LONG-TERM FOLLOW-UP OF NEONATES WHO HAVE BEEN IMMUNIZED WITH HEPATITIS B VACCINE

by

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ABSTRACT

Although the short-term effectiveness of hepatitis B vaccine has been well established, few long-term studies of the effectiveness of this vaccine in neonates who are born to HBsAg positive mothers have been undertaken. It is also not known if supplementary doses of vaccine are required to protect these children who are continuously exposed to the hepatitis B virus, and if so, how long after primary immunization the additional dose should be given.

To determine the hepatitis B vaccine effectiveness up to eight years, a cohort of 770 (66%) of 1166 children who had been immunized with HBIG and hepatitis B vaccine at birth, between 1984 and 1989 inclusive, were tested for the serological markers: anti-HBc, anti-HBs and HBsAg. (Anti-HBc indicates that viral replication has occurred at some time (infection); anti-HBs is the antibody to HBsAg and may appear after natural infection or following immunization; and HBsAg indicates presence of hepatitis B virus (HBV) infection.) The children in the cohort were immunized as part of the British Columbia Ministry of Health Program to prevent HBV infection in infants of carrier mothers.

Blood samples were obtained by finger-prick and tested at the Canadian Red Cross Society, Vancouver Centre, Blood Transfusion Service. The IMx assay kits were produced by Abbott Laboratories. Parents were interviewed for information on relevant variables.

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Associations were determined using chi-square tests, Mantel-Haenszel trend tests, linear and logistic regression procedures. Best prediction models for the outcomes: anti-HBc, anti-HBs and HBsAg were determined in a stepwise fashion.

Of the participants, 31% of the mothers were HBeAg positive as well as HBsAg positive. The overall attack rate for ages 2 to 8 years was 5.1%; the carrier rate was 2.3% and the seropositivity rate (>=10 mIU/ml) was 87.9%. The geometric mean titres of anti-HBs varied from a high of 272 mIU/ml at age two to a low of 23 mIU/ml at age seven. Vaccine efficacy for infection was 89%.

The best predictors for infection were the mother’s HBeAg status (P<0.0001), the age of the child when the first dose of vaccine was given (P=0.0001), the number of years the mother spent in her country of birth (P=0.001) and inversely the mother’s age (P=0.002). Anti-HBs titres were best predicted by the inverse age of the child (P=0.0001), the number of doses of vaccine (P=0.0007), the age of the child when the first dose of vaccine was given (P=0.01) and the number of months the child spent abroad (P=0.05).

Since the infection rate was relatively stable between age groups and the association between the child’s age and anti-HBc was not significant (P=0.15), waning immunity is not suggested by the findings of this study. This was in spite of the decline in anti-HBs titres with increasing age.
The conclusions of this study are: 1) That a booster dose of hepatitis B vaccine after the six month dose is not necessary at least up to age eight; 2) Because a delay in the first dose of vaccine resulted in increased infections, giving the first dose early is critical; 3) It could not be concluded from the data that HBIG was not important, therefore, continuation of HBIG at birth is recommended; 4) Since the timing of dose two later than two months after dose one was not found to be associated with increased infections, this dose could be incorporated with the regular immunization schedule at two months of age if administratively more feasible; 5) Susceptibility appears to arise earlier rather than later and may be due to non-response to the vaccine as a 14% non-response rate was found at 6 to 18 months of age.
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DEDICATION

I would like to dedicate this thesis to my husband, Giorgio, who was 'my rock' and who unfailingly supported and encouraged me throughout the two years, as well as provided many hours of technical computer support for the project. And to my daughter, Carmen, who was 'my bright shining star' and who also encouraged me, volunteered at the clinics and licked hundreds of envelopes!
CHAPTER ONE
INTRODUCTION AND GENERAL CONSIDERATIONS

1. DESCRIPTION OF THE PROVINCE-WIDE PROGRAM FOR THE PREVENTION OF HEPATITIS B VIRUS INFECTION IN INFANTS OF CARRIER MOTHERS

In July 1984, the British Columbia Ministry of Health extended its Province-Wide Program for the Prevention of Hepatitis B Virus (HBV) Infection in Infants of Carrier Mothers, (hereafter referred to as the Program), to include the provision of hepatitis B (HB) vaccine at birth, one month of age and six months of age. Prior to this, infants of mothers who were found hepatitis B surface antigen (HBsAg) positive when screened by the Canadian Red Cross Blood Transfusion Service had been given only hepatitis B immune globulin (HBIG) as a prophylaxis against the HBV. The provision of HBIG began in 1977, with one dose at birth. In 1982 the Program was expanded to include a dose at three months of age. When hepatitis B vaccine was added to the protocol, the second dose of HBIG was dropped. Initially, 10 ug of Heptavax, a plasma-derived vaccine produced by Merck, Sharp and Dohme was provided. In March 1988, Heptavax was replaced by Engerix, a yeast-derived 20 ug vaccine produced by SmithKline Beecham Biologicals. Both vaccines were administered intramuscularly. HyperHep, a preparation of 217 international units per millilitre HBIG produced by Cutter was also administered intramuscularly.

The HBIG, since it was a blood product, was issued to the birth hospital by the Canadian Red Cross Society, Vancouver Centre, Blood Transfusion Service (hereafter referred to as the Red Cross). With
the addition of the HB vaccine, the Red Cross continued to administer the Program as well as keep a central registry of the dates of vaccine delivery and dose administration.

The procedure of this on-going Program is as follows. Physicians provide a blood sample from pregnant women which is sent to the Red Cross. Among other tests, this blood sample is screened for the HBV surface antigen. If it is determined that an expectant mother is positive for HBsAg, the physician is advised by the Red Cross following which the HBIG and first dose of HB vaccine are sent to the birthing hospital along with recording forms. A notification letter is also sent to the family's area Health Department or Health Unit with the second and third doses of vaccine and recording forms. The respective hospital and Health Department or Health Unit are expected to return the recording forms to the Red Cross for recording of the dates of immunization on the central registry. If notification of immunization is not returned by approximately one month after the due date, reminder notices are sent to the respective agencies by the Red Cross.

2. OBJECTIVES OF THE STUDY

It was the purpose of this study to determine the effectiveness of the HB vaccine in the prevention of hepatitis B infection, as delivered by this Program, between July 1984 through 1989. Thus the study objectives were:

1. To determine the long-term attack rate of HBV infection for the children immunized at birth as part of the Program between 1984
and 1989 inclusive.

2. To determine antibody titres (anti-HBs) as related to time since birth in those children who were not infected.

3. To determine what factors were related to attack rates and anti-HBs titres in this cohort of children.

4. To evaluate the implications of infection rates and antibody status with regard to the (1) effectiveness of the HB vaccine, (2) evidence for waning immunity and (3) need for booster doses of vaccine.

"Effectiveness", for the sake of this study will be defined by comparing this Program’s rate of HBV infection against the expected rate of infection for this type of population without a HB vaccine immunization program.

3. JUSTIFICATION

Evaluation of the effectiveness of the HB vaccine in a mixed racial population of neonates born to HBV carrier mothers of mixed HBeAg status is not available in the literature for children up to eight years of age. It is not known if booster doses of HB vaccine are required for these children still at risk of HBV infection from household contacts. If booster doses are required, at what interval would they be most beneficial? This study attempts to answer these questions by focusing on attack rates and anti-HBs titres at differing lengths of time since immunization. In as much as certain population characteristics are similar and program delivery practices are similar, the information from this study is expected to be generalizable to other such programs.
4. RESEARCH QUESTIONS AND HYPOTHESES

With respect to the neonates in this Program, who were born in 1984 through to 1989 and who were immunized with HB vaccine immediately after birth:

1. What is the HBV attack rate over time?
2. What are the anti-HBs titres and how do they change over time?
3. Is there an association between attack rates and anti-HBs titres?
4. What are the associations between the HBV attack rates and various demographic, program intervention, and exposure factors?
5. What are the associations between anti-HBs titres and various demographic, program intervention, and exposure factors?

The null hypotheses corresponding to these research questions were as follows:

1. There is no association between infection rate and elapsed time since immunization with HB vaccine.
2. There is no association between infection rates and decreasing anti-HBs titres.
3. There are no associations between the attack rates and various demographic, program intervention and exposure factors.
4. There are no associations between the anti-HBs titres and various demographic, program intervention and exposure factors.

The study design chosen to test these hypotheses and answer these questions was a cohort study of children born between 1984 and
1989, inclusive, and tested cross-sectionally once in 1992. At the time of the single follow-up the ages of the children ranged from two to eight years.
CHAPTER TWO

LITERATURE REVIEW

1. BACKGROUND

Hepatitis B (HB) is a viral infection of the liver. It is characterized by three main serologic markers: HBsAg; which, if still present after 6 months, indicates the carrier state; anti-HBc; which indicates that viral replication (infection) has occurred at some time; and anti-HBs; which indicates immunity to hepatitis B virus (HBV). There is a greater likelihood of transmission of infection where HBeAg is present in association with HBsAg than when HBeAg is not present. The HBsAg, anti-HBc and anti-HBs serologic markers are defined in more detail in the method section, Chapter Three, page 20.

Hepatitis B virus is a major cause of acute and chronic hepatitis and cirrhosis. Over 300 million people throughout the world are persistent carriers of HBV (Garrison and Baker, 1991). The prevalence rate of current and past infection in many countries of Asia, Africa and Oceania is in the range of 30-100% (West et al. 1990). Although Canada is a country of low endemicity for hepatitis B infection, with a carrier rate of lower than 1% (West 1990), many risk factors for acquiring HBV, such as illicit drug use, homosexual activity and multiple sexual partners, are prevalent in our society. Furthermore, increased immigration from countries where the disease is highly endemic has been thought to be a factor in our rising incidence of hepatitis B (Canadian
Diseases Weekly 1987). Perinatal transmission from HBsAg positive mothers is one of the most common sources of HB infection. In Taiwan, approximately 40% of chronic carrier HBV infections are the result of perinatal transmission (Beasley et al. 1983). Up to 90% of infants who are born to carrier mothers also become HBsAg positive (Beasley et al. 1983). In British Columbia, prior to the initiation of HB prophylaxis in 1977, 23% of infants born to HBsAg positive mothers became carriers (Ballem et al. 1987).

The most serious consequences of the HBV carrier state are the long-term sequelae. The earlier in life that the infection from HBV occurs the more likely it will progress to the chronic carrier state, which in turn increases the risk of cirrhosis and cancer of the liver later in life (Polakoff and Vandervelde 1988). It is estimated that persons infected with the hepatitis B virus during the perinatal period have a 25% lifetime risk of death from liver cirrhosis or primary hepatocellular carcinoma (West et al. 1990).

2. SHORT-TERM EFFICACY OF HEPATITIS B VACCINE

When the hepatitis B vaccine became available, neonatal programs became a priority in many countries, including Canada. Many studies have established the short-term efficacy of HB vaccines in newborns. A summary of some of this research may be seen in Table 2.1.
### Table 2.1. Short-term efficacy hepatitis B vaccine

<table>
<thead>
<tr>
<th>Authors</th>
<th>Age Group</th>
<th>Months (age)</th>
<th>Number</th>
<th>Attack Rate(%) HBsAg+</th>
<th>Anti-HBs+ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beasley (1983)</td>
<td>neonates</td>
<td>9</td>
<td>159</td>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>Esteban (1985)</td>
<td>neonates</td>
<td>12</td>
<td>52</td>
<td>4</td>
<td>88</td>
</tr>
<tr>
<td>Stevens (1985)</td>
<td>neonates</td>
<td>18</td>
<td>113</td>
<td>14</td>
<td>96 &gt;50S/N</td>
</tr>
<tr>
<td>Stevens (1987)</td>
<td>neonates</td>
<td>12</td>
<td>122</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td>Lee (1987)</td>
<td>neonates</td>
<td>16</td>
<td>201</td>
<td>6</td>
<td>89&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ballem (1987) (British Columbia)</td>
<td>neonates</td>
<td>12</td>
<td>220</td>
<td>2</td>
<td>95&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Farmer (1987)</td>
<td>neonates</td>
<td>12</td>
<td>39</td>
<td>18</td>
<td>77</td>
</tr>
<tr>
<td>Hayashi (1987)</td>
<td>preschool</td>
<td>24</td>
<td>203</td>
<td>0</td>
<td>74</td>
</tr>
<tr>
<td>Polokoff (1988)</td>
<td>neonates</td>
<td>12</td>
<td>102&lt;sup&gt;(79)&lt;/sup&gt;</td>
<td>4</td>
<td>96</td>
</tr>
<tr>
<td>Poovorawan (1989)</td>
<td>neonates</td>
<td>13</td>
<td>55&lt;sup&gt;(46)&lt;/sup&gt;</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>The Gambian Study Group (1989)</td>
<td>infants &lt;1month</td>
<td>12</td>
<td>710</td>
<td>1</td>
<td>98</td>
</tr>
<tr>
<td>Waters (1989) (Alberta)</td>
<td>neonates</td>
<td>12</td>
<td>72&lt;sup&gt;(141)&lt;/sup&gt;</td>
<td>4</td>
<td>99</td>
</tr>
<tr>
<td>Theppisai (1990)</td>
<td>neonates</td>
<td>13</td>
<td>52</td>
<td>8</td>
<td>92</td>
</tr>
</tbody>
</table>

1 Denominator for attack and anti-HBs+ rates.
2 Attack and anti-HBs+ rates are rounded.
3 Denominator for anti-HBs+ rate.
4 Not stated in the article if N excluded HBsAg+ children.

In some of their earlier work, Beasley et al. (1983) studied a combination of HBIG and vaccine in different temporal combinations. They found that the combined attack rate at nine months, for infants of HBeAg positive mothers, was 5.7%. For those that did not become infected the seropositivity for anti-HBs was 100%.
Stevens et al. (1985) studied 113 infants of Asian-American mothers who were both HBsAg and HBeAg positive. They found that at 18 months of age, 14.2% of infants were infected. Two different vaccine schedules were employed, using HBIG at birth and plasma-derived Heptavax 20 ug. HB vaccine doses were given at either 0, 1 and 6 or at 1, 2 and 6 months of age. The combined seropositivity for anti-HBs of the two groups was 95.8%. Another study by Stevens et al. (1987) found similar results to Beasley (1983) when it compared two groups of neonates of HBeAg positive mothers: one received yeast-recombinant vaccine and the other plasma-derived vaccine. The overall attack rate for the two groups was 6.6% and the seropositivity rate was 100% for those that did not become HBsAg positive. A study by Poovorawan et al. (1989) in Thailand, found an even lower attack rate (3.6%) using 10 ug of recombinant DNA vaccine. At 13 months of age 100% of the children who did not become positive for HBsAg had anti-HBs titres >10 mIU/ml. The mothers were both HBsAg and HBeAg positive.

Several studies using low doses of plasma-derived HB vaccine are referred to in Table 2.1. Lee et al. (1987) reported an attack rate of 5.5% for 201 16-month-old infants who had either received three 2.5 ug doses of HB vaccine or a schedule including only two doses of 5 ug vaccine. Anti-HBs seropositivity over 10 mIU/ml for the two groups combined was 88.7%. Similarly, Theppisai et al. (1990) found an attack rate of 7.7% at 13 months for infants who had received four doses of either 2 or 5 ug of vaccine. The seropositivity rate for those not infected in the two groups was
100%. In contrast, in a study in New Zealand, a high attack rate (22%), was found in a group that received 5 μg of Hepavax vaccine alone. For the group that received vaccine combined with HBIG, the attack rate was 14.3% (Farmer et al. 1987). In the foregoing study, anti-HBs seropositivity was 72% for the vaccine-only group and 81% for the vaccine plus HBIG group at one year of age.

