had been vaccinated had more disease than the control group.^{48,49,50,51} Later trials reported up to 90 percent efficacy, but this was found only in the older Finnish children.¹⁶

Factors of the host, agent and environment, require consideration in determining the efficacy of a vaccine.⁴⁸ Factors related to study design also affect our understanding of the efficacy. A study by Sherry for the period 1977 to 1986⁵² showed that assessing efficacy over a short period of time cannot take into account the seasonal variations in disease patterns. Mortimer (1988)⁴⁸ discussed the possibility that because different strains of Hib diseases were prevalent in different geographic areas vaccines were less efficacious in some populations than in others.

2.3 Vaccine Trials

Development of an efficacious vaccine against Hib has been a slow process. It began with the production of an *Haemophilus influenzae* type b capsular polysaccharide vaccine, polyribosylribitol phosphate (PRP), which was first used in the early 1980s. There were problems with immune response to the vaccine, especially in infants and in certain high risk

groups, and with an increased risk of Hib disease reported during a brief period following immunization.^{48,49,50,53,54}

Continued investigation produced evidence that PRP, when coupled with a protein carrier, could produce a greater anti-Hib antibody response. The first such vaccine was Hib polysaccharide conjugated with diphtheria toxoid (PRP-D).¹⁶ Others that have been developed are the Hib oligosaccharide conjugated with a nontoxic, mutant diphtheria toxin (HbOC), Hib PRP conjugated with tetanus toxoid (PRP-T), and Hib PRP with an outer membrane protein complex of *Neisseria meningitidis* (PRP-OMP).⁵⁵

2.3.1 Immunogenicity and Efficacy Results: PRP

2.3.1.1 Finnish Studies

In 1974, 100,000 Finnish children, aged three months to five years, participated in a double-blind control trial of PRP vaccine.^{16,43} The results in this predominantly Caucasian population showed that protective levels of antibody were achieved in 90 percent of children 18 months of age and over. Very few children under 18 months had protective levels.

These Finnish children have been under continuing surveillance to determine long term efficacy. Using the data from this

surveillance it was estimated in 1984 that 50 to 60 percent of Hib disease in unvaccinated children over 18 months of age could have been prevented with this vaccine.²⁰ Children under 18 months continued to be at risk.⁴³

2.3.1.2 U.S. Studies

In 1985, based on data from the Finnish trial, the PRP vaccine was licensed in the U.S. for use in children eighteen months and over.⁵⁶ American case-control studies completed after licensure reported protective efficacy to be considerably lower than that of the 90 percent found in Finnish children. Results ranged from -58 percent to 88 percent.^{49,50,51,53,56} The -58 percent was considered by Ward⁵⁷ to be a problem of statistics, ie. small sample size, and not the vaccine. An earlier PRP immunogenicity trial in 1982 among the American Navajo, aged two, four and six months, achieved protective levels of ≥ 0.15 μ /mL in 55 percent of the infants, but only after the third dose.⁵⁸ Similar results were found among the Apache infants.^{59,60}

2.3.2 Immunogenicity and Efficacy Results: PRP-D

2.3.2.1 Finnish Trials

Conjugation of PRP with diphtheria (PRP-D) provided a vaccine believed to be more immunogenic than with PRP alone. In Finland, (1985 report), a small number of infants were first immunized at three, five and seven months.⁶¹ One month after the third dose (at eight months), 50 percent had achieved levels of ≥ 1.0 μmL and 92 percent achieved ≥ 0.15 μmL .

A larger trial, during 1985 and 1986, of 60,000 infants immunized at three, four and six months of age showed that 83 percent protective efficacy could be achieved with this new vaccine.⁶² At one month after the third dose 62 percent had achieved antibody levels of ≥ 0.15 μmL and 34 percent had achieved levels of ≥ 1.0 μmL , thus infants were considered protected from Hib disease by seven months of age.

This trial continued until a total of 114,000 infants were enrolled. The 1990 report of the final results indicated that a protective efficacy of 94 percent was achieved.⁶³ However, serum antibody levels after first and second doses of vaccine were low. After the third dose 70 percent of the infants had achieved ≥ 0.15 μmL and only 40 percent had achieved ≥ 1.0

μ /mL. It is of interest to note that 80 percent achieved a concentration of at least 0.10 μ /mL.

2.3.2.2 Alaskan Trials: Aborigines

Trials using PRP-D were conducted in Alaskan Aborigines aged two, four, and six months during 1981, and from 1984 to 1988.^{64,65} One small study reported immune responses which were similar to other non-Aboriginal populations.⁶⁵ The larger study with a predominantly Aboriginal sample and controls of the same ethnic background reported that only 48 percent achieved anti-Hib antibody levels of ≥ 1.0 μ /mL and an efficacy of 35 percent.⁶⁴

2.3.2.3 Northwest Territories Trial: Aborigines

Based on results of the 1985/1986 Finnish study of Caucasian infants⁶², but before the Alaskan results of Aboriginal infants⁶⁴ were available, N.W.T. health officials requested pre-licensure approval to immunize their high risk Aboriginal population with PRP-D vaccine. Because Finnish trial results were so overwhelmingly positive, the N.W.T. trial was requested by the Federal Bureau of Biologics only to confirm the safety and immunogenicity in their predominantly Aboriginal population. That is, they were to determine whether there was any difference in immune response to the

PRP-D Hib vaccine between the Inuit and Dene (Indian) populations of the N.W.T. and that of the Finnish infants.^{1,12}

The results of the N.W.T. trial, however, did not support the Finnish findings. They were similar to the results eventually reported among the Alaskan Aboriginal infants. In the N.W.T. trial, it was found that anti-Hib antibody response to the vaccine was low for both Inuit and Dene. Only 19 percent of the Inuit and 30 percent of the Dene achieved an antibody level of $\geq 1.0 \mu\text{mL}$, and 44 percent and 60 percent, respectively, achieved the $\geq 0.15 \mu\text{mL}$ antibody level.^{1,12} This low immune response by both groups was attributed to the vaccine being administered at a younger age than in the Finnish study and, for Inuit infants additionally, to a lower antibody responsiveness to PRP-D. It was assumed that all infants had the vaccine administered and blood specimens taken within the appropriate age ranges.

These results were disappointing as confirmation of immunogenicity was a pre-condition for a proposed attempt to demonstrate the effectiveness of the vaccine in preventing Hib disease in Aboriginal infants by immunizing all N.W.T. infants and young children between the ages of two and 18 months. It was particularly disappointing because the Inuit, who had the reported lowest immune response, also had the highest rates of disease in the N.W.T.^{1,12} (Kinloch, personal communication)

2.3.3 Immunogenicity and Efficacy Results: HbOC

2.3.3.1 U.S. Trials: Non-Aboriginals

From 1984 onwards, trials with several different conjugate vaccines were conducted. One vaccine was created by linking Hib components to a nontoxic diphtheria toxin variant CRM₁₉₇ (HbOC). Trials used a variety of schedules for immunization. Results supported the findings that in many populations HbOC vaccine provided relatively high, long term anti-Hib antibody responses after the second dose. Madore et al. reported in 1990 that 84 percent of U.S. infants, immunized at one, three and five months of age, achieved ≥ 1.0 μmL after the second dose, and 98 percent did so after the third dose.⁶⁶ Black et al. also found high levels after the second dose among U.S. infants immunized at two, four and six months of age; efficacy was shown to be only 26 percent after the first dose but 100 percent after both second and third doses.^{67,68,69}

2.3.3.2 U.S. Trials: Aboriginals

Aboriginal infants were not so fortunate. Studies among the Navajo and Apache of the U.S. showed that antibody levels did not reach even the minimally protective levels of ≥ 0.15 μmL until after the second dose (88%)^{60,70}; 100 percent did so after the third dose, with 92 percent achieving ≥ 1.0 μmL . At

older ages, the vaccine was more immunogenic, but that was of little use in a population in which disease occurred in younger infants.

2.3.4 Immunogenicity and Efficacy Results: PRP-OMP

2.3.4.1 U.S. Trials: Non-Aboriginals

Another vaccine was created by linking Hib components to the outer membrane protein complex of *Neisseria meningitidis* group B (PRP-OMP). PRP-OMP proved to be a very immunogenic vaccine in infants immunized under 6 months of age, even after the first dose; and only two doses were required in the series. In a study reported in 1986 on a small group of U.S. non-Aboriginal infants, two thirds achieved levels of $\geq 1.0 \mu\text{mL}$ serum antibody after one dose, and 80 percent did so after two doses.⁷¹ A second study, reported in 1987 on a similar group of infants, used a larger amount of Hib polysaccharide in the vaccine and an interval of two months instead of one between doses, and reported similar findings.⁷²

Several age groups have been studied using several different dosages of Hib (PRP) combined with the OMP.^{73,74} Overall, 98 percent of children in these studies achieved anti-Hib antibody levels of $\geq 0.15 \mu\text{mL}$ after the first dose regardless of age or PRP dosage; 99 percent of those two to six months of

age had results of ≥ 0.15 μmL after the first dose and 78 percent had achieved ≥ 1.0 μmL .⁷⁵ Multi-country trials reported that 98 percent of infants two to six months of age met the ≥ 0.15 μmL level, and 77 percent the ≥ 1.0 μmL level, after one dose. An efficacy study showed infants two months of age to be well protected.⁷⁶

2.3.4.2 U.S. Trials: Aborigines

Apache and Navajo infants in the U.S. were immunized with PRP-OMP in the hope that this vaccine would produce better results than previous vaccines (reports 1991).^{77,78} Ward had already found it to be so in Alaskan aboriginal infants.⁷⁹ They were not disappointed. Protective anti-Hib antibody levels, ≥ 1.0 μmL were reported to be high (66-75% after 2 doses). The vaccine was highly immunogenic after one dose (60-69%), which was better than HbOC had been in that high risk group under six months of age.⁷⁷ Follow-up reports from the Navajo showed that efficacy was 93 percent over all ages after the second dose, and 100 percent after the first dose in children under the age of 15 months.⁷⁸

2.3.5 Immunogenicity and Efficacy Results: PRP-T

Hib tetanus toxoid conjugate (PRP-T) was tested in Swedish infants immunized at three, five and 12 months of age, using

two different dosages. After one dose in both groups, tested at five months, 81 percent reached levels of $\geq 0.15 \mu/\text{mL}$, and 99 percent did so after two doses.⁸⁰

A review of several international PRP-T trials by Fritzell in 1992 indicated that the vaccine, regardless of the schedule for the primary series, prompted a protective antibody response in 70 to 100 percent of the infants after two doses, and 98 to 100 percent after three doses.⁸¹ Lower responses were noted among the Alaskan infants, 75 percent of whom achieved levels of $\geq 1.0 \mu/\text{mL}$ after the third injection.

