The Incidence of Invasive *Haemophilus influenzae* Disease in Northwestern Ontario

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A thesis presented to Lakehead University

in fulfillment of the

thesis requirement for the degree of

Master of Public Health

Supervised by Dr. Marina Ulanova, MD, PhD

Thunder Bay, Ontario, Canada, 2009

Thank you to my supervisor, Dr. Marina Ulanova, for her expert guidance and support in completing my thesis. Thanks also to the Propers, Browns, and dear friends who provided their encouragement and support during this process.

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Abstract

Introduction

Prior to the late 1980s, *Haemophilus influenzae* type b (Hib) was the most common cause of meningitis, epiglottitis, and other invasive bacterial infections in young children. The introduction of the conjugate Hib vaccines dramatically decreased the incidence of invasive Hib disease in young children and is thus recognized as a great public health achievement. Since the vaccine's implementation, there have been concerns about capsule switching and serotype replacement, which may allow non-type b *H. influenzae* to fill the ecological niche previously occupied by Hib. Recently, reports identifying non-type b serotypes of *H. influenzae* causing invasive diseases have been published. Moreover, there appears to be a large number of Indigenous people affected by non-type b *H. influenzae*. This study examined the incidence of invasive *H. influenzae* disease in Northwestern Ontario, Canada.

Methods

Health records from 2002 to 2008 from Thunder Bay Regional Health Sciences Centre (TBRHSC; Thunder Bay, ON) and Meno-Ya-Win Health Centre (Sioux Lookout, ON), were examined to determine demographic and clinical characteristics of cases of invasive *H. influenzae* disease. The Ontario Public Health Laboratory (Toronto, ON) provided data on serotypes of clinical *H. influenzae* isolates.

Results

Thirty-eight cases of invasive *H. influenzae* disease were identified in Northwestern Ontario from 2002 to 2008. Twenty cases (52.6%) occurred in Aboriginal people and 23.7% (n=9) of cases were $\geq$ 60 years of age. Moreover, 44.7% (n=17) of cases in this study with detailed clinical information had at least one significant underlying medical condition. Only five

cases had no underlying conditions: four of whom were children ≤ 5 years of age and one was an adult. Of the isolates in this study, 81.6% were serotyped. Out of the serotyped isolates, 41.9% were *H. influenzae* type a, 29% were nonserotypeable, 25.9% were type f, and 3.2% were type e. No Hib was identified; however, not all isolates were serotyped. The incidence of invasive *H. influenzae* disease in Northwestern Ontario was higher than the incidence of invasive Hib disease in Ontario in the pre-vaccine era, reaching 2.98/100,000 in 2006 and 2007. In 2006, the incidence in children (0-4 years) was outstandingly high, 38.7/100,000; 100% of these children were Aboriginal. For unclear reasons, Aboriginal people in Northwestern Ontario appear to be disproportionately affected by invasive non-type b *H. influenzae* disease.

Conclusions

Invasive *H. influenzae* disease is a public health concern for Northwestern Ontario. Over time, the incidence of invasive *H. influenzae* disease appeared to be high – surpassing the officially recorded incidence rate of invasive Hib disease in the pre-vaccine era for the entire province of Ontario. The prevalence of non-type b serotypes in invasive *H. influenzae* disease and the proportion of Aboriginal children affected calls for enhanced surveillance of this pathogen now and in the future.

Introduction

Haemophilus influenzae: characteristics of the pathogen

Haemophilus influenzae is a Gram-negative, non-motile coccobacillus which colonizes the nasopharynx of humans (Morris *et al.*, 2008) and is present in approximately 3-5% of the human population worldwide (Kroll & Langford, 1994). *H. influenzae* can colonize the nasopharynx of apparently healthy individuals without causing any harm to the individual. However, in the presence of inadequate host immune defences, *H. influenzae* colonization can result in severe invasive diseases, such as meningitis, epiglottitis, septic arthritis, and septicemia (Peltola, 1993). Transmission of *H. influenzae* is achieved through direct contact and nasopharyngeal droplets and secretions (Kroll & Langford, 1994; World Health Organization, 2006). *H. influenzae* is considered to be invasive when it enters a normally sterile site in the body (i.e. blood, cerebrospinal fluid, pleural fluid, synovial fluid, etc). When *H. influenzae* is present in areas of the body that are not normally sterile (i.e. the nasopharynx), it is considered to be non-invasive (Centres for Disease Control and Prevention, 2005).

There are seven types of *H. influenzae*: six encapsulated serotypes (a, b, c, d, e, & f) and one non-encapsulated type (nonserotypeable, NST). The encapsulated serotypes are defined by the antigenic properties of their respective polysaccharide capsules (Pittman, 1931). Prior to the late 1980s, *H. influenzae* type b (Hib) – the most virulent serotype – was the most common cause of meningitis, epiglottitis, and other invasive bacterial infections in children (Kaye, 1982). *H. influenzae* is a diverse organism which can be further classified into eight biotypes, defined by their production of indole, urease, and ornithine decarboxylase (Doern & Chapin, 1987). Biotype I is most often observed in invasive diseases (Kroll & Langford, 1994).

Invasive Hib infections primarily infect young children, especially those < 2 years old and have a case-fatality rate of 5%. In individuals who survive invasive Hib disease, severe sequelae can result – approximately 10-15% of Hib survivors experience severe neurologic disorders and another 15-20% experience hearing loss (Public Health Agency of Canada, 2006). Non-encapsulated or nonserotypeable (NST) *H. influenzae* are less virulent and infrequently cause invasive disease, but can cause otitis media in children as well as community-acquired pneumonia, especially in the elderly and in patients with chronic obstructive pulmonary disease (COPD; Murphy, 2005).

The virulence of *H. influenzae* is conferred by its polysaccharide capsule, IgA protease, and fimbriae. Specifically, the polysaccharide capsule provides the organism with a protective, hydrophilic environment, which is intrinsically anti-phagocytic. The capsule promotes inactivation of the C_3b component of the complement system – one of the immune system's first defences against infectious pathogens (Rother *et al.*, 1998). In addition, the capsule may be continuously shed from the organism, thus binding antibodies and preventing them from neutralizing *H. influenzae* (Kroll & Langford, 1994). Similarly, *H. influenzae* produces IgA protease, an enzyme which promotes degradation of IgA antibodies – immunoglobulins which are present on mucosal surfaces, such as the nasopharynx where *H. influenzae* colonizes (Vitovski *et al.*, 2002). Finally, *H. influenzae* has fimbriae, which allow the organism to firmly adhere to and selectively target non-ciliated cells, thereby promoting the organism's colonization of the nasopharynx. Once attached to non-ciliated cells, *H. influenzae* wedges between cellular tight junctions, thus enabling it to enter normally sterile body sites and cause severe invasive diseases (Kroll & Langford, 1994).

Immunological basis for vaccination against Hib

Hib has been identified as the cause of severe, potentially fatal invasive diseases in many young children (Kaye, 1982). Consequently, there is a great deal of interest in preventing individuals from contracting Hib. To date, vaccines remain one of the most cost-effective methods to prevent the development of infectious diseases (Halder, 2001) and the conjugate Hib vaccines are no exception (Swingler *et al.*, 2008). Before delving into the specifics of conjugate Hib vaccines, this paper will discuss the immunological basis for vaccination against Hib. First, some basic immunology and the principles behind vaccination will be reviewed.

Basic Immunology

To begin, the immune system is a collection of specialized cells, tissues, organs, and other components which function together to protect the body from invasion of foreign pathogens. It can be further sub-divided into the innate and adaptive immune systems. The innate immune system is the body's first defence against pathogens; it provides an immediate, non-specific response to eliminate an infectious organism from the body. The innate immune system primes the adaptive immune system to develop a pathogen-specific immune response. Often, the innate immune system is effective, but not sufficient to clear an infection from the body. In this case, the adaptive immune system will initiate a highly specific response to eliminate the pathogen. The adaptive immune system mounts an immune response through the use of tightly regulated lymphocytes, B and T cells (Janeway *et al.*, 2005).

B cells are one of the two major types of lymphocytes present in the adaptive immune system. When the immunoglobulin receptor on the surface of a B cell is activated by an antigen (a molecule to which a specific antibody can bind), the B cell differentiates into an antibody-producing cell, releasing antibodies with the same specificity as its immunoglobulin receptor. T

cells are the other major type of lymphocytes active in the adaptive immune system. They can be further sub-divided into CD4 T cells and CD8 T cells, based on the co-receptor protein they carry on their cell surface. CD4 T cells, or helper T cells, recognize peptides derived from intravesicular sources of antigen-presenting cells and differentiate to become either T_H1 or T_H2 CD4 T cells. T_H1 CD4 T cells release many cytokines to activate macrophages and T_H2 CD4 T cells activate B cells. CD8 T cells, or cytotoxic T cells, recognize antigens that are synthesized in the cytoplasm of a particular cell, such as viral antigens (Janeway *et al.*, 2005).

Antigen-presenting cells, dendritic cells, macrophages, and B cells, process antigens, and the epitopes contained therein. Epitopes are sites on antigens which are recognized by antibodies, as such, antigens may contain multiple epitopes. Once antigen-presenting cells process antigens, they display them on their cell surface for lymphocytes in the adaptive immune system to recognize and subsequently mount a response against them. When a protein has more epitopes, it will be recognized by more antibodies than if it had fewer epitopes, thus stimulating a greater antibody response (Janeway *et al.*, 2005).

Unlike the innate immune system which provides immediate protection against pathogens, the adaptive immune system requires 4-7 days to generate an effective response. Another difference between the two systems is that once a pathogen has been cleared from the body, the innate immune system remains unchanged from its encounter with the infectious agent. In contrast, after the adaptive immune system has been activated, memory B and T cells are created. These memory cells are more sensitive to antigen exposure than naive B and T cells. They do not require priming from the innate immune system and as such are able to respond more rapidly to a foreign pathogen should it enter the body again. The induction of immunological memory after the activation of the adaptive immune system is the reason that

vaccination is effective. After vaccination, immunized individuals develop memory cells that are able to activate when the individual is re-exposed to the particular pathogen against which they have been vaccinated (Janeway *et al.*, 2005).

Early Hib Vaccines

Early versions of the Hib vaccines in the 1970s were composed of Hib's capsular polysaccharide, polyribosylribitol phosphate (PRP). These vaccines did induce a protective antibody response against Hib, but not in the target population of children < 2 years old. Despite the administration of the vaccine, children < 2 years old do not mount immune responses to polysaccharide antigens. This is because the B cells responsible for responding to polysaccharide antigens, the marginal zone B cells, do not fully mature until an individual reaches approximately 2 years of age (Timens *et al.*, 1989; Kroll & Langford, 1994; Mond *et al.*, 1995; Swingler *et al.*, 2008). In addition, the activation of B cells in response to polysaccharide antigens differs from their response to protein antigens. Specifically, the polysaccharide antigen response requires a co-stimulatory signal from CD21 (a type 2 complement receptor). Children < 2 years old have reduced expression of CD21 on their B cells, thus explaining why they are relatively unresponsive to polysaccharide antigens (Rijkers *et al.*, 1998).

In order for the PRP vaccine to become effective in the target population, it was necessary to make the polysaccharide antigen more immunogenic. One such method is to couple a protein carrier to a polysaccharide antigen, a process first described by Goebel & Avery (1929). On their own, polysaccharides are usually T-independent antigens – antigens that can directly stimulate B cells to produce antibodies without the assistance of T cells. Proteins, on the other hand, are usually T-dependent antigens – antigens that require T cells to stimulate antibody production by B cells (Janeway *et al.*, 2005). When the immune system encounters a protein

antigen, a robust, polyclonal antibody response is generated, even in young infants. In contrast, when the immune system of a young child encounters a polysaccharide antigen, such as PRP, only a few B cell clones are triggered to begin producing antibodies (Kroll & Langford, 1994). By conjugating the protein carrier to a polysaccharide, the polysaccharide becomes a T-dependent antigen, thereby stimulating an immune response against both the polysaccharide and protein antigens (Rijkers *et al.*, 1998). This leads to increased anti-polysaccharide antibody levels. In addition, T-dependent antigens stimulate isotype switching – the process by which an antibody's effector function is altered (Janeway *et al.*, 2005). Isotype switching produces antibodies with a greater affinity and avidity for Hib (Kroll & Langford, 1994). In this way, children < 2 years old are able to respond to the Hib polysaccharide capsule and immunological memory to PRP is created. Thus, individuals in the target population are protected from developing invasive Hib disease (Kroll & Langford, 1994; Rijkers *et al.*, 1998).

Hib conjugate vaccines

The conjugate Hib vaccines have been recognized as a great public health achievement due to their high level of effectiveness and relatively low number of vaccine failures. Conjugate Hib vaccines work to reduce the disease burden caused by Hib by decreasing the carriage rate of Hib in young children, thereby reducing the pool of potentially infectious children in the community. In this way, the conjugate Hib vaccines confer a degree of herd immunity to all individuals in a given community regardless of whether or not they have been vaccinated against Hib (Kroll & Langford, 1994; Barbour, 1996). Herd immunity refers to the epidemiological phenomenon in which a population is resistant to an infectious agent because a large proportion of the population is immune to the aforementioned pathogen (Gordis, 2004).

There are three types of conjugate Hib vaccines in use today: PRP conjugated to *Neisseria meningitidis* outer membrane protein (PRP-OMP), PRP conjugated to tetanus toxoid (PRP-T), and PRP conjugated to cross-reacting mutant (CRM) diphtheria protein oligosaccharide 197 (CRM_{197} or PRP-HbOC; Swingler *et al.*, 2008). Each of these vaccines contains differing amounts of PRP as well as the specific protein component contained within the particular vaccine (Kroll & Langford, 1994; Dellepiane *et al.*, 2000). All three of these vaccines stimulate a robust antibody response after the full immunization series has been administered; however, PRP-OMP is the only vaccine with a unique serologic response. PRP-OMP is unique because it is the only one of the conjugate Hib vaccines that provides significant immunity after the first dose; however, when administered for the full immunization series, peak antibody concentrations are lower than when other forms of the Hib vaccine are given (Decker *et al.*, 1992). PRP-OMP is also different from the other conjugate Hib vaccines because OMP is not a single protein, but a combination of proteins. It is capable of inducing an immune response after only one dose because it engages Toll-like receptor 2 (TLR2), stimulating the production of interleukin-8 and thereby enhancing the immune response induced by this particular conjugate Hib vaccine formulation (Kroll & Langford, 1994; Latz *et al.*, 2004).

In 1991, the conjugate Hib vaccine was introduced as part of the routine childhood vaccination schedule in Canada – one dose at 2, 4, 6, and 18 months. The conjugate Hib vaccine used in Canada is PRP-T, as it achieves protective serum antibody concentrations in 99% of children who receive the full vaccination series (Public Health Agency of Canada, 2006). PRP-T is believed to be effective because it contains a larger protein with more epitopes (Kroll & Langford, 1994).

In Canada, the target vaccination coverage rate for Hib is 97%; however, in 2004 the Canadian Hib vaccination coverage rate was estimated to be much lower, only approximately 73% (Public Health Agency of Canada, 2006). It is quite possible that the vaccination coverage rate is an underestimate due to differences in data collection at the provincial level and the absence of a national immunization reporting system. For example, in Ontario the vaccination coverage rate for Hib was 96.7% in 2002 (Ontario Ministry of Health and Long-term Care, 2004). Overall, the conjugate Hib vaccine is widely used and is considered to be highly effective in Canada (Scheifele *et al.*, 2005).

In countries outside of Canada, the conjugate Hib vaccines are also considered to be effective in preventing the development of invasive Hib disease. In Finland, Eskola *et al.* (1990) examined the effectiveness of the conjugate Hib vaccine PRP-D (PRP conjugated to diphtheria toxoid) in 114,000 infants. This study randomized infants to receive one dose of the PRP-D vaccine at 3, 4, and 6 months, as well as one booster dose between the ages of 14 and 18 months. Infants in the control group received only one dose of the vaccine at 24 months. In the group that received the early vaccinations, there were only four documented cases of bacteremic Hib disease; in comparison, the control group had 64 cases between the ages of 7 and 24 months. This study estimated the efficacy of the vaccine to be 94% among young Finnish children.

Researchers in the United Kingdom (UK) showed similar results in a study that evaluated the effectiveness of the conjugate PRP-T Hib vaccine among infants. In this study, infants in certain health districts were given one dose of the PRP-T vaccine at 2, 3, and 4 months; the control group did not receive the vaccination. Eighteen months after the first doses had been administered to the treatment group; none of the infants given the PRP-T vaccine had developed invasive Hib disease. In comparison, 11 infections had occurred in the control population during

this time period – an incidence rate of 101.8/100,000. This study demonstrated 100% effectiveness for the conjugate PRP-T Hib vaccine among UK infants (Booy *et al.*, 1994).

Considerations for vaccine design

The efficacy of the conjugate Hib vaccines is closely related to the stringent quality control measures in place during the time in which the vaccine is manufactured. Most importantly, the covalent bond between the polysaccharide component and the protein carrier must be firmly established – the polysaccharide component provides antigenic specificity to Hib, while the protein component provides T cell stimulation to enhance the immune response and eventually lead to the development of immunological memory (Kroll & Langford, 1994). Any unconjugated polysaccharide and protein components in the vaccine have the potential to cause anergy of lymphocytes – render them ineffective due to the lack of co-stimulation at the time of antigen-binding (Janeway *et al.*, 2005). Failure to remove these unconjugated components can render the vaccination ineffective, thereby leaving the vaccinated individual still vulnerable to invasive Hib disease. In addition to removing unconjugated polysaccharide and protein components, it is important for any other contaminants to be removed from the final vaccine product in order for it to be considered safe. Pyrogens are particularly important to remove during the manufacturing process as they are capable of causing high fevers and even death when they enter the body (Kroll & Langford, 1994; Janeway *et al.*, 2005).

In addition to maintaining a good safety profile, conjugate Hib vaccines must be evaluated to determine their potency or immunogenicity. The immunogenicity of any given conjugate Hib vaccine is tested both before it is administered to individuals in the target population as well as after administration has begun. Each lot of a conjugate Hib vaccine that is manufactured must be tested to ensure that it is as effective as the previous lots produced by the

manufacturer. In addition, despite the fact that each of the conjugate Hib vaccines in use today is different from one another, quality control testing assures that no matter which conjugate Hib vaccine is being used, they are all equally effective in preventing the development of invasive Hib disease in the population of interest (Kroll & Langford, 1994; Dellepiane *et al.*, 2000).

Co-administration of other vaccines with conjugate Hib vaccine

The conjugate Hib vaccine can be successfully administered either separately or in conjunction with other regular childhood immunizations (Swingler *et al.*, 2008). Considering that children are now being immunized against an increasing number of infectious diseases, co-administration of multiple vaccines is advantageous to ensuring children receive their full complement of vaccinations (Dagan *et al.*, 1997). A study in Thai infants by Kerdpanich *et al.* (2007) found that when the PRP-T conjugate Hib vaccine was administered in the same syringe with the diphtheria-tetanus and whole-cell pertussis (DTPw) vaccines, the anti-PRP antibody titers were much lower than when the vaccinations were administered separately in opposite arms. While statistically significant, this result was not deemed to be clinically relevant as the anti-PRP antibody titers are still high enough to confer protection against Hib. Similar results were observed in a randomized control trial conducted in the UK. This study found that when the PRP-T vaccine was administered in the same syringe as the DTPw vaccine, the titers of anti-PRP antibodies were lower than when the vaccinations were administered in separate syringes. Again, anti-PRP antibody levels were still high enough to protect the immunized individuals against Hib, making this observation clinically irrelevant (Jones *et al.*, 1998). These studies demonstrate that it is possible to administer regular childhood immunizations either separately or in the same syringe. Co-administration of vaccinations is considered to be advantageous because it requires fewer numbers of injections, thus reducing the opportunity for sequelae related to the

administration of the vaccine. In addition, being able to combine vaccinations means children require fewer injections, thereby reducing the number of appointments necessary for children to acquire their vaccinations. This also reduces the amount of time that health care providers need to spend immunizing patients and further improves patient compliance by simplifying the immunization schedule (Dagan *et al.*, 1997; Jones *et al.*, 1998; Kerdpanich *et al.*, 2007).