In 1989, The Alberta Neonatal HB Vaccination Program, which had been in operation since 1985, reported a 4% (3/72) attack rate by one year of age (Waters, 1989). The seroconversion rate in those tested was 98.6% (140/142). In an earlier report of the British Columbia Program, for the years 1983 to 1986, only 2% of 220 infants were reported HBsAg positive and 95% were anti-HBs positive after 12 months of age (Ballem et al. 1987). The immunization schedule for both the Alberta and British Columbia programs included HBIG given immediately after birth. In Alberta the HB vaccine was given at birth, two and six months of age, whereas, in British Columbia the HB vaccine was given at birth, one and six months of age.

The HBV infection attack rates for infants in the short-term studies listed in Table 2.1. ranged between 0.28% for the Gambian Hepatitis Study Group (1989) and 18% for the study done by Farmer et al. (1987). The weighted-average attack rate was 3% for these studies which varied in length of follow-up from 9 to 24 months. The two studies with low attack rates by Hayashi et al. (1987) and the Gambian Group (1989) did not necessarily involve HBsAg positive
mothers. It should also be noted that the attack rate statistic included only those children who were HBsAg positive. Children who were anti-HBc positive were not included in the calculation of the attack rates since their antibodies could have been those acquired in utero, and therefore would not necessarily have been induced by subclinical infection. The weighted-average short-term anti-HBs positive rate for the studies in Table 2.1. was 94%. The range was between 74 and 100%. The denominator for calculating the conversion rates did not include those children positive for HBsAg. (Possible exceptions are Lee et al. and Ballem et al., where seroconversion rates were given with no indication of actual numbers.)

3. LONG-TERM EFFICACY OF HEPATITIS B VACCINE
After birth, when the vertical risk of maternal transmission of HBV infection is over, there is a continuing risk of horizontal transmission in the household. According to Tong (1989), 40% of carriers acquire the virus as a result of perinatal transmission, and another 35-40% become infected during the preschool years. Six to ten percent of infections are acquired after the fifth or sixth year of life (Maynard et al. 1989). Franks et al. (1989) reported that nearly half the cases of HBV infection among the U.S.-born children of refugees were not attributable to perinatal transmission from a mother with infectious disease. Thus continued immunoprophylaxis during the early years of life is important to prevent the chronic carrier state.
Most long-term trials on the efficacy of HB vaccine have focused on the protection of adults, anti-HBs seropositivity rates and titres of antibodies. A summary of attack rates and anti-HBs seropositivity rates for long-term studies may be seen in Table 2.2.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Age Group</th>
<th>Years after 1st dose</th>
<th>Number</th>
<th>Attack Rate HBsAg &amp;/or anti-HBc</th>
<th>Anti-HBs+ &gt;10 mIU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wainwright (1984)</td>
<td>all ages</td>
<td>5</td>
<td>1114</td>
<td>1</td>
<td>81</td>
</tr>
<tr>
<td>Coursaget (1986)</td>
<td>infants</td>
<td>4</td>
<td>37</td>
<td>3</td>
<td>98 (any level)</td>
</tr>
<tr>
<td>Coursaget (1986)</td>
<td>infants</td>
<td>7</td>
<td>135</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Seven year study</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jilg (1988)</td>
<td>adults</td>
<td>6</td>
<td>39</td>
<td>-</td>
<td>66</td>
</tr>
<tr>
<td>Horowitz (1988)</td>
<td>adults</td>
<td>3</td>
<td>245</td>
<td>-</td>
<td>62</td>
</tr>
<tr>
<td>Mannucci (1989)</td>
<td>1-54yrs</td>
<td>4</td>
<td>52</td>
<td>-</td>
<td>100</td>
</tr>
<tr>
<td>Ip (1989)</td>
<td>neonate</td>
<td>3</td>
<td>188</td>
<td>38</td>
<td>81</td>
</tr>
<tr>
<td>Moyes (1990)</td>
<td>neonate</td>
<td>4</td>
<td>70</td>
<td>0</td>
<td>78</td>
</tr>
</tbody>
</table>

1 Denominator for attack and anti-HBs+ rates.
2 Attack and anti-HBs+ rates are rounded.
3 Of those who had an initial response of 10 SRU's or greater.

Coursaget et al. (1986) undertook two long-term HB vaccine efficacy studies with infants in a hyperendemic area. A series of three injections of 5 ug Pasteur vaccine at one month intervals as well as a booster at 12 months were administered in both studies. The three-year study resulted in an attack rate of 2.7%, with one child of 37 tested, becoming positive for anti-HBc and none for HBsAg. After three years 97.5% of the children were still anti-HBs positive. The second study was of seven years duration following administration of the first dose of vaccine. Nine of the 135
children had HBsAg or anti-HBc events after this time demonstrating an attack rate of 6.7%. Another study of infants in an endemic area was undertaken in New Zealand but in this case it was determined that all mothers were non-carriers (Moyes et al. 1990). Three injections of 2 µg of plasma-derived vaccine were administered and 4 years later none of the 70 infants tested positive for anti-HBc. Seventy-nine percent had anti-HBs titres greater than 10 mIU/ml. Wainwright et al. (1989) also reported a very low attack rate in a study of all ages of an Eskimo population in Alaska. An attack rate of 0.36% was found after five years and 81% of 1114 individuals had anti-HBs titres of 10 SRU or greater. In contrast, Ip et al. (1989) reported a combined attack rate of 38% in Hong Kong neonates of HBeAg-carrier mothers after three years. Three immunization schedules were used in this study, combining HBIG and HB vaccine. The average of the seropositivity rates (>10 mIU/ml) for the three groups after three years was 80.5%.

The attack rates for the long-term studies listed in Table 2.2 ranged between 0.36% and 38%. Although only the study by Ip et al. (1989) involves follow-up of neonates, the adult and "all age" studies were included in our calculation, for an average attack rate for long-term studies, in order to be conservative in our estimate. Thus the weighted-average attack rate for the long-term studies was 5.5%, somewhat higher than the short-term attack rate of 3%. Four of the above studies undertaken in endemic areas demonstrated low attack rates even after seven years. However, the
attack rate of 38% in the perinatal study was in sharp contrast to the rates in most of the other long-term studies. This raises concern that the protection afforded by vaccine against HBV is of limited duration, particularly in neonatal populations that are exposed to a continued risk of infection. This long-term effectiveness study in a low prevalence population reassesses that risk.

4. LONG-TERM DURATION OF ANTIBODIES

Results of long-term follow-up studies in adults showed evidence of decline in anti-HBs titres in these subjects. Jilg et al. (1988) reported that 34% of health-care workers had antibody titres that dropped below 10 mIU/ml after six years. Horwitz et al. (1988) reported that 38% had antibody titres less than 10 mIU/ml after three years. These findings are not confirmed in a study of HIV negative hemophiliacs (Mannucci et al. (1989). After four years, this group, with a mean age of nine years, had a 100% seropositivity rate for anti-HBs. Wainwright et al., 1989 reported a rate of 81% for an Eskimo population of all ages after 5 years. The weighted-average seropositivity rate after three to seven years for all the long-term studies listed in Table 2.2. is 79%. For infants and neonates alone, anti-HBs seropositivity rates after three to four years were in the range of 78.2 to 97.5%.

There are studies that claim that protection against HB infection clearly parallels the persistence of anti-HBs (Coursaget et al., Seven-year study, 1986). Hadler et al. (1986) in a study of
homosexual men, found that the HBV infection was inversely related to the maximal antibody response to vaccine and that this risk increased when antibody titres fell below 10 SRU. As a secondary objective, this study looked for an association between antibody titres and HBV attack rates and explored the issue of susceptibility to HBV disease due to waning immunity.
CHAPTER THREE

METHOD

1. SAMPLE SPECIFICATION

The target population for this study was all neonates born to mothers who tested hepatitis B surface antigen positive as part of the Province-Wide Program for the Prevention of Hepatitis B Virus Infection in Infants of Carrier Mothers and who commenced the HB vaccine at birth during the years 1984 through 1989. There were a total of 1398 records of children at the Red Cross who were born during these years. Of these, 1166 (84%) children qualified for inclusion, 224 (16%) were excluded and 8 (0.57%) records were missed.

1.1. Included children

The sample was comprised of all children from the target population who could be located in British Columbia and for whom parental consent was given to participate in the study. Of the 1166 children who qualified for inclusion and were included in the data base, 770 (66%) participated in that the parents or guardians were interviewed and the child provided a blood sample. The disposition of included children can be seen in Table 3.1.

Included in the 770 children were two children with evidence of having commenced the HB vaccine series outside of British Columbia, but who had completed the vaccine series in British Columbia as part of the Program. Also included were three children who were born in early 1984, before the Program officially started. For
these children, there was evidence in the Red Cross records of the mother being HBsAg positive and there was a record of HB vaccine for each child, although some of the doses were given late.

<table>
<thead>
<tr>
<th>Disposition of included children</th>
<th>Number</th>
<th>Percent of included children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants</td>
<td>770</td>
<td>66.0</td>
</tr>
<tr>
<td>Unable to locate</td>
<td>231</td>
<td>19.8</td>
</tr>
<tr>
<td>After inclusion determined to have moved out of B.C.</td>
<td>30</td>
<td>2.6</td>
</tr>
<tr>
<td>After inclusion, determined to have died</td>
<td>1</td>
<td>.1</td>
</tr>
<tr>
<td>Agreed to participate but did not show for an appointment</td>
<td>16</td>
<td>1.4</td>
</tr>
<tr>
<td>Agreed to participate but an appointment could not be arranged</td>
<td>32</td>
<td>2.7</td>
</tr>
<tr>
<td>Refused to participate</td>
<td>86</td>
<td>7.4</td>
</tr>
<tr>
<td>TOTAL INCLUDED</td>
<td>1166</td>
<td>100</td>
</tr>
</tbody>
</table>

### 1.2. Excluded children

There were a total of 1398 records of children who were born in 1984 through 1989 in the Red Cross registry for the Program. Categories and numbers of children excluded from the study can be seen in Table 3.2. Children excluded were those who had not received the HB vaccine, had moved out of British Columbia or had mothers with no evidence of being HBsAg positive. One child was excluded because of death. Another child who had died was initially included in the data base since the Red Cross was unaware of this child’s death.
Fifty seven (4%), of the 1398 children, were given the HB vaccine and had mothers who were anti-HBc positive but HBsAg negative. A further 22 (1.6%) were given the vaccine but there was no indication on the Red Cross record that the mother was either HBsAg or anti-HBc positive. These children were excluded since exposure to the HBV at birth was not verified. Children who lived in the Yukon or had moved out of British Columbia were excluded because of cost constraints.

<table>
<thead>
<tr>
<th>Reasons for exclusion</th>
<th>Number</th>
<th>Percent of total records</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moved out of B.C. (Red Cross records)</td>
<td>41</td>
<td>2.9</td>
</tr>
<tr>
<td>Children given vaccine but Mother HBsAg negative and Anti-HBc positive</td>
<td>57</td>
<td>4.0</td>
</tr>
<tr>
<td>Children received vaccine but no record of HB markers for the mother</td>
<td>22</td>
<td>1.6</td>
</tr>
<tr>
<td>Mother converted to HBsAg negative before child’s birth</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>Mother HBsAg positive but no record of HB vaccine given to the child (85 born in 1984 before the Program started in July 1984)</td>
<td>86</td>
<td>6.2</td>
</tr>
<tr>
<td>Out of Province births in B.C. (Yukon Territories)</td>
<td>7</td>
<td>0.5</td>
</tr>
<tr>
<td>Parent ill with acute HB while child an infant</td>
<td>2</td>
<td>0.14</td>
</tr>
<tr>
<td>Adopted, adopting parents and address unknown</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>Death as a newborn</td>
<td>1</td>
<td>0.07</td>
</tr>
<tr>
<td>TOTAL EXCLUSIONS</td>
<td>224</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Two female children who were born in British Columbia hospitals,
one in 1986 and one in 1988, were excluded as no record of immunization with HB vaccine could be found at the Red Cross, Physician's office, Health Unit or in the Health Passport. These two children, for whom there was no record in the Red Cross registry, were identified through participating siblings, therefore, the mothers were known HBV carriers.

2. STUDY VARIABLES

2.1. Purposes for collection of variables

Baseline information was collected for all 770 participating children. The same information was collected on non-participants to allow comparison for basic differences which might indicate bias in the sample obtained. These variables included the child's age and sex and the mother's age and HBeAg status. Information on three other variables was collected to allow assessment for differences between specific groups. Firstly, the language spoken in the home was asked of those who refused to join the study in order to compared the languages of this group with the languages spoken by participants. A difference between groups when language is in some way associated with infection may indicate the results are biased. Secondly, information on another social variable, history of moving, was collected for all participants, to compare moving history with infection status. If there was a difference in infection rates between those who had moved (representing those cases we could not locate) and those who had not moved, then there may be bias in the results. If, for example, we were to find that those people who had moved had a higher infection rate, then our
results may give an underestimate of the true rate. Thirdly, to assess if there was a difference in the effort to contact those children infected compared to those not infected, the number of phone calls made to locate each participant was recorded. Information on other demographic, Program intervention and exposure variables was collected to search for associations with outcome variables and to build prediction models for these outcomes.

2.2. Definitions for Dependent Variables

1. **Hepatitis B core antibody (anti-HBc)** is a specific antibody to the hepatitis B core antigen that develops during acute infection and persists for the lifetime of the previously-infected person (Evans ed. 1989). Presence of this antibody in a child’s serum was considered evidence of vaccine failure. This dependent variable was categorized as positive or negative. Results of the serological test for anti-HBc were taken from Red Cross records for the 6 to 18 month testing and from the 1992 testing of samples collected for the study. Specimens were not tested for anti-HBc IgM because of limitations on the amount of serum available.

2. **Antibody to the surface antigen (anti-HBs)** is the specific antibody to the hepatitis B surface antigen and appears after exposure to this antigen either following naturally occurring infection or through immunization (Evans ed. 1989). For this study, and in the absence of anti-HBc, presence of this serologic marker was considered evidence of response to the HB vaccine if titres were greater than or equal to 10 milli-
international units per millilitre (mIU/ml). Anti-HBs was a continuous dependent variable. Anti-HBs titres taken from the Red Cross records for 6 to 18 month tests were measured in 'ratio of sample counts per minute to mean counts per minute of negative control' (S/N). Since this unit of measurement was not comparable to the mIU/ml unit of measurement which was used in this study, results were categorized as positive for 10 S/N's or greater and negative for less than 10 S/N's.

3. **Hepatitis B surface antigen (HBsAg)** is the glycoprotein coat of HBV and exists both associated with the complete virion and independently. If found in the serum of an individual, HBsAg indicates acute or chronic HBV infection (Evans ed. 1989). For this study, a child was classified as a carrier if he or she had a positive serologic test for HBsAg. We did not attempt to distinguish between chronic and acute carriers since it was not possible to obtain a second blood sample within the study period to confirm the chronic carrier status. The variable, HBsAg, was categorized as positive or negative for both 6 to 18 month results and study blood test results. The serologic test for HBsAg was done only if there was sufficient blood sample after completing tests for anti-HBc and anti-HBs since HBsAg was not included in the primary hypotheses.

2.3. Definitions for Independent Variables

2.3.1. Program intervention variables

1. **Number of doses** of HB vaccine received was a continuous variable measured from 1 to 6. No distinction was made between
the types of HB vaccine administered since there was overlap between products. Heptavax (Merck Sharp & Dohme), a plasma derived-product, was used at the program inception. Engerix (SmithKline Beecham) a recombinant vaccine, was initiated in March 1988, and was issued until after the study period.