When the PRP-T vaccine was mixed in the same syringe as DPT at two, four and six months it was reported to provide protective immune levels with 94 percent of the infants reaching $\geq 0.15 \mu/\text{mL}$ after the second dose and 65 percent reaching $\geq 1.0 \mu/\text{mL}$; 43 percent achieved $\geq 0.15 \mu/\text{mL}$ after the first dose.⁸²

2.3.6 Trials Comparing Vaccines

Finland has continued full scale immunization against Hib disease in all infants since 1988, first during a period in which HbOC was given to one half of the infants and PRP-D to the other half (1988/89), and then with PRP-T being given to all, commencing in 1990.^{83,84} Continuing analysis of the data from the study of the earlier period has shown that both

PRP-D and HbOC were protective, although HbOC was slightly better.

A Finnish study (report 1991) of infants immunized at four and six months of age, found no differences among PRP-T, HbOC and another Hib vaccine.⁸⁵ A British study reported in 1992, which immunized with PRP-T at two, three, and four months of age, found that 98 percent of infants achieved antibody levels ≥ 0.15 μ /mL after the third dose (tested at 5 months of age) and stated that this vaccine was better than either PRP-D or HbOC in this age group. Ninety one percent reached the ≥ 1.0 μ /mL level.⁸⁶

2.3.7 Factors Affecting Immune Response and Efficacy

Risk factors influencing susceptibility to Hib disease were mentioned earlier. Many of these same factors appeared to be related to the inability of different populations to develop high levels of protection to different vaccines. Many of the authors have controlled for potential influences in their studies and have reported inconsistent results.

Factors thought to be responsible for diminished immune response and low efficacy were observed as potential confounders and controlled for in many of the vaccine trials. These can be divided into host factors (such as the age of the

infant at immunization and follow-up testing^{20,25,43,56,61,65,72,86,87}, pre-immunization antibody levels^{65,88}, genetics^{59,89,90,91}, ethnicity^{12,53,59,60,64,65,90,92}, increased exposure to the organism⁶⁵, and sex⁵⁸), agent factors (such as the vaccine lot potency⁶⁵ and differing amounts of Hib protein in the vaccines⁴⁹), environmental factors (such as breast feeding^{63,93}, day care attendance^{51,53,63}, parental smoking⁵⁵, geographical location^{49,50,51,67,88}, and household composition^{49,51}) and study strategies (such as differences in study design^{49,69}, differences in antibody assays^{59,60,65}, and frequency of immunization^{61,94}). Most of these factors have been shown to negatively affect immune response and efficacy; breast feeding was the one factor positively associated.

The most frequently mentioned factors were age, ethnicity and genetics. Genetics can be considered for the individual, such as with a particular genetic deficiency, or for the population at large, where ethnicity is often used as a surrogate. Differences between the sexes have also been studied. The design of the studies had the potential to affect the findings. Case-control studies required great care in ensuring that the rates of immunization of study participants were similar to those of the general population. Control of confounders/effect modifiers was often difficult.

Age of the infant and timing of the vaccine injections are very closely related. The response of infants to any vaccine is considered to be better during the latter part of the first year of life as the ability of the infant to develop antibodies increases, that is, those who receive the vaccine later respond better.^{58,72,95,96}

Ethnicity, as it is often defined, is a variable which is associated with several other factors, such as genetics and immunoglobulin subclasses. It has also been associated with socioeconomic status, which is itself a composite of many variables. Immune response, however, has been reported to be linked to ethnic differences specifically^{1,59,90}, although "ethnicity" has not been clearly defined. Ethnicity is often used as a surrogate for race.

Different studies have investigated the potential confounding effect of ethnicity. Popejoy (1985) conducted a study on a multi-ethnic population and found that ethnicity did not affect the immunologic response²⁵; Shapiro (1985-87) reported a similar lack of confounding.⁵¹ A large study (1985-87) in California adjusted for ethnicity and found that it did not alter the results.⁵³ On the other hand, some black and Hispanic children in a separate study did not respond well at all.⁹⁰ A study among Apache infants reported a ten-fold

poorer response than their white counterparts, which was thought to be partially related to genetic factors.⁵⁹

Reports of vaccine associated response in infants with immunoglobulin subclass deficiency and particular allotype deficiencies have indicated that the response was dependent on the age of the child at the time of vaccination and the type of vaccine used.^{89,91,96,97} It may also be associated with ethnicity in those who did not have the Km(1) allotype.⁸⁹

In a study among Navajo infants by Coulehan et al.⁵⁸ males were reported to generally respond better to the vaccine, and to achieve protective levels of antibody more frequently, than females. This was not the case in other trials where the opposite or no effect was reported.^{73,98,99}

The differences between the assays used to determine antibody results has been frequently noted.^{87,88,100,101} This interassay variation has prompted authors to comment on the lack of comparability between studies, and to attempt to use a single laboratory for multi-centre trials. Several authors have suggested that the results of past immunogenicity studies have been "influenced" by the different laboratories used to do the antibody assays, and that the differences were not due to the vaccine itself but to the different laboratories used.⁸⁷

The one factor which has been shown to positively affect immune response is breastfeeding. Breastfeeding was common and prolonged in Finland.⁶¹ Eskola et al. noted that in Finland 80 percent of women breast fed their infants for over 6 months.⁶³ A Canadian (Alberta) study by Pabst provided evidence that response to conjugate vaccine was influenced by the type of infant feeding method used. Infants who had been breast fed had significantly higher anti-Hib antibody levels, when they were tested after their third dose of HbOC at 7 months of age, than the formula fed group.⁹³

There are many questions which seem to require further study in order to provide a definitive answer as to why some groups respond to a particular vaccine better than others. Two variables frequently considered as confounders are age and ethnicity. Both variables have been considered in studies which have resulted in differing conclusions, as previously noted. The definition of sex is clear and results which differ may be related to some other influence. The use of ethnicity as a variable, however, seems less clear, as definitions are not as clearly evident. The use of ethnicity as a variable in epidemiology and vaccine related studies may be questionable unless clearly defined.

2.3.8 Summary: Antibody Response and Efficacy

The findings of several studies, because of their large sample sizes and their positive results, have been generalized to other, dissimilar populations. Many variables influence antibody response and vaccine efficacy. It is imperative to clearly define variables, such as race, ethnicity and socioeconomic status, so that studies can be compared and evaluated. There has not been enough evidence to suggest that high levels of antibody correlate with protection from disease, or conversely, that low levels do not provide some protection.^{69,102}

2.4 Ethnicity as a Research Variable Defined

Researchers often link study subjects to a particular racial, ethnic or cultural group for study purposes because the results may be influenced by some factor of that group. The problem arises, however, when the group as a variable is not well defined.

According to the Encyclopedia Britannica (EB), a race is a "biological grouping" determined by "genetically transmitted differences". Problems related to race differentiation arise, suggests EB, when attempts to determine racial groups are not based on genetics.

Races have adapted to their environment and some of those genetic characteristics have changed over time. Today's scientific definitions of race depend on precisely measured inherited characteristics, such as blood type and inherited enzyme deficiencies.

"Race" today may also mean a group defined by language, nationality, religion or some other characteristic. These, as EB states, "are biologically and scientifically meaningless".

Ethnicity is often used interchangeably with race. It, too, is fraught with inconsistencies and often lacks scientific or biological definition.^{47, 105,103,104} Ethnobiology is, according to one medical dictionary, "the study of the biological characteristics of various races".¹⁰⁴

Ethnicity is a difficult concept to define, and often is not defined in measurable terms. Several authors have discussed the problems of using ethnicity as a research variable.^{105,106,107,108} Edwards particularly mentioned the problems associated with lack of definition in secondary analysis.¹⁰⁶

Ethnicity is difficult to define because it is complicated. It does contain biological components. Ethnicity is explained in terms of historical, linguistic and psychological factors,

each of which has biological components as prerequisites. All of these influence cultural norms, and are in turn influenced by culture.¹⁰⁷

In the Northwest Territories, "ethnicity" is used to ascertain status for political, health and other reasons. The definitions for Indian and Inuit have been determined by the Federal Department of Indian and Northern Affairs (DIAND), in the Federal Indian Act, and Medical Services Branch (MSB) of Health and Welfare Canada, and relate to treaty status and/or parentage. In spite of the difficulties of defining ethnicity, for the purpose of this thesis, this is the definition that is used and all study subjects fall into either of the two defined groups, Dene (Indian) or Inuit.

2.5 The New Epidemiology of *H. influenzae* type b Disease

More recent epidemiological observations, since research into this paper began, should be noted here. Scandinavia has experienced a marked decrease in Hib disease. Iceland commenced general immunization with PRP-D in 1989, and Hib disease has decreased from 135 cases reported in children under five years of age to no cases of meningitis and two cases of bacteraemia to the end of 1991.¹⁰⁹ Finland, which has used several conjugate Hib vaccines since 1986, has

reported a steady decline from 27 cases reported in 1985 to no cases of Hib disease reported in 1991.¹¹⁰

In the U.S., studies from several states have reported decreases in disease ranging from 80 percent to 93 percent.^{111,112} Studies conducted among multi-ethnic American Army personnel have also reported a decline in rates of Hib disease.^{113,114}

All reports noted that the decline began in children under eighteen months of age after the vaccine was commenced in the over-eighteen age group and before it was available for infants. Anderson and May have discussed the benefits of herd immunity and the factors upon which the organisms' reproduction rates depend in order to make a significant difference in the amount of disease.^{115,116}

Although the N.W.T. did not experience the same early herd effects, the current disease rates have dropped significantly with the introduction of newer conjugate vaccines. (Kinloch, personal communication; unpublished data, Infectious Disease Control, Government of N.W.T.)

2.6 Relevance of the Research

The N.W.T. Government, whose infants have had an exceedingly high rate of Hib disease, was anxious to obtain the vaccine (PRP-D) which had proven so highly immunogenic and efficacious in Finland. Because the populations were dissimilar, an investigation was required by the Federal Bureau of Biologics to ascertain if the infants of the N.W.T. would react similarly to the vaccine. The results of the 1986 N.W.T. study indicated that this particular group did not. The preliminary analysis, which reported that the Inuit infants responded poorly, was conducted on a small sample and provided no detailed consideration of the differences between the Inuit and the Dene infants who had received the Hib vaccine. There could have been many reasons for these results other than ethnicity, including chance, study bias, and other factors which will be explored in this thesis.