Despite the obvious advantages of combination vaccines, there can be consequences when different vaccines are co-administered. For example, after the introduction of the conjugate Hib vaccines in the UK in 1992, Hib vaccine efficacy was estimated to be 98% and the incidence of invasive Hib disease was relatively low – 0.65/100,000. In 1999, however, an increase in the number of cases was observed and by 2002 the incidence rate of invasive Hib disease in the UK was 4.6/100,000 (Ramsay *et al.*, 2003). Two reasons for this increase have been suggested. First, when the Hib vaccine immunization schedule was initially introduced in the UK, it contained only three doses of a conjugate Hib vaccine (one at 2, 3, and 4 months of age). Second, around the time of the increase in the incidence of invasive Hib disease, the combination vaccine in use included a conjugate Hib vaccine as well as the diphtheria-tetanus and acellular-pertussis (DTPa) vaccine. Similar to what was observed by Kerdpanich *et al.* (2007) and Jones *et al.* (1998), some studies conducted in the UK demonstrated decreased anti-PRP antibody levels when the conjugate Hib vaccine was given in combination with the DTPa vaccine. However, despite lower antibody titers, the antibody levels were still high enough to confer protection against invasive Hib disease (Goldblatt *et al.*, 1999; Poolman *et al.*, 2001).

In contrast, other studies have reported that the decreased anti-PRP antibody response as a result of the combination Hib-DTPa vaccine does not provide adequate protection against invasive Hib disease. McVernon *et al.* (2003) suggested that the combination of the lower

antibody titers induced by the Hib-DTPa vaccine and the UK's accelerated immunization schedule (lacking a booster dose) may be sufficient to cause an increase in the incidence of invasive Hib disease. Johnson *et al.* (2006) reported that the Hib-DTPa combination also affects the development of invasive Hib disease by reducing anti-PRP antibody avidity for Hib. Low anti-PRP antibody avidity decreases the functional activity of anti-PRP antibodies, leading to an increased likelihood of vaccine failure and thus increased susceptibility to invasive Hib disease (Lee *et al.*, 2008).

Global epidemiology of invasive *H. influenzae* disease

Since the introduction of the conjugate Hib vaccines in the early 1990s, the burden caused by invasive Hib disease has been substantially reduced; however, use of the conjugate Hib vaccines is not uniform around the world. The World Health Organization (WHO) estimated that in 2005, 119 countries had routine Hib immunization programs and 92% of the eligible population in the developed world were vaccinated against Hib. In contrast, WHO estimated that Hib vaccination coverage was about 42% in developing countries and only 8% in the world's poorest nations (Morris *et al.*, 2008), see Figure 1 (WHO, 2008). Worldwide, Hib is estimated to cause 3 million cases of invasive disease and approximately 386,000 deaths each year. Individuals in many countries are affected, but the most devastating effects of invasive Hib disease are seen in the poorest countries (WHO, 2005). This section will discuss the incidence of invasive Hib disease and the efficacy of the conjugate Hib vaccines in different geographic regions of the world. (Canadian data are not included in this section as they are considered on their own later in the paper).

North America (excluding Canada)

Prior to the introduction of the conjugate Hib vaccines in the United States, the incidence rate of invasive Hib disease in children < 5 years old was 88/100,000 (Cochi *et al.*, 1985). In 2007, the number of Hib cases had decreased by 99.8% and the number of deaths due to invasive Hib disease in the US had decreased by 99.5% compared to the pre-Hib vaccine era (Roush & Murphy, 2007). Despite the overall success of the Hib vaccination campaign in the US, it is important to note that its effects were not uniform across the country. In particular, the incidence rate of invasive Hib disease in the pre-vaccine era was > 700/100,000 in Native Alaskan children < 5 years old (Ward *et al.*, 1986). Singleton *et al.* (2006), demonstrated that after the introduction of the PRP-OMP Hib vaccine, the incidence rate of invasive Hib disease in children < 5 years old decreased to 18.3/100,000 in 1995 – still a higher incidence rate compared to other populations. In 1996, the conjugate Hib vaccine in Alaska was changed from PRP-OMP to PRP-HbOC and a subsequent resurgence in invasive Hib disease (47.6/100,000) was observed in children < 5 years old. This increase was partially attributed to the lower antibody responses PRP-HbOC induces after one dose compared to the relatively higher anti-PRP antibody responses after one dose of PRP-OMP (Singleton *et al.*, 2006). It was also partially attributed to a high oropharyngeal carriage of Hib in Native Alaskan children. Specifically, a cross-sectional study by Galil *et al.* (1999) found that despite widespread vaccination against Hib, 9.3% of Native Alaskan children aged 1-5 years old carried Hib in their oropharyngeal cavities. This is especially surprising considering that the vaccine reduces the carriage of Hib in immunized individuals and during this time period, 95.6% of Native Alaskan children had received three or more doses of a conjugate Hib vaccine. In comparison, only 87.1% of non-Aboriginal Alaskan children had received three or more doses of a conjugate Hib vaccine during this period. Despite

the high vaccination coverage among the Native Alaskan children, the incidence rate of invasive Hib disease was still five times higher than it was among the non-Aboriginal Alaskan children (Singleton *et al.*, 2006).

South America

The studies summarized in this section demonstrate the effectiveness of the conjugate Hib vaccines in young children in middle-income countries in South America: Brazil and Chile (Morris *et al.*, 2008). In Brazil, the conjugate Hib vaccines were made publicly available in 1999. Prior to the introduction of the vaccine, the incidence of Hib meningitis in children < 1 year old was 60.9/100,000. Five years after the introduction of the conjugate Hib vaccine, the incidence rate of Hib meningitis in Salvador, Brazil had decreased to 3.1/100,000 – a 95% decrease, nearly eliminating Hib meningitis in this region (Ribeiro *et al.*, 2007).

The situation in Chile was similar to that of Salvador, Brazil. In 1996, the conjugate Hib vaccine was introduced into the routine vaccination schedule in Chile. At the time of the vaccine's introduction, the incidence rate of Hib meningitis in children < 5 years of age was 40/100,000. By 1998, just two years after the vaccine was first offered, the incidence rate of Hib meningitis had dropped dramatically to 2/100,000 – representing 90% vaccine effectiveness against invasive Hib disease (Lagos *et al.*, 1996).

Europe

In Europe, invasive Hib disease has been monitored by the European Union Invasive Bacterial Infections Surveillance Network (EU-IBIS) since 1999. The countries included in the network are: Austria, Belgium, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Malta, Netherlands, Norway, Poland, Portugal, Slovak Republic, Slovenia, Sweden, UK; in addition, Australia and Israel also

participate in EU-IBIS (EU-IBIS Network, 2006). Prior to the introduction of the conjugate Hib vaccine, the incidence rate of invasive Hib disease in Europe was similar to what was observed in North America (Morris *et al.*, 2008). By the early 1990s, most European countries had introduced the conjugate Hib vaccine. In 2004, the incidence rate of Hib meningitis in children < 5 years old was 0/100,000 in Finland, Iceland, Italy, Slovenia, and Sweden (EU-IBIS Network, 2006). In Denmark, the incidence of invasive Hib disease prior to the introduction of the Hib vaccine was 49/100,000; by 2002 the incidence rate had decreased to 0/100,000 (Hviid & Melbye, 2004). For the countries included in EU-IBIS, the incidence rate of invasive Hib disease in 2004 was 0.64/100,000 in children < 5 years of age. The only notably higher incidence rate was observed in Estonia – 20.52/100,000 – likely attributable to the fact that the Hib vaccine was not introduced in the country until 2005 (EU-IBIS Network, 2006).

Two countries that were exceptions to the overall decreasing incidence of invasive Hib disease in Europe were the UK and the Netherlands. The situation in the UK has already been described previously in this paper. In brief, the combination of an accelerated vaccination campaign (only 3 doses of the conjugate Hib vaccine), when co-administered with the DTPa vaccination lead to an increase in the incidence of invasive Hib disease. These results demonstrated the importance of providing a fourth booster dose of the vaccination to young children (Berrington *et al.*, 2006) and initiated the implementation of a booster catch-up campaign (Trotter *et al.*, 2003). In the Netherlands, the situation is similar to that of the UK. Specifically, the vaccine was introduced in 1993, after which the number of cases of invasive Hib disease decreased to a minimum of 12 cases in 1999. In 2004, the number of cases had increased to 49, apparently due to an increase in the number of vaccination failures in the country. The reason behind the increase is unknown; however, the authors suggested that the

reduction in Hib carriage caused by the conjugate Hib vaccine reduces the exposure of the general population to Hib. If the conjugate Hib vaccine was not in use, the carriage of Hib among individuals in the population would be higher. In this way, individuals would periodically be exposed to Hib over the course of their lifetime. Intermittent exposure to Hib would result in boosting of the natural immune response to Hib, thereby enhancing the individual's protection against Hib. The use of the conjugate Hib vaccine reduces the natural boosting that would occur over an individual's lifetime if the vaccine was not in use. As a result, the immune response to Hib wanes and the individual becomes more susceptible to Hib. This demonstrates the importance of booster doses of the vaccine to prevent this bacterial infection (Spanjaard *et al.*, 2005).

In addition to gathering information on the incidence of invasive Hib disease in Europe, the EU-IBIS Network also garners information on the incidence of non-type b invasive *H. influenzae* disease. From 1999 to 2003 they found that invasive infections due to non-serotypeable *H. influenzae* occurred frequently among children $\geq$ 15 years of age, though the highest incidence rate was still detected among children < 5 years old. Of the non-type b *H. influenzae* strains, serotype f was most frequent, followed by some cases of serotype e. In Europe, invasive Hib disease tends to cause meningitis, while non-type b invasive *H. influenzae* disease tends to cause pneumonia and septicaemia. Overall, the European case fatality rate due to all types of invasive *H. influenzae* infections was 8.34%, and was highest in invasive disease caused by non-encapsulated bacteria (EU-IBIS Network, 2006).

Africa

The majority of countries in Africa have not yet introduced the conjugate Hib vaccine into their routine immunization schedules. In fact, as of December 31, 2007, only 19 of 46

African countries had included the vaccine in their routine immunizations (Morris *et al.*, 2008). This section will examine the effects of the conjugate Hib vaccine in Angola, The Gambia, Uganda, South Africa, and Sudan.

In 2004, 555 children (median age 11 months) were treated for bacterial meningitis at the hospital in Luanda, Angola; Hib was responsible for 60% of all cases of bacterial meningitis. The case fatality rate for all causes of bacterial meningitis was 35% and of the survivors, 24% had developed severe neurological sequelae. Due to a lack of appropriate antibiotics, the treatment regimen for bacterial meningitis in this region is to give the affected patient a blood transfusion. Those who receive the blood transfusion have a case fatality rate of 23%; in comparison, those who do not receive the procedure have a case fatality rate of 39% (Pelkonen *et al.*, 2008). Data were not available on the effectiveness of the Hib vaccine in this region.

Prior to the introduction of the conjugate Hib vaccine in The Gambia in 1997, the annual incidence of invasive Hib disease was 274/100,000 in children < 1 year old (Adegbola *et al.*, 2005) and 60/100,000 in children < 5 years old (Howie *et al.*, 2007). In 2002, five years after the vaccine was introduced in The Gambia, the incidence rate had dropped to 0/100,000 for children < 5 years old (Howie *et al.*, 2007). Unfortunately, Hib surveillance was stopped in The Gambia in 2007 so it is impossible to know if the incidence rate of invasive Hib disease in this region has changed since then (Morris *et al.*, 2008).

In Uganda, the incidence rate of Hib meningitis before the introduction of the conjugate Hib vaccine was 88/100,000 in children < 5 years of age (Felkin *et al.*, 2004). The vaccine was introduced into Uganda's regular immunization schedule in 2002 and the incidence rate of Hib meningitis dropped to nearly zero cases by 2007. As a result of the conjugate Hib vaccine in Uganda, 5,000 deaths and 1,000 cases of severe sequelae due to meningitis are prevented each

year. Since Uganda's population is largely composed of displaced peoples from war torn regions in the surrounding area, it will be a challenge to maintain vaccine availability and coverage for this transient population (Lewis *et al.*, 2008).

The success of the Hib vaccination campaign in Uganda as described by Lewis *et al.* (2008) is encouraging; however, the use of the conjugate Hib vaccine has decreased Hib carriage and has opened up an ecological niche for other serotypes of *H. influenzae* to cause severe invasive diseases, such as pneumonia. Pneumonia is a serious infection accounting for 10-30% of childhood deaths in Uganda. Typically pneumonia caused by *H. influenzae* is due to NST strains (Murphy, 2005). A study conducted over a four month period from 2005-2006 identified 157 children (aged 2-59 months) with severe pneumonia; the case fatality rate was 15.5%. Of the 157 cases of pneumonia, 23.5% of cases were caused by *H. influenzae* isolates. Each one of the *H. influenzae* isolates was resistant to chloramphenicol, the first-line antibiotic used to treat pneumonia in Uganda. Factors that were predictive of death included severe malnutrition, hypoxaemia, and pneumonia classified by WHO standards as "very severe" (Nantanda *et al.*, 2008).

In South Africa, the PRP-T Hib vaccine was introduced in 1999; WHO estimated the vaccination coverage rate to be 92% in 2004 (WHO, n.d.). Martin *et al.* (2004) found that the Hib conjugate vaccine had no effect on the number of cases of bacterial meningitis in the region. The high rates of HIV-1 infection in South Africa likely affect the efficacy of the conjugate Hib vaccine; HIV-1-infected children have a 35 times greater risk of experiencing Hib vaccine failure than children in the same region who are not infected with HIV-1 (Madhi *et al.*, 2005).

Finally, in Sudan the conjugate Hib vaccine has not yet been introduced; however, since 1998, seasonal epidemic meningitis has been a serious public health problem for this country. A

study conducted during the 2004-2005 epidemic season found 1,830 cases of suspected meningitis in children, nearly 10% of which were caused by Hib. Children who developed meningitis caused by invasive Hib infection were 9 months old on average, much younger than children who developed meningitis caused by *Streptococcus pneumoniae* (1.5 years of age) or *Neisseria meningitidis* (5 years of age; Afifi *et al.*, 2008).

Middle East

Many countries in the Middle Eastern region of the world are considered low or low-middle income countries and therefore do not have regular conjugate Hib vaccine immunization programs. Those countries that have introduced the Hib vaccine do not necessarily have Hib surveillance data readily available (Morris *et al.*, 2008). The best evidence comes from Saudi Arabia – one of the high income countries in this region. Al-Mazrou *et al.* (2004) conducted a study identifying the incidence of meningitis in Saudi Arabia from 1999 to 2001. They found 208 cases of meningitis during this time period, 28% of which were attributable to Hib. The median age of patients who contracted Hib meningitis was 8 months of age; the incidence rate was 16.88/100,000. Another study conducted at the University Hospital of Riyadh, Saudi Arabia from 1996 to 2007, identified 80 cases of invasive Hib disease in children < 18 years of age during the first half of the study period (1996 to 2000). In 2000, the conjugate Hib vaccine was introduced in Saudi Arabia; since then there has been a steady drop in the number of cases. Between 2001 and 2007, only 36 cases of invasive Hib disease were identified in the country (Al-Zamil, 2008).

In Israel, the PRP-OMP vaccine was introduced into the national immunization schedule in 1994 and achieved greater than 90% coverage. Dagan *et al.* (1999) estimated that the vaccine effectiveness was 95% for invasive Hib disease and up to 97% for Hib meningitis. In addition,

their study demonstrated a reduction in Hib infection in infants who were too young to be vaccinated, a strong sign of the effect of herd immunity in this country. Similarly, in Qatar in 1992, one year before the conjugate Hib vaccine was introduced, 14 cases of Hib meningitis were observed. In both 1995 and 1996 only one case was reported, thus demonstrating the effectiveness of the vaccine (Wenger *et al.*, 1999). Kuwait introduced the Hib conjugate vaccine in 1997 and by 2000 had reached 98% vaccination coverage (no data were available on the vaccine's effectiveness; Wenger *et al.*, 1999).

The Pacific Region and Asia

After the introduction of the Hib vaccine in Australia & New Zealand, the decrease in the incidence of invasive Hib disease was comparable to what was observed in North America and Western Europe (Morris *et al.*, 2008). In Australia, the incidence of invasive Hib disease declined from 15/100,000 in 1993, the year the vaccine was introduced, to 1.2/100,000 in 2000 (Horby *et al.*, 2003). The biggest difference, however, was among the Indigenous children. Prior to the introduction of the Hib vaccine, Australian Aboriginal children had the highest incidence rate of invasive Hib disease in the world – 991/100,000 (Hanna, 1990). In addition to having the highest incidence of invasive Hib disease, Indigenous Australian children were also more likely to develop the disease at an earlier age – 6 months of age, compared to 12 months of age for non-Indigenous Australian children (Guthridge *et al.*, 2000). After the PRP-OMP vaccine was introduced in Australia in 1993, the incidence of invasive Hib disease decreased. The effectiveness of the conjugate Hib vaccine was estimated to be 97.5% (Markey *et al.*, 2001).

In New Zealand, the conjugate Hib vaccine was introduced in 1994 and resulted in a 92% decrease in hospital admissions from Hib meningitis in children < 5 years old (Wilson, Wenger, Mansoor, Baker, & Martin, 2002). Fiji introduced the Hib vaccine in 1995, but inadequate

supply led to irregular vaccinations until 1999, thus no good surveillance data are currently available for this country (Wilson *et al.*, 2003).

In Asia, it was believed that the burden of invasive Hib disease was lower than in other geographic regions due to the rarity of isolating Hib from clinical specimens; thus the Asian Hib data are somewhat limited (Morris *et al.*, 2008). Peltola (1999) examined data from 100 reports on Hib produced by Asian countries. In this paper Asia is considered to be the geographic expanse from the Mediterranean Sea in the west to the Pacific Ocean in the east, excluding Russia, Turkey, and Papua New Guinea. Peltola identified an incidence rate of 40/100,000 for invasive Hib diseases in children 0-4 years old. Despite the widespread success of the conjugate Hib vaccines around the world, the misconception that invasive Hib disease does not affect Asian populations is a major barrier to its effective implementation in this region (Peltola, 1999).

In India, preliminary data on the incidence of invasive Hib disease have only recently become available. Minz *et al.* (2008) conducted a prospective surveillance study from 1997 to 1999 to determine its incidence in children in this region. In children 0-59 months of age, the incidence rate of Hib meningitis was 7.1/100,000; in children 0-11 months of age, the incidence rate was much higher – 32/100,000. These data are significant because India is home to 30% of all the children in the world, thus representing an enormous population susceptible to invasive Hib disease (Minz *et al.*, 2008).

Many Asian countries have not yet introduced the conjugate Hib vaccine into their national immunization schedules, thus is it not possible to comment on the vaccine's effectiveness on this continent (see Figure 1; Morris *et al.*, 2008).