2. **Age when Dose one was received** was a continuous variable consisting of the number of days of age the child was when the first dose of vaccine was received.

3. **Dose two timing** was a dichotomous categorical variable. 'Yes', indicated the child had received the second dose of the HB vaccine within two months of dose one, and 'No' meant the child did not receive the HB vaccine within two months of dose one.

4. **Dose three** was a categorical variable, 'Yes/No', indicating whether or not the child had received the third dose of the HB vaccine series.

5. **Dose four and Dose five** were categorical variables, 'Yes/No', indicating whether or not the child had received additional doses of HB vaccine beyond the protocol of the Program.

6. **Hepatitis B immune globulin** or HBIG was a categorical variable classified as 'Yes' when the child had received HBIG at birth and 'No' if HBIG was not received. For the multivariate analyses, HBIG was classified as 'Yes/Unknown/No' and treated as a continuous variable. HBIG, if administered, was given within 48 hours of birth.

### 2.3.2. Demographic variables

1. The **child's age** was a continuous variable when used in the
multivariate analyses and categorical when used in univariate analyses. As a categorical variable, age was grouped by year of age at the last birthday (2 to 8 years). Age was calculated from the day of birth to the day the blood sample was taken. For non-participating children the midpoint day (July 31, 1992) of the blood sample taking period was used as the sample day in the calculation of age.

2. **Child’s year of birth** was a categorical variable grouped according to year of birth (1984 to 1989).

3. **Child’s sex** was a categorical variable, male/female.

4. **Mother’s age** was used as a continuous variable in the multivariate analyses and as a categorical variable in the univariate analyses. The categorical variable was grouped according to quartiles with the age categories from 1 to 4 being 15.7 to 26.7; 26.8 to 30.0; 30.1 to 33.1 and 33.2 to 42.1 respectively.

5. **Mother’s hepatitis B e antigen status** was a measure of the mother’s infectivity and was a categorical variable measured as positive or negative. Negative was the reference level. This information, when available, was taken from the Red Cross registry for mothers who were tested before the birth of the child.

6. **Mother’s country of birth** was a categorical variable and was used only as a grouped variable. Countries were grouped according to the most common mode of transmission of the HBV in that country (vertical or horizontal). Vertical transmission referred to perinatal transmission and horizontal
transmission referred to transmission by close association with other persons after birth. Classification was made based on geographic patterns of frequency of mode of transmission of HBV infection as given by Maynard, Kane and Hadler (1989) with the exception of Africa. The World Health Association report of Maternal and Perinatal Infections, 1991, suggested that HBV infections in children in Africa are associated with high horizontal transmission rates in early childhood. The countries grouped according to vertical transmission were: China, Hong Kong, Viet Nam, Philippines, South East Asia, Taiwan, North and South Korea, and Macaw. Countries grouped according to horizontal transmission were Canada, India, Pakistan, the Middle East countries, East and North Africa, Sub-Sahara Africa, Eastern Europe, Japan, Western Europe, South and Central America, United States of America, Australia and New Zealand.

7. Father's country of birth was categorized in the same way as mother's country of birth.

8. Years mother spent in her country of birth was grouped according to quartiles and used in the univariate analyses as a categorical variable and as a continuous categorical variable in the multivariate analyses. Categories 1 to 4 were: 1 to 13 years; 14 to 20 years; 21 to 25 years; and 26 to 40 years respectively. So as to measure the effect of the number of years spent in a foreign birth country, this variable considered those mothers born in Canada as having zero years in her country of birth.
9. Years father spent in his country of birth were coded and grouped the same way as 'Mother's years in birth country' with quartiles categories 1 to 4 being: 1 to 12 years; 13 to 20 years; 21 to 26 years; and 27 to 44 years respectively.

10. Language was a categorical variable grouped by vertical or horizontal transmission according to the countries represented by the languages spoken. Chinese, Korean, Vietnamese, South East Asian and Philippine languages were included in the vertical transmission group. English, Japanese, European, African, Middle Eastern, and East Indian languages were included in the horizontal transmission group. This variable was also used to assess for selection bias in the sample as discussed in Chapter Three, page 19.

2.3.2. Illness and exposure variables

Variables 1 to 12 below were categorical variables classified as 'Yes' or 'No' for both univariate and multivariate analyses.

1. Hepatitis illness was considered 'positive' if the child had been diagnosed by a physician or health care workers. Evidence of laboratory confirmation was not requested.

2. Jaundice was yellowing of the skin and eyes due to hepatitis. Physiological jaundice due to birth or breast feeding were excluded.

3. History of breast feeding was defined as breast feeding for at least a week in duration and at least one feeding a day.

4. Ear piercing was defined as puncture of the ear for the purpose of wearing an ear ring.
5. **Acupuncture** was defined as the Chinese practice of puncturing the body with needles to cure disease or relieve pain.

6. **Tattooing** was defined as puncturing the skin for the insertion of pigment to mark the skin.

7. **Surgical procedures** were defined as procedures that involved an incision or suturing as performed by a health professional. Dental procedures were excluded.

8. **Blood transfusions** were defined as the child receiving blood intravenously in any amount.

9. **Blood products** were defined to include plasma, serum, thrombin, fibrinogen, packed red blood cells and cryoprecipitate. They did not include immune globulin, heat-treated plasma protein fraction, albumin or fibrinolysin.

10. **Daycare** was defined as any out-of-home, formal, paid-for daycare in a group setting, for example, group or family daycare. It did not include in-home baby sitting, whether done on a regular or occasional basis.

11. **Hospitalization** was defined as overnight admission at least once. Visits to the Emergency Room not followed by admission were not included.

12. **Travel** included only trips to overseas continents or to Mexico, Central or South America. Trips to the United States or within Canada were not included.

13. **Minimum age at travel abroad** was a categorical variable indicating the youngest age at which the child had travelled and was grouped according to the child's age at last birthday. In the multivariate analyses, minimum age at travel was treated
as a continuous variable.

14. **Total months abroad** was a continuous variable representing the total time, in months, for each child, for all trips abroad. This variable was also grouped according to: 'no months abroad/less than three months abroad/three months or more abroad'. The grouped variable was used in the multivariate analyses and treated as a continuous variable.

15. **Contacts** was a continuous variable indicating up to 10 the number of household contacts the child had had since birth. To qualify as a household contact, the person had to have shared the same kitchen as the child and lived in the same house for at least one month. For the multivariate analyses, contacts were grouped as 1, 2, 3, 4, 5, 6, and 7+ but treated as a continuous variable.

### 2.3.4. Variables collected to assess for selection bias

1. **Moved** residences since the child was born was a categorical variable classified as 'yes' or 'no'. A change in permanent address after the child was first brought home from the hospital at birth constituted a move for that child.

2. **Phone calls** was a continuous variable indicating the number of contact attempts made by phone for the purpose of locating a specific case. Calls included those made to the family’s home, place of business, physician and/or health department.

3. **Language** was discussed above under demographic variables.
3. DATA COLLECTION PROCEDURES

The main goals of the data collection procedures were:

1. To collect blood samples from as many of the target population as possible.

2. To collect information on independent variables by conducting a questionnaire interview with parents and by recording data from children’s records at the Red Cross.

3.1. Study Documents

The documents prepared for the study were the following:

1. Physician information letter
2. Parent information letter
3. Telephone introduction guide
4. Consent for participation form
5. Questionnaire and interviewer’s guide
6. Laboratory test report form and testing protocol
7. Result letter forms for parents and physicians.

Copies of the above documents may be seen in APPENDIX 1.

3.1.1. Physician information letter

The purposes of the physician letter were as follows:

1. To inform the physician of the goals of the study and what participation in the study would involve.

2. To inform the physician that his/her patient would be contacted and may wish to consult with their physician before agreeing to participate.

3. To advise the physician that they would receive a copy of the results of the tests for their patient if the subject’s parents consented.

4. To request change of address or telephone information on their
patient to assist in locating of prospective subjects.

5. To give the physician an opportunity to request that his patient not be contacted by the study team in the case of death of a child or other extenuating circumstances.

3.1.2. Parent information letter

The purposes of the parent information letter were as follows:

1. To advise parents of the study; its goals, who the researchers were and what participation in the study would involve.

2. To invite parents to consider allowing their child/ren to participate in the study and inform them that a member of the study team would contact them by telephone.

3. To give parents a name and phone number to call for more information.

3.1.3. Telephone introduction guide

The purposes of the telephone introduction guide were as follows:

1. To provide a format for phone contact with parents of prospective subjects in order to invite permission for their child/ren to participate in the study and to arrange an appointment.

2. To reinforce the importance of using the same format for all calls in order to prevent selection bias.

3. To provide instruction on how to respond to a parent who is uncertain about participation, has questions about the study or refuses to participate.

4. To provide guidance in the case of language barrier.
3.1.4. Consent for participation form

The purposes of the consent for participation form were as follows:
1. To review the purposes of the study with parents and give them an opportunity to ask questions.
2. To describe what participation in the study would involve; that is, providing a blood sample and completing a questionnaire.
3. To advise parents of the confidential nature of study records and the voluntary nature of their participation.
4. To advise parents that blood test results for their child would be sent to them.

There were three places for parent signatures on the consent form. As well as giving permission for their child to participate in the study, parents, if they wished, could give consent for a copy of the results of the blood tests to be sent to their physician and/or could indicate willingness to be contacted for future studies on the effectiveness of HB vaccine in their child.

3.1.5. Interview questionnaire

The purpose of the questionnaire was to provide a tool for the collection of data on demographic variables and exposure risk factors. The questionnaire development and questionnaire guide are discussed in Chapter Four.

3.1.6. Laboratory test report form and protocol

The purposes of the laboratory test report form and protocol were:
1. To provide a record of the study outcome results (anti-HBc, anti-HBs and HBsAg) for recording on the Red Cross registry.
form and for entry into the study database.

2. To provide instructions on the method for taking blood samples and shipping procedures.

3. To provide a protocol for testing to ensure sufficient blood was available for the primary outcome (anti-HBc) tests and to guide necessary retesting.

3.1.7. Parent and physician result letters

The purposes of the parent and physician result letters were as follows:

1. To provide parents and physicians with the results of the child's blood test along with an interpretation of the results.

2. To give recommendations if follow-up was required as a result of blood test outcomes.

3. To thank the parents and children for their participation in the study.

4. PROCEDURES IN PREPARATION FOR DATA COLLECTION

4.1. Informing the community

Before beginning the study, the goals and procedures of the study were discussed with the Health Officers Council of British Columbia. Physicians in British Columbia were informed of the study in a protocol published in the British Columbia Medical Association Journal two months before the study began. A letter was sent by Dr. John Farley, Director, Division of Communicable Disease Control, British Columbia Centre for Disease Control (BCCDC), to all Provincial Health Units and City Health Departments
to advise them of the goals of the study and their possible involvement. Health Departments and Health Units were requested to assist by providing current addresses for the prospective participants and by providing space for clinics where possible.

4.2. Updating of records

Staff were hired to assist with the computer entry of 'locating data' for the 1166 children eligible for inclusion. These data, including the child's and mother's birth dates, the mother's name, address and phone number, and, when available, the father's and physician's name and address, were taken from the Red Cross registry form and entered onto a dBase database. A copy of the Red Cross registry form may be seen in APPENDIX 2. Once these 'locating data' were recorded in the computer, a printout for each child was made. This printout provided a form for the possible correction of locating information, for the recording of contact attempts and for the disposition of the case.

Since it was expected that many of the addresses on the Red Cross records would be out of date, updating of the children's 'locating information' was done prior to sending out the physician and parent information letters. The Provincial Health Units and City Health Departments played a key role in this updating process. They did so in three ways: (1) by providing computer printout lists of children who had been on the Program; (2) by providing access to computer or hard records to study staff; and (3) by providing corrections to printouts of children's, parents' and physicians'
names, addresses and telephone numbers. In spite of the updating process 167 (14%) of the parent letters and 32 (2.7%) of the physician letters were none the less returned after the first mailing. The response rate of subsequent mailings was enhanced with the assistance of physicians’ office records and telephone directories.

4.3. Contacting the clients

Once the 'locating information' in a particular area was updated, the physician information letter was sent out. After one week plus five days delivery time, the parent information letters were mailed out. In the meantime, arrangements were made regarding locations and times for clinics in Health Units, Health Departments and community service rooms. (Home visits were made when the numbers in a community were too few to warrant a clinic or when clients were without transportation.) Once clinic times were scheduled and approximately one week had passed since the parent letters were mailed, attempts were made to contact the parents of the children to request participation in the study and to set up appointment times.

Five research assistants, two of whom spoke Chinese, and two Vietnamese volunteers assisted with telephoning prospective subjects. Over 70% of the phone contacts were made by one research assistant. Orientation to the contacting procedure included the following:

1. Review of the telephone introduction guide and format.
2. Stressing the importance of using the format wording and following guidelines consistently.
3. Stressing the importance of not coercing the parents.
4. Demonstration of the phoning procedure.
5. Return demonstration of the phone procedure and feedback.

5. CLINIC STAFFING AND DATA COLLECTION PROCEDURES

Clinic appointments were set up at approximately 30 minute intervals. Appointments began with review and signing of the consent form. Care was taken to make sure the parent could read the face sheet. If they couldn't, the consent form was read to them or explained to them. The three signature sections were also explained, and questions about the study were answered. If the parent did not speak English, interpretation was provided.

After parents or guardians signed the consent form, giving permission for the child's participation, the questionnaire was completed by interview by the research staff. Finally, a finger prick blood sample was obtained from the child.

Blood samples were taken by three nurses experienced in taking finger blood samples. These nurses were provided by TASC Research Services on a fee-for-service basis. The study coordinator and one Chinese-speaking research assistant were trained by the TASC nurses in the finger blood sampling technique as well. Research staff who took blood samples were immunized against the HBV.
A second Chinese speaking research assistant interviewed parents but did not take blood samples. Volunteers assisted with the reception of clients and interpretation. In seven cases it was necessary to complete the interviews by telephone, due to language problems. In three other cases interviews were also completed by telephone. A special clinic was held for Vietnamese-Canadians with Vietnamese-language interpreters. Chinese interpretation was provided at most clinics.

5.1. Orientation of staff to clinic procedures was as follows:
1. A package of information including a list of clinic duties, study documents and background information on hepatitis B disease and vaccine was given to clinic staff.
2. The study coordinator reviewed the documents with the staff, giving an opportunity for questions.
3. The consent form completion and questionnaire interview were demonstrated by the coordinator.
4. A return demonstration was observed by the coordinator followed by feedback.

5.2. The aims of the questionnaire-orientation session were:
1. To ensure the interviewer understood the meaning of the questions and the terms used. The questionnaire guide was used to assist in clarification of terms.
2. To ensure the interviewer understood how to complete the questionnaire with the parent responses.
3. To stress the importance of using the exact words of the
questions consistently to avoid bias. For example, rewording the question about hepatitis history in a leading manner such as "Your child hasn’t had hepatitis has he?" should not be done.

4. To stress the importance of completing all the questions, thereby avoiding missing data. (It was required that all questionnaires be checked for completeness by the interviewer at the end of each interview.)