It is imperative that a vaccine used in the N.W.T. be one which is effective in the highest risk population. If the risk factor for diminished immune response is one over which there is no control, such as ethnicity or genetics, then it behooves the government to choose the vaccine which will provide protection to that population, and not base their vaccine choice on administrative or financial reasons. If, however, response to a vaccine is related to problems outside

of the innate characteristics of the Inuit, then the immunization program for the N.W.T. can more readily follow the rest of the country, and the problems related to lack of efficacy, such as socioeconomic status, can be dealt with by government and other agencies.

There are concerns with the spread of infection because of the increasing use of child care facilities which concentrates the susceptible age groups, and the overcrowded living conditions often found in Northern areas. Immunization and chemoprophylaxis are methods of prevention which may be cost beneficial in terms of the health care of the acutely ill and their contacts. Those with sequelae are an important burden on the health care and social welfare systems. The potential for drug resistance by the organism enhances the need for the use of an effective vaccine for this population.

The relevance of this study is to the Aboriginal people of the N.W.T., particularly the Inuit who have the highest rates of Hib disease in the country. The immunogenic properties of the vaccines could be enhanced by recognition of risk factors related to immune response.

It is possible that by exploring the factors available in this particular sample there will be some explanation of why these Inuit appeared to respond less well to the PRP-D vaccine than

the Dene. It is not reasonable to promote ethnicity as a factor in diminished immune response with this small, and possibly biased, sample. Chance also may have made a contribution. This research could provide factors other than ethnicity which should be considered in vaccine related studies or in public health decision-making.

3. SECONDARY ANALYSIS OF DATA AS A RESEARCH METHOD

3.1 Description of Secondary Analysis

The data used for this thesis were originally collected in order to determine the safety and immunogenicity of the PRP-D vaccine in Inuit and Dene infants of the N.W.T.. The data were made available to me in order that I might explore the question of what factors, other than ethnicity, could explain the observed differences in antibody response between the Inuit and Dene infants. This thesis examined the data for factors which might provide reasonable doubt that the reported differences in immune response occurred because of ethnicity. It is recognized that the differences may have occurred entirely by chance. According to Hearst and Hulley¹¹⁷ this examination of existing data for purposes other than originally intended, defines the research method of secondary analysis of data.

There are two ways in which secondary analysis can be used as a research method. The first is to formulate a research question, consider which variables are necessary and then proceed to find a dataset that will be complete enough to test the hypothesis which will answer the question. The search for such a dataset may be difficult and time consuming, and also costly, as the original research may not have collected data

on the variables that are now of interest. It is also possible that the design of the original study is not the most appropriate one to test the present hypothesis.

Secondary analysis may also be used when a dataset is readily available and appears to support further analysis in order to provide answers to questions which were not considered previously. Although the dataset may contain the necessary variables for the new research, the quality of the data is still of concern and must be carefully examined.

Quality is also of concern in the type of dataset to be used. This thesis uses individual data, one of the two types of data available for secondary analysis. The other type is provided in aggregate form. The advantage of using data on individuals is that more information is available for analysis, and associations can be measured between individuals, rather than only between groups.

Confidentiality is a concern when using the data of individuals. When the data were provided to me on the infants of the N.W.T. study, it was necessary to maintain confidentiality by removing all identifiers, and analyzing and reporting in a way which would meet with the approval of the people of the N.W.T. and the people who provided the data.

The identifiers which were removed included community information as well as personal.

3.2 Strengths of Method

Secondary analysis of data is a more economical and quicker method than collection of new data. It is often advantageous to examine data more closely in order to determine additional findings which may have been missed in the analysis of the original question, and which may reflect on the outcome of the original results.

It was implied in the 1986 N.W.T. study that ethnicity was a factor in the Inuit infants' results. This may not be the case, however, as the differences between the Dene and Inuit in the intervention groups were not addressed. Further analysis will possibly provide some insight into those differences. It is possible that the differences occurred entirely by chance.

3.3 Limitations of Method

Limitations of the method can be serious in that data quality cannot always be assured. Ideally, the researcher would have access to the original study team and reports so that some knowledge of the sources of error may be acquired. In many

cases though, this resource is not available and the new research must be conducted with some scientific guesswork.

Quality of the dataset is predetermined by the original investigators. The variables chosen, the data collection methods, the data entry procedures and the database development may not be of a quality which will provide the researcher with that which is required for additional analysis. In this particular case, I was part of the team which conducted the 1986 N.W.T. study, and have a reasonable knowledge of all that occurred.

The value of the dataset to the new researcher is also determined by the purpose of the original study. The purpose of the N.W.T. study was a limited one. It had only to support the findings of another PRP-D vaccine study, which were that the vaccine was safe and highly immunogenic. Because of this, many variables believed to affect immune response and efficacy were not considered, such as breast feeding and smoking.

It is not enough to pose a question and know that a database exists which will answer it. The database must be accessible to the researcher. Although I had been working for the same organization which did the 1986 N.W.T. Hib study, and had been given permission to use the data by the principal investigator, it was still necessary to request use of the

data from the organization which had supported the research. Access may be related to cost as well, as funds are required to search for and purchase use of the data.

Chapter 4 will describe the 1986 N.W.T. study in more detail and the limitations which require consideration in the secondary analysis.

4. 1986 N.W.T. STUDY

4.1 Purpose of Study

The purpose of the *Haemophilus influenzae* type b (Hib) immunization study in the Northwest Territories (N.W.T.) was to determine whether there were any differences in safety or immune response to the Hib vaccine between the Inuit and Dene (Indian) populations of the N.W.T.¹ and that of the infants in a Finnish study⁶² which had demonstrated a protective response using the same vaccine.

4.2 Study Population

In order to more fully describe the study from which the data for this thesis were taken, it is appropriate to mention the composition of both the intervention and control groups, and to include the "others", who were part of the 1986 N.W.T. analysis. The "others" were excluded from the secondary analysis, however, because of very small numbers and because of the multi-ethnic components of the group.

The 1986 N.W.T. study enrolled a total of 267 infants from 11 communities across the N.W.T.: 167 (62%) Inuit, 74 (28%) Dene, and 26 (10%) "Other". "Others" were a mix of non-status Indians, Metis and other races, mainly Caucasian.^{1,118}

These communities were a convenience sample chosen because of their large number of births per year. They came from three predominantly Inuit and two predominantly Dene Regions. All infants in a community were invited to be enrolled. The required number for enrolment was 60 Inuit and 30 Dene in each of the intervention and control groups, for a total sample of 180 infants. The sample size for the Inuit was to be twice that of the Dene, which were the approximate proportions in the general population.

The study took place between January and December, 1988. Intervention group infants received regularly scheduled diphtheria, pertussis and tetanus (DPT) vaccine at two, four and six months, and at the same time, but at a different injection site, Hib conjugate vaccine (PRP-D). The Control group received DPT only. Blood samples were drawn from both groups just before the first dose of vaccine and one month after the third dose. Both groups also received oral polio vaccine as per infant schedule.

4.3 Allocation and Exclusions

Infants who were hospitalized with severe illness or who had known impairment of the immune system were excluded from enrolment. Infants who were enrolled, but were ill at the time of immunization, were delayed until well. Illness was defined

as having a temperature over 38.5 °C, or other indications of significant generalized illness. It did not include minor illnesses, such as the common cold. The infants were excluded or deferred by the nurses involved in the study, in consultation with the study co-ordinator as necessary.

There were two methods of assigning infants to intervention or control groups. Those who were already born (birth to four months of age, less one day) were randomly assigned to either group, and those yet to be born were systematically allocated to either group, as they were born, in an alternating fashion. Infants were allocated within their ethnic group in their own community to either the intervention or control group.

There were 148 infants enrolled in the intervention group and 119 in the control group (Table 1). After exclusions because of loss to follow-up, deviation from the protocol, incomplete immunization series, lack of blood results (including those who had blood taken but of insufficient quantity), there remained 106 (72%) in the intervention group and 67 (56%) in the control group whose blood specimens could be analyzed. Of those enrolled, there were 42 (28%) infants for whom there were no results in the intervention group: 33 Inuit (78%), 5 Dene (12%), 4 Others (10%), and 52 (44%) in the control group: 32 Inuit (61%), 14 Dene (27%), 6 Others (12%).

4.4 Intervention and Outcome Measure

The vaccine provided by Connaught Laboratories (Canada) was Hib polysaccharide conjugated with diphtheria toxoid (PRP-D). The vaccine had been proven to be safe, very immunogenic and efficacious in Finland. This vaccine was administered along with DPT to one group of infants, while the other received only DPT. Half way through the study Connaught replaced the original vaccine lot with a newer and enhanced version, thus providing results from two different lots for the analysis.

Two blood specimens, one immediately before the first immunization and a second one month after the third immunization, were obtained for 191 (72%) infants and serum analysis for anti-PRP antibody titre was possible for 173 (65%): 106 (72%) assigned to intervention and 67 (56%) control infants. Those for which blood results were not available for statistical analysis included 8 samples which were of insufficient quantity for serum analysis and a further 10 which were excluded because the infants had received different vaccine lots within the series.

4.5 Evaluation of Results

4.5.1 Overall Results

In the N.W.T. study, immunogenic response (antibody levels $\geq 0.15 \mu\text{mL}$) to the vaccine was reported to be low for all infants and was attributed to the vaccine having been administered at a younger, less responsive age, than in the Finnish study. The young age at first immunization was required in order to respond to the earlier onset age of Hib disease in N.W.T. infants. Inuit infants were reported to have responded least well of all groups. This was considered to be because of some factor related to their ability to respond rather than to the vaccine itself.

It is possible that the study results were a consequence of the sample available for analysis and did not adequately reflect the true immune response of N.W.T. infants, especially the Inuit. The minimum sample size was determined by the Bureau of Biologics, Health and Welfare Canada, Ottawa, in order to consider the request for general use of an unlicensed vaccine in the N.W.T.. They required that a small study be done prior to their approval, and that at least 60 Inuit and 30 Dene be enrolled in each of the intervention and control groups; total, 120 Inuit and 60 Dene. It is not known how this sample size requirement was determined. The "Others"

were enroled and their blood specimens analyzed, but no minimums were set for their enrolment as it was assumed that they would respond similarly to the Finnish infants.