H. influenzae in Canada

Prior to the introduction of the conjugate Hib vaccine in Canada, the 5 year annual incidence rate of invasive Hib disease across the country (1986 to 1990) was 22.7/100,000 (Public Health Agency of Canada, 2007). From 1985 to 1990 a total of 2,095 invasive Hib cases were found across Canada (Scheifele, 1996); however, regional differences were observed. For example, a study conducted by Hammond *et al.* (1988) from 1981 to 1984 identified the rates of *H. influenzae* meningitis in Manitoba and the Keewatin District of the Northwest Territories (NWT). In Manitoba, the overall incidence rate of *H. influenzae* meningitis for the whole population was 2.5/100,000; in children < 5 years of age the rate was much higher – 32.1/100,000. In comparison, the overall incidence rate of *H. influenzae* meningitis in the Keewatin District of NWT for the overall population was 69.6/100,000. For children < 5 years of age in this region, the incidence rate was dramatically higher – 530/100,000. This study also found that the onset of *H. influenzae* meningitis was earlier for Native Indian and Inuit children than it was for non-Native children in these regions (Hammond *et al.*, 1988).

The conjugate Hib vaccine was introduced in Canada in 1992, after which the incidence rate of invasive Hib disease significantly decreased – dropping to 0.3/100,000 in 2004 (Public Health Agency of Canada, 2006). Between 1992 and 1993, there was a 63.7% decrease in invasive Hib disease, the greatest inter-annual decrease since the conjugate Hib vaccine was introduced in Canada (Scheifele, 1996).

Since the vaccine was first included in the routine Canadian immunization schedule, Hib surveillance has been undertaken by researchers across the country. One group that has paid close attention to the rates of invasive Hib and *H. influenzae* disease is the Immunization Monitoring Program, Active (IMPACT) group, a network of 12 pediatric care hospitals

responsible for approximately 90% of tertiary pediatric care beds across Canada (McConnell *et al.*, 2007).

In 2000, the IMPACT group identified only four cases of invasive Hib disease across the country – a historic figure for Canada at the time. In each of the four cases, the child had received at least one dose of the conjugate Hib vaccine. Two of the children had received the vaccine doses at 2 and 4 months, but not their 6 month dose despite being at the appropriate age to receive the third injection. The other two children had received the appropriate numbers of vaccinations for their respective ages and thus were considered to be true vaccine failures. This study highlighted the importance of children receiving their vaccinations according to the recommendations of the Canadian immunization schedule (Scheifele *et al.*, 2001).

In 2005, IMPACT published a study identifying the incidence of Hib in vaccinated and unvaccinated children in Canada. After reviewing patient records from each of the 12 IMPACT hospitals, they were able to identify 29 cases of invasive Hib disease from 2001 to 2003. Overall, there was a gradual decrease in the number of cases, from 16 in 2001, to 10 cases in 2002, and only three cases in 2003. There were many reasons why the children in this study contracted Hib, including: the child was too young to have completed the primary immunization series, the child had not received the full vaccination series due to parental refusal, or the child had been fully vaccinated, but was immunocompromised. In addition, two of the children who contracted Hib had completed the full vaccination series and were previously healthy prior to infection with Hib. It is also interesting to note that of the 29 cases of invasive Hib disease, seven were identified in children from the Yukon, Northwest Territories, and Nunavut – regions of Canada where a large proportion of the population is of Aboriginal heritage (Scheifele *et al.*, 2005).

Since 1998, all the Canadian provinces have been uniformly using the PRP-T conjugate Hib vaccine, thus allowing the overall efficacy of this vaccine to be assessed at the national level. In 2008, IMPACT published a paper examining the incidence of Hib infections in Canadian children. From 2004 to 2007, 25 cases of invasive Hib disease were reported, representing an incidence rate of 0.10/100,000. Out of the 25 cases, four occurred in unimmunized children, 11 were in incompletely immunized children, and 10 had received their age-appropriate doses of the conjugate Hib vaccine, thereby representing vaccine failures. One such case was a 17 year old who had received one dose of the PRP-D conjugate Hib vaccine. At the time the conjugate Hib vaccine was introduced, this was the protocol for protecting children > 5 years of age against invasive Hib disease (Scheifele *et al.*, 2008). This vaccine is considered to be of lower efficacy than the other conjugate Hib vaccines, thus is no longer available for use in Canada (Decker *et al.*, 1992). The overall rate of PRP-T vaccine failure observed by this study was 0.05/100,000. In 2007, only two cases of invasive Hib disease were observed by IMPACT, representing a new historic rate for Hib disease in Canada.

This study also commented that between 2002 and 2005, all the provinces introduced a pneumococcal conjugate vaccine into the routine immunization schedule; however, this new vaccination did not appear to reduce the efficacy of the PRP-T vaccine for the short-term. In addition, the rarity of vaccine failures in children > 6 years of age (no longer within the peak range for Hib disease) demonstrated the long-term effectiveness of the PRP-T vaccine in Canada (Scheifele *et al.*, 2008).

In addition to the national data published by IMPACT, some provincial data which support the IMPACT's results have also been recorded. In Ontario, the conjugate Hib vaccine was introduced for use in infants in 1992, resulting in a dramatic decrease in the number of cases

of invasive Hib disease. One study examined the incidence of invasive Hib disease in Ontario from 1994 to 1998 and identified 46 confirmed cases; 56.5% of cases occurred in children ≤ 5 years of age. Only one child observed in this study was too young to have received at least one dose of the PRP-T vaccine. Over the course of this study, the number of cases observed decreased each year, from 13 cases in 1994 to only six cases in 1998. The incidence rate of invasive Hib disease in Ontario in 1998 was 0.06/100,000 (Sciberras, 1999).

Another study investigated the incidence of invasive Hib disease in children in three Canadian provinces: British Columbia, Alberta, and Ontario. This study, conducted from 1995 to 1997, was concerned with whether the PRP-T vaccine would still be effective when administered in conjunction with diphtheria-pertussis-tetanus inactivated polio vaccine (DPT-IPV). For all three provinces, only 38 cases of Hib were detected, with only 12 of these cases occurring among children eligible to receive the PRP-T and DPT-IPV vaccines. In 1995, 20 cases of invasive Hib disease were observed; in 1997, this number dropped to only seven cases. The incidence rate of invasive Hib disease among the three provinces was 0.6/100,000 (Scheifele *et al.*, 2000), the same incidence rate that Sciberras (1999) observed during the same time period in the province of Ontario alone. Only four cases of invasive Hib disease were considered to be vaccination failures, indicating a failure rate of 0.28/100,000 child-years of observation. This study demonstrated the effectiveness of the PRP-T vaccine given in conjunction with the DPT-IPV vaccine in Canadian children (Scheifele *et al.*, 2000).

In addition to the aforementioned Canadian data on the incidence of invasive Hib disease, recent studies have also discussed the incidence of non-type b invasive *H. influenzae* disease in this country. Specifically, IMPACT conducted a retrospective chart review at their 12 hospitals from 1996 to 2001 to determine the demographic and clinical information of children who had

contracted invasive *H. influenzae* disease during this time period. This study identified 166 cases of *H. influenzae* disease, 35% caused by Hib, 54% by non-type b serotypes, and 11% which were not serotyped. Out of the non-type b serotypes 53% were NST, 28% were Hia, 12% were Hif, 4% were Hid, and 2% were Hie. For each year of the study, non-type b isolates outnumbered Hib isolates (McConnell *et al.*, 2007).

The majority of patients (86%) who contracted Hib had no underlying medical conditions before developing invasive Hib disease; 36% of cases (n=21) were considered to be vaccine failures (14 had received PRP-T, and the remaining 7 had received either PRP-D or an unconjugated PRP vaccine). Just over half of patients with Hib were Caucasian and 19% were Aboriginal; ethnic background was not recorded for 22% of the patients. Overall, the case fatality rate associated with Hib was 3.2%. After the infection, 13 patients experienced hearing impairments and other neurological sequelae (McConnell *et al.*, 2007).

Next to Hib, the second most common serotype of *H. influenzae* observed was nonserotypeable (NST). NST isolates accounted for 54% of non-type b serotypes identified in this study. Unlike those patients who contracted Hib, only 34% of patients with invasive NST *H. influenzae* disease had no underlying medical conditions prior to their infection. Ethnic background was not recorded for 51% of these cases; in the cases where it was documented, eight patients were Caucasian and eight were Aboriginal. The case fatality rate for invasive NST disease was 2.1% – very close to the case fatality rate observed in invasive Hib disease. Only a few patients experienced hearing impairment and neurological sequelae at the time of hospital discharge (McConnell *et al.*, 2007).

After Hib and NST *H. influenzae*, the third most common serotype observed was Hia. Just over two-thirds of patients with Hia had no underlying medical conditions prior to

contracting the infection. In comparison to the Hib cases, individuals with Hia had a 16% case fatality rate. This is much higher than the 5% case fatality rate associated with invasive Hib disease in the pre-vaccine era (Public Health Agency of Canada, 2006; McConnell *et al.*, 2007). Of the patients who survived their Hia infection, 12% had hearing impairments and another 12% had neurological sequelae at the time of discharge from the hospital (McConnell *et al.*, 2007).

In addition to the high case fatality rate, 76% of patients (n=19) with Hia in this study were of Aboriginal descent, defined as having at least one parent of First Nations, Métis, or Inuit heritage. Most of these patients (n=14) were First Nations or Métis children from Manitoba, Saskatchewan, Alberta, or British Columbia; the remaining five patients were Inuit, four of whom were from the Keewatin Region of Nunavut Territory, and one was from the Northwest Territories (McConnell *et al.*, 2007).

To put the Hia data in perspective, it is useful to compare the incidence rates of Hia for different regions in this study. For Inuit children < 5 years old from the Keewatin Region, the incidence rate of invasive Hia disease in 2001 was 418.8/100,000. In comparison, the mean incidence rate for First Nations and Métis children < 5 years old from 1996 to 2001 in the four western provinces was 3.7/100,000. Additionally, the incidence rate of Hia in non-Aboriginal children < 5 years old in the four western provinces during this time period was 2.3/100,000. The results of this study demonstrate that non-type b invasive *H. influenzae* disease exists in Canadian children and is a significant cause of morbidity and mortality (McConnell *et al.*, 2007).

In addition to the data by IMPACT, a research group in Manitoba has also published on the incidence of invasive non-type b *H. influenzae* disease. This study characterized all isolates of *H. influenzae* that caused invasive disease in Manitoba from 2000 to 2006. Over the study period they found 122 isolates of *H. influenzae* responsible for invasive diseases. Out of these

isolates, 69 were NST, 36 were Hia, five were Hib, and the few remaining isolates were types c, d, e, and f. This study demonstrated an incidence rate of non-type b *H. influenzae* serotypes that is very similar to the rates of Hib in the pre-Hib vaccine era (see Figure 2). Interestingly, most of the patients in this study that contracted invasive Hia disease were from northern Manitoba and Nunavut Territory – regions of Canada where a high proportion of the population is Aboriginal. Another interesting finding from this study included the observation that 56% of invasive *H. influenzae* disease occurred in individuals > 10 years of age – compared to only 10% of isolates occurring in this age group in the pre-Hib vaccine era (Tsang *et al.*, 2007).

In a subsequent study analyzing the same 122 isolates, it was discovered that 17% were resistant to ampicillin due to β-lactamase production, 10.7% were resistant to trimethoprim-sulfamethoxazole, 2.5% were resistant to amoxicillin-clavulanic acid, and 1.6% were resistant to clarithromycin. Resistance was most common in the genetically-diverse NST strains; 37.7% of NST strains were resistant to at least one antibiotic compared to 15% of the other *H. influenzae* serotypes (Sill *et al.*, 2007). These studies demonstrate that despite the Hib vaccination program in Manitoba, *H. influenzae* disease represents a major public health concern for this province.

In addition to the work of the IMPACT group and Tsang *et al.* (2007), the International Circumpolar Surveillance group has also examined the incidence of invasive *H. influenzae* disease in Canada. Surveillance conducted from 2000 to 2005 in the arctic Canadian Territories as well as Alaska, observed 138 cases of invasive *H. influenzae* disease. Among the serotyped isolates, nearly half were Hia, 92% of which occurred in Aboriginal people. The annualized incidence rate of invasive Hia disease in children < 2 years old for both Alaska and northern Canada was 19.7/100,000. When northern Canada was considered on its own, the incidence rate for Hia in this age group was 79.1/100,000. Similarly, high invasive Hia disease incidence rates

were reported for the Aboriginal population in this region. The annualized incidence rate for invasive Hia disease in northern Canada for all Aboriginal people was 5.9/100,000; in Aboriginal children < 2 years of age, the incidence rate of invasive Hia disease was 101.9/100,000 (Bruce *et al.*, 2008).

A similar study by Degani *et al.* (2008) also investigated the incidence of invasive *H. influenzae* disease in the Canadian arctic region from 2000 to 2005. They identified 62 cases of invasive *H. influenzae* disease, 59 of which were serotyped. Of the serotyped isolates, 59% were Hia, with nearly 75% of these isolates identified in children < 2 years old. In addition to the cases of Hia, eight cases of Hib were also observed. Seven of these cases occurred in children and the remaining case occurred in an adult. Since none of the children were old enough to have received the complete vaccination series, none of these cases were considered to be conjugate Hib vaccine failures. Of the people who contracted invasive *H. influenzae* disease in this study, 92% were of Aboriginal heritage (Degani *et al.*, 2008).

As well as the studies identifying the incidence of invasive *H. influenzae* disease, some recent studies have also examined the antibiotic susceptibility of invasive *H. influenzae* isolates in Canada. Since vaccines do not exist for any *H. influenzae* serotypes other than Hib, antibiotics are the only means of treating patients with non-type b invasive *H. influenzae* disease, thus it is important to know which antibiotics are most effective. Sill and Tsang (2008) published a study which analyzed isolates from provincial public health laboratories across Canada found from 1990 to 2006. A total of 236 isolates were identified over the study period: 62.3% were NST, 21.6% were Hia, and 7.6% were Hib, the remaining isolates were type c, d, e, and f. Of all the isolates, 42 were resistant to ampicillin due to β-lactamase production. Nearly half of the Hib isolates were resistant to ampicillin and one Hib isolate was resistant to three antibiotics

(ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole). In comparison, only one Hia isolate and 30 (20.5%) of NST isolates were resistant to ampicillin. More NST isolates were resistant to trimethoprim-sulfamethoxazole than any of the other serotypes tested (Sill & Tsang, 2008).

Similarly, a study conducted from 2006 to 2007 in two Canadian as well as 18 European centres, identified the antibiotic susceptibility of 536 invasive *H. influenzae* isolates. In particular, they found that 25% of the isolates were resistant to ampicillin; however, the addition of clavulanic acid to ampicillin therapy made the bacteria more susceptible to the medication. This represents an increase in the number of ampicillin resistant isolates compared to previous work conducted by the same group in 2004 and 2005 (Jansen *et al.*, 2008). In light of the number of ampicillin-resistant isolates, it is recommended that antibiotics of the third-generation cephalosporin family, such as cefixime and cefpodoxime, as well as antibiotics of the fluoroquinolone family, such as ciprofloxacin and levofloxacin, be used to treat invasive *H. influenzae* disease (Sill & Tsang, 2008; Jansen *et al.*, 2008).

Aboriginal health in Canada

In Canada, the term Aboriginal is used to describe Indigenous or Native peoples. There are three main groups of Aboriginal people in Canada: First Nations, Métis, and Inuit. First Nations people are individuals who are considered to be North American Indians, a term which is associated with a great deal of ambiguity and cultural insensitivity, thus is not uniformly used. Among First Nations people, there are geographic, linguistic, and cultural differences, making this group extremely diverse (Indian and Northern Affairs Canada, 2009). Alternatively, the Métis people are Indigenous people considered to be of mixed ancestry. Originally, the Métis were the offspring of Indian women and European men during the time of the fur trade in

Canada. Over time, however, the intermarriages of Métis people resulted in the development of distinct Métis communities, complete with their own language, identity, and cultural traditions (Métis National Council, n.d.; Indian and Northern Affairs Canada, 2009). Finally, the Inuit are Aboriginal people who live in the arctic regions of Canada and much like the First Nations and the Métis people, are a diverse group with regional, linguistic, and cultural differences (Indian and Northern Affairs Canada, 2009).

The federal government has legislative authority over "Indians and land reserved for Indians" as defined by the *Constitution Act, 1867* (Hurley, 1999). In addition to the *Constitution Act*, the *Indian Act, 1876* granted the federal government the ability to determine who could be considered "Indian", where these individuals could reside, and the activities in which they were permitted to engage. Ultimately, the purpose of the Act was to assimilate the Aboriginal population into the dominant European society; however; this legislature also provided certain protections to Aboriginal people. Specifically, many treaties signed by the Crown and Aboriginal tribes, make reference to medicine being supplied free of charge. For example, in Treaty 6, an agreement of the Crown and the Cree of Fort Carlton, Fort Pitt, and Battle River, there is a clause which reads: "that a medicine chest shall be kept at the house of each Indian Agent for the use and benefit of the Indians at the direction of such agent" (Morris, 1880). This clause has been interpreted in court to mean that "all medicines, drugs, or medical supplies are to be supplied free of charge to treaty Indians" (*Dreaver v. The King*, 1935). The *Indian Act* remains a key piece of Canada's constitution, thus the provision of medical services to Aboriginal people is entrenched in the country's legislature (Boyer, 2003).

Despite the fact that the federal government is responsible for the health of Aboriginal people in Canada, health care in this country remains within the jurisdiction of the provincial

governments. In the case of Aboriginal people, the federal government finances their access to health care; however, the province is responsible for the delivery of this care. Unfortunately, this has created a fragmented system of health care delivery for those of Aboriginal descent (Health Canada, 2005). Like any Canadian citizens, Aboriginal people have access to publicly-financed physician and hospital services as defined by the C*anada Health Act, 1984* (Government of Canada, 1984). In addition to these basic health services, Aboriginal people also have access to non-insured health benefits (i.e. dental care, optometry, and pharmaceuticals) financed by the federal government. When the non-insured health benefits were first implemented in the late 1970s, the cost to administer the program was $36 million. Over time, the costs have sky-rocketed – by the early 1990s the program was estimated to cost $442 million annually (Waldram *et al.*, 1995).

Although Aboriginal people have access to a wide range of health care services in Canada, there is a disparity between the health of Aboriginal and non-Aboriginal people in this country (Kunitz, 1990; Adelson, 2005). This is clearly demonstrated by the discrepancies in both mortality and morbidity between these populations. In terms of mortality, Aboriginal people have been shown to have shorter life expectancies than non-Aboriginal people. For non-Aboriginal Canadian men, life expectancy is 76.3 years, compared to the 68.9 years seen among Aboriginal men, a gap of 7.4 years on average. The situation is much the same for Aboriginal women. Non-Aboriginal Canadian women live to be approximately 81.8 years, compared to approximately 76.6 years for Aboriginal women in Canada, a gap of 5.2 years on average (Adelson, 2005).

In terms of morbidity, Aboriginal people experience a greater disease burden attributed to both infectious and chronic diseases when compared to their non-Aboriginal counterparts. In

particular, Indigenous people in Canada have significantly higher rates of tuberculosis (TB), diabetes, suicide and unintentional injury, and alcohol and substance abuse than non-Indigenous Canadians (Young *et al.*, 2000; Clark *et al.*, 2002; Health Canada, 2005).