5.3. Procedure for the finger-prick blood sample

Before taking the blood sample the child’s hand was warmed using a heating pad or warm water in order to increase the flow of blood to the hand. All research staff wore gloves while taking blood samples. After cleansing the skin with an alcohol swab and drying it, with sterile gauze, the side of the finger next to the nail bed was pricked with a sterile single-use spring-loaded lancet. The skin was pulled as this was done to assist in penetration. As the blood flow began, the hand was gently squeezed and released to allow blood flow to return. The nail bed was gently pumped after each drop. Too much pressure was avoided so as to prevent haemolysis of the blood sample. Nurses were requested to obtain at least two microtainers of blood and more if possible. Once the blood sample was obtained, a bandaid was put on the child’s finger and the child was given a sticker as a reward. Blood samples were labelled with I.D. numbers which were double checked against I.D. numbers on the questionnaire and laboratory form. Samples were kept cool until delivery to the Red Cross, which was, in most
cases, within 24 hours. (Please see Chapter Five for a discussion of the laboratory testing procedures.)

In all, 770 blood samples were collected and 770 interview questionnaires were completed between the beginning of May and the end of October 1992. In order to do this, 57 clinics were held and 47 home visits were made. Eight trips were made around the province of British Columbia and 31 communities were visited apart from Vancouver.

Results of blood tests were merged with form letters appropriate to the respective outcomes and sent to parents and physicians. Samples of these letters may be seen in APPENDIX 1.

6. DATA COMPILING PROCEDURES
A spreadsheet was constructed in Lotus 1-2-3 for the input of data. Blood test results, program intervention variables and demographic variables were initially entered into a dBase IV database and then imported into the Lotus spreadsheet. Questionnaire data were entered directly into the Lotus spreadsheet. Finally, these data were cleaned, transferred to the University of British Columbia mainframe computer and prepared for the analysis.

7. COMPUTER SOFTWARE USED FOR THE ANALYSIS
The SPSS statistical package was used for most of the analysis procedures. Where Pearson exact statistics were required because greater than 20% of cells had expected frequencies less than five,
the SAS program was used. The Egret and Statxact testing packages were used where the exact trend tests or exact confidence intervals were required. Finally, where numbers could be taken from previously calculated tables in SPSS, Epi Info version 5, Statcalc Mode, was used. In this instance the uncorrected chi-square or Mantel-Haenszel statistic was used.
CHAPTER FOUR
DEVELOPMENT OF THE QUESTIONNAIRE

1. PURPOSE OF THE QUESTIONNAIRE
The purpose of the questionnaire was to gather information on the presence of various risk factors that might be associated with HBV infection in the study cohort. The information gathered would then be used to answer the third research question: "How are attack rates associated with various risk factors?" By including risk factor variables in the model building process, the analysis could determine if there was an association between these risk factors and the HBV attack rate. In the development of the questionnaire, content and format were the main factors considered and will be discussed in the following. (A sample of the questionnaire may be seen in APPENDIX 1.)

2. CONTENT OF THE QUESTIONNAIRE
The first step was to determine what risk factors for the transmission of the hepatitis B virus (HBV) were known to be associated with HBV infection and which of these risks were relevant to the age-group of the study cohort. The literature was reviewed and experts in Public Health and Communicable Diseases were consulted. There were no questionnaires regarding this age group available in the reviewed literature. Long-term studies previously undertaken indicated that data collected were limited to demographic or perinatal information (Coursaget et al., Seven-year study, 1986; Ip et al. 1989).
There is evidence that HBV infections have been transmitted by percutaneous and permucosal exposure to infected blood or body fluids (Benenson ed. 1990). Thus questions were designed to include assessment of opportunities for transmission via intravenous, intramuscular, subcutaneous, intradermal, or mucous membrane means. Opportunities for blood exposure include blood transfusion, receipt of blood products, surgical procedures and other procedures which might occur during hospitalization. Procedures in the community could include acupuncture, tattooing and ear piercing. Use of illicit intravenous drugs as a means of transmission was excluded as this was not a likely means in the two-to-eight-year-old age group. Acupuncture and tattooing were included on the recommendation of the British Columbia Centre for Disease Control.

Evidence also suggests that the virus can be spread via nasal secretions, saliva or skin ulcers or rashes that exude serum (Taylor et al. 1989). According to Davis et al. (1989) contamination of ulcers or abrasions with saliva or small amounts of blood may be a means of transmission. Thus questions about the presence of close contacts (specifically household contacts), and the use of day care for the child were included. Transmission between children in day care in developed countries has been infrequent; however, occurrence has been documented (Shapiro et al. 1989). Questions about history of sores and abrasions were not asked since almost all children experience these. Similarly, almost all children visit the dentist; thus, these exposures would not be useful in discriminating between groups.
Prevalence rates in some overseas countries suggest that the main mechanism of transmission between the ages of one to fourteen is horizontal spread (Taylor et al. 1989). Furthermore, travel to some of these countries may increase the risk of exposure. For this reason a question on foreign travel was included. Some countries have higher prevalence rates for HBV infection and HBeAg carriage; therefore, demographic information, such as country of birth, for the mother and father, was asked in the questionnaire.

There is conflicting evidence on breast feeding as a mechanism of transmission of HBV. Beasley et al. (1975) found no relationship between breast feeding and the subsequent development of antigenaemia in babies. Valjro et al. (1985) found that breast-feeding enhances the clearance of HBsAg in infants with HBV infections. Because of this difference in results, it was decided to include breast feeding as a variable, to examine its association with infection in this study cohort.

Sexual contact, although a possible means of transmission in this age group, would likely be rare, and therefore was excluded as a question item. The sensitivity of this type of question might also reduce compliance. Finally, since faecal-oral transmission of HBV has not been demonstrated, questions related to eating practices were not included.

3. FORMAT OF THE QUESTIONNAIRE
All children enrolled in the study would have to be seen to obtain a blood sample. It was therefore decided that the questionnaire
would be conducted as a personal interview with the parents. For this reason, the questionnaire was designed to be easily used by the interviewer. Exact phrases to be used by the interviewer when asking parents the questions were included, as well as sample questions and instructions to clarify how answers should be recorded. A questionnaire guide was also prepared which attempted to anticipate problems or questions that might arise. (Please refer to APPENDIX 1 for a sample of the questionnaire guide.)

To facilitate recording, easy checking for completeness and quicker coding and data entry, adequate space for answers was placed on the right hand side of the page or in tables. Most answers simply involved the circling of "Yes" or "No". A dichotomous scale was suitable for most questions as the information sought was simply whether a child had been exposed to the named risk or not. Question reduction was not a large issue as the first draft had only 12 questions. More time was spent on ensuring completeness.

4. PILOT STUDY OF THE QUESTIONNAIRE

4.1. Objectives of the pilot study

A pretest of the questionnaire was conducted with the following objectives:

1. To identify ambiguous terms.
2. To identify problems with question wording and understanding.
3. To identify problems with question scaling.
4. To identify problems with lay out.
5. To identify problems in administering the questionnaire.
6. To determine the length of time it would take to complete the
questionnaire.

4.2. Results of the questionnaire pilot study

A sample of ten families was chosen to participate in the piloting of the questionnaire. Of these, 6 agreed to be interviewed, 3 could not be reached after 3 attempts and 1 refused.

1. The terms 'hepatitis', 'jaundice' and 'breast feeding' were found ambiguous. The wording was improved and clarifications were included in the guide.

2. The options given for relationship of 'contact' to the child were not adequate. Therefore, it was decided to provide space in a table in which the interviewer could list the relationship of contacts and code numbers could be increased as found necessary.

3. To make coding less confusing, length of breast feeding and the time spent overseas would be measured in months and fractions of months rather than months and weeks.

4. It was found that the lay out could be improved with tables for the questions on household contacts and trips abroad.

5. Repetition was reduced by listing blood exposure risks in one question.

6. There were no problems with refusal to answer or inability to answer questions during the interviews. However, the following concerns were expressed:
   
   Will this be confidential?

   Will my child's name be made public?

   (The above questions were answered in the actual study by the parent information letter and the consent form.)
Can I give hepatitis to my children or other people?  
How is the virus spread?  
Why would my child get hepatitis if he/she had received the vaccine?  
Why do they use the vaccine if they are not sure it works?  
Are my children protected against the HBV?  
Do the eyes get yellow before the skin gets yellow?  

To answer some of the above questions and others that may arise, a pamphlet titled "ADVICE FOR HEPATITIS B CARRIERS" was obtained from the Canadian Liver Foundation and offered to each parent at the clinics and home interviews.

7. The length of time of appointments varied between 30-60 minutes and the actual questionnaire interview took 9 to 20 minutes with an average of 12.5 minutes.

5. RELIABILITY OF THE QUESTIONNAIRE

Since the subject of the questions represented factual information and not opinions, problems of timing and subject were not expected to be serious factors in reducing reliability of the questionnaire. For most of the information a criterion would be available or could be obtained; e.g., medical records for immunization, hospitalization and breast feeding history. Thus a true answer exists and "asking the same questions again and again ought to yield consistent results" or show high reliability (Oppenheim 1992). Kelly et al. (1990) found in their study of hospital patients that reliability was good to excellent for demographic factors such as birth place and medical information.
Following revisions to the questionnaire, two mothers were interviewed again. They gave the same answers to all the questions, thus indicating consistency of response, at least for two cases. (Improvements had been made only in the format and wording prior to re-piloting.)

Because of cost and time constraints, reliability studies using test-retest, split-half and inter-observer agreement studies were not carried out. However, to improve questionnaire accuracy, and to reduce variation due to the interviewer, various activities were planned as follows:

1. During training, interviewers were oriented to the questionnaire using a questionnaire guide. They were then expected to observe an interview and finally to conduct one under supervision. Use of the exact wording was stressed. Ongoing feedback was given to ensure clarity and promote completeness.

2. Parents were requested to bring certain information with them, such as Health Passports.

3. If the parent knew that certain information existed but could not remember it, he/she was allowed to call back with the information. With respect to hepatitis B blood test results for household contacts, parents often requested the study team to contact the family physician.

4. Terms such as 'hepatitis', 'breast feeding' and 'foreign travel' were defined in the questionnaire and guide.

5. Where language barriers might exist, parents were requested to bring along a family member who spoke English or an interpreter.
was provided. Chinese was the most frequent language requiring interpretation. Most of the interpretation was provided by a Chinese-speaking interviewer research assistant.

6. QUESTIONNAIRE WEAKNESSES

Recall bias needs to be considered in questionnaires. Some questions in this questionnaire may have been open to this bias. In particular, the questions pertaining to household contacts, eg., year of birth, year of HB vaccine immunization and history of hepatitis disease may have been most open to bias since parents were reporting on events which happened to other people. The number of exposures for the child such as hospitalizations, trips abroad and months of breast feeding might also be biased, but to a lesser degree, since the children were still quite young and these events, being important events, were more likely to be remembered by the parents.

Another possible weakness pertained to the history of hepatitis B vaccine immunization in siblings who were born before 1984. Before the Hepatitis B Vaccine Program started for neonates, hepatitis B immune globulin (HBIG) was given without the vaccine. Although interviewers attempted to clarify the difference, some parents may have interpreted the receipt of HBIG as being the hepatitis B vaccine. The information on household contacts, such as relationship to the child and history of hepatitis disease was subsequently not used in the analyses. The total number of 'contacts' was used as a surrogate measure of exposure for the child to other household members with hepatitis B disease.
7. VALIDITY OF THE QUESTIONNAIRE

The validity of the questionnaire is dependent on face and content validity. No 'gold standard' for measuring risk for HBV infection is available to establish criterion validity. Therefore, known theory about HBV transmission was used in its construction.

7.1. Face Validity

"Face validity involves the common acceptance by all concerned that a particular measure indeed measures what it purports to measure" (Shortell and Richardson 1978). Five experts in the field of Public Health and Communicable Diseases reviewed the questionnaire and agreed it would measure what it was supposed to measure.

7.2. Content Validity

For content validity, the scale must have enough items and adequately cover the domain under investigation (Streiner and Norman 1992). The items in this questionnaire included all known means of HBV transmission that are relevant to children and that can reliably be measured.
CHAPTER FIVE
LABORATORY PROCEDURES FOR THE TESTING OF BLOOD SPECIMENS

1. IMMUNOASSAYS USED TO TEST FOR STUDY OUTCOMES

1.1. Introduction

All of the blood specimens collected by study personnel were tested in the Transmissible Diseases Laboratory of the Red Cross Society, Vancouver Centre, Blood Transfusion Service. The assays used to detect markers were from IMx kits produced by Abbott Laboratories: IMx Core for anti-HBc, IMx AUSAB for anti-HBs and IMx HBsAg for HBsAg.

Hepatitis IMx assays are based on a microparticle enzyme immunoassay (EIA) technology. The IMx are fully automated tests requiring only the initial pipetting of the specimen into a sample well and the use of one control per run of 23 patient samples (Eble et al. 1991). Different hepatitis assays are performed in separate runs.

1.2. Assessing the characteristics of the IMx immunoassays

For the purpose of setting retest protocols for the study assay procedures, the characteristics of each screening test were calculated. These characteristics were the sensitivity, specificity and posttest likelihoods. Likelihood ratios were used for calculation of the posttest likelihood values. The following formulas were used:

\[
\text{Sensitivity} = \frac{a}{a+c} \quad \text{Specificity} = \frac{d}{b+d}
\]

Where \(a\) equals true positives; \(b\) equals false positives; \(c\) equals
false negatives and \( d \) equals true negatives.

\[
\text{Likelihood ratio(+) : } LR(+) = \frac{\text{sensitivity}}{1-\text{specificity}}
\]

\[
\text{Likelihood ratio(-) : } LR(-) = \frac{1-\text{sensitivity}}{\text{specificity}}
\]

Posttest likelihood of a positive outcome given a positive result:
\[
\text{PTL}^+ = \frac{\text{Prevalence} \times LR(+) \times (1-\text{Prevalence}) + \text{Prevalence} \times LR(+)}{\text{Prevalence} \times LR(+) \times (1-\text{Prevalence}) + \text{Prevalence} \times LR(+)}
\]

Posttest likelihood of a positive outcome given a negative result:
\[
\text{PTL}^- = \frac{\text{Prevalence} \times LR(-) \times (1-\text{Prevalence}) + \text{Prevalence} \times LR(-)}{\text{Prevalence} \times LR(-) \times (1-\text{Prevalence}) + \text{Prevalence} \times LR(-)}
\]

The prevalence values used in the formulas were the rates found in this study: 5.1% for infection rates (anti-HBc); 87.9% for the seropositivity rate (>=10 mIU/ml anti-HBs); and 2.3% for the HBV carrier rate (HBsAg). The numbers in the contingency tables are based on previous research and compare IMx assay results to a "gold standard".

Where information was available, IMx assays were compared to the radioimmunoassay (RIA). For detecting HBsAg, the sensitivity of the RIA has been shown to be 99.2% and the specificity, 98.9% (Holland in Gerety ed. 1985). Although considered the most accurate test, the RIA is often impractical because of high expense, complexity and the problem of disposal of radioactive reagents (McCready et al. 1991).

2. IMx CORE FOR THE ASSESSMENT OF ANTI-HBc OUTCOMES

The sensitivity of IMx Core in picking up anti-HBc has been found to be between 0.4 to 0.5 Paul Erhlich I (PEI) units per ml compared to 0.6 to 0.7 for RIA (Spronk et al. 1991). Spronk reports results of five clinical sites testing 4,841 specimens in
parallel with IMx Core and Corzyme, a previously licensed EIA assay. Overall agreement between IMx Core and Corzyme was 99.1% (4794/4841). In the above research, 148 of 162 anti-HBc positive specimens were correctly identified by the IMx Core giving a sensitivity of 91.4%. The specificity of IMx Core could not be determined from Spronk’s information as results for 3 of the 44 discordants were not stated in the report. IMx Core compared favourably, however, to Corzyme in that the seven known discordants were reactive only in IMx Core and these seven specimens were positive also for other HB markers.