Dropouts and deviations from the protocol must also be considered. The intervention group decreased from 148 enroled to 135 (91%) who completed the immunization series, to 106 (72%) with blood results in the analysis. The ethnic breakdown of the intervention group who provided blood results, from the total enrolled, was 59 Inuit (64%), 37 Dene (88%) and 10 Others (71%) (Table 1). The control group dwindled from 119 enroled to 99 (83%) who completed the immunization series, to 67 (56%) whose blood results were included in the statistical analysis. The ethnic breakdown of those in the control group who provided blood results, from the total enrolled, was 43 Inuit (57%), 18 Dene (56%) and 6 "Others" (50%). Such low percentages of results for analysis severely limits generalization of the findings to the total population.

4.5.2 Participation by Inuit and Dene

Nurses working at health centres in communities where the study was to take place were offered the opportunity of incorporating the study protocol into their immunization program or of having a special project nurse come into their

community and deal exclusively with the project infants. In the Inuit communities the nurses chose the former; in the Dene communities, with one exception, the latter was chosen. The choices may have influenced the results by creating opportunities for bias related to participant selection and withdrawal.

4.5.2.1 Inuit

Inuit families knew well the morbidity and mortality, as well as sequelae, associated with Hib disease. They were very eager to have their infants immunized with the new vaccine to prevent meningitis. Thus, those in the intervention group were more likely to finish the Hib immunization series (82/92; 89%) than the DPT alone series (63/75; 84%) of the control group, although the difference was small. It was more difficult to get those who completed the series to come back for the second blood test. Only 59 of 92 (64%) in the intervention group and 43 of 75 (57%) in the control group provided blood specimens (Table 1).

Blood results were available for 69 out of 92 Inuit infants (75%) who completed the intervention group, however, the results of ten were eliminated because they had been given two different vaccine lots in the series, resulting in 59 of 92 (64%) with measurable blood results. Forty-three out of 75

(57%) of the Inuit controls who finished their series produced results. It was difficult for the nurses to maintain intensive control over the project in addition to their other duties.

4.5.2.2 Dene

The Dene, whose rates of meningitis were very low, required more convincing to be enrolled than the Inuit, but once enrolled, 40 out of 42 (95%) in the intervention group completed the series. There were blood results for 37 out of 42 Dene (88%). There was only one case of mixed vaccine lots in the series. On the other hand, only 24 out of 32 (75%) of the controls completed their series and 18 out of 32 (56%) produced blood samples. The fact of having a project nurse to oversee every detail contributed to the response of the intervention group, but the control group were less convinced to return for follow-up.

4.5.3 Effect of Participation on Interpretation of Results

The potential for selection bias in the sample was high in the intervention group. Nurses in Inuit communities, who incorporated the study into their daily routine and started using the protocol first, were provided with the less immunogenic first lot of vaccine. Well into the study they

were provided with the "enhanced" second lot. The study nurse, hired for the purpose, started the Dene communities later and had less time to use the first lot before the second became available. Thus the lots were more evenly distributed among the Dene infants.

Sixty-four percent of Inuit infants in the intervention group provided blood results for analysis, while 88 percent of the Dene infants did so. Compare this to 57 percent and 56 percent, respectively, who provided results among the control group (none of whom had anti-Hib antibody results at seven months of age). The more potent vaccine, lot 8J91140, was administered to 51 percent of the Dene and 42 percent of the Inuit who provided results.

The overall difference in anti-PRP antibody response between the study and control groups in the 1986 N.W.T. study was reported as being statistically significant at the 95% confidence level, as were the differences when stratified by ethnic groups ($P < 0.001$).

The total Inuit cohort in the intervention group that completed the series was 82, of which 59 had blood results. The observed success rate, success being defined as having ≥ 0.15 $\mu\text{g/mL}$ antibody level, was 44 percent (Table 2). The best case scenario, if all of those missing blood results had

achieved acceptable antibody levels, would be 60 percent; the worst case, 32 percent success if none did.

The total Dene cohort in the same group was 40, of which 37 were followed up. The observed success rate was 60 percent. The best case scenario, if all were accounted for, would be 63 percent; the worst case, 55 percent success.

Thus, if all had had blood specimens analyzed, the proportion of each group achieving success in the best case would have been nearly equal. The worst case would have been quite different, as nearly twice the Dene would have achieved success as the Inuit. As it was, with the results available, the Dene in this sample showed considerably higher success. This difference may be a result of confounding or of chance.

4.5.4 Conclusion

Three groups of infants were administered the PRP-D vaccine in this study. The Inuit and Dene anti-PRP antibody responses were the primary focus, while the "Others" were included because they were part of the community.

The antibody results for all groups in this sample did not come close to matching the success found in a similar study among infants in Finland using PRP-D vaccine. Inuit infants

appeared to be less successful at achieving protective antibody levels than the Dene infants, leading to the reported assumption that being Inuit was a barrier to success.

Problems with recruitment, infants lost to follow-up, replacement of the original vaccine lot with an allegedly more enhanced lot, and the logistics associated with two methods of staffing (study nurse, clinic nurse), as well as a small sample size, contributed to uncertainty about the response from the Inuit infants. It seemed reasonable to ask the question, "What factors other than ethnicity could have explained the observed difference between the Inuit and Dene infants?". There were data from 106 aboriginal infants that would be examined to answer the question.

5. STUDY DESIGN

5.1 Research Question

The purpose of this thesis was to explore differences between Inuit and Dene infants of the Northwest Territories (N.W.T.), in terms of possible risk factors, or risk markers^{47,119}, confounders or effect modifiers, which might have been associated with the reportedly lower antibody response to Hib conjugate vaccine (PRP-D) among the Inuit infants. It was implied in the 1986 N.W.T. study that ethnicity was considered to be a contributing factor.

The question was, "What factors, other than ethnicity, might explain the observed differences in anti-PRP antibody response between the Inuit and the Dene infants?".

As mentioned in section 2.5, the dataset was of questionable quality and sample size was a problem. However, the purpose of the research was to provide evidence that reasonable doubt exists regarding the validity of ethnicity as the reason for the observed difference between Inuit and Dene results.

5.2 Research Design

A literature review (chapter 2) was conducted of Hib vaccine studies on Aboriginal people, particularly North American, and on Circumpolar countries, with special reference to Finland, to determine if ethnicity was considered to be a factor in antibody response to Hib vaccines. Other factors associated with lowered anti-Hib antibody response were reviewed, such as sex and age, as well as nutrition, smoking in the home, and interference from viral infections prior to or at the time of immunization.

The research design used in this exploratory study was mainly descriptive in nature, and using the terminology of Kleinbaum, Kupper and Morgenstern², was a "follow-up prevalence study", and included cohort and cross-sectional design. The study design could be considered "cohort" in that the major study factor, ethnicity, could be identified in the beginning and the outcomes determined on follow-up. However, some of the children already had the outcome (protective levels of antibody) at the beginning of the study, contrary to the requirements for a pure cohort design. In addition, several other risk factors that were considered, e.g. vaccine lot, could only be determined during the study period. Since the prevalent cases at the onset of the study were not removed

from the study, it could be considered cross-sectional in nature.

Data from the Northwest Territories' PRP-D vaccine trial were obtained and secondary analysis of data from the intervention group was employed. The analysis considered the differences between the proportions of Dene and Inuit infants who achieved protective anti-PRP antibody results when several available factors, potentially associated with the outcome reported in the 1986 N.W.T. study, were included in the analysis.

5.3 Study Population

There were 92 Inuit (M=53, F=39) and 42 Dene (M=21, F=21) infants (N=134) who commenced the Hib PRP-D vaccine series. They represented communities across the Northwest Territories chosen for their high number of annual births. The ethnic composition of individual communities was considered homogeneous as Inuit and Dene infants lived in separate and distinct communities (Table 3). The non-Inuit, non-Dene "Others" (N=14) were not considered in the secondary analysis because of their small sample size and their multi-ethnic backgrounds.

The Dene experienced relatively little Hib disease compared to the Inuit. However, examination of the PRP-D pre-series

antibody results, taken at two months of age, provided no evidence that they had a better early natural immune response to disease than the Inuit. Dene seemed less likely to have serum anti-Hib antibody levels of $\geq 0.15 \mu\text{mL}$ than Inuit infants, although the test was not statistically significant. The percentages of infants with pre-immunization protective levels were: Dene 26%, Inuit 30%.

The infants received Hib PRP-D vaccine from one of two production batches, either Lot 22000-3 (n=60, 45%) or lot 8J91140 (n=50, 37%). Some received a mixture of the two lots (n=12, 9%), as the second lot was introduced mid-way through the study causing some confusion. Twelve infants (9%) did not complete the series.

After eliminations for various reasons (eg. incomplete series, no blood), there were 34 Inuit (n=34/53, 64%) and 19 Dene (n=19/53, 36%) who received the first lot (22000-3), 25 Inuit (n=25/43, 58%) and 18 Dene (42%) who received the second lot (8J91140), and there remained nine (90%) Inuit and one (10%) Dene who received the mixed lots (Table 4). The total was 106 infants.

There were 20 Inuit and 11 Dene males who received the first lot, as did 14 Inuit and 8 Dene females. The second lot was

administered to 13 Inuit and 6 Dene males, as well as to 12 Inuit and 12 Dene females (Table 9).

Generally, all infants commenced their PRP-D vaccine series at, or shortly after, two months of age. The schedule was not maintained, however, as the series progressed. The age range at the time of the third vaccine dose was from 144 to 423 days for the Inuit and from 191 to 403 for the Dene. Blood was taken for analysis between 186 and 439 days for the Inuit and 228 and 359 days for the Dene, with a median age of 259 and 278 days respectively.

5.4 Study Variables

Risk factors are variables that are believed to have a causal relationship to an outcome, whereas, risk markers are variables that are associated with an outcome, but are not necessarily causal. Confounders and effect modifiers are variables which distort the relationship between the factor of interest and the outcome, thus preventing a true picture.⁴⁷

Variables considered to be potential confounders and/or effect modifiers in the relationship between ethnicity and achievement of successful antibody levels were sex, vaccine lots, and the biological effects of age at the time of vaccine administration and age at the collection of the second blood

specimen. Data related to other important variables, such as breastfeeding and smoking in the home, were not available.