As previously mentioned, TB has been a particularly devastating disease within Aboriginal communities in Canada. At the beginning of the 20th century, the death rate from TB was approximately 700/100,000. Key factors found to contribute to this high death rate were poor sanitation, malnutrition, household overcrowding, and a lack of immunity to the TB bacillus (Wherrett, 1977). Clark *et al.* (2002) conducted a study that examined the incidence rate of TB in Canadian First Nations communities, as well as the association between TB, housing density, and isolation of communities; thereby, unearthing a strong link between rates of TB and housing density. Specifically, in communities where the average persons per room (ppr) was 0.4-0.6, the rate of TB was 18.9/100,000. In comparison, in communities with 1.0-1.2 ppr, the rate of TB infection was 113/100,000 – nearly six times higher. Moreover, this study determined that for every increase of 0.1 ppr, the risk of more than two cases of TB occurring in the community increased by 40%. Moreover, those living in an isolated community (defined as one in which there are flights and good telephone service, but no road access) were 2.5 times more likely to contract TB than individuals in non-isolated communities (defined as those with road access < 90 km to physician services). This study demonstrated that overcrowded housing in First Nations communities has the ability to increase the exposure of susceptible individuals to TB; a risk compounded by a community being isolated from basic health care services (Clark *et al.*, 2002).

Similar to TB, diabetes mellitus is another health condition which disproportionately affects Aboriginal people in Canada. In 1997, the prevalence of diabetes mellitus was 3.6 times higher for Aboriginal men and 5.3 times higher for Aboriginal women when compared to their

Canadian non-Aboriginal male and female counterparts (Young *et al.*, 2000). The highest rates were observed in First Nations men ages 65-69 years (3,088/100,000) and in First Nations women ages 70-74 years (4,833/100,000) – prompting many public health officials to declare diabetes a chronic disease of epidemic proportions in this population (Health Canada, 2005). In addition to the considerable number of Aboriginal people affected by diabetes, the number of deaths due to diabetes among Aboriginal women on reserves is five times greater than the Canadian mortality rate for diabetes (Mao *et al.*, 1992). Moreover, studies have shown that First Nations people often develop diabetes mellitus at a younger age. Alarmingly, 5% of all First Nations people have developed diabetes by the age of 30, and by 65 years of age, one-third of the population have developed the disease (Assembly of First Nations, 2004; Young *et al.*, 2000). It is interesting to note that although rates of diabetes are extremely high in First Nations population, they are not nearly so high among the Inuit. For example, in 1998 the First Nations and Inuit Regional Health Survey reported that 4% of the Inuit in Labrador have diabetes, while the prevalence of diabetes among the general Canadian population during the same time period was 4.1% (Labrador Inuit Health Commission, 1999).

Other issues that affect many Aboriginal communities in Canada are those of injury and suicide. Injury and poisoning are significant problems for many Aboriginal communities in Canada and are the leading cause of death among all Aboriginal people in this country (Adelson, 2005). In 1997, First Nations populations experienced a rate of injuries and poisonings, including suicide, three times higher than the general Canadian population (Health Canada, 2005).

Suicide rates among the Aboriginal population are higher than for the general Canadian population (Spaulding, 1986; Health Canada, 2005). For First Nations people, the suicide rate is 2.1 times higher than that of their Canadian counterparts (Adelson, 2005). In comparison, the

Inuit suicide rate is more than 5.5 times greater than the First Nations suicide rate, and over 11 times higher than the overall Canadian suicide rate (Health Canada, 2005). In 1999, suicide accounted for 38% of deaths among Aboriginal youth ages 10-19 and 23% of deaths among Aboriginal people 20-44 years of age (Adelson, 2005). Moreover, adolescent status Indian women are 7.5 times more likely to commit suicide than adolescent women in the general Canadian population (Grace, 2003). The factors that contribute to an individual committing suicide are numerous and complex, though poverty, depression, powerlessness, physical, emotional, and sexual abuse, and lack of economic, social, or cultural resources have been explored as possible contributors (Kirmayer *et al.*, 1993; Clifton, 1994; Adelson, 2005).

In addition to the aforementioned health problems, alcohol and other substance abuse remain a serious concern for Aboriginal people in Canada. In 1991, 73% of First Nations people who participated in the Aboriginal Peoples Survey reported that alcohol was a problem in their communities (Statistics Canada, 1993). The 2003 report from the Ontario First Nations Regional Health Survey demonstrated that over half of the respondents were considered to be heavy drinkers – individuals who consume more than five alcoholic beverages on one occasion at some point during the last year (MacMillan *et al.*, 2003). In Canada, Aboriginal youth are two to six times more likely to experience alcohol-related problems than non-Aboriginal youth in the Canadian population (Scott, 1994). Moreover, the number of deaths due to alcohol is higher in First Nations people than it is in non-Aboriginal Canadians. In British Columbia, a study conducted from 1991 to 2001 demonstrated that nearly one-quarter of all First Nations deaths in the province were directly or indirectly related to alcohol. The age-standardized alcohol-related mortality rate was six times higher for First Nations people than the rest of the population in British Columbia (Health Canada, 2002).

The situation is similar when considering abuse of substances other than alcohol. For example, a 1996 study from the Northwest Territories reported that marijuana and hashish use among Aboriginal residents ≥ 15 years was three times higher than among non-Aboriginal residents of the same age. Moreover, Aboriginal youth in this region were 3.5 times more likely to have used cocaine, crack, heroin, speed, or lysergic acid diethylamide and 11 times more likely to have ever sniffed solvents (Northwest Territories Bureau of Statistics, 1996). In Canada, one in five Aboriginal youth has used solvents and approximately one-third of these users are < 15 years old (Scott, 1994). In British Columbia, 6% of deaths in the entire population from 1991 to 2001 were drug-induced. The age-standardized drug-related mortality rate was three times higher in the First Nations population than in all residents of the province (Health Canada, 2002).

As demonstrated by the preceding evidence in this paper, the state of health of Aboriginal people in Canada is dramatically different from that of non-Aboriginal Canadians. The health disparities experienced by Aboriginal people in Canada can, at least partially, be explained by examining the role of historical experiences and culture in the health of Canada's Indigenous population. First, the role of colonization in creating health inequities will be examined. Soon after arriving in North America, Europeans began the process of colonization and the world of Indigenous people was permanently altered. This alteration involved three main phases: demographic collapse, cultural dispossession and assimilation, and historical trauma. The first phase, demographic collapse, was one in which a number of epidemics, such as smallpox, typhoid fever, and cholera, were unknowingly brought over to North America by the Europeans. They swept throughout the continent, killing a large proportion of the Aboriginal population. This enormous decrease decimated the traditional social structures of Indigenous societies, and had a profound effect on the mental

health of an Aboriginal population that relied on close ties to their communities and an extended kinship system (Waldram *et al.*, 1995; Duran & Duran, 1995; Mitchell & Maracle, 2005).

The second phase of colonization, cultural dispossession and assimilation, involved a time when Indigenous people lost their autonomy due to the restrictive policies of the colonizing nation. Europeans viewed Aboriginals from a Judeo-Christian perspective, believing that Indigenous people were devil-worshippers and had to be saved (Duran & Duran, 1995; Braveheart, 1998). This difference in religious and spiritual beliefs provoked the Europeans to impose a number of restrictions on Aboriginal society, including legal, spiritual, and social limitations. One such restriction involved removing Indigenous people from their traditional lands and placing them on reserves. Since all aspects of an Aboriginal person's life, including physical, emotional, and spiritual facets, are tied to the land, this change was devastating (Duran & Duran, 1995; Mitchell & Maracle, 2005). In addition, Europeans made it illegal for Aboriginals to practice their traditional culture – essentially making it illegal to be an Indigenous person. This had major implications for Indigenous mental health because at the time when they were grieving for the loss of many people in their communities and their connection with traditional homelands, their grieving rituals and other cultural customs had been outlawed (Duran & Duran 1995; Braveheart, 1998).

In addition to the political changes implemented by the Europeans, the colonizing nation developed social machines of destruction, namely residential schools. The role of residential schools was to destroy the reproducibility of Indigenous culture by stripping Aboriginal children of their identity. Children were forcibly removed from their families and sent great distances from their home communities to attend the schools. Once at the residential schools, the children were required to adopt traditional colonial dress and behaviour, were forbidden from speaking their native language, associating with members of the opposite sex, and practicing their traditional culture (Duran &

Duran, 1995; Waldram *et al.*, 1995; Smylie, 2000). These children were malnourished, overworked, and traumatized by physical, emotional, and sexual abuse (Smylie, 2000). The normal social development of children in residential schools was "mutilated beyond recognition by the traumas of loss and grief, danger and fear, hatred and chaos" (Duran & Duran, 1995, p. 31).

Following the demographic collapse and social engineering associated with colonialism, Indigenous people began to suffer from "historical trauma – the collective and compounding emotional and psychic wounding both over the life span and across generations" (Braveheart, 1998). Historical trauma is often the result of unresolved grief and manifests itself in a few key ways, including: "withdrawal and psychic numbing, anxiety and hypervigilance, guilt over one's own survival, identification with ancestral pain and death, and chronic sadness and depression" (Braveheart, 1998). The existence of historical trauma among Indigenous people can be explained by examining the many acculturation stressors placed on their population during the colonization process.

One acculturation stressor was environmental shock. During the process of colonization, Europeans began systematically destroying the land on which Aboriginal people and their ancestors had lived for centuries. This was of particular importance because all aspects of an Indigenous person's life had a close connection with the land. Another stressor was economic competition. Traditionally, Aboriginal people lived off the land, thus when Europeans took control over the land, including all of the plants and animals on it, the economy of the Indigenous people was marginalized (Duran & Duran, 1995; Mitchell & Maracle, 2005).

Other acculturation stressors included the invasion of the land and forced relocation of Aboriginal people. During the process of colonization, Europeans removed Indigenous people from their traditional lands and placed them on reserves. Those who were unwilling to leave their land

were killed (Waldram *et al.*, 1995; Duran & Duran, 1995). As a result of this invasion, many Indigenous people died, causing the survivors to develop "refugee syndrome" – a mindset where the remaining people in the population become focused on doing the bare minimum to survive (Braveheart, 1998). In addition, Aboriginal people had to deal with the stress associated with their culture being deemed by the dominant European society to be a "point of view", considered mystical, and therefore less valid than the Western concept of the world. Finally, the dominant society has denied the trauma Aboriginal people experienced during colonization, thereby compounding their grief (Braveheart, 1998; Mitchell & Maracle, 2005).

The concept of historical trauma is important because it continues to have major effects on the health of Aboriginal people today. During the time when Indigenous culture was being annihilated, their traditional grieving processes were outlawed, thus creating the opportunity for a "soul wound" to develop (Duran & Duran, 1995, p. 24). This soul wound has been passed on from generation to generation largely due to the policies that were created and have been maintained to subjugate Indigenous people and their culture (Duran & Duran, 1995; Braveheart, 1998; Mitchell & Maracle, 2005).

Along with the historical events that impacted and continue to impact Aboriginal health in Canada today, there are many other factors that play a role in an individual's health, such as educational attainment, employment, housing and community infrastructure, traditional language and culture, and the remoteness of the community in which one lives (Health Canada, 2005). First, educational attainment is a socio-economic determinant related to health status, as Canadians who have completed less education tend to report having poorer health (Federal, Provincial, and Territorial Advisory Committee on Population Health, 1999). In 2001, the Canadian census reported that on-reserve registered Indians rated lower than their off-reserve Canadian counterparts for all

educational attainment indicators. In particular, registered Indians on-reserve had lower secondary school completion rates, fewer admissions to postsecondary education institutions, and a lower university degree completion rate. Moreover, registered Indians were more likely to take longer to complete their postsecondary education than other Canadians (Hull, 2000). In the case of the Inuit, the rates for most educational attainment indicators are comparable to that of registered Indians in Canada, or are lower (Health Canada, 2005).

Similar to educational attainment, employment status has the capability to impact a person's health. In 2001, the unemployment rate of on-reserve registered Indians was four times higher than the general Canadian population. For all Aboriginal groups in Canada (including First Nations, Inuit, and Métis), unemployment rates were lowest among those in the 25-44 year age group and among those Aboriginal people who had completed postsecondary education. As might be expected, higher rates of unemployment were consistent with lower average income levels in the Aboriginal population in Canada (Hull, 2000; Health Canada, 2005).

Another factor which influences the health of Aboriginal people in Canada is housing. In 2001, 36% of Aboriginal homes on reserves were in need of major repairs, compared to only 8.2% of non-Aboriginal homes surveyed during the same year (Health Canada, 2005). Moreover, 19% of residences on reserves had more than one person per room, a factor which can increase the risk of transmitting infectious diseases, such as tuberculosis. In contrast, 2% of non-Aboriginal Canadian homes had more than one person per room (Indian and Northern Affairs Canada, 2000). Furthermore, the presence of mould has been detected in many Aboriginal dwellings. For example, Lawrence and Martin (2001) determined that over 50% of homes in an Aboriginal community in British Columbia had excessive mould growth, representing a potentially serious health problem.

Similar to housing conditions, community infrastructure can play a key role in the health of individuals. One indicator of strong community infrastructure is water adequacy. In 2000/01, Health Canada evaluated 98.2% of First Nations homes to determine if they had an adequate water supply. The results demonstrated that less than half of the First Nations communities studied had piping to centralized water treatment plants for the majority of homes in their respective communities (Health Canada, 2005). More importantly, the quality of the water is a significant factor in the health of individuals. Infectious diseases, such as giardiasis and shigellosis, are associated with the consumption of poor quality water (Rosenburg *et al.*, 1997). In 1999, 65 First Nations communities had an average of 183 days of boil water advisories; over one-quarter of these advisories were in place from six months to one year (Health Canada, 2005).

In addition to water adequacy and quality, adequate sewage disposal systems are crucial to the health and wellbeing of individuals in any community. In 1999, 80% of dwellings in Aboriginal communities had sewage disposal systems that were acceptable according to provincial and territorial Canadian standards; however, 14% had systems that were unacceptable and the remaining 6% had no sewage disposal system at all (Health Canada, 2005). Other key community services may also be lacking in Aboriginal communities. Specifically, in 1999, 40% of Aboriginal communities in Canada had no solid waste disposal programs and nearly half had either no fire protection services or inadequate fire protection services (Health Canada, 2005).

Other factors which impact the health and wellbeing of Aboriginal people in Canada are traditional language and culture. For example, Healey and Meadows (2008) conducted an exploratory qualitative study to understand Inuit women's perception of their health and wellbeing. They found that the women believed it was important to speak Inuktitut (one of the Inuit languages in arctic Canada) and to teach the language to their children because it reinforced their traditional ties

to Inuit culture. The loss of language and culture in the Inuit community has caused many women to grieve, as this loss is also associated with identity, social inclusion, and wellness problems. During the time between the 1981 and the 2004 Canadian censuses, the number of children whose mother tongue was an Indigenous language decreased (Health Canada, 2005).

Finally, remoteness, the distance from physician services and access to other communities through different modes of transportation, can influence an individual's health. Of the 626 First Nations communities in each of the 10 Canadian provinces, 64.2% are "non-isolated" (< 90 km from physician services). Just over 14% are "semi-isolated" (have road access, but physician services are >90 km away); 17.9% are "isolated" (have scheduled flights and good telephone service, but no road access). Only 3.5% are considered to be "remote-isolated" (no scheduled flights or road access and minimal telephone and radio service). Geographic location of Aboriginal communities not only impacts their access to health care services, but also their culture, economy, and community infrastructure (Armstrong, 1999; Health Canada, 2005).

As is demonstrated by the preceding evidence, the state of health of Aboriginal and non-Aboriginal people in Canada is very different. When compared to their non-Aboriginal Canadian counterparts, Aboriginal people have higher rates of both chronic and infectious diseases (Adelson, 2005). In particular, Aboriginal people experience a disproportionate burden of disease caused by TB (Wherrett, 1977; Clark *et al.*, 2002), HIV (Plitt *et al.*, 2009), and hepatitis A, B, and C (Minuk & Uhanova, 2003) when compared to the non-Aboriginal Canadian population. Prior to the introduction of the conjugate Hib vaccine, certain Aboriginal populations experienced the highest recorded rates of invasive Hib disease in the world (Hanna, 1990). Despite the introduction of the conjugate Hib vaccine, Aboriginal people remain more susceptible to invasive Hib disease than non-Aboriginal people (Guthridge *et al.*, 2000; Singleton *et al.*, 2006).

Statement of problem

As indicated by the aforementioned evidence, Hib is a significant cause of invasive diseases in many countries around the world (WHO, 2005). Though individuals of all ethnic backgrounds are affected by Hib, evidence suggests that Aboriginal people are most susceptible to invasive Hib disease (Hanna, 1990; Guthridge *et al.*, 2000; Singleton *et al.*, 2006). The introduction of the conjugate Hib vaccine has decreased the incidence of invasive Hib disease in many countries around the world; however; it may have opened up an ecological niche for non-type b serotypes to cause invasive disease (EU-IBIS, 2006; Dworkin *et al.*, 2007; McConnell *et al.*, 2007; Tsang *et al.*, 2007). To date, no studies have identified the incidence of invasive *H. influenzae* disease in Northwestern Ontario – a region of Canada where 19.6% of the population is of Aboriginal descent (Statistics Canada, 2008).

Study questions

This study was initiated to address the following questions:

1. What is the current incidence rate of invasive *H. influenzae* disease in Northwestern Ontario?
2. What serotypes are causing invasive *H. influenzae* disease in this region?
3. Do Aboriginal people in Northwestern Ontario experience a disproportionate burden of invasive *H. influenzae* disease compared to people of other ethnic backgrounds?
4. What factors influence the development of invasive *H. influenzae* disease in this region?
    a. Does age play a role in susceptibility to invasive *H. influenzae* disease?
    b. Do people who contract invasive *H. influenzae* disease have underlying medical conditions or does the infection primarily occur in healthy individuals?

c. What effect, if any, does living in a rural or urban area in Northwestern Ontario have on the likelihood of developing invasive *H. influenzae* disease?

d. What clinical presentations are common for individuals with invasive *H. influenzae* disease?

e. What are the outcomes of individuals who contract invasive *H. influenzae* disease?

f. For children who have been vaccinated against Hib, what role does this play in the development of invasive *H. influenzae* disease?

Study objectives

The purpose of this study is to determine the current incidence rate of invasive *H. influenzae* disease in Northwestern Ontario as well as to examine the factors that contribute to the development of this infectious disease. This study will focus on the role individual host factors, such as age, gender, place of residence, ethnic background, underlying medical conditions, and vaccination status play in the development of invasive *H. influenzae* disease. As well, the clinical presentations of invasive *H. influenzae* disease and the subsequent disease outcomes for the cases identified in this study will be considered. Additionally, the serotype of the isolate will be compared to the individual host factors, as well as the clinical presentation to determine its association with the development of invasive *H. influenzae* disease in the region. All of the data presented in this study comes from city centres in Northwestern Ontario, such as Thunder Bay Regional Health Sciences Centre (TBRHSC), the regional hospital of Northwestern Ontario, and from the Meno-Ya-Win Health Centre (Sioux Lookout, Ontario). Some data from the Lake of the Woods Hospital (Kenora, Ontario) will also be presented.

Relevance to public health

Although the conjugate Hib vaccines have been effective in reducing the carriage and incidence rates of invasive Hib disease, *H. influenzae* is still a significant public health concern in Canada. To our knowledge, the epidemiology of invasive *H. influenzae* disease has not yet been examined in Northwestern Ontario. As was previously mentioned, a recent paper by Tsang *et al.* (2007) identified a large number of invasive *H. influenzae* isolates in Manitoba, predominantly Hia and NST. In particular, many of the isolates were identified in patients in northern Manitoba and Nunavut – regions with large Aboriginal populations. Since Northwestern Ontario is geographically proximal to Manitoba and also has a large population of Aboriginal people (19.6%; Statistics Canada, 2008), it is reasonable to suggest that a similar situation may be occurring in this region. This paper provides the first known examination of the epidemiology of invasive *H. influenzae* disease and its associated factors in Northwestern Ontario in the post-Hib vaccine era.