In-house data were provided by Abbott Laboratories Technical Service Division and can be seen in Table 5.1. These data represent assay results from 1989 on a mixed population of blood bank, reference laboratories and hospital serum samples.

<table>
<thead>
<tr>
<th>IMx Core</th>
<th>Corab (RIA)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>171</td>
<td>4</td>
<td>175</td>
</tr>
<tr>
<td>-</td>
<td>7</td>
<td>2396</td>
<td>2403</td>
</tr>
<tr>
<td></td>
<td>178</td>
<td>2400</td>
<td>2578</td>
</tr>
</tbody>
</table>

The sensitivity of the IMx assay according to data in Table 5.1. was 96.1% (95% confidence interval; 93.3%, 98.9%) and the specificity was 99.8% (95% confidence interval 99.6%, 99.98%). Using the point estimate for sensitivity and specificity and a study prevalence of 5.1%, the PTL+ was 96.3% and the PTL- was 0.21%.
Since the PTL- was very low it was not necessary to retest negative results. To improve the specificity and increase the PTL+, positive assay results were repeated if sufficient specimen was available.

2.1. Results of Anti-HBc positive retests for this study

In this study, 769 specimens were tested for anti-HBc. Of these, 39 were found positive. Sixteen of the 39 anti-HBc positive specimens were positive for HBsAg as well. Of the remaining 23, 14 specimens were retested and found positive while 9 were not retested because of insufficient specimen. Two borderline results not included in the 39 anti-HBc positive results were retested using a second blood sample and found to be negative.

3. IMx AUSAB FOR THE ASSESSMENT OF ANTI-HBs OUTCOMES

The assay, IMx AUSAB, was developed for the detection and quantitation of antibody against hepatitis B surface antibody. "Anti-HBs concentrations in specimens are calculated automatically by comparison of the specimen rate to values determined from a stored standard curve" (Ostrow et al. 1991). IMx AUSAB is able to detect anti-HBs in concentrations as low as 2 mIU/ml which is equivalent to a previously licensed assay AUSAB EIA (a non-automated enzyme immunoassay) and AUSAB RIA, a direct solid phase radioimmunoassay. Both tests are used routinely to measure anti-HBs concentrations using a reference standard established in December 1977 (Barker et al. 1978; Courouce 1990). Ostrow (1991) has found that agreement of IMx AUSAB with AUSAB EIA and AUSAB RIA was 97.8% (1265/1293) and 99.1% (1281/1293) respectively.
In the research done by Ostrow (1991) 1293 specimens taken randomly from hospital sera and plasma, a sexually transmitted disease clinic, a volunteer blood bank and a plasmapheresis centre were tested in parallel by IMx AUSAB, AUSAB RIA and EIA. Sensitivity and specificity characteristics for IMx AUSAB were calculated using numbers given in the report by Ostrow and can be seen in Table 5.2. IMx AUSAB is compared to AUSAB RIA results in this table and 10 mIU/ml is used as the cutoff point for positive results.

Table 5.2. Comparison of IMx AUSAB and RIA assays for anti-HBs

<table>
<thead>
<tr>
<th></th>
<th>IMx AUSAB</th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td></td>
<td></td>
<td>169</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td></td>
<td></td>
<td>2</td>
<td>1121</td>
</tr>
<tr>
<td>AUSAB</td>
<td></td>
<td></td>
<td></td>
<td>170</td>
<td>1122</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>171</td>
<td>1123</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1293</td>
<td></td>
</tr>
</tbody>
</table>

The sensitivity of the IMx AUSAB according to the numbers in table 5.2. was 98.8% (95% confidence interval, 97.2%, 100%) and the specificity was 99.9% (95% confidence interval, 99.7%, 100%). Using the estimated sensitivity and specificity and the study prevalence of 87.9%, the PTL+ was 99.98% and PTL- was 8.03%.

Ostrow et al. (1991) also report results of sera tested by IMx AUSAB, AUSAB RIA and EIA in 85 individuals previously vaccinated with HB vaccine. The vaccines used were Heptavax or Recombivax (n=74) (Merck Sharp and Dohme); Engerix B (n=6) (Smith Kline) or Hevac B (n=5) (Pasteur). There was 100% agreement observed between
assays and 81 (95.3%) were reactive for anti-HBs. Although this study indicated 100% sensitivity and specificity, the ages of the individuals tested and duration from immunization were not stated.

Retesting of positive results was not recommended for anti-HBs on the basis of the high PTL+ for the IMx AUSAB assay, especially in immunized individuals. However, it was laboratory policy to retest low level reactive results (<6 mIU/ml) for reliability reasons related to concerns about picking up false positive results. Since the 8% PTL- is a false negative concern, the ideal would have been to retest all samples with results below 10 mIU/ml since this was the cutoff point chosen for this study between positive and negative results. The PTL- of 8% suggests that 8% of study results below 10 mIU/ml, or seven cases, were actually positive, and therefore the seroprotection rate of 87.9% for the current study was an underestimate. The 100% positive and negative predictive values for the immunized population described above would tend to support the 8% PTL- as being high. However, if the assumption were made that there were in truth seven more positive cases, then the seropositivity rate for this study would have been 88.9% rather than the 87.9% found.

3.1. Results of Anti-HBs negative retests for this study

Of the 756 children tested for anti-HBs, 64 (8.5%) were found to have 0 or <6 mIU/ml. There was sufficient sample to retest 33 of these specimens. Of the 33, the result sequence of 25 was negative/negative; four were positive/positive and four specimens were discordant between the first and second run. One of two
specimens which were initially positive was positive on the third test and one was negative. One specimen initially negative was negative on the third test. The fourth discordant sample could not be resolved as there was insufficient specimen for a third test. The initial result for that specimen was 3.3 mIU/ml and the second was 0 mIU/ml. For this case, the second test result was the one used. Of all the repeat assays on negative or low level results, none were 10 mIU/ml or greater on retest. Thus, there were no false negatives found by study samples being retested with the same test.

4. IMx HBsAG FOR THE ASSESSMENT OF HBsAG OUTCOMES

The IMx HBsAg assay has been shown to be able to detect concentrations of HBsAg less than 0.5 ng/ml or 0.2 PEI units/ml, which is equivalent to the AUSZYME Monoclonal assay, a previously licensed standard method (Ebel et al. 1991). A total of 9,700 specimens were assayed in clinical studies reviewed by Eble. Specimens tested included samples from blood donors, hospital and a public health patients, obstetric and gynaecology patients, dialysis patients, acute and chronic HBV patients, and patients with diseases other than active hepatitis B. The overall agreement between IMx HBsAg and the licensed reference method was 99.77% in this research. The results for which numbers were given in the report by Eble (1991) can be seen in Table 5.3. The reference standard, AUSZYME, was a previously licensed method with a reported sensitivity of 97.8% and specificity of 97.9% when compared to RIA (McCready et al. 1991).
Table 5.3. IMx HBsAg results compared to AUSZYME

<table>
<thead>
<tr>
<th></th>
<th>AUSZYME</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>IMx</td>
<td>331</td>
<td>15</td>
<td>346</td>
</tr>
<tr>
<td>HBsAg</td>
<td>4</td>
<td>8467</td>
<td>8471</td>
</tr>
<tr>
<td></td>
<td>335</td>
<td>8482</td>
<td>8817</td>
</tr>
</tbody>
</table>

The sensitivity calculated from Table 5.3. was 98.8% (95% confidence interval, 97.63%, 99.97%) and the specificity was 99.8% (95% confidence interval, 99.7%, 99.9%). Using these sensitivity and specificity estimates and a study prevalence of 2.3%, the PTL+ was 92.1% and PTL- was .03%. Since the likelihood of a negative HBsAg test result being false was so low this study’s protocol recommended a retest of only the positive results. Also, it was felt that the possible consequences of misdiagnosing a child as a carrier could be serious enough to justify confirmation.

4.1. Results of HBsAg positive retests for this study

Of the 16 study specimens that were HBsAg positive, nine were confirmed positive by retest. There was insufficient specimen for retesting seven others. Three of the children for whom there was insufficient sample were known to be HBsAg positive at one year of age.

Five children who were positive for HBsAg were also positive for the anti-HBs marker. Two of these children were HBsAg positive by 12 months of age. Two of the remaining three children remained positive on retest for anti-HBs and one was found to be negative.
5. TECHNICAL PROBLEMS ENCOUNTERED IN TESTING THE BLOOD SAMPLES

There were 76 specimens for which technical problems prevented obtaining test results: one anti-HBc; 14 anti-HBs; and 67 HBsAg. Technical problems were those due to inability to obtain a sufficient volume of blood sample or failure of test runs. Occasionally a child or parent was unwilling for a second attempt to obtain a sample if the first prick clotted before the required amount was obtained. Test run failures were due to bubbles or granules in the sample, machine failure or power surge. Haemolysis affected four samples, only one of which was severe enough to prevent doing the third test.
CHAPTER SIX

RESULTS

1. STATISTICAL ANALYSIS

The analysis was begun by producing descriptive statistics on all of the variables. Univariate chi-square analyses of all variables, separately, against the outcome variables were then performed. The Pearson chi-square statistic was used for 2 by 2 tables and for not-ordered categorical variables. The Mantel-Haenszel trend statistic was used for ordered categorical variables.

Prediction models for the infection (anti-HBc) and carrier state (HBsAg) outcomes were developed using logistic regression procedures. Using logistic regression was necessary since these outcomes were dichotomous. The stepwise method, including forward selection and backward elimination, was used to handle the confounding between variables. The criterion for entry of a variable into the model was a significance level of 0.05 using the score statistic. The likelihood-ratio test was used for removing terms from the model using a criteria of 0.10 significance level. Interaction terms were assessed in the final model.

Linear regression procedures were used for the analysis of the anti-HBs outcome, since this variable was continuous. Interaction terms were assessed for the final model.

To look for evidence that necessary assumptions were violated, both studentized residuals and standardized delta betas were calculated.
for the final models. These variables were then plotted. A normal probability plot of studentized residuals and a histogram of the standardized residuals were produced for the anti-HBs outcome as well.

To avoid the effect of influential observations, some variables were grouped and, where appropriate, included in the modelling procedures as continuous variables.

Statistical significance, unless otherwise qualified, refers to a P value of <= 0.05.

2. BASELINE DISTRIBUTIONS AND COMPARISON OF PARTICIPANTS AND NONPARTICIPANTS

Descriptive statistics for some of the baseline demographic variables are discussed in the following in order to compare participants with nonparticipants. A summary of these statistics may be found in Table 6.4.

Child’s sex

Of the 1166 children who met the inclusion criteria, 44.8% were male and 44.3% were female. The sex of 11% was unknown and these were all nonparticipants. Of 770 participants, 390 (50.6%) were male, and 380 (49.4%) were female. Of the nonparticipants with known sex group, 132 (49.3%) were male and 136 (50.7%) were female. The sex composition of the two groups was thus very similar and was not statistically different (P=0.69).
Child’s age

For the children who participated, ages were grouped in two ways, age at last birthday and year of birth. The distributions were as illustrated in Table 6.1. and 6.2.

Table 6.1. Child’s age at last birthday
(on day blood sample taken)

<table>
<thead>
<tr>
<th>Age</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>75</td>
<td>9.7</td>
</tr>
<tr>
<td>3</td>
<td>163</td>
<td>21.2</td>
</tr>
<tr>
<td>4</td>
<td>147</td>
<td>19.1</td>
</tr>
<tr>
<td>5</td>
<td>133</td>
<td>17.3</td>
</tr>
<tr>
<td>6</td>
<td>125</td>
<td>16.2</td>
</tr>
<tr>
<td>7</td>
<td>114</td>
<td>14.8</td>
</tr>
<tr>
<td>8</td>
<td>13</td>
<td>1.7</td>
</tr>
<tr>
<td>Total</td>
<td>770</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 6.2. Child’s year of birth

<table>
<thead>
<tr>
<th>Year</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1984</td>
<td>58</td>
<td>7.5</td>
</tr>
<tr>
<td>1985</td>
<td>121</td>
<td>15.7</td>
</tr>
<tr>
<td>1986</td>
<td>132</td>
<td>17.1</td>
</tr>
<tr>
<td>1987</td>
<td>144</td>
<td>18.7</td>
</tr>
<tr>
<td>1988</td>
<td>160</td>
<td>20.8</td>
</tr>
<tr>
<td>1989</td>
<td>155</td>
<td>20.1</td>
</tr>
<tr>
<td>Total</td>
<td>770</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Because year of birth was highly correlated with age when the blood sample was obtained, no attempt was made to separate the two. Results are generally presented for age at last birthday.

The mean age for the total group, participants and nonparticipants, was 5.1 years with a range of 2.5 to 8.6 years. The mean ages and ranges for participants and non-participants were 5.1 (2.5-8.6) and 5.2 (2.6-8.3) respectively. The Mantel-Haenszel trend test for differences in ages between participants and non-participants
indicated that the groups were similar for this variable (P=0.46).

Mother's age
Mothers' ages were also comparable between participants and nonparticipants with an overall mean age of 29.8 years and a range of 15.7 to 42.1 years. The mean ages and ranges for participants and nonparticipants were 29.9(15.7-42.1) and 29.7(15.8-39.8) respectively. The Mantel-Haenszel trend test P value for differences in mother's ages between participants and nonparticipants was 0.56.

Mother's HBeAg status
Results of the mothers' HBeAg status were recorded on Red Cross records for 883 eligible children. Of the participants, 588 (76%) mothers were tested for HBeAg. The distribution of results between participants and nonparticipants was as follows in Table 6.3.

<table>
<thead>
<tr>
<th></th>
<th>positive</th>
<th>negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Participants</strong></td>
<td>182(31%)</td>
<td>406(69%)</td>
</tr>
<tr>
<td><strong>Non-Participants</strong></td>
<td>85(29%)</td>
<td>210(71%)</td>
</tr>
</tbody>
</table>

Table entries are counts with row percentages.

The difference between participants and non-participants was not significant (P=0.51; odds ratio, 1.11; exact 95% confidence interval, 0.81, 1.53).
3. VARIABLES COLLECTED TO ASSESS FOR BIAS IN THE SAMPLE

Language

Information on which language was spoken in the home was asked of those people who participated as well as those people who refused to participate. The languages (grouped by horizontal or vertical transmission countries) represented by those who refused was then compared with those language groups of the participants. The difference between the two groups was not significant (P=0.33).

Moving residences after the birth of the child (Moving)

Sixty-three percent of participants were found to have moved. Of those that did not participate, 66 percent were known to have moved or could not be located at their last known address. For participants who had moved, the infection rate was 0.06 while for those who had not moved it was 0.04. This difference was not statistically significant (P=0.26).