The Government of the N.W.T. definition of ethnicity, stated in section 2.4, is the definition used for this paper. All study subjects fall into either the Dene or the Inuit ethnic group.

There were two definitions of "ideal age" for immunization which should be noted for this study. The first is for the ideal age at vaccine administration in relation to immune response to the vaccine. Infants who are older at the time of immunization respond with higher levels of anti-PRP antibody than do younger infants.

The second definition is for the ideal age at which vaccines are administered in relation to routine immunization program schedules. The routine schedule for DPT and Hib vaccines, which are given concurrently, is two, four and six months of age. It was this definition that was referred to when observed age at vaccine administration was compared to the "ideal age" in this study.

The dependent outcome variable was the anti-PRP antibody response to the Hib PRP-D vaccine, defined as being either protective ($\geq 0.15 \mu\text{mL}$) or not.

5.4.1 Sex Differences

Females, for the most part, are believed to have lower rates of Hib disease in infancy than males, particularly of meningitis, although the findings vary from study to study.^{17,18,23,24,34} Two studies reported that females also responded better to vaccines than did males^{98,99}, although most vaccine efficacy trials did not specifically mention sex differences in relation to results. It is possible that sex also had an effect on the results of this study.

5.4.2 Lot Differences

Two different Hib PRP-D vaccine lots (22000-3, 8J91140) were administered in the 1986 N.W.T. study. The second lot (8J91140), introduced midway through the study, was assumed by the vaccine manufacturer, Connaught Laboratories (Canada), to have enhanced immunogenic capabilities.

5.4.3 Age Differences

It is accepted that infants respond better to vaccines as they grow older.¹²⁰ Doses administered later in the series prompt a reinforcing response that is initiated by the "memory" that the body develops as a person is immunized.³ The age at the time of the last immunization of the series,

and the subsequent blood specimen one month later, were important to the antibody response in this study.

5.5 Data Collection Methods

The entire dataset for the intervention group was contributed to my study by the principal investigator of the 1986 N.W.T. study and the Department of Health, Government of the Northwest Territories. The data were provided in the software database program Reflex^R, which was transferred to the SPSS^R statistical package for analysis, after unnecessary fields were eliminated.

The outcome measure for this secondary analysis was the post-immunization anti-PRP serum antibody levels from specimens collected one month after the third vaccine dose. Results, as determined by a single laboratory using the Farr assay method, were provided for all infants who had contributed the pre-series and post-series blood samples. In this analysis of the post-series results, a cutoff value of $\geq 0.15 \mu\text{mL}$, indicating short-term immunity, was used, with infants considered to have either reached minimal levels of protection or not.

The vaccines were administered and the blood specimens taken, prepared and stored by trained nurses who were either employed in the communities or were part of study personnel. The

specimens were then collected by a study nurse and transported to Connaught Laboratories (Canada).

5.6 Quality of the Data

There were several important delimiters associated with the dataset which would influence the reliability and validity of the results. Two major differences between the Inuit and Dene, which appeared to influence their participation in the 1986 N.W.T. study, were their experience with Hib disease and the choice of nursing personnel to do the follow-up in their communities.

Morbidity and mortality associated with Hib disease were higher among the Inuit than the Dene. The former were eager to have their infants participate in the study, but the latter required more convincing, thus creating a potential for participant bias.

The second difference created a potential for bias associated with the nurses who were enrolling participants, immunizing them and following them. In the Inuit study communities, there were several nurses involved in the education, enrolment, immunization and follow-up of the infants. They lived in the communities and provided the medical/nursing care for those communities. They had considerable influence with

the parents, who may have enrolled to please the nurses. They also had more opportunity to approach the parents to enroll. On the other hand, they were often very busy and were unable to repeatedly contact the parents to complete the second blood test.

There was one study nurse to enroll infants among the Dene. She lived outside of the study communities and was required to travel to them. She travelled when she could capture a number of infants within a specific period of time to save travel costs. She did not know the communities as well as someone who lived there and the parents required more encouragement to enroll their infants. It took time to hire the study nurse, which meant that the Dene started into the project later than did the Inuit. Many also had their immunizations given and their blood specimens taken at older ages.

Both of these factors created problems with enrollment and infants lost to follow-up. As well, using community nurses resulted in the exclusion of a few participants from the study because mixing of the vaccine lots occurred in their series.

The second vaccine lot, which replaced the first lot as new infants started their series, was expected to produce better results than the first. Introduction of the second lot meant that lots must be considered as a factor in analysis of the

results, further restricting the sample size. The assumption was also made that there were factors in addition to the vaccine lots which influenced the observed difference between the groups because of the age-related frequencies noted.

It was not possible to determine the effect of other important variables, such as breast feeding and household smoking, on the antibody levels because they had not been required for the 1986 N.W.T. study.

5.7 Data Analysis Methods

The secondary analysis was conducted on the serum anti-PRP antibody results of 106 infants from the 11 ethnically homogeneous communities across the N.W.T. who received the Hib PRP-D immunization series.

Ten infants who received both Hib vaccine lots in their immunization series were excluded from the analysis in the 1986 N.W.T. study. It was noted that when the investigators analyzed the lots separately, however, no significant difference was found between the ethnic groups in that analysis.¹ It was decided, therefore, that the ten would be included in the general analyses for this investigation.

Data were prepared for transfer from the 1986 N.W.T. study database in Reflex^R to SPSS/PC^R statistical package, version 3.0. Frequencies were determined and the data evaluated for completeness.

Descriptive analyses of the serologic results, completed to determine if results could meet the usual assumptions regarding normal distribution, showed that the shape of the distribution was skewed.

The blood serum anti-PRP antibody levels were dichotomized into success, defined as achieving ≥ 0.15 $\mu\text{g/mL}$, or no success. Results were dichotomized because the sample size was not large and the sample did not approximate a normal distribution. Histograms, means, medians and modes all reflected non-normal distributions of results, as well as of several of the variables of interest. Non-normal distributions are best handled with non-parametric analysis. In addition, clinically it is more useful to know if the infants achieve protection rather than what the actual antibody level is.

The crude odds ratio was calculated to consider the overall association between the ethnic groups, in relation to their outcomes.

Stratification was performed in order to evaluate confounders and remove their effect, and to evaluate effect modifiers and describe their effect, on the relationship between ethnicity and anti-PRP antibody levels. Stratified analysis more readily illustrated the magnitude and direction of the effect when crude and adjusted odds ratios were compared. When the assumption of uniformity across strata was met, the Mantel-Haenszel pooled odds ratio (OR) was determined. The Mantel-Haenszel test was used to determine statistical significance. Strata were considered to be uniform when the adjusted and crude odds ratios showed little difference.

Several variables were collapsed into more manageable form for the purpose of stratified analysis. Inuit communities were combined into their Regions, and age at third vaccine dose and age at second blood specimen were collapsed into months of age.

Serologic responses to the Hib vaccine were compared:

- among Inuit Regions, controlling for sex, to explore if the distribution of antibody response in Inuit infants was the same for all Regions so that the regions could be analyzed together as a single unit; distribution among the Regions was unequal for cases and sex.

- between the ethnic groups, controlling for sex, lots and ages at third dose and at second blood, in order to explore the effect of each of these factors on the association between ethnicity and immune response; for reasons as described in section 5.4.
- between ethnic groups, controlling for lot and sex simultaneously in order to explore the possibility of a combined influence on the observed levels of immune response. Age variables could not be controlled for with other variables because cell numbers would be too small.

Stratified analysis was chosen as a method over the more commonly used logistic regression because the latter relies on the assumption that the estimate of effect is uniform across the different levels of the confounding variables (ie, no effect modification).¹²¹ It was not known if the outcome would be modified by some of the variables and a closer scrutiny of the data was necessary. Determining the strata-specific odds ratios would provide a greater understanding of the data and the strength of the associations between factors and outcome than logistic regression would do. In addition, the sample sizes and the design of the study make the use of logistic regression analysis questionable.¹²²

Odds ratio (OR) was chosen over relative risk because it is less subject to the constraints of the design (i.e. OR can be computed for case-control, cohort, as well as cross-sectional designs). It approximates the relative risk, and many statistical comparisons are equally valid for the two measures. Relative risk assumes a direct estimate of risk and requires a well designed follow-up study. As mentioned earlier, the effect of the prevalence of pre-immunization antibody levels and their duration were not considered in the analysis. Infants of the two ethnic groups were not evenly distributed in terms of lots, sex or ages at third vaccine and second blood. There were several potential biases related to enrollment and follow-up. Considering all of these problems, I decided to use OR as the statistical measure of choice.

The Mantel-Haenszel test of significance is considered to be acceptable even when the odds ratios of the strata are varied, as long as each stratum has sufficient numbers.¹²³ The exact test for confidence limits was used. Odds ratios (ORs), and Mantel-Haenszel ORs and significance and confidence intervals were calculated using the EpiInfo 5.0 software.

6. RESULTS

The central question was, "What factors, other than ethnicity, might explain the observed differences in anti-PRP antibody response between the Inuit and Dene infants?". The purpose of the analyses was to identify those factors.

6.1 Crude Results

The proportions of infants in this sample who achieved successful levels (≥ 0.15 μmL) of anti-PRP antibody after vaccination, stratified by ethnicity and sex, are shown in Table 5. The overall success was 52 percent; among the Inuit it was 46 percent and among the Dene it was 58 percent. These percentages differ from those of the 1986 N.W.T. study (Inuit 44%, Dene 60%) because of the inclusion of infants who received mixed lots in their series.

Forty-seven percent of the study sample were female ($n=50/106$). Successful antibody levels were achieved by 62 percent of females ($n=31/50$) and 39 percent ($n=22/56$) of males.

The crude analysis suggested that in this sample, with 31 of 68 (46%) Inuit infants and 22 of 38 (58%) Dene infants having achieved protective anti-hib antibody levels, being Dene

increased the likelihood of achieving protective levels from the PRP-D vaccine, with an odds ratio (OR) of 1.64 (95% CI 0.68<1.64<3.97, $P=0.31$). Statistical significance was not achieved and the role of chance in the results could not be ruled out.

Age was one variable believed to have an effect on the antibody results. Table 6 illustrates the median age in days at which the three doses of vaccine in the series were given and at which the second blood specimen was taken, and compares them to the age at which they were scheduled to be administered. The median age at the taking of the second blood specimen for the Inuit was 259 days (range of 186 - 439), and for the Dene it was 278 days (range of 228 - 359).