Methodology

Data Collection

This study was conducted in three distinct phases. Cases of invasive *H. influenzae* disease were defined according to the Centers for Disease Control and Prevention (2005) definition: a clinically compatible case that is laboratory confirmed (isolation of *H. influenzae* from a normally sterile site – for example, blood, cerebrospinal fluid (CSF), or, less commonly, joint, pleural, or pericardial fluid). Data were collected from the major regional hospital in Northwestern Ontario, TBRHSC as well as the Meno-Ya-Win Health Centre (Sioux Lookout, Ontario). Additionally, serotype data for clinical isolates of invasive *H. influenzae* disease were provided by the Ontario Public Health Laboratory (PHL, Toronto, Ontario). The charts of

patients from whom an isolate was identified in a sterile site were retrieved for data abstraction. Each chart was reviewed and the following data recorded: patient's gender, age, postal code, source from which *H. influenzae* was isolated, clinical presentation, disease outcome, underlying medical conditions, ethnic background, vaccination status, and serotype of isolate.

Phase I

The Health Records staff at TBRHSC searched through their records from 2002-2007 for diseases that could be indicative of invasive *H. influenzae* disease (i.e. bacterial meningitis, epiglottitis, periocular cellulitis, septicaemia/bacteremia, empyema, septic arthritis, pyelonephritis, and pericarditis). Patient records were reviewed to determine if any of the infections had been caused by *H. influenzae*; for those individuals with invasive *H. influenzae* disease, relevant data were abstracted from the patient's record.

Phase II

The Clinical Laboratory staff at TBRHSC searched through their records to identify all laboratory-confirmed isolates of *H. influenzae* found at the institution from 2002 to 2008. For all isolates identified in normally sterile body sites (i.e. blood, cerebrospinal fluid, synovial fluid, pleural fluid, etc.), the patient's chart was reviewed and the relevant data abstracted.

Phase III

Additional regional data for cases of *H. influenzae* identified throughout Northwestern Ontario from 2002 to 2008 were provided by the Ontario PHL. Cases of invasive *H. influenzae* disease recorded by the Ontario PHL were originally identified in individuals in one of three health care centres in Northwestern Ontario: Thunder Bay, Sioux Lookout, and Kenora. The Ontario PHL data from Thunder Bay were cross-referenced with the data collected from TBRHSC in Phase I and II to eliminate duplication of cases.

Data Analysis

Due to the small number of cases, no statistical analyses were conducted on the data. Each case was reviewed individually and in comparison to the other cases. Percentages were calculated to determine ethnic origin (where possible), serotype of *H. influenzae*, and underlying medical conditions. Incidence rates were calculated by dividing the number of cases of invasive *H. influenzae* disease per year in a specific age group by the total population for the aforementioned age group in Northwestern Ontario (per 100,000). The population for each age group used in the incidence calculations came from Canada's 2006 census data (Statistics Canada, 2008).

For underlying medical conditions, secondary immunodeficiencies were defined as non-genetic conditions that developed as a result of exposure to a variety of factors including infectious agents, metabolic diseases, and environmental conditions (i.e. HIV, diabetes mellitus, chronic renal failure, radiation therapy to treat various cancers, etc.; Chinen & Shearer, 2008).

Age groupings of the Public Health Agency of Canada's Notifiable Diseases were used (<1, 1-4, 5-9, 10-14, 15-19, 20-24, 25-29, 30-39, 40-59, 60+); however, for our analysis, some of the age groups were combined because of the small numbers in our data set.

Statistics Canada (2008) defines Aboriginal people as those persons who identify with at least one Aboriginal group, North American Indian (First Nations), Métis or Inuit, and/or those who report being a Treaty Indian or a Registered Indian, as defined by the *Indian Act* of Canada, and/or those who report being members of an Indian band or First Nation. Patients were considered to be of Aboriginal descent if they self-identified as an Aboriginal person or had a postal code which corresponded to a remote Aboriginal reserve in Northwestern Ontario. This

study was approved by the Research Ethics Boards at Lakehead University and TBRHSC (Thunder Bay, Ontario) as well as at the Meno-Ya-Win Health Centre (Sioux Lookout, ON).

Results

Demographics

From January 2002 to December 2008, there were 449 isolates of *H. influenzae* identified by the Clinical Laboratory at TBRHSC. Of these isolates, there were 25 individual cases of invasive *H. influenzae* disease. During the same period, an additional 13 cases of invasive *H. influenzae* disease were identified elsewhere in Northwestern Ontario. The demographic information for all the cases of invasive *H. influenzae* disease is presented in Tables 1-2. When the data were combined, there was no remarkable difference in gender, 42.1% (n=16) male and 57.9% (n=22) female. The age of patients ranged from 0 (newborn) to 89 years of age. Nineteen cases out of 38 (50%) were children (0-9 years of age). Among children, six were < 1 year old (15.8% of all cases), ten were 1-4 years old (26.3%) and three were 5-9 years old (7.9%). Ten out of 38 cases were ≥ 60 years old (23.7%).

For 25 cases identified at TBRHSC, postal code data were available: 19 (76%) lived in an urban centre in Northwestern Ontario, five (20%) lived in a rural area in Northwestern Ontario, and one (4%) lived at no set address (data not shown). Since only a few cases were located in rural locations, it was not possible to determine what effect this might have had on serotype or disease outcome.

Ethnicity

For nearly half of the cases (18, or 47.4%), the ethnic background was not recorded. Due to the sensitive nature of this information, it is not officially required to be documented in the health records of patients treated at hospitals in Ontario. Nevertheless, using the aforementioned

criteria, 20 cases in this study were identified to be of Aboriginal descent (52.6%). For children 0-9 years of age, 73.7% of children were Aboriginal; for children < 1 year of age (n=6), 100% were Aboriginal.

Source of isolation

The majority of *H. influenzae* isolates (86.8%) were from blood (n=33), 1 isolate was from pleural fluid, and the remaining 4 were from other sites: synovial fluid, ethmoidal cells, abdominal cavity, and placenta (Table 3). As shown on Table 2, of the 38 isolates, 31 (81.6%) were serotyped; out of the serotyped isolates, 41.9% were Hia (n=13), 29% were NST (n=9), 25.9% were *H. influenzae* type f (Hif; n=8), and 3.2% were *H. influenzae* type e (Hie; n=1), see Figure 3. No cases of invasive Hib disease were observed.

Clinical presentation and disease outcome

Detailed clinical information was available for 28 of the 38 cases. The most common clinical presentations were pneumonia/empyema (28.6%; n=8) and sepsis (28.6%; n=8), as well as epiglottitis (10.7%; n=3). In addition, septic arthritis, meningitis, pyelonephritis, urinary tract infection, pharyngitis, tonsillitis, periocular cellulitis, and an abdominal wall abscess were observed in individual cases (Table 2).

The most common outcome of invasive *H. influenzae* disease was full recovery (75%; n=21), with no apparent sequelae at the time of hospital discharge. Of the cases with detailed clinical information, three patients died; two had severe underlying medical conditions and one patient was born at 24 weeks of gestation. In addition, one patient was placed on long-term antibiotic therapy after discharge, two patients were transferred to another hospital for further medical care, and the outcome for one patient was unknown.

Underlying medical conditions

As is evident in Table 2, the many of the 28 cases with detailed clinical information had at least one significant underlying medical condition (44.7%; n=17); these could be categorized into one of two groups: conditions causing secondary immunodeficiencies and congenital conditions/prenatal exposure to toxins. Out of 17 patients with underlying medical conditions, 58.8% (n=10) belonged to the first category (radiotherapy or chemotherapy for treatment of cancer, chronic renal failure, diabetes mellitus, etc) and 41.2% (n=7) were associated with some congenital or prenatal conditions. In only 5 cases, there were no significant underlying medical conditions – four of whom were children ≤ 5 years old and one was an adult.

Vaccination status was reviewed in each child's chart; however, it was only recorded for six cases. Four of the six children had up to date immunizations (66.7%), while two children had delayed vaccinations. (For one child the delay was due to serious underlying medical conditions). Since two-thirds of the children in this study had no information available on their vaccination status, it was not possible to determine what role, if any, vaccination against invasive Hib disease had on the development of invasive *H. influenzae* disease in this region.

Incidence of invasive H. influenzae disease

Based on our findings, the incidence rates of invasive *H. influenzae* disease in Northwestern Ontario were calculated for each year of the study (see Figure 4). For comparison, the incidence of invasive Hib disease for the entire province of Ontario (1989 to 2004) is also shown (data by the Public Health Agency of Canada, 2006). This graph depicts the incidence of invasive Hib disease in Ontario beginning in 1989, prior to the introduction of the conjugate Hib vaccine into the routine Canadian immunization schedule. In 1989, the incidence rate of invasive Hib disease in Ontario was 1.42/100,000; this decreased steadily to 0.07/100,000 in 2004.

In comparison, the incidence rate of invasive *H. influenzae* disease in Northwestern Ontario in 2003, 1.71/100,000, was higher than the incidence rate of invasive Hib disease in Ontario in the pre-conjugate Hib vaccine era, 1.42/100,000 in 1989. Moreover, the incidence rate of invasive *H. influenzae* disease in Northwestern Ontario remained high, reaching 2.98/100,000 in 2006 and 2007. In 2008, the incidence rate decreased to 1.71/100,000, likely attributable to normal year-to-year variability. It is important to note that despite this apparent decrease, the incidence rate in 2008 was still higher than the incidence rate of invasive Hib disease in the province of Ontario in the pre-conjugate Hib vaccine era.

To determine the incidence of invasive *H. influenzae* disease in specific age groups, incidence rates were calculated per age group for each year of the study (see Figure 5). The incidence rates of invasive *H. influenzae* disease in Northwestern Ontario were outstandingly high for young children. Most notably, the 0-4 age category experienced an incidence rate of 15.5/100,000 in 2002, 30.9/100,000 in 2004, increasing up to 38.7/100,000 in 2006. In comparison, the incidence rates of invasive Hib disease in the whole province of Ontario for children < 1 year old was 1.55/100,000 in 2002 and 0.78/100,000 in 2004. For Ontario children 1-4 years old, the incidence rate for 2002 was 0.18/100,000 and 0.37/100,000 in 2004 (data are not currently available for invasive Hib disease in Ontario after 2004, Public Health Agency of Canada, 2006). This comparison indicates that from 2002 to 2004, the incidence of invasive *H. influenzae* disease among young children in Northwestern Ontario was much higher than the officially recorded incidence of Hib for the whole province of Ontario.

Discussion

Emergence of non-type b serotypes of Haemophilus influenzae

The occurrence of invasive non-type b *H. influenzae* disease in Canada over the last 10 years has been reported in recent literature (Tsang *et al.*, 2007; McConnell *et al.*, 2007; Degani *et al.*, 2008; Bruce *et al.*, 2008). However, to our knowledge, the overall incidence of invasive *H. influenzae* disease in Northwestern Ontario, a large region where 19.6% of the population is Aboriginal (Statistics Canada, 2008), has not been investigated. A recent study published by IMPACT identified 25 cases of invasive Hia disease from 1996 to 2001. These researchers found that Aboriginal children accounted for 76% of patients with Hia infection (McConnell *et al.*, 2007). In comparison, in this study Aboriginal children accounted for 70% (7 out of 10) of pediatric cases of invasive Hia disease. Since the ethnic background of the remaining three cases of invasive Hia disease was not recorded, our findings may underestimate the actual number of Aboriginal children affected by Hia. Importantly, although IMPACT encompasses nearly 90% of the pediatric tertiary care beds in Canada, this program does not cover Northwestern Ontario and its population of approximately 235,000 people. The closest IMPACT hospitals are in Winnipeg, Manitoba (~700 km to the west of Thunder Bay) or in Toronto and Ottawa (~1400 km to the east). This leaves the vast geographic expanse of Northwestern Ontario (an area nearly the size of France) unexamined.

An increase in non-type b invasive *H. influenzae* disease has also been recently observed in other regions of North America (Tsang *et al.*, 2007; Dworkin *et al.*, 2007). Tsang *et al.* (2007) identified 122 cases of invasive *H. influenzae* disease in Manitoba, Canada from 2000 to 2006, with a proportional increase in non-Hib strains: 57% were NST and 29% were Hia. Moreover, the incidence of invasive *H. influenzae* nearly matched the rate of invasive Hib disease in the

pre-vaccine era. In the United States, Dworkin *et al.* (2007) observed an increase in the incidence of invasive *H. influenzae* disease in adults ≥ 65 years old from 1.1/100,000 in 1996 to 3.9/100,000 in 2004. The latter study identified 770 cases of invasive *H. influenzae* disease in Illinois, 52.4% of isolates were NST and 18.3% were Hif. In contrast, in our study, we observed greater prevalence of Hia (41.9%) compared to other serotypes of encapsulated *H. influenzae* as well as to NST *H. influenzae*. A higher incidence of Hia among patients with invasive *H. influenzae* disease has been observed in geographical regions with significant proportions of Indigenous people in Canada, the United States, and Australia (Gratten *et al.*, 1994; Moloughney, & Chan, 1997; Millar *et al.*, 2005; Hammitt *et al.*, 2005; Degani *et al.*, 2008; Bruce *et al.*, 2008). Thus, it is reasonable to suggest that Aboriginal people may have an increased susceptibility to invasive Hia disease.

Since the introduction of the conjugate Hib vaccines into the routine childhood immunization schedule, there have been concerns that serotype replacement could occur, resulting in an increase in the incidence of non-type b invasive *H. influenzae* disease. For example, among encapsulated pneumococcal bacterial strains against which there is now a vaccine, there is evidence of capsule switching and serotype replacement (Long, 2005; Musher, 2006). Specifically, there are many serotypes of *Streptococcus pneumoniae*; however, the conjugate pneumococcal vaccine only confers protection against a small number of pneumococcal serotypes. The introduction of the 7-valent conjugate *S. pneumoniae* vaccine (protective against serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) has reduced the incidence of invasive pneumococcal disease caused by these serotypes. Recently, it has been shown that since the introduction of the conjugate pneumococcal vaccine, non-vaccine strains have been observed to cause invasive pneumococcal disease (Byington *et al.*, 2002; Huang *et al.*, 2005). Since the

introduction of the 7-valent pneumococcal vaccine, a 23-valent pneumococcal vaccine has been introduced, which protects against the 7 aforementioned serotypes as well as 16 other serotypes. As bacteria are rapidly evolving organisms, the introduction of vaccinations to eliminate certain strains of bacteria opens up an ecological niche which may be filled by non-vaccine serotypes. In this way bacteria may evolve so that non-vaccine strains replace those contained in the vaccination (Long, 2005; Musher, 2006).

Since Hib are also encapsulated bacteria, it is possible that capsule switching or serotype replacement could occur with *H. influenzae* (Tsang, 2007). Evidence does exist which suggests that the introduction of the conjugate Hib vaccines has led to an increase in the number of cases of invasive non-type b *H. influenzae* disease. For example, Bajanca and Canica (2004) examined the presence of *H. influenzae* in Portugal from 1989 to 2001. They found 11 cases of invasive NST *H. influenzae* disease from 1989 to 1993. In 2000, the conjugate Hib vaccine was introduced; causing a decrease in the number of Hib isolates and an increase in the number of NST *H. influenzae* isolates (up to 20 cases). Similarly, a study conducted in Brazil demonstrated an increase in non-type b *H. influenzae* after the introduction of the conjugate Hib vaccine in this country. Ribeiro *et al.* (2003) demonstrated that prior to the introduction of the Hib vaccine in Brazil, the incidence of Hia meningitis was 0.02/100,000. One year after the vaccine's introduction, the incidence of Hia meningitis increased to 0.16/100,000 – an eight-fold increase. Data presented by the EU-IBIS have also demonstrated an increase in non-type b *H. influenzae* after the introduction of the vaccine in many European countries. The EU-IBIS report demonstrated that from 2000 to 2004, the incidence of invasive NST *H. influenzae* disease exceeded the incidence of invasive Hib disease. Moreover, the case fatality rate for all invasive

H. influenzae disease in Europe at the time was 8.34%; the fatality rate was highest among cases with invasive disease caused by NST *H. influenzae* (EU-IBIS, 2006).

In contrast, there is also evidence which does not support the capsule switching and serotype replacement hypothesis. Recent comments from European researchers suggests that some of the evidence in support of capsule switching and serotype replacement is derived from studies with a small number of cases and is a result of normal year-to-year variability (Ladhani *et al.*, 2008). Since the incidence of non-type b invasive *H. influenzae* disease was not recorded prior to the introduction of the conjugate Hib vaccines, it is difficult to determine if *H. influenzae* capsule switching or serotype replacement is indeed occurring in the post-Hib vaccine era. Lipstitch (1999) suggests that an increase in non-type b *H. influenzae* may be due to one of two possibilities. First, there are actually a greater number of non-type b *H. influenzae* serotypes causing invasive disease. Alternatively, the prevalence of Hib in the pre-vaccine era effectively masked the presence of the non-type b *H. influenzae* serotypes causing invasive disease. The introduction of the conjugate Hib vaccines has reduced the prevalence of Hib in the population, thereby potentially allowing the non-type b *H. influenzae* serotypes to have the opportunity to cause invasive diseases (Waggoner-Fountain *et al.*, 1995; Lipstitch, 1999).

The emergence of non-type b *H. influenzae* raised an important question of whether any alterations in the genetic structure of these bacteria may be responsible for the changes in the epidemiology of invasive *H. influenzae* disease. Adderson *et al.* (2001) observed several cases of severe invasive disease caused by Hia with striking similarity to the clinical and epidemiologic features of invasive Hib disease. These particular isolates were found to have *IS*1016-*bex*A deletion within the capsule gene cassette, potentially responsible for their high virulence. Another explanation for the changing epidemiology of invasive *H. influenzae* disease could be

that the decrease in Hib carriage caused by the Hib vaccine has allowed for non-type b *H. influenzae* to colonize the nasopharynx. In this way, the ecological niche previously occupied by Hib has been filled. De Almeida *et al.* (2008) who recently identified a case of septic arthritis due to Hia in Brazil hypothesized that the ability of Hia to cause severe invasive disease depends not only on its virulence, but also on its ability to colonize the nasopharynx in individuals vaccinated against Hib. *In vitro* data suggests that Hib produces a bacteriocin which inhibits the growth of non-type b serotypes of *H. influenzae*; the reduction in Hib carriage has eliminated the bacteriocin which previously prevented non-type b serotypes from colonizing the nasopharynx (Venezia & Robertson, 1975).

Whether or not the use of the conjugate Hib vaccine has indeed caused capsule switching and serotype replacement, resulting in the emergence of non-type b invasive *H. influenzae* disease, it does effectively prevent the significant disease burden previously caused by Hib prior to the vaccine's introduction. Thus, it is important to maintain the use of the conjugate Hib vaccine; however, it is also important to monitor the presence of all types of invasive *H. influenzae* disease to determine if non-type b serotypes are emerging (WHO, 2006; WHO, n.d.; Tsang, 2008).

H. influenzae and Aboriginal people

A recent study conducted by Bruce *et al.* (2008) in conjunction with the International Circumpolar Surveillance Hia Working Group also identified a remarkably high incidence of invasive Hia disease among Indigenous people in the North American arctic. While the incidence rate of invasive Hia disease in the arctic was 0.9/100,000 for the whole population, the rates significantly differed between Indigenous and non-Indigenous people, 2.9/100,000 and

0.2/100,000, respectively. The reported incidence rate of invasive Hia disease was highest among Indigenous children < 2 years of age, 52.6/100,000 (Bruce *et al.*, 2008).