Number of phone calls made contacting participants (Phone calls)

The average number of phone calls made per participating child was three. The median was also three. The minimum was one and the maximum was 17. There was no significant difference in the attempts to contact those children infected as opposed to children not infected (P=0.69). The association was also absent for carrier children (P=0.33).
Table 6.4. Baseline characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total group</th>
<th>Partic's</th>
<th>Non Partic's</th>
<th>Partic's/Non Partic's</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child sex</td>
<td>M 44.8</td>
<td>M 50.6</td>
<td>M 33.3</td>
<td>P value 0.69(^1)</td>
</tr>
<tr>
<td></td>
<td>F 44.3</td>
<td>F 49.4</td>
<td>F 34.3</td>
<td>RR 1.01</td>
</tr>
<tr>
<td></td>
<td>Unknown 11.0</td>
<td>Unknown 32.3</td>
<td>Unknown 32.3</td>
<td>CI 0.94, 1.09</td>
</tr>
<tr>
<td>Child age</td>
<td>mean 5.13</td>
<td>mean 5.10</td>
<td>mean 5.18</td>
<td>P value 0.46(^2)</td>
</tr>
<tr>
<td></td>
<td>range 2.45-8.62</td>
<td>range 2.45-8.62</td>
<td>range 2.58-8.30</td>
<td></td>
</tr>
<tr>
<td>Mother's age</td>
<td>mean 29.81</td>
<td>mean 29.88</td>
<td>mean 29.67</td>
<td>P value 0.56(^2)</td>
</tr>
<tr>
<td></td>
<td>range 15.67-42.06</td>
<td>range 15.67-42.06</td>
<td>range 15.82-39.78</td>
<td></td>
</tr>
<tr>
<td>Mother's HBeAg status</td>
<td>P value 0.51(^1)</td>
<td>RR 1.03</td>
<td>CI 0.94, 1.14</td>
<td></td>
</tr>
<tr>
<td>Phone calls</td>
<td>Anti-HBC Pos/Neg P value 0.69(^2)</td>
<td>HBsAg Pos/Neg P value 0.33(^2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moved</td>
<td>0.26(^1)</td>
<td>0.34(^1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language</td>
<td>Partic's/Refusal P value 0.33(^1)</td>
<td>RR 1.33</td>
<td>CI 0.74, 2.38</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Uncorrected Pearson's chi-square test

\(^2\) Mantel-Haenszel test for trend
4. MAIN OUTCOME VARIABLES - DESCRIPTIVE INFORMATION

4.1. ANTI-HBc

Of 769 participants for whom anti-HBc results were available, 39 (5.1%; 95% confidence interval, 3.5, 6.7) tested positive. For one child who participated, results for the anti-HBc antibody marker could not be obtained. Infection rates for each age group are illustrated in Figure 6.1. and by year of birth in Figure 6.2.

Figure 6.1
INFECTION AND CARRIER RATES BY AGE

![Graph showing infection and carrier rates by age with two lines: one for Anti-HBc positive and another for HBsAg positive.](image-url)
4.2. ANTI-HBs

Of the 717 children for whom results of anti-HBs testing were obtained and who were also anti-HBc negative, 87.9% (630) (95% confidence interval, 85.5, 90.2) showed evidence of seropositivity (>=10 mIU/ml). Fifty-five (7.7%) of the 717 children had 0 antibody titres. By age group cohort, seropositivity varied from a high of 99.4% for age three to a low of 70.4% for age seven. Figure 6.3. illustrates the seropositivity rates by age group and Figure 6.4. shows the distribution of anti-HBs titres for all ages.
Table 6.5. also illustrates the distribution of anti-HBs titres but in larger groups.

Table 6.5. Anti-HBs titres in mIU/ml

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;10</td>
<td>87</td>
<td>12.1</td>
</tr>
<tr>
<td>10-49</td>
<td>144</td>
<td>20.1</td>
</tr>
<tr>
<td>50-99</td>
<td>106</td>
<td>14.8</td>
</tr>
<tr>
<td>100-499</td>
<td>264</td>
<td>36.8</td>
</tr>
<tr>
<td>500-999</td>
<td>58</td>
<td>8.1</td>
</tr>
<tr>
<td>&gt;=1000</td>
<td>58</td>
<td>8.1</td>
</tr>
</tbody>
</table>
The mean level of anti-HBs was 242 mIU/ml with a standard deviation of 303 mIU/ml. The minimum and maximum titres were zero and 1000 mIU/ml. The geometric mean was 79.7 mIU/ml with a standard deviation of 7.2 mIU/ml. The difference in geometric mean titres between age group cohorts is illustrated in Figure 6.5. A decline in titres between age groups, until age eight, occurs as age increases.
The difference is less than 40 mIU/ml (12%) between age groups two and three followed by a sharp drop between the three and four year old groups, from a geometric mean of 238 to 79 mIU/ml (67%). The difference is small again between age groups four to seven when the geometric mean drops from a level of 79 to 23 mIU/ml (71%), however, a sharp rise occurs again in the age eight group. This increase in titres for eight year old children from a geometric mean of 23 to 106 mIU/ml is partly due to additional doses and one late third dose (1991). However, as can be seen in Figure 6.6.,
there was still an increase in anti-HBs titres at age eight after excluding these cases. Males and females follow a similar pattern in titres except for age two and age eight groups when males have higher titres by approximately 100 mIU/ml. (Please see Figure 6.7. for geometric means by sex.)

![Figure 6.6]

**Figure 6.6**

**GEOMETRIC MEAN LEVELS BOTH SEXES**

>3 DOSES and LATE 3RD DOSE CASES EXCLUDED

**Legend**

- geometric mean
- lower conf. bound
- upper conf. bound

**4.3. HBsAG**

Of the 770 children seen, sufficient blood sample was obtained to test 703 (91.3%) children for the HBV surface antigen. It was found that 16 (2.3%; 95% confidence interval 1.2, 3.4) of those tested were HBV surface antigen positive and considered carriers,
while 687 (97.7%) were negative and not considered to be carriers. All of the children who tested positive for HBsAg were also anti-HBc positive.

5. COMPARISON OF STUDY OUTCOMES WITH OUTCOMES AT 6 TO 18 MONTHS OF AGE

Children born between 1984 and 1987 were recalled by the Program for HBV blood marker testing at around 12 months of age. Those tested between 6 to 18 months of age were included in the analysis. The results had been recorded on Red Cross records as follows in Table 6.6.
Table 6.6. Results of 6 to 18 month tests

<table>
<thead>
<tr>
<th></th>
<th>No. tested</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTI-HBc</td>
<td>169</td>
<td>33.7</td>
</tr>
<tr>
<td>ANTI-HBs</td>
<td>183</td>
<td>85.8 (&gt;=10 S/N’s)</td>
</tr>
<tr>
<td>HBsAg</td>
<td>196</td>
<td>3.0</td>
</tr>
</tbody>
</table>

The comparisons between results of cases tested between 6 to 18 months and study results for this group of children are illustrated in the two by two tables in Table 6.7. below.

Table 6.7. Comparisons between 6 to 18 month results and study results

<table>
<thead>
<tr>
<th></th>
<th>Anti-HBc (study)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc</td>
<td>+</td>
<td></td>
<td>7</td>
<td>51</td>
</tr>
<tr>
<td>6-18mos</td>
<td>-</td>
<td></td>
<td>2</td>
<td>109</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9</td>
<td>160</td>
</tr>
<tr>
<td>HBsAg (study)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HBsAg</td>
<td>+</td>
<td></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>6-18 mos</td>
<td>-</td>
<td></td>
<td>1</td>
<td>168</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5</td>
<td>169</td>
</tr>
<tr>
<td>Anti-HBs (study)</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>+</td>
<td></td>
<td>130</td>
<td>20</td>
</tr>
<tr>
<td>6-18 mos</td>
<td>-</td>
<td></td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>140</td>
<td>33</td>
</tr>
</tbody>
</table>

Associations between each of the study outcomes against the 6 to 18 month outcomes can be seen in Table 6.8. and are described in the following.
Study anti-HBc results were significantly positively associated with anti-HBc results at 6-18 months (P=0.008). (Of those children anti-HBc positive in infancy a large proportion (88%) were no longer positive at study testing, suggesting that some children may have been circulating maternal anti-HBc.) There was also a highly significant association between study anti-HBc results and HBsAg results at 6 to 18 months (P<0.0001). The association between study anti-HBc and 6 to 18 month anti-HBs results was not significant (P=0.12).

Study HBsAg results were positively associated with both anti-HBc (P=0.02) and HBsAg results (P<0.0001) at 6 to 18 months and negatively associated with anti-HBs titres (P=0.008).

Study anti-HBs results were strongly associated with infancy anti-HBs results (P<0.0001). However, there was no association between study anti-HBs results and anti-HBc (P=0.25) or HBsAg (P=0.36) results at 6 to 18 months.

Of the 107 children who were known to be both anti-HBc negative and anti-HBs positive at 6 to 18 months, two were anti-HBc positive at study testing.
<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>RR &amp; CI</th>
<th>Direction of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Study Anti-HBc outcomes (N=169)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc status at 6-18 months (pos/neg)</td>
<td>0.008¹</td>
<td>7.5³</td>
<td>positive at 6-18 months</td>
</tr>
<tr>
<td>Anti-HBs status at 6-18 months (pos/neg)</td>
<td>0.12¹</td>
<td>0.30³</td>
<td>negative at 6-18 months</td>
</tr>
<tr>
<td>HBsAg status at 6-18 months (pos/neg)</td>
<td>&lt;0.0001¹</td>
<td>105³</td>
<td>positive at 6-18 months</td>
</tr>
<tr>
<td>2. Study HBsAg outcomes (N=151)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc status at 6-18 months (pos/neg)</td>
<td>0.02¹</td>
<td>O.R. infinite³</td>
<td>positive at 6-18 months</td>
</tr>
<tr>
<td>Anti-HBs status at 6-18 months (pos/neg)</td>
<td>0.008¹</td>
<td>0.05³</td>
<td>negative at 6-18 months</td>
</tr>
<tr>
<td>HBsAg status at 6-18 months (pos/neg)</td>
<td>&lt;0.0001¹</td>
<td>672³</td>
<td>positive at 6-18 months</td>
</tr>
<tr>
<td>3. Study Anti-HBs outcomes (N=159)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study anti-HBc positive cases excluded</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-HBc status at 6-18 months (pos/neg)</td>
<td>0.25²</td>
<td></td>
<td>higher titres if negative at 6-18 months</td>
</tr>
<tr>
<td>Anti-HBs status at 6-18 months (pos/neg)</td>
<td>&lt;0.0001²</td>
<td></td>
<td>higher titres if positive at 6-18 months</td>
</tr>
<tr>
<td>HBsAg status at 6-18 months (pos/neg)</td>
<td>0.36²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Fisher's exact test  
² Mantel-Haenszel test for trend  
³ Odds ratio and exact 95% confidence interval

6. ANTI-HBc OUTCOME - RESULTS OF ANALYSES

6.1. Association of covariates with Anti-HBc

In this section the associations of each of the variables with the
anti-HBc outcomes will be described. A summary of these associations with anti-HBc outcomes can be seen in Table 6.10.

6.1.1. Baseline characteristics – associations with Anti-HBc

No significant associations were found between the anti-HBc outcome and the child’s sex (P=0.56), age (P=0.15) or year of birth (P=0.12). However, there was a highly significant association between occurrence of infection and the mother’s age (P=0.0009) and the mother’s HBeAg status (P<0.0001; relative risk, 7.97; 95 percent confidence interval, 3.51 to 18.08).

6.1.2. Demographic variables – descriptive information and associations with Anti-HBc

Mother’s country of birth

Grouped according to countries with primarily vertical or horizontal transmission, the number and percent of mothers born in each country was as follows in Table 6.9.

<table>
<thead>
<tr>
<th>Vertical transmission</th>
<th>No.</th>
<th>Percent</th>
<th>Horizontal transmission</th>
<th>No.</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>China</td>
<td>212</td>
<td>27.5</td>
<td>Canada</td>
<td>74</td>
<td>9.6</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>140</td>
<td>18.2</td>
<td>India, Pakistan, Mid.E.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Viet Nam</td>
<td>119</td>
<td>15.5</td>
<td>E. and N. Africa</td>
<td>21</td>
<td>2.7</td>
</tr>
<tr>
<td>Philippines</td>
<td>65</td>
<td>8.4</td>
<td>Eastern Europe</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>S.E. Asia</td>
<td>49</td>
<td>6.4</td>
<td>Japan</td>
<td>10</td>
<td>1.3</td>
</tr>
<tr>
<td>Taiwan</td>
<td>15</td>
<td>1.9</td>
<td>Western Europe</td>
<td>8</td>
<td>1.0</td>
</tr>
<tr>
<td>N. &amp; S. Korea</td>
<td>15</td>
<td>1.9</td>
<td>Sub Sahara Africa</td>
<td>7</td>
<td>.9</td>
</tr>
<tr>
<td>Macaw</td>
<td>6</td>
<td>.8</td>
<td>S. &amp; Central America</td>
<td>6</td>
<td>.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>U.S.A. Aust. N.Z.</td>
<td>3</td>
<td>.4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>621</td>
<td>80.6</td>
<td><strong>Total</strong></td>
<td>149</td>
<td>19.4</td>
</tr>
</tbody>
</table>

73
<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>RR &amp; CI</th>
<th>Direction of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child's Sex</td>
<td>0.561</td>
<td>1.20, 0.65, 2.22</td>
<td>females</td>
</tr>
<tr>
<td>Child year of age (2-8)</td>
<td>0.152</td>
<td>older ages</td>
<td></td>
</tr>
<tr>
<td>Child birth year</td>
<td>0.122</td>
<td>earlier birth years</td>
<td></td>
</tr>
<tr>
<td>Mother's age (quartiles)</td>
<td>0.00092</td>
<td>younger mothers</td>
<td></td>
</tr>
<tr>
<td>Mother's birth year</td>
<td>0.122</td>
<td>birth years</td>
<td></td>
</tr>
<tr>
<td>Mother's birth country (vertical/horizontal transmission)</td>
<td>0.061</td>
<td>2.88, 0.90, 9.24</td>
<td>vertical</td>
</tr>
<tr>
<td>Mother's years in birth country (quartiles)</td>
<td>0.092</td>
<td>longer time</td>
<td></td>
</tr>
<tr>
<td>Father's birth country (vertical/horizontal transmission)</td>
<td>0.141</td>
<td>1.88, 0.80, 4.41</td>
<td>vertical</td>
</tr>
<tr>
<td>Father's years in birth country (quartiles)</td>
<td>0.082</td>
<td>longer time</td>
<td></td>
</tr>
<tr>
<td>Language (by countries of vertical/horizontal transmission)</td>
<td>0.031</td>
<td>2.36, 1.06, 5.28</td>
<td>vertical</td>
</tr>
<tr>
<td>Mother's HBeAg status (positive/negative)</td>
<td>&lt;0.00011</td>
<td>7.97, 3.5, 18.08</td>
<td>positive</td>
</tr>
<tr>
<td>Number of doses HB vaccine</td>
<td>0.052</td>
<td>fewer doses</td>
<td></td>
</tr>
<tr>
<td>Dose 1 (age received)</td>
<td>0.00052</td>
<td>&gt;7 days</td>
<td></td>
</tr>
<tr>
<td>Dose 2 timing (within 2 months of dose 1, no/yes)</td>
<td>0.523</td>
<td>1.44, 0.03, 10.0</td>
<td>not within 2 mos</td>
</tr>
<tr>
<td>Dose 3 (no/yes)</td>
<td>0.183</td>
<td>2.26, 0.42, 7.90</td>
<td>no 3rd dose</td>
</tr>
<tr>
<td>Dose 4 (no/yes)</td>
<td>1.003</td>
<td>infinite, 0.10,</td>
<td>infinite</td>
</tr>
<tr>
<td>Dose 5 (no/yes)</td>
<td>1.003</td>
<td>infinite, 0.01,</td>
<td>infinite</td>
</tr>
<tr>
<td>HBIG (not given/given)</td>
<td>0.083</td>
<td>5.22, 0.52, 27</td>
<td>not given and unknown</td>
</tr>
<tr>
<td>(given/unknown/not given)</td>
<td>0.0012</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>Estimate</td>
<td>Standard Error</td>
<td>P-value</td>
</tr>
<tr>
<td>-------------------------------------------------</td>
<td>------------</td>
<td>----------------</td>
<td>---------</td>
</tr>
<tr>
<td>Moved since child’s birth (yes/no)</td>
<td>0.26^1</td>
<td>1.47</td>
<td>0.74, 2.91</td>
</tr>
<tr>
<td>Jaundice after first month (yes/no)</td>
<td>1.00^3</td>
<td>0^4</td>
<td>0.46, 0.06</td>
</tr>
<tr>
<td>Breast feeding (yes/no)</td>
<td>0.33^1</td>
<td>0.74</td>
<td>0.40, 1.36</td>
</tr>
<tr>
<td>Daycare (yes/no)</td>
<td>0.09^1</td>
<td>0.38</td>
<td>0.12, 1.22</td>
</tr>
<tr>
<td>Earpiercing (yes/no)</td>
<td>0.33^1</td>
<td>1.43</td>
<td>0.70, 2.95</td>
</tr>
<tr>
<td>Acupuncture (yes/no)</td>
<td>1.00^3</td>
<td>0^4</td>
<td>0, 28.95</td>
</tr>
<tr>
<td>Surgery (yes/no)</td>
<td>0.40^1</td>
<td>0.67</td>
<td>0.27, 1.70</td>
</tr>
<tr>
<td>Blood transfusion (yes/no)</td>
<td>1.00^3</td>
<td>0^4</td>
<td>0, 20.90</td>
</tr>
<tr>
<td>No. of household contacts (grouped 1,2,3,4,5,6,7+)</td>
<td>0.01^5</td>
<td>0.04^2</td>
<td>&gt;# contacts 7-10 cont’s</td>
</tr>
<tr>
<td>Hospitalization (yes/no)</td>
<td>0.29^1</td>
<td>0.58</td>
<td>0.21, 1.61</td>
</tr>
<tr>
<td>Travel (yes/no)</td>
<td>0.52^1</td>
<td>0.78</td>
<td>0.37, 1.67</td>
</tr>
<tr>
<td>Total months of travel</td>
<td>0.90^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Months abroad</td>
<td>0.53^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimum age at travel</td>
<td>0.56^2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^1 Pearson’s chi-square  
^2 Mantel-Haenszel test for trend  
^3 Fisher’s exact  
^4 Odds ratio and exact confidence interval  
^5 Exact test for trend