6.2 Inuit Regions

Differences in vaccination protection between regions appeared to be the case among this sample taken from the three Inuit regions (Table 7). Stratifying by sex did not appear to alter the variability. Sex, however, seemed to modify the effect of the infants' response. A summary Mantel-Haenszel (M-H) summary odds ratio (OR) was not calculated because of one stratum which provided no stratum-specific results and because of the apparent modifying effect of sex. The M-H summary OR is based on the consideration that each stratum result may be

thought of as an estimate of the overall association, and modified results would not provide such an estimation.

Comparing males of the Baffin and Kitikmeot Regions, the results were statistically significant, but not meaningful, as the OR was "undefined" and the upper confidence limit noted as being "very large" (calculation by EpiInfo), due to one cell of zero. No other results were statistically significant.

Regional differences in protective antibody success were difficult to assess because of the small cell sizes, and larger total number of infants in the Baffin Region. In the Keewatin and Kitikmeot Regions, the number of males was twice that of females, while in the Baffin Region the sexes were the same.

6.3 Vaccine Lots

Comparison of vaccine lots by antibody levels showed that those given lot 22000-3 were less likely to achieve protective levels than those given the enhanced lot 8J91140 (Crude OR 0.29, 95% CI 0.11<0.29<0.74, $P = 0.007$). Fifty-eight percent of the Inuit infants had received lot 22000-3, as did 51 percent of Dene. (Table 8)

The results, when stratified by ethnicity, demonstrated that ethnicity did not modify or confound the results, and that lot 8J91140 was the better vaccine for both groups, although the result for the Dene was not statistically significant (OR 0.35, 95% CI 0.07<0.35<1.63, Yates corrected, $P=0.229$). The results were statistically significant using the Mantel-Haenszel summary odds ratio (M-H OR 0.30, 95% CI 0.12<0.30<0.76, Yates corrected, $P=0.009$) and for the Inuit (OR 0.27, 95% CI 0.08<0.27<0.90, Yates corrected, $P=0.032$). (Table 8).

Vaccine results stratified by vaccine lot and sex (Table 9) revealed that for lot 8J91140 Inuit and Dene outcomes seemed not to be modified or confounded by sex, but for lot 22000-3, sex did appear to modify and confound the outcome. Female Dene infants appeared to be more likely to have protective levels among those who had received lot 22000-3. None of these tests were statistically significant. There were more males who received the first lot, and more females who received the second lot.

6.4 Sex

A greater proportion of females than males had protective antibody levels among both the Inuit (50% vs 42%) and the Dene (80% vs 33%), although among the Inuit the proportions were

closer to equal (Table 5). Statistically significant results among the Dene (OR 0.13, 95% CI 0.02<0.13<0.66, $P=0.009$) showed that sex did play a role in the results, with females achieving greater success than males. Among the Inuit, the females appeared to be better protected but this test was not statistically significant (OR 0.73, males to females). The Mantel-Haenszel summary odds ratio was significant, showing that males were overall less successful (OR 0.42, 95% CI 0.17<0.42<0.96, $P=0.04$). The ratio of females to males was greater among the Dene than among the Inuit.

The sex distribution between the two vaccine lots was inconsistent (Table 9). There were twice as many males given the first lot than the second, whereas the females were evenly distributed between the lots. Lots were shown to have a effect on the results.

6.5 Age at First Vaccine Dose

The "ideal age" for first scheduled vaccine dose was between 60 - 89 days (2 months of age). Eighty-six of 106 (81%) infants had their first vaccine dose during that time, 55 of 68 (81%) Inuit and 31 of 38 Dene (81%) (Table 10).

6.6 Age at Third Vaccine Dose

The "ideal age" for the third vaccine dose was between 180 - 209 days (6 months of age). Although the majority of the Inuit and Dene infants commenced their immunization series at the appropriate age (Table 10), the third vaccine dose did not follow the schedule for either Inuit or Dene. A higher proportion of Inuit (44%) had their third immunization in the "ideal", but younger, age period than the Dene (21%) (Table 11).

Comparison of serology results between the Dene and Inuit infants, when stratified by age at third vaccine dose, provided a variety of ORs which made consideration of an overall comment impossible regarding association between ethnicity and age at third vaccine dose. There was no pattern of effect from which to draw any conclusions. The observations in the individual stratum indicated the possibility of Inuit achieving better results at some age levels, Dene at others. Sample numbers between strata were uneven. The crude OR was 1.64 (95% CI 0.68<1.64<3.97). (Table 12) Statistical significance was not achieved. The sample available was too small. The Mantel-Haenszel summary odds ratio (OR) was not calculated as the strata ORs were not similar.

6.7 Age at Second Blood Specimen

The blood samples used to determine the antibody response to the vaccine were to be drawn one month after the third dose of vaccine at the scheduled "ideal age" of between 210 - 239 days (7 months). This meant that the Dene infants generally had the post-immunization blood sample drawn at an older age than the Inuit because of the delayed third vaccine dose (Table 13).

Comparison of serology results between the Dene and Inuit infants, when stratified by age at the time of the second blood specimen, was similarly affected by the inequality of proportions of Inuit and Dene across the strata. There appeared to be a pattern of increasing Inuit disadvantage as the age at second blood specimen increased. This may just reflect the numbers available in each stratum for analysis. Both groups in the stratum ≥ 300 days, however, appeared to be close to equal, as they should be at that age, although Inuit appeared to be the better protected group. The crude odds ratio was 1.17 (95% CI 0.46<1.17<2.93 when comparing the Dene to the Inuit blood levels for the age at second blood. (Table 14)

6.8 Summary of Results

There were very few of the analyses for this sample that provided statistically significant results, constantly reinforcing that the role of chance could not be eliminated. Crude analysis suggested that Dene were better protected than Inuit, but was not statistically significant.

Analyses concerning the two vaccine lots provided evidence that there was a difference in response to each of them. Those who received lot one were less likely to achieve protective levels than those given the enhanced second lot (statistically significant), and a greater percentage of Inuit received the first lot. Results were the same for both ethnic groups, although for Dene they were not statistically significant.

Sex was the other factor which seemed to have an influence on the results. Although not statistically significant, when vaccine results were stratified by vaccine lot and sex, those having the first lot appeared to be more likely to achieve protection if they were female and Dene.

When the lots were combined, females achieved statistically significantly better results among the Dene. This also appeared to be so among the Inuit, but not significantly so.

The role of the different lots requires consideration in interpretation of these results.

No conclusions could be drawn about the effect of age at third dose and age at second blood specimen, nor about the differences between Inuit regions. None of the tests were statistically significant.

7. DISCUSSION

The 1986 Hib PRP-D vaccine safety and immunogenicity study of infants of the Northwest Territories (N.W.T.) concluded that, while neither Inuit, Dene nor non-Aboriginal infants responded well to the vaccine, the Inuit responded least well, implying that ethnicity may have been a factor in the response. Analyses in this study found, however, that the distribution of the sexes between groups and the use of two vaccine lots modified or confounded the results. The differences in ages at third vaccine and second blood specimen were also believed to influence the results. However, the roles of inadequate sample size and general lack of statistically significant results, strongly suggesting that chance was a factor, could not be ignored. It was clear that any results from this sample could not be generalized to the larger population.

7.1 Ethnicity as a Research Variable

In the Northwest Territories, ethnicity is used to determine status for political, health and other reasons. The definitions that are used were determined by the Federal Department of Indian and Northern Affairs (DIAND) and Medical Services Branch (MSB) of Health and Welfare Canada, and are based on treaty status and parentage. Generally, anyone who was not Dene or Inuit was considered to be an "Other".

For the purpose of this thesis, all study subjects fell into either of the two groups, Dene or Inuit. Dene were defined by the Government of the N.W.T. using the Federal Indian Act definition for Indian. Inuit were defined using MSB guidelines.

Although considered to be two different ethnic groups, in fact, each group was composed of similar yet different subgroups, who may have followed similar yet different lifestyles. Combining all Dene into one group and all Inuit into another group for research purposes may have biased the results, depending how many other factors, such as cultural practices, socioeconomic status or breastfeeding practices, were similar within the groups.

Certain socioeconomic factors, often strongly related to particular ethnic groups, are thought to be related to Hib disease and to decreased vaccine efficacy in particular groups.³⁸ It is important to consider cultural and socioeconomic factors as well as ethnicity, but they too must be well defined. The gold standard to which early Hib vaccine trials were compared was the Finnish PRP trial, and later their PRP-D trials. These provided results from a predominantly middle-class "Caucasian" population.⁹

7.2 Results

The crude analysis indicated that Dene infants in this sample were more likely to achieve protective levels of anti-PRP antibody than Inuit infants, but the results were not statistically significant (OR 1.64, $P=0.31$). This difference between the groups would seem to support the idea that the Inuit demonstrated diminished immune response because of their ethnicity. The lack of power, however, created an uncertainty around the findings. Differences, or equality, of the two groups in relation to antibody response could neither be supported nor rejected. The role of ethnicity was still in question.

Some studies have reported results in which ethnicity/race was believed to be a factor in antibody response to vaccine. Siber et al.⁵⁹ did not specifically consider the reasons for the low immune response of the Apache infants, but did state that either ethnicity or epidemiologic factors could be responsible. This ambiguous comment did not provide much support that ethnicity was a factor in antibody response. Other studies of ethnic groups clearly reported no differences related to that factor.^{25,91,97} Investigators in the trials of Alaskan Aboriginal infants concluded that the infants' lack of response was not related to the population's ethnicity, but to other factors.⁶⁴ Other researchers also concluded that

differences in response were not related to genetics and ethnicity, but rather to factors such as socioeconomic status.³⁸ Ethnicity was not defined in these studies.

It was unfortunate that the second vaccine lot (8J91140) was introduced into the study. It created problems with analysis in that it was necessary to divide even further an already small sample. Although Dene infants were evenly distributed between the two lots, more Inuit received the first lot (22000-3) than the second. There were more males who received the first lot and slightly more females who received the second. Overall, females had a significantly greater chance of achieving a protective response than did males. Dene females also had a significantly greater chance of achieving success. Inuit females seemed to have had a better chance than males (not significant). It would seem from these results that sex may have been a factor in immune response. Sex has been mentioned in other studies as a factor related to immune response.^{98,99}

Although the ages at third immunization and second blood specimen could not be justified as variables of influence in vaccine response, it is worth noting that while 64 percent of infants administered the first lot had their post-series blood specimen taken at greater than "ideal age", 86 percent receiving the second lot had their specimen taken after the

"ideal age". Overall for both lots, 88 percent of Dene and 60 of Inuit had their blood specimens taken after the "ideal age". Age is considered an important factor in relation to response to vaccine,³⁹ although it could not be proven in this study.