Prior to the introduction of the conjugate Hib vaccine, some Aboriginal populations exhibited an increased susceptibility to invasive Hib disease (Wotton *et al.*, 1981; Losonsky *et al.*, 1984; Ward *et al.*, 1990). A study conducted in 1981 to 1984 in a region of northern Canada with a large Aboriginal population, demonstrated an incidence rate of 69.6/100,000 for *H. influenzae* meningitis; in children < 5 years old the incidence rate of *H. influenzae* meningitis was 530/100,000 (Hammond *et al.*, 1988). Despite the widespread use of the conjugate Hib vaccines, recent reports indicate that Aboriginal people remain disproportionately affected by invasive Hib disease (Guthridge *et al.*, 2000; Singleton *et al.*, 2006; Menzies *et al.*, 2008).

Our study also demonstrated an increased prevalence of invasive *H. influenzae* disease among Aboriginal children – 75% (n=12) of 0-4 year old and 66.7% (n=2) of 5-9 year old children were of Aboriginal descent. All six infants < 1 year of age with invasive *H. influenzae* disease were Aboriginal. Overall, Aboriginal patients identified according to the criteria used in our study accounted for 20 of 38 cases (52.6%). Given that Aboriginal people in Northwestern Ontario account for 19.6% of the population (Statistics Canada, 2008), our findings imply that this group is disproportionately affected by invasive *H. influenzae* disease. The reason for this is currently unclear.

The health of Canadian Aboriginal people is essentially worse compared to the general population, which is most notably demonstrated by the 5 to 7.5 year difference in life expectancy between Aboriginal and non-Aboriginal people in the country. In particular, Aboriginal people have higher rates of both infectious and chronic diseases than their non-Aboriginal counterparts (Adelson, 2005). Studies suggest that the increased incidence of invasive Hib disease in the post-

vaccine era among some Indigenous populations, such as in Australia and Alaska, is due to the social determinants of health (Guthridge *et al.*, 2000; Singleton *et al.*, 2006). It is entirely possible that Aboriginal people in Northwestern Ontario are also experiencing an increased incidence of invasive *H. influenzae* disease due to the social determinants of health (Galanis *et al.*, 2002). However, it could also be reasonably suggested that some genetic factors may determine whether an individual has an increased susceptibility to invasive *H. influenzae* disease. Of interest, an allelic polymorphism in the VκA2 gene encoding the variable part of the kappa light chain antibody to PRP was detected in Navajos (Feeney *et al.*,1996). Since 60% of the total anti-PRP antibody repertoire is encoded by this gene, and these antibodies exhibit a high avidity for Hib, it was suggested that such a genetic polymorphism may play a role in an increased susceptibility to invasive Hib disease, as well as in less effective antibody responses to the vaccine in some Aboriginal populations (Feeney *et al.*, 1996). It remains to be determined whether any specific genetic factors present in Indigenous populations may account for insufficient immune defence against non-type b *H. influenzae*.

Non-type b H. influenzae vaccine developments

Currently, the only available *H. influenzae* vaccine is for Hib. For clinicians treating patients with invasive non-type b *H. influenzae* disease, the only effective therapy at this time is antibiotics. Despite the fact that antibiotics are available to treat non-type b invasive *H. influenzae* disease, recent studies have demonstrated that antibiotic resistance is prevalent, especially among NST *H. influenzae* isolates (Ladhani *et al.*, 2008; Sill & Tsang, 2008). For effective antibiotic therapy of infections caused by *H. influenzae,* it is recommended that clinicians use third generation cephalosporins, especially in cases in which *H. influenzae* has

caused meningitis, as these antibiotics provide central nervous system penetration (Curtis *et al.*, 2003).

As an alternative to antibiotic therapy, some recent studies have experimented with developing a vaccine that will be effective against NST *H. influenzae*. Although there is more than one target for the development of NST *H. influenzae* vaccines, this paper will consider the Protein D and P6 antigens as there is strong evidence supporting their use (Prymula *et al.*, 2006; Forsgren *et al.*, 2008; Nomura *et al.*, 2008). Protein D is a highly-conserved 42 kDa protein found in all types of *H. influenzae*, including the unencapsulated or NST types. It is involved in the pathogenesis of respiratory tract infections by impairing ciliary function. As well, Protein D is involved in the development of non-invasive otitis media (Forsgren *et al.*, 2008). Protein D's virulence derives from its glycerophosphodiesterase activity, which triggers host cells to release phosphorylchlorine, thereby promoting the establishment of stable biofilm colonies of NST *H. influenzae* (Hong *et al.*, 2007). Both rat and chinchilla otitis media models have demonstrated protection from NST *H. influenzae* after receiving Protein D vaccination (Poolman *et al.*, 2001; Novotny *et al.*, 2006).

Recently, clinical trials have demonstrated the efficacy of vaccination with Protein D in preventing NST *H. influenzae* infection in humans (Prymula *et al.*, 2006; Prymula *et al.*, 2008). In a study in the Czech Republic and Slovakia, nearly 5,000 children were randomized to receive either the pneumococcal Protein D conjugate vaccine or the hepatitis A vaccine. The pneumococcal Protein D conjugate vaccine contains 11 serotypes of *S. pneumoniae* (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F) all individually conjugated to Protein D. The group who received the pneumococcal protein D conjugate vaccine was protected against otitis media caused by both *Streptococcus pneumoniae* and NST *H. influenzae* (Prymula *et al.*, 2006). Later,

the same researchers demonstrated that the reactogenicity and safety of the pneumococcal protein D conjugate vaccine was similar to that of the licensed combination diphtheria-tetanus-acellular pertussis-hepatitis B virus-inactivated polio virus-Hib (DTPa-HBV-IPV-Hib) vaccine and the 11-valent pneumococcal conjugate vaccine (Prymula *et al.*, 2008).

In addition to the protein D conjugate vaccines, a recent paper also identified NST *H. influenzae* P6 outer membrane protein as a potential vaccine target. P6 is a highly-conserved 16,000 Da protein present on the outer membrane of all types of *H. influenzae* (Nomura *et al.*, 2008). It plays a key role in maintaining the structural integrity of the outer membrane, thus it is critical for the viability of *H. influenzae* organisms (Murphy *et al.*, 2006). The immune response to P6 is often poor among patients with recurrent NST *H. influenzae* infections, such as in children with recurrent otitis media and adults with COPD (Yamanaka & Faden, 1993; Abe *et al.*, 2002). Nomura *et al.* (2008) used a computer algorithm to identify peptide-specific T cell lines which would respond to P6, thus representing new potential therapeutic vaccination targets.

The development of NST *H. influenzae* vaccine candidates are promising therapeutic strategies; however, there are some challenges to their development. One challenge is to develop vaccine candidates that do not mimic molecular patterns that naturally occur in the body, thereby preventing the development of autoimmune diseases and inappropriate inflammatory immune responses (Cripps *et al.*, 2002). An additional challenge is ensuring that individuals of all ethnic backgrounds develop protective immune responses after the administration of the vaccine candidate. Previous studies of Aboriginal children have demonstrated an increased susceptibility to invasive Hib disease, as well as decreased antibody responses to the conjugate Hib vaccines (Siber *et al.*, 1990; Guthridge *et al.*, 2000). Another challenge for consideration is sustaining immunity after immunization against NST *H. influenzae*. As was the case with Hib, the carriage

of Hib in the nasopharynx of healthy individuals represented a pool from which individuals immunized against the pathogen could be re-exposed to it, thereby boosting the immune memory response to Hib. The widespread use of the conjugate Hib vaccine has led to decreased carriage rates, and therefore fewer opportunities for vaccinated individuals to come into contact with Hib, thus resulting in less boosting of their memory response. In order to account for the reduced boosting opportunities, immunization schedules recommend including a fourth dose of the conjugate Hib vaccine to stimulate an enhanced memory response against this pathogen. The NST *H. influenzae* vaccine candidates may require a similar boosting dose in order to sustain immunity to NST *H. influenzae* (Blanchard-Rohner & Pollard, 2008).

The role of age and underlying medical conditions

The incidence rate of invasive *H. influenzae* disease in Northwestern Ontario was remarkably high among 0-4 year old children, especially in 2006 (38.7/100,000). This was higher than the incidence rate of invasive Hib disease for the entire province of Ontario, prior to the implementation of conjugate Hib vaccine. Indeed, the incidence of invasive Hib disease in 1989 was 38.21/100,000 for children aged 0-1 year and 11.69/100,000 for those 1-4 years of age (Public Health Agency of Canada, 2006). Among young children with invasive *H. influenzae* disease, we observed the prevalence (41.2%) of certain congenital or prenatal conditions, such as a congenital heart defect or prenatal exposure to toxic substances.

It is interesting to note that of the patients who developed epiglottitis, all three were adults. This is a noticeable change from the pre-vaccine era when invasive Hib disease often caused epiglottitis in children (Garpenholt *et al.*, 1999). Similarly, the one cases of meningitis identified by this study was observed in an adult. This is also different from the pre-vaccine era when invasive Hib disease often caused meningitis in children (Cochi *et al.*, 1985). Since many

patients in Northwestern Ontario are sent to Winnipeg, Manitoba for treatment, it is possible that cases of invasive *H. influenzae* disease causing meningitis among children were missed by this study. A more detailed description of the cases identified in this study is included in the Appendix.

Despite the occurrence of invasive *H. influenzae* disease in Northwestern Ontario, this infection remains relatively uncommon among the general population of the region. As we found in our study, 44.7% (n=17) of patients had some underlying medical conditions which potentially contributed to the development of the invasive disease. Most of the adult patients had conditions which may have affected the ability of their immune system to effectively prevent invasive *H. influenzae* disease, such as diabetes mellitus, malignancy, autoimmunity, or COPD. In addition, approximately one fourth of cases in this study were ≥ 60 years old and had potentially developed immune senescence, the immune deficiency and dysregulation that accompanies aging (Ben-Yehuda & Weksler, 1992).

H. influenzae type b

Although no single case of Hib was detected by this study, the presence of Hib in the region cannot be completely ruled out. Only 81.6% of the isolates in this study were serotyped and were identified as Hia, Hif, Hie, and NST. Since not every isolate was serotyped, it is possible that some isolates were Hib; if all isolates had been serotyped, the results of this study could potentially be significantly different.

Interestingly, although this study did not identify any isolates of Hib, an interview with the manager of Infectious Diseases at the Thunder Bay District Health Unit revealed that one case of invasive Hib disease was identified in Northwestern Ontario in 2003 (D. Binnett, personal communication, October 26, 2007). Since this case was not isolated by the laboratory at

TBRHSC, it was not identified by our study. To protect the identity of the individual who contracted invasive Hib disease, none of the demographic and clinical information about this individual can be presented in this paper.

Limitations of this study

While this study demonstrates the importance of invasive *H. influenzae* disease in Northwestern Ontario, it does have some limitations. Although many patients in the region are sent to TBRHSC, Meno-Ya-Win Health Centre, or the Lake of the Wood District Hospital for treatment of severe diseases (such as those caused by invasive *H. influenzae*), a number of patients are sent to Winnipeg, Manitoba for treatment, thus we are likely under-estimating the total number of cases in the region. In addition, many patients in remote northern communities who develop invasive infectious diseases are treated with antibiotics at local nursing stations before being transferred to a larger hospital, such as TBRHSC. By the time diagnostic tests are conducted, *H. influenzae* may no longer be present in the body or it may only be present at very low levels, thus the clinical laboratory staff may be unable to culture it. In this way, invasive diseases attributable to *H. influenzae* may be overlooked.

Moreover, not all isolates were serotyped, thus providing an incomplete picture of the seroepidemiology of invasive *H. influenzae* disease. In Ontario, the decision to send an invasive isolate of *H. influenzae* away for serotyping is left up to the attending physician treating the patient in question. Since only Hib is required to be reported and is relatively rare, many physicians choose to treat the patient without determining which serotype has caused the invasive infection. The overwhelming success of conjugate Hib vaccination may have created a false sense of security for many health care providers as they may be unaware of the emergence of non-type b *H. influenzae* as a cause of invasive diseases.

Similarly, due to its sensitive nature, ethnic background information is not routinely collected in Canada, thus we were unable to determine the role ethnicity may play in susceptibility to invasive *H. influenzae* disease. Nevertheless, of the patients with invasive *H. influenzae* disease identified in our study, 52.6% were of Aboriginal descent. Since ethnic background was unknown for approximately half of the patients, it is possible that we under-estimated the proportion of our study population that was of Aboriginal heritage. It is equally possible that we may have missed identifying individuals of other ethnic backgrounds who may also be susceptible to invasive *H. influenzae* disease.

Finally, this study was limited as detailed clinical information was not available for each case. For some of the cases identified at TBRHSC, the relevant information was not included in the patient's chart. One possible explanation could be that TBRHSC opened February 22, 2004, in the middle of the study period. Prior to the opening of the regional hospital, patients examined in Thunder Bay were treated at one of two smaller hospitals in the city. These two hospitals amalgamated in 2004 to create TBRHSC. During the process of the hospitals joining, it is entirely possible that records may have been lost or misplaced. In addition, some of our cases were identified by the PHL in Thunder Bay. Cases sent to the PHL could have be sent from nearly anywhere in Northwestern Ontario, such as individual doctor's offices, walk-in clinics, etc., and thus we were unable to obtain any detailed clinical information for these patients.

Recommendations for practice, policy, and research

After completing this study, some key recommendations for practice, policy, and research have been developed. These recommendations have been created with Northwestern Ontario in mind; however, they are applicable to other regions of Canada as well as other countries around the world.

Practice

In terms of practice, clear guidelines for health care providers to detect, diagnose, and report all cases of invasive *H. influenzae* disease should be developed and implemented. To enhance surveillance of these potentially dangerous pathogens, all invasive isolates of *H. influenzae* should be routinely serotyped, reported to the appropriate PHLs, and then communicated to Public Health Agency of Canada (PHAC). Moreover, health care providers need to be educated on the continuing presence of invasive *H. influenzae* disease in Canada, and the need to remain vigilant against this infection despite the widespread success of the conjugate Hib vaccine in this country. Providers should also be made aware that an increase in invasive disease caused by non-type b *H. influenzae* strains has been observed in many regions of Canada, thus making it an important public health concern.

Policy

In terms of policy, all serotypes of *H. influenzae* causing invasive disease should be made reportable to the PHAC. In regions in which an unusually high number of cases of invasive *H. influenzae* disease have been observed, investigation into the responsible serotypes should be conducted. In this way, populations with apparent susceptibilities to invasive *H. influenzae* disease may be identified and further policies developed to protect these individuals from these serious infections. In addition, policies should explicitly support the work of *H. influenzae* researchers in this country so that they may be helpful in identifying potential therapeutic targets and protecting vulnerable populations.

Research

In terms of research, it is recommended that researchers continue to identify the incidence of invasive *H. influenzae* disease due to all encapsulated and non-encapsulated types of *H.*

influenzae, not just Hib. Where increases in invasive *H. influenzae* disease are observed, research should be conducted to determine the important factors behind the aforementioned increase. If particular serotypes are determined to be responsible for an increasing number of cases of invasive *H. influenzae* disease, then researchers should develop new vaccines to better control emerging serotypes of invasive *H. influenzae* disease. Research should also focus on determining whether the isolates of invasive *H. influenzae* disease are resistant to antibiotics and develop new antibiotics to treat resistant invasive isolates. It would also be valuable if research was conducted to determine which serotypes have colonized the nasopharynx of people in a given region. By identifying which serotypes are present among non-invasive isolates, clues may be given as to which serotypes are likely to be observed in invasive disease. Similarly, it would be worthwhile for researchers subject isolates to PCR and molecular analysis to determine if there is anything unique to the isolates identified in a particular region. Finally, research should be used to determine what role ethnic background plays in susceptibility to invasive *H. influenzae* disease. If ethnic background is associated with susceptibility to invasive *H. influenzae* infections, researchers should identify the factors related to this susceptibility. These factors should be relayed to policy-makers to determine how modifiable factors might be addressed to reduce the incidence of invasive *H. influenzae* disease in affected populations.

Conclusion and significance of findings

The results of this study demonstrate that despite the widespread use of the conjugate Hib vaccine, invasive *H. influenzae* disease remains an important public health concern in Northwestern Ontario. Over the course of this study, the incidence of invasive *H. influenzae* disease in this region was high. In 2006 and 2007, the incidence of invasive *H. influenzae* disease in Northwestern Ontario surpassed the officially recorded incidence rate of invasive Hib disease

in the pre-vaccine era for the entire province of Ontario. This study is the first known examination of the incidence of invasive *H. influenzae* disease in this region of Canada. Our findings point to the changing seroepidemiology of invasive *H. influenzae* disease in Northwestern Ontario, echoing recent observations by others in Canada and the United States (Tsang *et al.*, 2007; McConnell *et al.*, 2007; Dworkin *et al.*, 2007; Bruce *et al.*, 2008). The cases of invasive *H. influenzae* disease identified by this study were caused by non-type b serotypes, especially Hia and NST. Despite the presence of non-type b invasive *H. influenzae* disease in this region, it does not appear to equally affect all populations. In particular, the elderly, Aboriginal children, and individuals with conditions causing secondary immunodeficiencies seemed to be most affected by invasive *H. influenzae* disease. These results demonstrate the importance of enhancing disease surveillance for all serotypes of this pathogen and remaining vigilant for emerging serotypes of invasive *H. influenzae* disease in the post-Hib vaccine era. The findings from this study may be used to support the development of new *H. influenzae* vaccines to protect individuals from invasive disease caused by non-type b *H. influenzae* serotypes. In addition, they may be used to support the development of policies which protect vulnerable populations from developing invasive *H. influenzae* disease in the future.

Acknowledgements

This study would not have been possible without the contribution of Dr. Raymond Tsang (National Microbiology Laboratory, Winnipeg, Manitoba), Dr. Frances Jamieson (Ontario Agency of Health Protection and Promotion), Dr. Sharen Madden, and Dr. Len Kelly (Northern Ontario School of Medicine, Sioux Lookout, Ontario). We also acknowledge Heidi Greenwell, Wendy Gouliquer, Bev Junnila, Evelyn Maclean (TBRHSC), and Christopher Abbey for their assistance in designing the study methodology and during the data collection process, Prasad

Rawte, Shirley Brown, and Elizabeth Pszczolko (Ontario Agency of Health Protection and Promotion) for laboratory assistance, Dr. Greg Gamble for his valuable suggestions and comments on the study methodology, and Dr. Garry Ferroni (Northern Ontario School of Medicine, Sudbury, Ontario) for his important suggestions and support throughout the study process. This study was also supported by the Ontario Graduate Scholarship.