The difference between the two groups of countries in terms of infection outcomes just missed significance (P=0.06; relative risk, 2.88; 95 percent confidence interval, 0.90, 9.24). The trend indicated that children of mothers born in primarily vertical transmission countries were more likely to be infected.
Father's country of birth

The distribution for father's country of birth was similar to that for mothers with most fathers born in countries more commonly associated with vertical transmission of HBV (75%). There was no significant association between father's country of birth and anti-HBc outcomes (P=0.14).

Language

Sixty-six percent of the participants spoke languages of countries where vertical transmission of HBV is most common. Cantonese (43%) and English (29%) were the most common languages spoken. The chi-square test of association showed a significant relationship between the language spoken and a positive outcome for anti-HBc (P=0.03). Children who spoke languages of countries where method of transmission is more likely to be vertical were more likely to become infected.

Mother's years in birth country

The minimum number of years that the mother spent in her country of birth (other than Canada) was one year and the maximum was 40 years. The average was 21.6 years with a standard deviation of 7.5 years. When grouped in quartiles the mode was between 21 to 25 years. There was a trend toward more infections with increasing number of years spent in the mother's country of birth but it did not reach statistical significance (P=0.09).
Father's years in birth country

The number of years fathers spent in their country of birth (other than Canada) averaged 19.2 with a range of 1 to 44 years. The mode was in the age group 21 to 25 years. As with the mother, there was a trend towards increasing number of HBV infections with increasing number of years fathers spent in their country of birth but this tendency was not statistically significant (P=0.08).

6.1.3. Program intervention variables - descriptive information and associations with Anti-HBc

Doses of hepatitis B vaccine (Number of Doses)

The number of doses of vaccine received by the participating children ranged from one to six with a distribution as illustrated in Table 6.11.

Table 6.11. Number of doses of HB vaccine received

<table>
<thead>
<tr>
<th>No. of Doses</th>
<th>No. Children</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9</td>
<td>1.2</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>2.6</td>
</tr>
<tr>
<td>3</td>
<td>732</td>
<td>95.1</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>.9</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>.1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>.1</td>
</tr>
</tbody>
</table>

There was an association between number of doses of hepatitis B vaccine and anti-HBc outcome (P=0.05). Children with fewer doses were more likely to have become infected.

Age of child at first dose (Dose one)

Most children received 'Dose one' on their first (452, 58.7%) or
second (268, 34.8%) day of life. Ninety-eight percent had received Dose one by the end of their first 7 days. A few children had Dose one extremely late and to avoid dependence of statistics on these few (influential) observations, this variable was grouped as indicated in Table 6.12.

Table 6.12. Child's age at Dose one

<table>
<thead>
<tr>
<th>Age in days</th>
<th>No. children</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-3</td>
<td>743</td>
</tr>
<tr>
<td>4-7</td>
<td>12</td>
</tr>
<tr>
<td>8-61</td>
<td>9</td>
</tr>
<tr>
<td>62-307</td>
<td>6</td>
</tr>
</tbody>
</table>

There was strong evidence to show that the age of the child when 'Dose one' was administered is associated with the risk of infection (Mantel-Haenszel trend test, P=0.0005), with children having received Dose one after seven days of age more likely to have become infected.

HBIG

Of the 770 children seen, 745 (97%) had documented evidence of having received HBIG. Ten children had not received the injection and for 15 it was unknown if HBIG had been administered. When the unknown cases were removed and this variable was classified as 'given/not given', there was a tendency toward more infections in those who did not receive the HBIG but it was not statistically significant (P=0.08; odds ratio 5.22; exact 95 percent confidence interval, 0.52 to 27.47). However, when HBIG was classified as 'given/unknown/not given', there was a statistically significant trend test result for anti-HBc outcomes (P=0.001) in the same
Timing of Dose two of hepatitis B vaccine (Dose two timing)
Seven hundred forty two (96.4%) children received their second dose of hepatitis B vaccine within two months of Dose one. Fifteen (1.9%) received Dose two after this time. The date was unknown for four of the remaining, while nine children did not receive a second dose. No association was found between Dose two timing and occurrence of infection ($P=0.52$).

Dose three of hepatitis B vaccine (Dose three)
Seven hundred forty one (96.2%) children received three doses of hepatitis B vaccine and 29 (3.8%) did not. The association between obtaining a third dose of vaccine and infection was not significant ($P=0.18$).

Doses four to six of hepatitis B vaccine
There were only nine children who were given four or more doses of vaccine and none of these children became anti-HBc positive, thus the power was inadequate to determine whether there was an effect or not.

6.1.4. Association of anti-HBc with illness and exposure variables

History of illness
No children were reported by the parents to have had any illnesses due to hepatitis or to have had hospitalizations related to hepatitis or reactions to hepatitis B vaccine. Deaths of two children who were born during the study period years were due to
causes other than those related to hepatitis.

**Jaundice**
According to the parents, three (0.4%) children had a history of jaundice starting after the first month of life. There were no associations found between this variable and any of the dependent variables. None of the 39 infected children had a history of jaundice reported.

**History of breast feeding (Breast feeding)**
Of the children seen, 395 (51.3%) were breast fed. No significant association was found between breast feeding and infection (P=0.33).

**History of ear piercing (Ear piercing)**
Of 770 children seen 133 (17.3%) were reported to have had their ears pierced. No association was found between this variable and the occurrence of infection (P=0.33).

**Acupuncture**
Only 4 (0.5%) of the children seen had experienced acupuncture and none of these had become infected. Thus no associations were found between acupuncture and outcome variables.

**Surgery**
Of the 770 children seen, 138 (17.9%) had had surgery or experienced suturing. There was no significant association found with infection (P=0.40).
Blood transfusion and blood products (Blood transfusions)

Only 5 children in the study were reported to be recipients of blood transfusions. One of these children had also received other blood products. These children were not among the children who had become infected and therefore no associations were found with the anti-HBc or HBsAg outcomes.

Hospitalization

Of the 126 (16.4%) children in the study who were hospitalized at least once, 101 (80%) were admitted once and the remainder between two to ten times. There was no significant association found with the anti-HBc outcome (P=0.29).

Daycare

The number of children in the study who had attended daycare was 138 (17.9), while 632 (82.1%) were reported to have had no daycare experience. The association between daycare and the anti-HBc outcome (P=0.09) was not statistically significant; however, there was a tendency for daycare attendance to be protective.

Number of household contacts (Contacts)

All children were reported to have had at least one household contact. The number of household contacts per child is illustrated in table 6.13.
Table 6.13. Distribution of number of household contacts

<table>
<thead>
<tr>
<th>Number contacts</th>
<th>Number children</th>
<th>Number contacts</th>
<th>Number children</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>58</td>
<td>2</td>
<td>258</td>
</tr>
<tr>
<td>2</td>
<td>191</td>
<td>3</td>
<td>110</td>
</tr>
<tr>
<td>4</td>
<td>61</td>
<td>5</td>
<td>61</td>
</tr>
<tr>
<td>5</td>
<td>10+</td>
<td>10+</td>
<td>3</td>
</tr>
</tbody>
</table>

Statistically significant association was found between the child’s number of household contacts and the anti-HBc outcome (Mantel-Haenszel exact trend test P=0.01). The effect was for more infections when larger numbers of contacts had been present in the household.

Travel abroad (Travel)

Of the 191 (25%) children reported to have travelled overseas, 73% had spent less than 3 months abroad and 27% more than 3 months abroad. The minimum time spent abroad was 2 days and the maximum length of time was 32 months. The mode was one month. Eight children who had travelled overseas tested anti-HBc positive. However, no significant association was found between travelling abroad and HBV infection (P=0.52).

Minimum age for travel

Forty-four percent of the children who had travelled were under two years of age when first taken abroad. A further 21 percent were two years of age and 35 percent were three years or older on their first trip abroad. No significant association was found between earliest age of travel and the anti-HBc outcome (P=0.56).
Months abroad
The association between the amount of time spent abroad and the occurrence of infection was not significant (P=0.53).

6.2. Multiple logistic regression analysis for the Anti-HBc outcome
6.2.1. Modelling procedure introduction
In the foregoing section, the associations of the study variables with anti-HBc were described as analyzed singly. In this section groups of variables are analyzed together for associations with the anti-HBc outcomes using multiple logistic regression procedures. As part of the logistic regression procedures, prediction models are built for the anti-HBc outcome. One component of the model is the group of significantly associated variables which best explains the variation in the anti-HBc outcomes. The other component is the coefficient. The variables in the model are chosen using a forward stepwise process, and what was found to occur in this stepwise process will be described. The modelling procedure, which involves simultaneous adjustment of all included variables, produces coefficients for each variable selected to be in the model. Finally, the variables and coefficients are combined in the logistic regression formula which is used to predict the probability of infection.

For ease of reading, variable names will be placed in quotation marks and in some cases will be abbreviated as indicated above in the results of associations with anti-HBc.

Two models were built for the anti-HBc outcome. Because of 190
missing values for the variable 'Mother's HBeAg status', the first model was built without including 'Mother's HBeAg status' thus retaining as much information as possible. 'Mother's HBeAg status' was included in the second modelling procedure. The variables excluded from both procedures because of negligible associations were the following: 'Dose two timing', 'Dose three, 'Dose four', 'Dose five', 'Moving', 'Jaundice', 'Acupuncture', 'Blood transfusions', 'Months abroad', 'Minimum age of travel', 'Father's country of birth' and 'Father's years in birth country'.

6.2.2. Model one Anti-HBc (without 'Mother's HBeAg status')
The variables considered in the first model were: 'Child's age', 'Child's sex', 'Mother's age', 'Mother's country of birth', 'Mother's years in birth country', 'Language', 'Number of Doses' of HB vaccine, age of the child when 'Dose one' was received, receipt of 'HBIG', 'Contacts', histories of 'Moving', 'Breast feeding', 'Ear piercing', 'Surgery', 'Daycare', 'Hospitalization' and 'Travel'. The final variables selected, by the forward stepwise process, to remain in the first model were as follows: 'Dose one' (P=0.01); 'Mother's age' (P=0.0001); and 'Mother's years in birth country' (P=0.003).

The stepwise process revealed that 'Dose one' was highly confounded with 'HBIG' and also explained some of the variation due to 'Number of doses' while being negatively confounded with 'Mother's age'. 'Mother's age' was positively confounded with 'Number of doses', but negatively confounded with 'Mother's country of birth' and 'Mother's years in birth country'. 'Mother's years in birth
country', being more highly statistically significant, acted as a surrogate for 'Mother’s country of birth' and 'Language' as well as explaining some variation due to 'Daycare'. 'Daycare' was the only remaining variable with a significance level less than 0.10 but entry into the model was denied because the criterion for entry was 0.05. 'Child’s age' reached a significance level of only 0.56.

6.2.3. Model two Anti-HBc (with 'Mother’s HBeAg status')

When 'Mother’s HBeAg status' was included in the procedure for model two, positive confounding was apparent between 'Mother’s HBeAg status' and 'Mother’s age', 'Language', 'Number of contacts' and 'Mother’s country of birth'. 'Child’s age' was negatively confounded with the 'Mother’s HBeAg status'. This resulted in a score test P value of 0.04 for 'Child’s age' after 'Mother’s HBeAg status' was the first variable to be chosen for the model. But statistical significance was lost for 'Child’s age', after 'Dose one', 'Mother’s years in birth country' and 'Mother’s age' were selected for entry into the model. There remained a tendency for 'Daycare' (P=0.09) to be associated with anti-HBc outcomes. A relative risk of 0.27 (95 percent confidence interval, 0.05 to 1.35) indicated that this trend toward association with HBV infection was protective. Thus, the final model with statistically significant variables which best explained the variation in the anti-HBc outcomes were 'Mother’s HBeAg status' (P<0.0001), 'Dose one' (P=0.0001), 'Mother’s years in birth country' (P=0.001) and 'Mother’s age' (P=0.002). The final significance level reached by 'Child’s age' was only 0.24 (score test).
\( R^2 \), the proportion of variation in outcome accounted for in the final model was \((247.63-188.37)/247.63=0.24 \). The interaction between 'Mother's HBeAg status' and each of the other variables in the model was assessed. None of the interaction terms reached statistical significance. A summary of statistics for the anti-HBc models may be seen in Table 6.14.