Consideration of several factors together indicated that differences in lots and sex influenced the results. Insufficient sample size contributed to the lack of consistently significant results.

Although both ethnic groups had poor results with lot one, more Inuit received lot one than lot two and more Inuit than Dene received lot one.

When stratified by lot and sex, Dene did better, or there was no difference, in all strata, except lot one for males. However, more Dene males received lot one than lot two.

The Dene advantage of achieving protective antibody results generally increased as their age increased. However, more Inuit received the third vaccine dose and provided blood at the "ideal age", but younger age, than did the Dene.

It is of some importance to mention that infants who had baseline protective antibody levels at two months of age were

included in the analysis. (Table 15) Among the Dene and Inuit of the control group in the 1986 N.W.T. study (DPT only), the percentage of those who had protective anti-Hib antibody levels at two months (baseline) was not known. At the second blood, however, neither group was reported to have had protective levels (0.0). Twenty-six percent of Dene infants and 30% of Inuit infants in the intervention group (Hib, DPT) had protective levels at baseline, and 59.5% and 44%, respectively, at one month after the series.

The antibody levels at two months were a reflection of the maternal antibodies and were expected to diminish over time. It was not clear from the literature whether the protection should disappear completely by seven months. Therefore, the observation that infants in the control group had no protection could be an error. If, however, this was a correct observation,, the observed proportion of infants in the intervention group with protective levels of antibodies was a real measure of protection of the vaccine.

On the other hand, if there was some residual protection at seven months, the observed proportion of infants with protection in the intervention group was a measure of the success of the vaccine on top of this natural protection. Nevertheless, it was this combined rate of protection that was of interest. Therefore, the comparisons presented here, using

the rate of protection at seven months, ignoring the antibody levels at two months, are valid.

7.3 Analytical Procedures and Power

Many of the wide confidence intervals reflected great variability in the results. The number of infants to be enrolled needed to be considered in relation to the variables believed to influence immune response. The fixed population size of this study eliminated the possibility of adequate numbers for other than basic exploratory analysis.

The statistical power was not great enough to substantiate the results. It was known from the start of this study that power would be a problem. However, what was required was to establish reasonable doubt that ethnicity should be considered as a factor for diminished immune response to Hib vaccine among Inuit of the N.W.T..

7.4 Limitations and Sources of Error

Chapter three introduced the use of secondary analysis of data as a research method, and noted some of the problems associated with using previously collected data. One of the most serious problems with the analyses in this study was that of small sample size, and related low statistical power. As

mentioned in sections 2.5 and 7.3, the purpose of this exploration was to establish reasonable doubt as to the validity of using ethnicity to explain the poorer immune response observed among the Inuit infants.

There were problems associated with pooling of results in that any variables influencing the association between ethnicity and antibody response remained hidden and unexplained, and thus encouraged a false supposition about the overall effect of ethnicity and antibody response. The assumption that the data from different Inuit regions and different lots could be pooled led to results which were not generally reliable, as pooling can create problems with confounders.¹²³ Although stratified analyses were completed for regions and lot differences, the frequencies of data available with such division was too small for adequate comparison. It was not possible to differentiate the Dene bands among the Dene sample, which also could have influenced results.

There were sources of bias in this analysis that were beyond my control. Researcher bias occurred because the nurses working in the Inuit communities were in the communities full time and encouraged participants to enrol. They did not, however, have the time to do adequate follow-up. This was reflected in the 89 percent completion of vaccine series when compared to 95 percent for the Dene, who were enrolled and

followed up by a study nurse who travelled into the communities.

As the study nurse prepared to give immunization and collect bloods in the Dene community she had to consider the travel costs and logistics, such as having several infants ready for follow-up. Infants who missed one visit were left until the next visit. This meant that they were followed up but often were delayed into the older ages for the procedures. Community nurses were more available to collect data from the Inuit infants, which meant that they were more often in the "ideal", but younger ages. The older aged infants would be more likely to achieve better antibody levels.

Participant bias occurred because the Inuit were anxious to receive the Hib vaccine but were not so diligent about the follow-up blood specimen. The Dene, who were encouraged by the study nurse to provide blood specimens, were more available.

This meant that there were more Dene who provided blood specimens for analysis. Also, because they were late to start enrollment, they were more likely to have received the second vaccine lot. The Inuit started enrollment earlier and received much of the first lot. The lack of Inuit blood

specimens further lessened their opportunity to show protective results.

This study considered the outcome to be achievement of protective anti-PRP antibody levels and the associated factor to be ethnicity. Selection of Dene infants as a comparison group for the Inuit was the only possibility, and yet there was bias inherent in that choice. The Dene had relatively little Hib disease, and this may have affected their response to the vaccine by some naturally occurring response mechanism which the Inuit did not possess or just by the fact that they were not very interested in participating in the study. This would be important because it could artificially affect their response to the vaccine. It was suggested in section 5.3, however, that Inuit seemed more likely to have pre-vaccine protection than the Dene. Other characteristics, such as socioeconomic conditions, housing, nutrition, and climate may have influenced their ability to react to the vaccine, but no data were available.

Assessment of studies related to infants response to vaccines by Ward et al.⁵⁷ indicated that characteristics of the selected comparison groups could strongly influence the outcome of the study because of their lack of similarity to the study group. They suggested that the range of study results were not necessarily related to poor vaccine, or

response to vaccine, but to the different characteristics of the groups being compared.

7.5 Recommendations for Future Research

Dealing with N.W.T. populations may always require consideration of small samples to achieve desired results. Travel to the many communities is costly and difficult, depending on the location and season. Only a few communities can provide the number of births required to achieve the desired sample size. Although they are not the nomadic population they once were, the Dene and Inuit lifestyle must be considered if future studies are to be successful.

It is of great importance to carefully define the variables to be used in research. The use of "ethnicity" as a surrogate for other factors, such as socioeconomic status, geographical location, or genetic variability, does little to enlighten the researchers, the study community or the population at large.

The usual biological analysis of vaccine related studies compares the geometric mean anti-Hib antibody titres of both groups. Greater power to detect differences can be obtained, and smaller sample sizes required, with the analysis of continuous rather than dichotomous data.

Hib vaccine immunogenicity studies are no longer feasible because there are now licensed vaccines for infants as young as two months of age. It should be remembered, however, that immunogenicity does not necessarily equate with vaccine efficacy or effectiveness and that the continuous collection of data related to disease outcomes is important. This surveillance should collect all variables which may be related to disease and exposure, as well as vaccine status. In the final analysis, it is the effectiveness of a vaccine that is important.

This study provided a good deal of information about the areas with which future vaccine immunogenicity studies in the N.W.T. must be concerned. When new vaccines are formulated, studies will be required. New research must carefully consider the sample size available, the similarity between groups, and meticulous adherence to the protocol (eg. vaccine lots and age at vaccine administration). Future studies should also include the ability to analyze for other risk factors, particularly those related to socioeconomic status and breastfeeding, which were not available to this study.⁵⁷

The study was designed to explore factors related to differences between Inuit and Dene immune response to the PRP-D vaccine. Many of the variables associated with antibody response were not available for analysis. Of those that were,

the sample size was inadequate for the statistical tests to determine associations that might have existed.

7.6 Implications for Practice

Many vaccines for the prevention of *Haemophilus influenzae* type b have been tested on children of different ages with varying results. This quotation is from one of the world's primary researchers of *Haemophilus influenzae* type b vaccines, "Precise knowledge of both the immunogenicity of the vaccine and the risk factors in each country indeed seems essential for successful planning of national vaccination strategies.⁶³

It is important to refine that statement to include not only the risk factors for a country but to determine those factors, or markers, for individual populations and groups which enhance or detract from optimal immune response and effectiveness of a vaccine.

Stieb et al.¹²⁴, in a review of twenty different studies, indicated that disease incidence, age at time of disease, and geographic variations of populations were important factors in determining immunization programs. These ideas were reinforced by several others.^{55,61,125,126,127}

Differences between Inuit and Dene infants may have occurred by chance or because of a real inability of the Inuit to respond to the vaccine. It would be of public health importance to know that there is no real difference between them because of the fact that vaccine programs tend to be generic for the masses. If the Inuit do not respond as well to vaccines as the Dene or the general Canadian population, adherence to a national, or even territorial/provincial, immunization program (schedule) may not have the desired effect on the population at highest risk.

8. CONCLUSION

This study did not entirely resolve the question of whether ethnicity, or other factors, might explain the observed differences between Inuit and Dene infants. This was due to problems with the data used in this secondary analysis, and with the resulting lack of power to detect differences in some of the stratified analyses. The importance of the study was that, by examining the data in more detail, the role of ethnicity in relation to immune response was put into question.

Only a few of the factors for which analyses were completed were statistically significantly associated with protective levels of anti-PRP antibody, mainly because of sample size. It seemed likely, however, that ethnicity was not a factor. Rather, it might be concluded that administration of different lots, sex, possible regional differences among the Inuit, as well as the possibility of age related factors (ages at third vaccine dose and second blood specimen), played some part in the infants' response.

In spite of the lack of reliability and validity of the results, I believe that there was reasonable doubt shown in the alleged role of ethnicity in the results of the 1986 N.W.T. study. It is not reasonable to continue to promote

ethnicity as a factor in the diminished immune response of the Inuit infants of this study sample.