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Figures and Tables

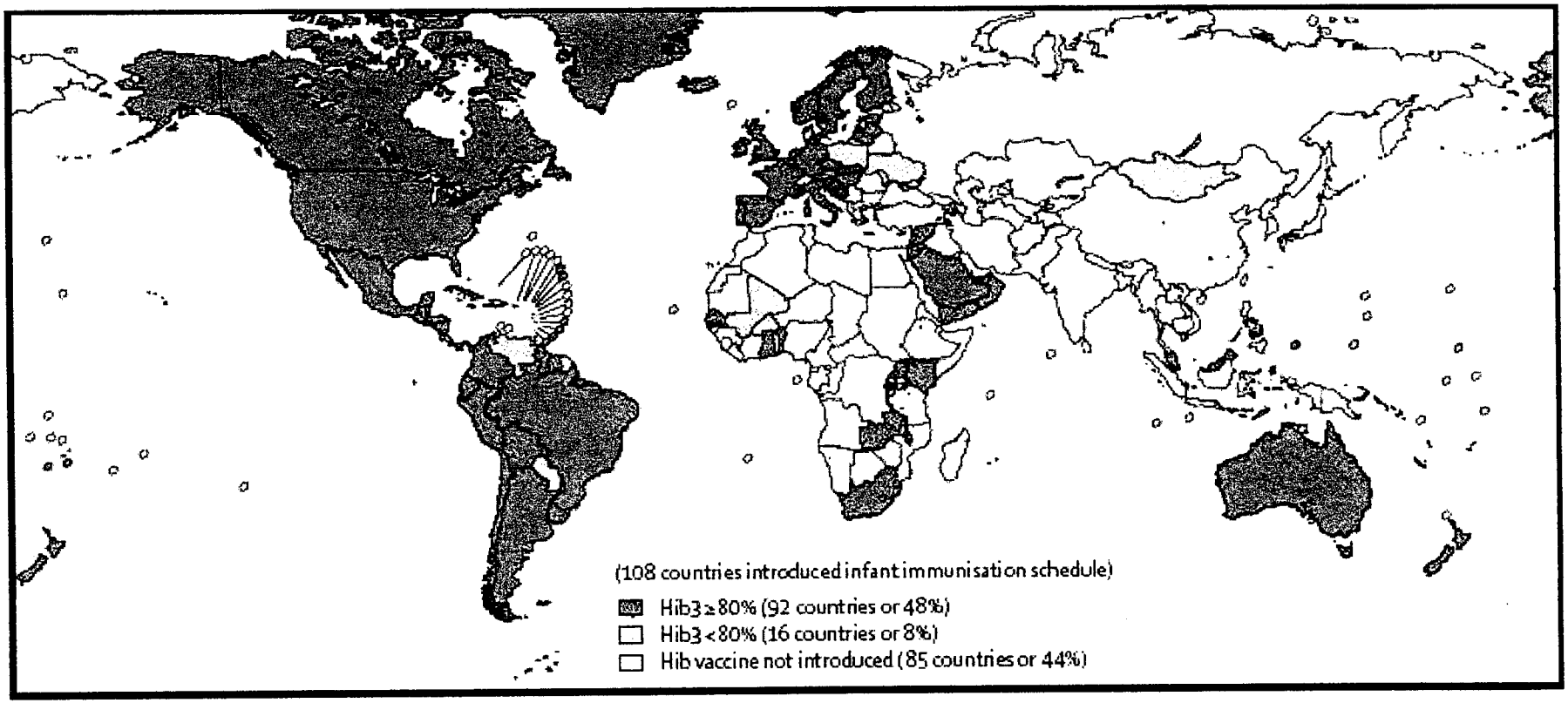

Figure 1 – Countries that have introduced *Haemophilus influenzae* type b (Hib) vaccine and infant Hib vaccine coverage, 2006 (WHO, 2008; Morris *et al.*, 2008).

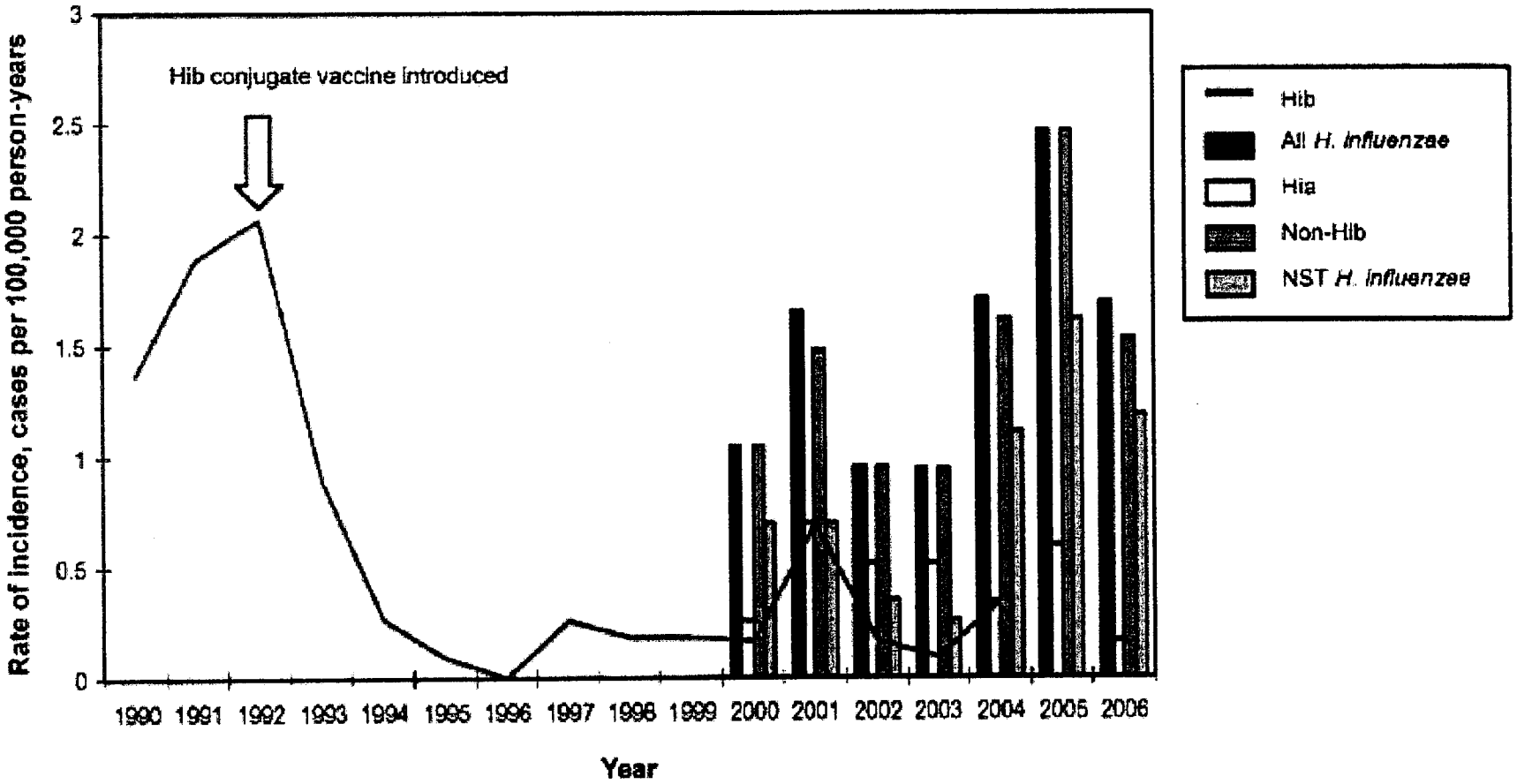

Figure 2 – Incidence rates of invasive *Haemophilus influenzae* disease in Manitoba from 2000 to 2006 (Tsang *et al.*, 2007).

Table 1. Age, gender, and Aboriginal heritage among 38 patients with invasive *Haemophilus influenzae* disease

Age Group, Years	No. (%) of patients			No. (%) Aboriginal Heritage
	All	Male	Female	
<1	6 (15.8%)	4 (66.7%)	2 (33.3%)	6/6 (100%)
1-4	10 (26.3%)	6 (60%)	4 (40%)	6/10 (60%)
5-9	3 (7.9%)	1 (33.3%)	2 (66.7%)	2/3 (66.7%)
10-24	1 (2.6%)	0 (0.0%)	1 (100%)	0/1 (0.0%)
25-59	9 (23.7%)	1 (11.1%)	8 (88.9%)	3/9 (33.3%)
≥60	9 (23.7%)	4 (44.4%)	5 (55.6%)	3/9 (33.3%)
Overall	38	16 (42.1%)	22 (57.9%)	20/38 (52.6%)

Table 2. Age, serotype, clinical presentation, disease outcome, and underlying conditions of 38 patients with invasive *Haemophilus influenzae* disease

Patient #	Age	Serotype	Clinical Presentation	Disease Outcome	Underlying Conditions
1	Newborn	NST	Sepsis	Infection Cleared	Born with jaundice, mother smoked during pregnancy
2	Newborn	NST	Not determined*	Death	Extreme prematurity
3	6 months	[a]	Pneumonia	Infection Cleared	Mother smoked during pregnancy
4	7 months	Hif	Sepsis	Long-term antibiotic therapy	Mother smoked during pregnancy
5	8 months	Hif	Sepsis	Infection Cleared	Congenital heart defect
6	11 months	[a]	Septic Arthritis	Infection Cleared	Mother abused alcohol, tobacco, solvents while pregnant
7	1 year	Hia	Pneumonia	Infection Cleared	None
8	1 year	Hia	[b]	[b]	[b]
9	1 year	Hie	[b]	[b]	[b]
10	1 year	Hia	Sepsis	Transferred to another hospital	None
11	2 years	Hia	Urinary Tract Infection	Infection Cleared	Mother had active systemic lupus erythematosus while pregnant
12	2 years	NST	Sepsis	Infection Cleared	None
13	3 years	Hia	Pharyngitis	Infection Cleared	Mild anemia
14	3 years	Hia	[b]	[b]	[b]
15	3 years	Hia	[b]	[b]	[b]
16	4 years	Hia	[b]	[b]	[b]
17	5 years	[a]	Periocular Cellulitis	Infection Cleared	None
18	7 years	Hia	Tonsilitis	Infection Cleared	Anemia
19	9 years	Hia	[b]	[b]	[b]
20	24	NST	[b]	[b]	[b]
21	33	[a]	Abdominal Wall Abscess	Infection Cleared	Clinical depression
22	34	Hia	Pneumonia	Infection Cleared	Diabetes

23	36	[a]	Epiglottitis	Infection Cleared	May have had epiglottitis before
24	40	NST	Pyelonephritis	Infection Cleared	Diabetes, alcoholism
25	45	Hif	Epiglottitis	Infection Cleared	None
26	45	Hif	[b]	[b]	[b]
27	47	Hia	Pneumonia	Infection Cleared	Remote acute rheumatic fever, cardiomyopathy, sleep apnea
28	53	[a]	Sepsis	Death	Lymphoma, Crohn's disease
29	57	NST	Sepsis	Death	Metastatic breast cancer
30	60	Hif	Epiglottitis	Infection Cleared	Multiple myeloma
31	62	NST	[b]	[b]	[b]
32	62	Hif	[b]	[b]	[b]
33	66	Hia	Pneumonia	Infection Cleared	Remote tuberculosis, chronic obstructive pulmonary disease (COPD)
34	72	Hif	Pneumonia	Infection Cleared	Multiple myeloma
35	74	Hif	Pneumonia	Transferred to another hospital	Hypertension, elevated liver enzymes of unknown etiology
36	77	[a]	Empyema	Unknown*	Tuberculosis, non-small cell lung carcinoma
37	79	NST	Meningitis	Infection Cleared	Clinical depression, hypertension, hypothyroidism
38	89	NST	Sepsis	Infection Cleared	COPD, chronic renal failure

[a] Isolate not serotyped. [b] Data not available. *No information was presented in the chart.

Table 3. Source of isolation of *Haemophilus influenzae*

	Blood	Pleural Fluid	Aspirates from normally sterile body sites
Hia	13	–	–
Hie	1	–	–
Hif	7	–	–
NST	8	–	1
Unknown	4	1	3
Total	33	1	4

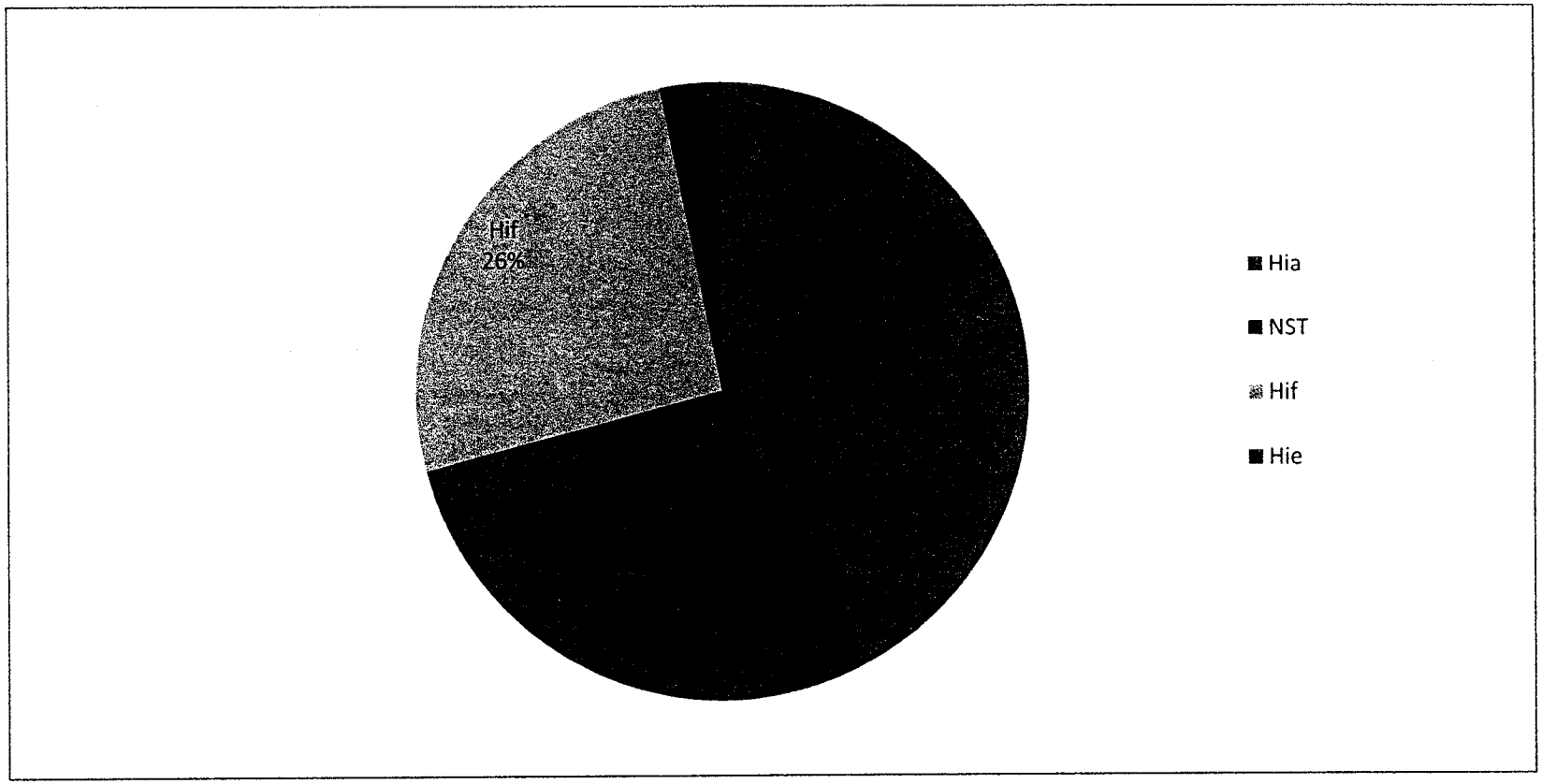

Figure 1 – Proportion of *Haemophilus influenzae* serotypes among 31 serotyped isolates.

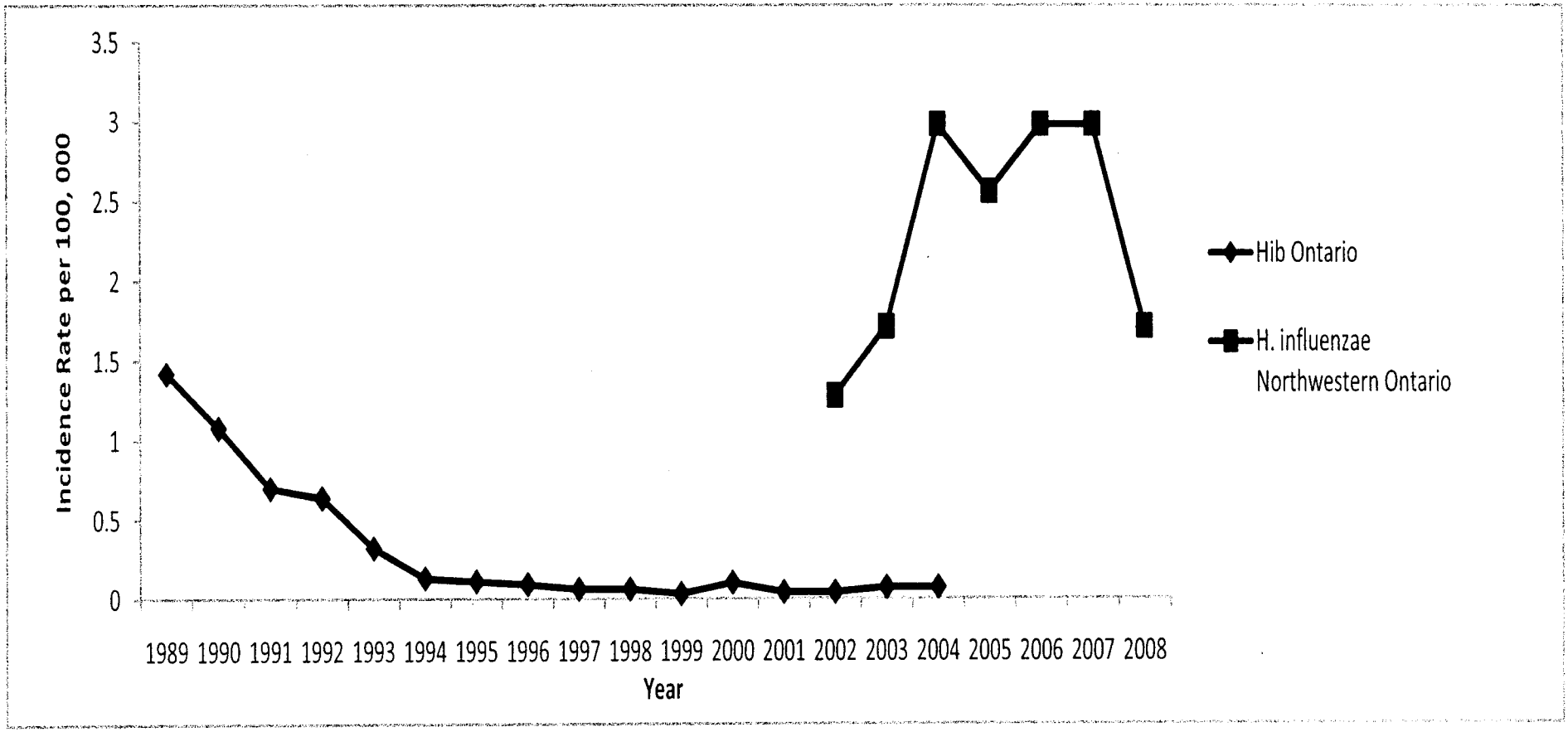

Figure 2 – Incidence of invasive *Haemophilus influenzae* disease in Northwestern Ontario, 2002 to 2008, compared to incidence of invasive *Haemophilus influenzae* type b (Hib) disease in Ontario per 100,000, 1989 to 2004 (all ages, both sexes, data provided by Public Health Agency of Canada Notifiable Diseases, 2006).

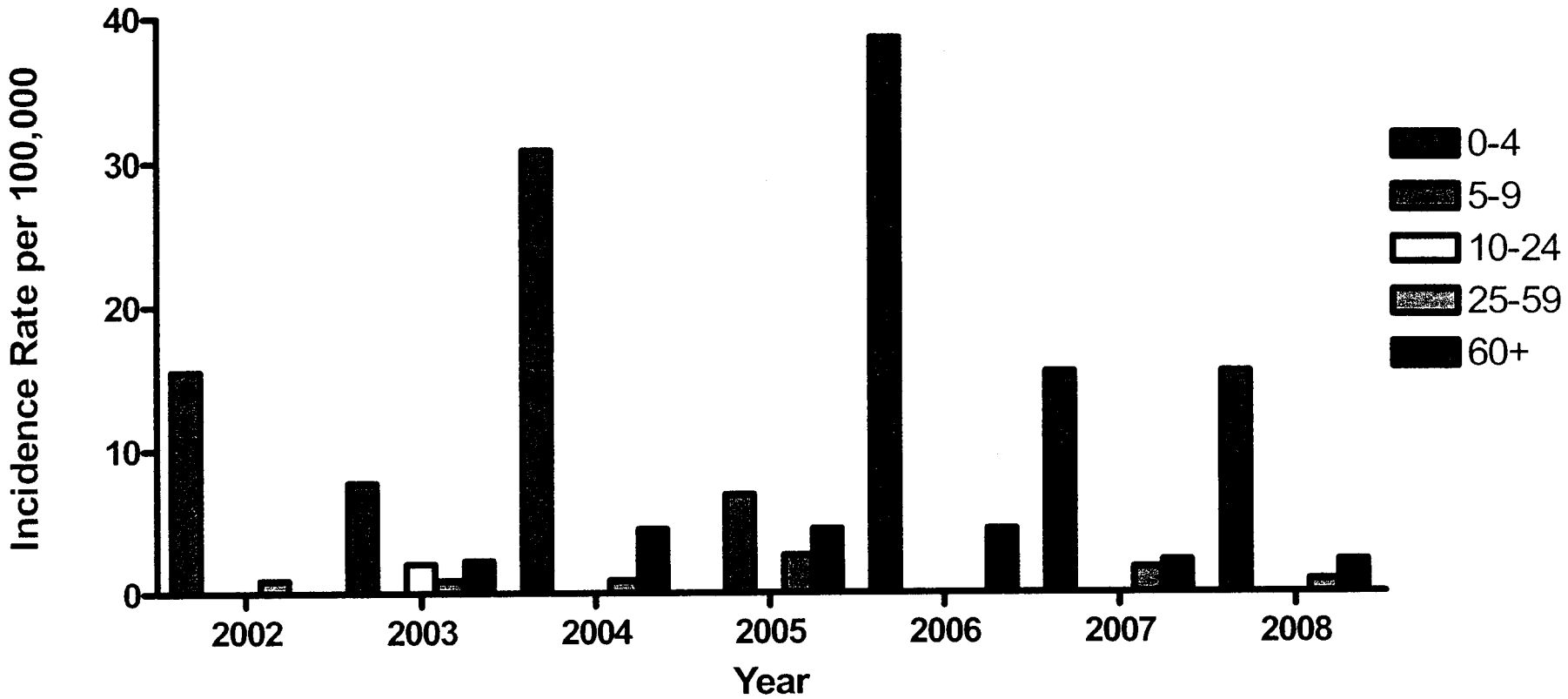

Figure 3 – Incidence of invasive *Haemophilus influenzae* disease in Northwestern Ontario, 2002 to 2008, by specific age groups per 100,000.

Appendix – Supplementary information for cases with detailed clinical information

Patient 1 – Patient #1 was a newborn, full-term Aboriginal female from a remote, rural community a few hundred kilometres northeast of Thunder Bay, who was born with sepsis caused by *H. influenzae*. As she was a newborn, she had no remarkable medical history; however, she was born with jaundice and her mother had smoked five cigarettes per day while pregnant. The isolate was identified in the patient's blood and was sent to the Ontario Public Health Laboratory (PHL) for analysis. It was determined to be NST, biotype IV, and susceptible to all routinely tested antibiotics (trimethoprim-sulfamethoxazole, ampicillin, ceftotaxime, cefuroxime, and chloramphenicol). The patient was treated with intravenous ampicillin, cleared the infection, and was discharged with no apparent sequelae. As a newborn, the patient had not yet been vaccinated.

Patient 2 – Patient #2 was a newborn Aboriginal male from whom NST *H. influenzae* was isolated in his placenta. No information was presented in the patient's chart on his clinical presentation, likely due to the fact that the patient lived for approximately one hour. His underlying medical history was significant for extreme prematurity as the patient was born at 24 weeks of gestation.

Patient 3 – Patient #3 was a 6 month old Aboriginal male, born full-term on a reserve northwest of Thunder Bay, who presented to TBRHSC with pneumonia caused by *H. influenzae*. His underlying medical history was significant for prenatal exposure to tobacco. The isolate was identified in the patient's blood and was not serotyped by the Ontario PHL. The patient was treated in hospital and the infection cleared with no apparent sequelae at the time of discharge. At the time of the infection, the patient had only received his first doses of the routine childhood immunizations.

Patient 4 – Patient #4 was a 7 month old Aboriginal male, born full-term, who presented to TBRHSC with sepsis caused by *H. influenzae*. His underlying medical history was significant for prenatal exposure to tobacco as well as a previous case of pneumonia when he was 22 days old. In addition, the patient had a family history of asthma. The isolate was identified in the patient's blood and was analyzed by the Ontario PHL. It was determined to be Hif, biotype I, and sensitive to all routinely tested antibiotics. The patient was treated for 10 days with intravenous cefuroxime in hospital. At the time of discharge, the patient was to receive long-term antibiotic therapy: 10 days of oral Zithromax, followed by five weeks of Cefprozil antibiotic therapy. No information was available on the patient's vaccination status.

Patient 5 – Patient #5 was an 8 month old, Aboriginal male who presented with post-operative sepsis following cardiac surgery. The patient was born full-term via a Caesarean section in remote rural reserve northeast of Thunder Bay. At birth it was discovered that the patient had a congenital heart defect which required corrective surgery. Post-operatively, the patient developed sepsis caused by *H. influenzae* (isolate not serotyped). The isolate was identified in the patient's blood and was sent away to the Ontario PHL. It was determined to be Hif and was sensitive to all antibiotics routinely tested by the Ontario PHL. The patient was treated with antibiotics and recovered from the infection with no apparent sequelae. Due to the patient's complex underlying

medical conditions, Hib vaccination had been delayed. Over the course of his treatment at TBRHSC the patient received three doses of the conjugate Hib vaccine.

Patient 6 – Patient #6 was an 11 month old Aboriginal male, born full-term, who presented to TBRHSC with septic arthritis caused by *H. influenzae*. He had been in foster care since birth as his mother was in jail. His underlying medical history was significant for prenatal exposure to alcohol, tobacco, and solvents. In addition to his *H. influenzae* infection, the patient was co-infected with *Pseudomonas aeruginosa.* The isolate was identified in the patient's synovial fluid and blood. It was not sent away for serotyping, but was determined to be susceptible to all routinely tested antibiotics. The patient was treated with intravenous cefazolin for two weeks, followed by three weeks of oral Cefixime therapy. At the time of discharge, the patient had cleared the infection and had no apparent sequelae. All of his vaccinations were up to date at the time of the infection.

Patient 7 – Patient #7 was a 1 year old Aboriginal male who presented to the Meno-Ya-Win Health Centre with pneumonia and pleurisy caused by *H. influenzae*. He had no remarkable medical history. The isolate was identified in the patient's blood and was sent to the Ontario PHL for serotyping. It was determined to be Hia. The patient was treated and the infection cleared. He was discharged home with no apparent sequelae. At the time of the infection, his vaccinations were up to date.

Patient 8 – Patient #8 was a 1 year old male from whom *H. influenzae* type a, biotype II was isolated in his blood. No detailed clinical information is available for this patient.

Patient 9 – Patient #9 was a 1 year old male from whom *H. influenzae* type e, biotype IV was isolated in his blood. No detailed clinical information is available for this patient.

Patient 10 – Patient #10 was a 1 year old Aboriginal female from whom *H. influenzae* type a, biotype II was isolated in her blood; she presented with sepsis. Her underlying medical history was not significant for any underlying medical conditions at the time of her infection. She was treated at TBRHSC and eventually transferred to a hospital in Winnipeg for further care. At the time of her infection, her vaccinations were up to date.

Patient 11 – Patient #11 was a two year old Aboriginal female, born at full-term, who presented with a urinary tract infection caused by *H. influenzae*. Her medical history was significant for anemia; while she was pregnant, the patient's mother had active systemic lupus erythematosus (SLE). In early childhood the patient received a pacemaker to cope with a high degree of secondary heart block as a result of her mother's active SLE while pregnant. As well, the patient was considered to be of low birthweight and was born with sepsis and feeding problems. It was noted in the chart that the patient had no significant immunodeficiencies. The isolate was identified in the patient's blood and was sent to the Ontario PHL for serotyping. It was determined to be Hia, biotype II, and sensitive to all routinely tested antibiotics. The patient was treated with Zithromax, at a dose appropriate for her weight. At the time of her discharge from the hospital, she had cleared her infection and had no apparent sequelae. No information was available on the patient's vaccination status.

Patient 12 – Patient #12 was a 2 year old, Caucasian female who presented with sepsis caused by *H. influenzae*. The patient was born full-term at TBRHSC and at the time of the infection had no remarkable medical history. The isolate was identified in the patient's blood and was sent to t Ontario PHL for serotyping. It was determined to be NST, biotype II, and resistant to ampicillin, ceftotaxime, and cefuroxime. The patient was treated with antibiotics and recovered with no apparent sequelae. No information was available on the patient's vaccination status.

Patient 13 – Patient #13 was a 3 year old Aboriginal male who presented to the Meno-Ya-Win Health Centre with pharyngitis caused by *H. influenzae*. His medical history was significant for mild anemia. The isolate was identified in the patient's blood and sent to the Ontario PHL for serotyping. It was determined to be Hia. The patient was treated, cleared the infection, and was discharged with no apparent sequelae. No information was available on the patient's vaccination status.

Patient 14 – Patient #14 was a 3 year old female from whom Hia, biotype II was isolated in her blood. No detailed clinical information is available for this patient.

Patient 15 – Patient #15 was a 3 year old Aboriginal male from whom Hia was isolated in his blood. He presented to the Meno-Ya-Win Health Centre. No detailed clinical information is available for this patient.

Patient 16 – Patient #16 was a 4 year old female from whom Hia, biotype II was isolated in her blood. She presented to the Lake of the Woods District Hospital (Kenora, Ontario). No detailed clinical information is available for this patient.

Patient 17 – Patient #17 was a 5 year old Aboriginal female from a rural, remote community several hundred kilometres north of Thunder Bay. The patient presented with periocular cellulitis caused by *H. influenzae*. She had no remarkable medical history. As the patient was not born at TBRHSC, it was not possible to determine whether the patient was born prematurely or at full-term. The isolate was identified surgically in the patient's retro-orbital cavity and was not sent to the Ontario PHL for testing. It was determined to be resistant to triethoprim-sulfamethoxazole. The patient was treated with Clavulin 250mg three times a day until the infection was resolved. She was discharged with no apparent sequelae. No information was available on the patient's vaccination status.

Patient 18 – Patient #18 was a 7 year old Aboriginal female who presented to the Meno-Ya-Win Health Centre with tonsillitis caused by *H. influenzae*. Her underlying medical history was significant for anemia. The isolate was identified in the patient's blood, sent for analysis and was determined to be Hia. The patient was treated and the infection cleared; she was discharged home with no apparent sequelae. At the time of the infection, the patient's vaccinations were up to date.

Patient 19 – Patient #19 was a 9 year old male from whom Hia, biotype II was isolated in his blood. No detailed clinical information is available for this patient.

Patient 20 – Patient #20 was a 24 year old female from whom NST *H. influenzae*, biotype II was isolated in her blood. No detailed clinical information is available for this patient.

Patient 21 – Patient #21 was a 33 year old female who presented with a post-operative infection. Initially the patient presented to TBRHSC with persistent abdominal pain. She was determined to have fluid accumulating in the pelvic cavity, which was then surgically aspirated. Post-surgery the patient developed a 10 cm X 8 cm abscess in her abdominal cavity caused by *H. influenzae*. The isolate was identified in the patient's abdominal cavity and was not sent away for serotyping. Antibiotic susceptibility testing was performed and found that the isolate was resistant to ampicillin. The patient was treated with Keflex and then Cloxacillin to clear the infection. At the time of discharge, the patient had cleared the infection with no apparent sequelae. In terms of underlying conditions, the patient had been previously diagnosed with major depressive disorder and had a history of bad post-operative infections.

Patient 22 – Patient #22 was a 34 year old Aboriginal female who presented to the Meno-Ya-Win Health Centre with pneumonia caused by *H. influenzae*. Her medical history was significant for diabetes mellitus. The isolate was identified in the patient's blood and sent away for serotyping. It was determined to be Hia. The patient was treated, the infection cleared, and she was discharged with no apparent sequelae.

Patient 23 – Patient #23 was a 36 year old female who presented with epiglottitis caused by *H. influenzae*. The patient's underlying medical history was only significant for a previous possible case of epiglottitis. The isolate was identified in the patient's bloodstream and was not sent away for serotyping. It was determined to be susceptible to all routinely tested antibiotics. While at TBRHSC, the patient was treated intravenously with ceftriaxone. She cleared the infection and was discharged home with a prescription for Ceftin. At the time she left the hospital, the patient had no apparent sequelae.

Patient 24 – Patient #24 was a 40 year old Aboriginal female with no set address who presented to TBRHSC with pyelonephritis caused by *H. influenzae*. Her underlying medical history was significant for diabetes mellitus and alcoholism. The isolate was identified in the patient's blood and was sent to the Ontario PHL for analysis. It was determined to be NST, biotype III, and was sensitive to all routinely tested antibiotics. The patient was treated intravenously with Levaquin and the infection cleared. At the time she was discharged, she had no apparent sequelae.

Patient 25 – Patient #25 was a 45 year old female who presented with epiglottitis caused by *H. influenzae*. She had no remarkable medical history. The isolate was identified in the patient's blood and sent to the Ontario PHL for serotyping. It was determined to be Hif, biotype I. The patient was treated intravenously with Ceftriaxone, cleared the infection, and was discharged home with no apparent sequelae.

Patient 26 – Patient #26 was a 45 year old female from whom Hif, biotype I was isolated from her blood. No detailed clinical information is available for this patient.

Patient 27 – Patient #27 was a 47 year old Aboriginal male who presented to the Meno-Ya-Win Health Centre with pneumonia caused by *H. influenzae*. His underlying medical history was

significant for remote acute rheumatic fever, cardiomyopathy, and sleep apnea. The isolate was identified in the patient's blood and was sent to the Ontario PHL for serotyping. It was determined to be Hia. He was treated, the infection cleared, and was subsequently discharged with no apparent sequelae.

Patient 28 – Patient #28 was a 53 year old Caucasian female who presented with sepsis caused by *H. influenzae*. Her underlying medical history was significant for lymphoma, Crohn's disease that had previously required a partial bowel resection, thrombocytopenia, and mineralocorticoid deficiency. The patient may have developed sepsis from many possible sources as she had pneumonia, ulceration of the urethra, and skin ulcers. The isolate was identified in the patient's blood and was not sent away for analysis. It was determined to be susceptible to all routinely tested antibiotics. The patient was treated with an antibiotic, but died shortly after therapy was started, likely due to her underlying medical conditions.

Patient 29 – Patient #29 was a 57 year old female who presented with sepsis caused by *H. influenzae*. The patient's medical history was significant for metastatic breast cancer. The isolate was identified in the patient's blood and was sent for serotyping. It was determined to be NST and resistant to trimethoprim/sulfamethoxazole. Shortly after developing the infection, the patient died, likely due to her underlying medical conditions.

Patient 30 – Patient #30 was a 60 year old male who presented with epiglottitis caused by *H. influenzae*. His underlying medical history was significant for multiple myeloma, an autologous peripheral blood stem cell transplant one year prior to the infection (as a result of multiple myeloma), prostate hypertrophy, and chronic pain. The isolate was identified in the patient's blood and was sent away for analysis. It was determined to be Hif. He was treated with Ceftriaxone and one dose of Decadron. At the time of discharge, the patient had cleared the infection and was sent home with a prescription for Ceftin 500mg twice daily for seven days to prevent the infection from recurring. He left TBRHSC with no apparent sequelae.

Patient 31 – Patient #31 was a 62 year old female from whom NST *H. influenzae*, biotype I was isolated in her blood. She presented to the Lake of the Woods District Hospital (Kenora, Ontario). No detailed clinical information is available for this patient.

Patient 32 – Patient #32 was a 62 year old female from whom Hif was isolated in her blood. No detailed clinical information is available for this patient.

Patient 33 – Patient #33 was a 66 year old Aboriginal female who presented to Meno-Ya-Win Health Centre with pneumonia caused by *H. influenzae*. Her medical history was significant for a previous tuberculosis infection and chronic obstructive pulmonary disease (COPD). The isolate was identified in the patient's blood and sent to the Ontario PHL for serotyping. It was determined to be Hia. The patient was treated; the infection cleared, and she was discharged home with no apparent sequelae.

Patient 34 – Patient #34 was a 72 year old male from a rural community just outside Thunder Bay who presented with pneumonia caused by *H. influenzae*. His underlying medical history was significant for multiple myeloma and palliative radiotherapy. The isolate was identified in the

patient's blood and sent to the Ontario PHL for analysis. It was determined to be Hif. Antibiotic susceptibility testing was conducted which revealed that the isolate was sensitive to ampicillin; however, no testing was done to determine if the isolate was sensitive or resistant to any other antibiotics. The patient was treated with ampicillin, cleared the infection and was subsequently discharged home with no apparent sequelae.

Patient 35 – Patient #35 was a 74 year old Aboriginal male from a remote, rural community several hundred kilometres north of Thunder Bay who presented with community-acquired pneumonia caused by *H. influenzae*. His underlying medical history was significant for hypertension and elevated liver enzymes of unknown etiology. It was difficult for staff to acquire a full medical history because the patient did not speak English. The patient had such a severe case of pneumonia while in hospital that he was required to be intubated. The isolate was identified in the patient's blood and was sent to the Ontario PHL for analysis. It was determined it to be Hif, and sensitive to all routinely tested antibiotics. This patient was treated with Cefuroxime and transferred to the Meno-Ya-Win Health Centre in Sioux Lookout for further treatment.

Patient 36 – Patient #36 was a 77 year old female who presented with empyema caused by *H. influenzae*. Her medical history was significant for tuberculosis and lung cancer. The isolate was identified in the patient's pleural fluid, but no other information was provided in her medical record.

Patient 37 – Patient #37 was a 79 year old female who presented with meningitis caused by *H. influenzae*. The patient's underlying medical history was significant for hypothyroidism, hypertension, anemia, depression, and chronic back and neck pain. The isolate was identified in the patient's blood and was sent to the Ontario PHL for analysis. It was determined to be NST and was susceptible to all routinely tested antibiotics. While in hospital the patient was treated with cefuroxime and was subsequently discharged with the same prescription. The patient was sent home in a weak state, but with no apparent sequelae.

Patient 38 – Patient #38 was an 89 year old Caucasian male who presented with sepsis caused by *H. influenzae*. The patient's medical history was significant for COPD, chronic renal failure, anemia, and unconfirmed multiple myeloma. The isolate was identified in the patient's blood and was sent away for analysis. It was determined to be NST, biotype II, and was sensitive to all routinely tested antibiotics. While at TBRHSC, the patient was treated with Norfloxacin 400mg twice daily for seven days. The patient subsequently cleared the infection and was discharged with no apparent sequelae.