Table 1. 1986 N.W.T. study population

Infants enroled 267 (11 communities)

167 Inuit (62%) 74 Dene (28%) 26 Other (10%)

INTERVENTION GROUP			CONTROL GROUP		
Commenced PRP-D and DPT			Commenced DPT only		
	n	(%)		n	(%)
Inuit	92	(62)	Inuit	75	(63)
Dene	42	(28)	Dene	32	(27)
Other	14	(10)	Other	12	(10)
Total	148	(100)	Total	119	(100)
Complete Series: 135/148 (91%)			Complete Series: 99/119 (83%)		
	n	(%)		n	(%)
Inuit	82	(60)	Inuit	63	(64)
Dene	40	(30)	Dene	24	(24)
Other	13	(10)	Other	12	(12)
Total	135	(100)	Total	99	(100)
Incomplete: 13/148 (9%)			Incomplete: 20/119 (17%)		
	n	(%)		n	(%)
Inuit	10	(77)	Inuit	12	(60)
Dene	2	(15)	Dene	8	(40)
Other	1	(8)	Other	0	(0)
Total	13	(100)	Total	20	(100)
Blood Results: 106/148 (72%)			Blood Results: 67/119 (56%)		
	n	(%)		n	(%)
Inuit	59	(56)	Inuit	43	(64)
Dene	37	(35)	Dene	18	(27)
Other	10	(9)	Other	6	(9)
Total	106	(100)	Total	67	(100)
Overall Group Results:			Overall Group Results:		
	n	N (%)		n	N (%)
Inuit	59	92 (64)	Inuit	43	75 (57)
Dene	37	42 (88)	Dene	18	32 (56)
Other	10	14 (71)	Other	6	12 (50)
Total	106	148 (72)	Total	67	119 (56)

Table 2. Success achieved with all blood results returned;
1986 N.W.T. study

Success defined as ≥ 0.15 μ /mL anti-PRP antibody

	INUIT INFANTS	DENE INFANTS
Completed Series	82	40
Blood Analyzed	59	37
Specimens Missing	23	3
Success Observed	44%	60%
Best Case Success	60%	63%
Worst Case Success	32%	55%

Success reported:

Inuit 44% = 26 infants of 82;

26+23 (if missing results ≥ 0.15) = 49/82 = 60%

26+0 (if missing results < 0.15) = 26/82 = 32%

Dene 59.5% = 22 infants of 37;

22+3 (if missing results ≥ 0.15) = 25/40 = 63%

22+0 (if missing results < 0.15) = 22/40 = 55%

Table 3. Homogeneity of Aboriginal population in N.W.T. study communities; number enrolled in intervention group (N=134)

Community	Inuit n (%)	Dene n (%)
Cambridge Bay	13 (14.1)	0 (0)
Fort Good Hope	0 (0)	6 (14.3)
Fort McPherson	1 (1.1)	5 (11.9)
Hall Beach	3 (3.3)	0 (0)
Igloolik	12 (13.0)	0 (0)
Iqaluit	32 (34.8)	0 (0)
Lac La Martre	0 (0)	2 (8.8)
Fort Rae	0 (0)	20 (47.6)
Rankin Inlet	31 (33.7)	0 (0)
Yellowknife	0 (0)	9 (21.4)
TOTAL	92 (100)	42 (100)

Table 4. Inuit and Dene infants who provided blood specimens for serum analysis, by vaccine lots

Lots	Inuit n (%)	Dene n (%)	Total n (%)
22000-3	34 (64)	19 (36)	53 (100)
8J91140	25 (58)	18 (42)	43 (100)
Mixed	9 (90)	1 (10)	10 (100)
Total	68 (64)	38 (36)	106 (100)

Table 5. Numbers and percentages of infants who achieved successful anti-PRP antibody levels after vaccination, as determined by antibody levels of $\geq 0.15 \mu\text{mL}$, stratified by ethnicity and sex; secondary analysis

		Antibody Levels		
Ethnicity	Sex	$\geq 0.15 \mu\text{mL}$	$< 0.15 \mu\text{mL}$	Total
Inuit	Males	16 (42)	22 (58)	38 (100)
	Females	15 (50)	15 (50)	30 (100)
	Total	31 (46)	37 (54)	68 (100)
Dene	Males	6 (33)	12 (67)	18 (100)
	Females	16 (80)	4 (20)	20 (100)
	Total	22 (58)	16 (42)	38 (100)

Inuit, sex, 95% CI 0.25<0.73<2.12, not significant

Dene, sex, 95% CI 0.02<0.13<0.66, $P=0.009$

M-H Summary Odds Ratio, sex, 95% CI 0.17<0.42<0.96, $P=0.04$

Table 6. Median age in days at the three immunization doses and second blood specimen compared to the "ideal age"

Vaccine Dose	Median Age	"Ideal age" range
First	72	60 - 89
Second	141	120 - 149
Third	216	180 - 209
Second Blood	253	210 - 239

Table 7. Serum antibody levels of Inuit infants stratified by sex and region, comparing Baffin Region to each of the others (n=67)*.

Sex	Region	≥ 0.15 n (%)	< 0.15 n (%)	< OR <
M	Kitikmeot	0	7 (100)	see @
	Keewatin	6 (46)	7 (54)	0.25<1.31<7.06
	Baffin	9 (53)	8 (47)	
F	Kitikmeot	2 (50)	2 (50)	0.07<1.10<17.8
	Keewatin	2 (40)	3 (60)	0.15<1.65<23.2
	Baffin	11 (52)	10 (48)	

* (Total Inuit sample is 68; one is not from these regions.)

@ 1.12<"undefined"<"very large", P=0.02 (EpiInfo)
No other results were statistically significant.

Sex together, Baffin and Kitikmeot, 95%CI 0.84<5.0<51.99
Baffin and Keewatin, 95%CI 0.39<1.39<5.02

Table 8. Serum antibody levels for lots 22000-3 and 8J91140 for each ethnic group

Eth	Lot	≥ 0.15 n (%)	< 0.15 n (%)	<OR<
Dene	22000-3	9 (47)	10 (53)	0.07<0.35<1.63 P=0.229
	8J91140	13 (72)	5 (28)	
Inuit	22000-3	11 (32)	23 (68)	0.08<0.27<0.90 P=0.032
	8J91140	16 (64)	9 (36)	

Crude OR, lots, 95% CI 0.11<0.29<0.74, P=0.007

MH Summary OR, lots, 95% CI 0.12<0.30<0.76, P=0.009

Table 9. Inuit and Dene serum antibody levels stratified by vaccine lot and sex

Lot	Sex	Eth	≥ 0.15 n (%)	< 0.15 n (%)	Strata <OR<	<OR<
22000-3	M	Dene	3 (27)	8 (73)	0.09<0.70<4.35	0.51<1.84<6.87
		Inuit	7 (35)	13 (65)		
	F	Dene	6 (75)	2 (25)	0.78<7.50<97.0	
		Inuit	4 (29)	10 (71)		
8J91140	M	Dene	3 (50)	3 (50)	0.11<1.17<12.3	0.20<1.09<5.77
		Inuit	6 (46)	7 (54)		
	F	Dene	10 (83)	2 (17)	0.06<1.0<16.39	
		Inuit	10 (83)	2 (17)		

Overall Crude OR 95% CI 0.68<1.64<3.97, P=0.31

MH Summary OR is not calculated for data with effect modification

Table 10. Age at administration of first vaccine dose for Inuit and Dene infants

Days	Inuit n (%)	Dene n (%)	Total n (%)
32-59	7 (10)	1 (3)	8 (83)
60-89*	55 (81)	31 (81)	86 (81)
90-119	6 (9)	5 (13)	11 (10)
120-122	0 (0)	1 (3)	1 (1)
Total	68 (100)	38 (100)	106 (100)

* "ideal age" as per schedule

Table 11. Age at administration of third vaccine dose for Inuit and Dene infants

Days	Inuit n (%) cum%	Dene n (%) cum%	Total n (%)
144-179	5 (7) (7)	0 (0) (0)	5 (5)
180-209*	30 (44) (51)	8 (21) (21)	38 (36)
210-239	20 (29) (80)	15 (40) (61)	35 (33)
240-269	4 (6) (86)	7 (18) (79)	11 (10)
≥270	9 (13) (99)	8 (21) (100)	17 (16)
Total	68 (99)	38 (100)	106 (100)

* "ideal age" as per schedule

Table 12. Age at administration of third vaccine dose for Dene and Inuit by level of antibody achieved

Days	Eth	≥ 0.15 n (%)	< 0.15 n (%)	OR CI
144-179	Dene	0 (0)	0 (0)	0
	Inuit	2 (40)	3 (60)	
180-209*	Dene	3 (38)	5 (62)	0.78 0.10, 4.96
	Inuit	13 (43)	17 (57)	
210-239	Dene	9 (60)	6 (40)	1.83 0.39, 8.87
	Inuit	9 (45)	11 (55)	
240-269	Dene	5 (71)	2 (29)	0.83 0.01, 24.09
	Inuit	3 (75)	1 (25)	
≥ 270	Dene	5 (62)	3 (38)	2.08 .021, 22.15
	Inuit	4 (44)	5 (56)	

* "ideal age" as per schedule

Crude OR 95% CI 0.68<1.64<3.97

Table 13. Age at which second blood specimen was taken for Inuit and Dene infants

Days	Inuit n (%) cum%	Dene n (%) cum%	Total n (%)
186-209	4 (6) (6)	0 (0) (0)	4 (4)
210-239*	22 (34) (40)	4 (12) (12)	26 (27)
240-269	23 (35) (75)	11 (33) (45)	34 (35)
270-299	5 (8) (83)	10 (30) (76)	15 (15)
≥300	11 (17)(100)	8 (24)(100)	19 (19)
Total	65 (100)	33 (100)	98**(100)

* "ideal age" as per schedule

** date of second blood specimen unavailable for eight cases

Table 14. Age at which second blood specimen was taken for Dene and Inuit infants by level of antibody achieved

Days	Eth	≥ 0.15 n (%)	< 0.15 n (%)	OR CI
186-209	Dene	0 (0)	0 (0)	0
	Inuit	2 (50)	2 (50)	
210-239*	Dene	1 (25)	3 (75)	0.58 0.01, 8.96
	Inuit	8 (36)	14 (64)	
240-269	Dene	5 (45)	6 (55)	0.76 0.14, 4.05
	Inuit	12 (52)	11 (48)	
270-299	Dene	7 (70)	3 (30)	1.56 0.08, 22.92
	Inuit	3 (60)	2 (40)	
≥ 300	Dene	4 (50)	4 (50)	0.83 0.09, 7.33
	Inuit	6 (55)	5 (45)	

* "ideal age" as per schedule

Crude OR, 95% CI 0.46<1.17<2.93

Table 15. Percent Infants with Protective Antibody Levels, First and Second Blood Specimens, Control and Intervention Groups of 1986 N.W.T. Study

Study Group	Ethnic Group	2MOS* %	7MOS* %
Control	Dene	?	0.0
	Inuit	?	0.0
Intervention	Dene	26	59.5
	Inuit	30	44.0

* Baseline blood specimen was taken before the first dose of vaccine at 2 months of age; 7 months of age represents the age at which the second blood specimen was scheduled to be taken, one month after the third dose

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