Table 6.14. Prediction models for the Anti-HBc outcome in children age 2 to 8

<table>
<thead>
<tr>
<th>Variables in model 1</th>
<th>P value</th>
<th>Coef.</th>
<th>RR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=758</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at Dose one</td>
<td>0.01</td>
<td>0.75</td>
<td>2.11</td>
<td>1.26, 3.53</td>
</tr>
<tr>
<td>Mother's age</td>
<td>0.0001</td>
<td>-0.17</td>
<td>0.85</td>
<td>0.78, 0.92</td>
</tr>
<tr>
<td>Mother's years in birth country</td>
<td>0.003</td>
<td>0.56</td>
<td>1.75</td>
<td>1.19, 2.57</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>-0.53</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Variables in model 2</th>
<th>P value</th>
<th>Coef.</th>
<th>RR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=580</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother's HBeAg status</td>
<td>&lt;0.0001</td>
<td>2.37</td>
<td>10.70</td>
<td>3.98, 28.78</td>
</tr>
<tr>
<td>Age at Dose one squared</td>
<td>0.0001</td>
<td>0.34</td>
<td>1.40</td>
<td>1.20, 1.62</td>
</tr>
<tr>
<td>Mother's years in birth country</td>
<td>0.001</td>
<td>0.75</td>
<td>2.13</td>
<td>1.29, 3.47</td>
</tr>
<tr>
<td>Mother's age</td>
<td>0.002</td>
<td>-0.15</td>
<td>0.86</td>
<td>0.77, 0.95</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>-2.37</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interaction terms (not in the model)

<table>
<thead>
<tr>
<th></th>
<th>P value</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother HBeAg*Dose one sq.</td>
<td>0.89</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother HBeAg*Mother's age</td>
<td>0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother HBeAg*Mother's years in birth country</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 \(-2\) Log likelihood Ratio
2 Score test

Because 'Dose one' was so highly confounded with HBIG in the foregoing procedures, it was decided to re-run the procedures for model one and model two, forcing HBIG into the model. In model one, once 'HBIG' was forced in, 'Dose one' was not significant
The P value for 'HBIG' when in the model was 0.02, (relative risk, 2.95; 95 percent confidence interval, 1.35, 6.48.). Forcing 'HBIG' into the model when 'Mother's HBeAg status' was also in the model did not change the final model. 'Dose one' remained significant (P=0.04, score test) and when included, rendered 'HBIG' non-significant.

6.2.4. Prediction formula for HBV infection (positive Anti-HBc)

The prediction of a positive anti-HBc outcome in this study sample may be modelled as follows:

\[
\text{Log odds Anti-HBc positive} = (-2.37 + 2.37 \times \text{MOM HBeAg} + 0.34 \times \text{DOSE ONE}^2 + 0.75 \times \text{MOM YEARS IN BIRTH COUNTRY} - 0.15 \times \text{MOMS AGE})
\]

If, for example, a child had a mother who was HBeAg positive (1) [0 if the mother was negative], the child's age at time of Dose one was two days (category 1, squared), the child's mother spent 5 years in her country of birth (category 1) and the mother's age was 25 years, the odds of this child being infected would be:

\[
\text{log odds anti-HBc positive} = (-2.37 + 2.37 \times 1 + 0.34 \times 1 + 0.75 \times 1 - 0.15 \times 25)
\]

odds anti-HBc positive = \(\exp(-2.66) = 0.07\)

If the mother were HBeAg negative the odds of infection for the child would be 0.007.

6.2.5. Anti-HBc outcome diagnostic procedures

Residuals

More large positive studentized residuals were found in the final prediction model for anti-HBc outcomes than would be expected by
chance. However, only one was outside the 3 standard deviation range. For the variable 'Mother’s age' the studentized residuals were spread evenly and with no particular pattern. The large residuals were also evenly dispersed between the four quartiles for number of years in mother’s country of birth. However, for 'Dose one', the nine large residuals fell in the first category which was one to three days. It appears, then, that the risk of infection is greater for those children who have their first dose of HB vaccine within three days of birth than the model would predict. These children were evenly spread between positive and negative status for 'Mother’s HBeAg'. (The plot of residuals against 'Dose one' may be seen in APPENDIX 3, page 161.)

In an attempt to improve the fit of the model, the variable 'Dose one' was squared such that category one was $1^2$, category two was $2^2$ and so forth. The model was re-run with both 'Dose one' and 'Dose one squared' in the model as well as the other final model variables which were 'Mother’s HBeAg status', 'Mother’s age' and 'Mother years in birth country'. With 'Dose one squared' in the model, 'Dose one' was no longer significant ($P=0.26$, likelihood ratio test). Thus, the model with significant variables that was best able to explain the variation in anti-HBc outcomes was 'Mother’s HBeAg status' ($P<0.0001$); 'Mother’s age' ($P=0.002$); 'Mother’s years in birth country' ($P=0.001$); and 'Dose one squared' ($P=0.0001$).

Influential observations
The changes in beta coefficients (delta betas) when cases were
removed one at a time from the analysis were calculated and plotted. It was found that the delta betas for 'Mother’s age', 'Mother’s years in birth country', 'Dose one squared' and 'Mother’s HBeAg status' were all within the standard error for that coefficient. Thus, there were no influential cases.

7. ANTI-HBs OUTCOME - RESULTS OF ANALYSES

7.1. Introduction
Before looking for associations with covariates or building a prediction model for the anti-HBs outcome, cases which were anti-HBc positive were excluded from the analysis. This was done since anti-HBs titres in children who are anti-HBc positive may have been boosted by natural infection rather than be due to immunization with the hepatitis B vaccine.

7.2. Associations of covariates with Anti-HBs titres
Associations of study variables with the anti-HBs outcome may be seen in Table 6.15. An inverse association was found between anti-HBs titres and the 'Child age' (P<0.0001). The opposite was true for the direction of effect with 'Mother’s age', but the association was not statistically significant (P=0.06). The 'Number of doses' of hepatitis vaccine was significantly associated with anti-HBs outcomes (P=0.02), with more than two doses resulting in higher titres. 'Dose one' was also associated with anti-HBs titres (P=0.01), the effect being that titres were higher if 'Dose one' was given later. 'HBIG', classified as given/not given (P=0.28), was not associated with anti-HBs titres, nor was it when classified as given/unknown/not given (P=0.93).
A significant association was found between anti-HBs titres and 'Minimum age of travel' (P=0.001). This was not the case, however, for the variable 'Months abroad' grouped as: no time/ <3 months/ >=3 months (P=0.70). There was also no significant association between anti-HBs titres and the 'Number of contacts' (P=0.22) or 'Breast feeding' (P=0.85).

<table>
<thead>
<tr>
<th>Table 6.15. Association of covariates with anti-HBs titres</th>
</tr>
</thead>
<tbody>
<tr>
<td>variable</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Child’s sex</td>
</tr>
<tr>
<td>Child’s age in years (2 to 8 years)</td>
</tr>
<tr>
<td>Mother’s age in quartiles</td>
</tr>
<tr>
<td>Number doses HB vaccine</td>
</tr>
<tr>
<td>Dose one (age rec’d grpd 1-3, 4-7, 8-61, 62+ days)</td>
</tr>
<tr>
<td>Dose 2 (received within 2 months of dose 1, Y/N)</td>
</tr>
<tr>
<td>HBIG (given/unk/notgiven)</td>
</tr>
<tr>
<td>No. of household contacts (grouped 1,2,3,4,5,6,7+)</td>
</tr>
<tr>
<td>Breast fed (Y/N)</td>
</tr>
<tr>
<td>Months abroad (grouped- no time/ &lt;3mos/ &gt;3mos)</td>
</tr>
<tr>
<td>Minimum age at travel</td>
</tr>
</tbody>
</table>

¹ Pearson’s chi-square
² Mantel-Haenszel trend test
³ Mantel-Haenszel exact test
7.3. Multiple regression analysis for the Anti-HBs outcome

7.3.1. Introduction
The natural logarithm of the anti-HBs titres resulted in a distribution which closely resembled a normal distribution apart from a cluster of 55 cases at the low end (log of 0 + 0.5) and 58 cases at the high end (log of 1000 + 0.5). The testing procedures did not allow identification of actual values above 1000 mIU/ml. It was calculated that there should be 18 cases in the right hand tail beyond 2 standard deviations and there were actually 17. Thus the distribution of the logarithm of the anti-HBs titres resembled the normal distribution. A histogram of the log anti-HBs titres may be seen in Figure 6.8.

7.3.2. Model building procedure
The model for the prediction of anti-HBs titres was built by backward elimination. In the first analysis, all of the study variables which could have some biologically plausible relationship to anti-HBs titres were included. These were ‘Child’s sex’, ‘Child’s age’, ‘Number of doses’, ‘Dose one’, ‘Dose two timing’, ‘HBIG’, ‘Breast feeding’, ‘Number of contacts’ and ‘Months abroad’. After the first procedure those variables which had missing values and did not reach a significance level of 0.05, and other variables which did not reach a significance level of 0.05 were removed. The procedure was then repeated for the final model. The final model with statistically significant variables that best predicted anti-HBs titres was ‘Child’s age’ (P<0.0001), ‘Number of doses’ (P=0.001), ‘Dose one’ (P=0.01) and ‘Months abroad’ (P=0.05). There
was positive confounding between 'Dose one' and 'Dose two timing' while 'Child's age' and 'Months abroad' were highly negatively confounded. In the univariate analysis there was no association between anti-HBs and 'Months abroad' (P=0.70), but after adjusting for 'Child's age' in the multivariate model it was apparent that longer time abroad was significantly associated with higher titres of anti-HBs. None of the interaction terms tested, ('Child's age' with 'Number of doses' (P=0.51), 'Child's age' with 'Dose one' (P=0.11) and 'Child's age' with 'Months abroad' (P=0.20)), reached
statistical significance when entered into the final model. R squared for the final anti-HBs model was 0.20. Summary statistics for the anti-HBs model may be seen in Table 6.16.

### Table 6.16. Final prediction model for Anti-HBs titres in children age 2 to 8

<table>
<thead>
<tr>
<th>Variables in the model</th>
<th>P value</th>
<th>Coef.</th>
<th>Ratio</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child's age</td>
<td>&lt;0.0001(^1)</td>
<td>-0.53</td>
<td>0.59</td>
<td>0.54, 0.64</td>
</tr>
<tr>
<td>Number doses vaccine</td>
<td>0.0007(^1)</td>
<td>0.75</td>
<td>2.12</td>
<td>1.38, 3.26</td>
</tr>
<tr>
<td>Dose one (age rec'd)</td>
<td>0.01(^1)</td>
<td>0.53</td>
<td>1.71</td>
<td>1.14, 2.56</td>
</tr>
<tr>
<td>Months abroad</td>
<td>0.05(^1)</td>
<td>0.22</td>
<td>1.25</td>
<td>1.002, 1.56</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td></td>
<td></td>
<td>4.22</td>
</tr>
</tbody>
</table>

**Interaction terms (not in the model)**

- Child’s age*Number doses: 0.51\(^1\)
- Child’s age*Dose one: 0.11\(^1\)
- Child’s age*Months abroad: 0.20\(^1\)

\(^1\) t test

### 7.3.3. Prediction formula for Anti-HBs titres

The exponential of the coefficients obtained from the final model provided the ratios for calculating the prediction of anti-HBs titres. For every one year increase in the 'Child's age', anti-HBs titres are reduced by a factor of 0.59. Each additional dose of vaccine results in a 2.12 times increase in anti-HBs titres. Giving 'Dose one', one category of time later results in a 1.71 times increase over the previous level and a one category increase for 'Months abroad' increases anti-HBs titres by a factor of 1.25. For the study sample, the prediction formula derived from this model can be depicted as follows:
Log anti-HBs + 0.5 =

\[(4.22 - 0.53 \times \text{CHILD'S AGE} + 0.75 \times \text{NUMBER DOSES} +
0.53 \times \text{DOSE ONE category} + 0.22 \times \text{MONTHS ABROAD category})\]

Where 'Dose one' categories are: 1= 1-3 days; 2= 4-7 days; 3= 8-61 days and 4= >=62 days and
'Months abroad' categories are: 0= no time abroad; 1= <3 months abroad; 2= >=3 months abroad.

Thus, a five year old child who had 3 doses of hepatitis B vaccine, had the first dose within 3 days of birth and travelled at least three months abroad can be expected to have an anti-HBs level of:

\[
\text{Log Anti-HBs + 0.5 = exp}(4.22 - 0.53 \times 5 + 0.75 \times 3 + 0.53 \times 1 + 0.22 \times 2)
\]

\[
\text{Anti-HBs = exp}(4.79) - 0.5
\]

\[
= 120.3 - 0.5
\]

\[
= 119.8 = 120 \text{ mIU/ml}
\]

If the same child had not travelled abroad, the predicted anti-HBs titres would be 77 mIU/ml.

### 7.3.4. Diagnostic procedures for the Anti-HBs model

**Residuals**

The histogram of studentized residuals resembled the normal curve with a small amount of increased concentration of cases around the -1 to -2 standard deviation area and a small cluster beyond +2 standard deviations. The normal probability plot approximated a straight line and was fairly linear. Studentized residual plots showed that 42 out of 707 cases plotted fell outside the 2 standard deviation range. For the variable 'Child’s age' there did not appear to be any pattern to these cases. However, the residuals
for 'Number of doses' were concentrated with the third dose below -2 standard deviations indicating the model over predicted the level of antibodies for some children who had three doses of vaccine. The first category of 'Dose one' (1-3 days) contained all of the residuals below 2 standard deviations. The model predicted a higher level of anti-HBs for these 42 children than was actually the case. The large residuals for 'Months abroad' were spread between the three categories but the category of 'no months abroad' had 34 residuals indicating an over prediction of anti-HBs titres for some children who did not travel. Residual plots for 'Number of doses' and 'Dose one' may be seen in APPENDIX 3.

Influential observations

The studentized beta coefficients (delta betas) were within 0.2 standard deviations for 'Child's age'; within 0.6 standard deviations for both 'Number of doses' and 'Dose one', and within 0.3 standard deviations for 'Months abroad'. This indicates there were no influential observations.

8. HBsAg OUTCOME - RESULTS OF ANALYSES

8.1. Introduction

Although it was not in the primary hypotheses to look for factors associated with the development of the hepatitis B virus carrier state as opposed to infection, it was decided to examine the study variables in this respect for hypothesis generation purposes. Univariate associations with HBsAg were first explored followed by multivariate analyses, which was done in two ways. Firstly, logistic regression procedures were run with the denominator being
'all cases for which results for the HBsAg tests were obtained' (N=703). Missing values reduced this number to 525 when mother's HBeAg status was added. Secondly, the procedures were run when the denominator was restricted to 'those cases which were anti-HBc positive' (N=39). There were only 32 cases when the procedure was run including the variable 'Mother's HBeAg status'.

8.2. Associations of covariates with HBsAg

Statistics for the associations of covariates with the HBsAg outcome are summarized in Table 6.17. There were no significant associations found between HBsAg outcomes and the following variables: 'Child's sex', 'Child's age', 'Mother's country of birth', 'Mother's years in birth country', 'Father's country of birth', 'Father years in birth country', 'Number of doses', 'Dose two timing', 'Language', history of 'Moving' since the child's birth, 'Jaundice', 'Breast feeding', 'Ear piercing', 'Acupuncture', 'Surgery', 'Blood transfusions', 'Daycare' or 'Hospitalization'. Significant associations were found between the HBsAg outcome and the following variables: 'Mother's age' (inverse), 'Dose one', 'HBIG', 'Mother's HBeAg status', 'Number of contacts', 'Travel' and 'Months abroad' (inverse).

When the analysis was restricted to anti-HBc positive cases only, there were significant associations only with the following variables: 'Number of contacts', 'Travel' (inverse), 'Total months abroad' (inverse), and 'Months abroad' grouped (inverse). A summary of associations between variables and the HBsAg outcome, excluding anti-HBc negative cases, may be seen in Table 6.18.
Table 6.17. Association of covariates with HBsAg

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>RR &amp; CI</th>
<th>Direction of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child's sex</td>
<td>0.59^1</td>
<td>1.30</td>
<td>females</td>
</tr>
<tr>
<td>Child's age in years (2 to 8)</td>
<td>0.23^2</td>
<td>0.49, 3.46</td>
<td>older ages</td>
</tr>
<tr>
<td>Child's birth year</td>
<td>0.21^2</td>
<td></td>
<td>earlier years</td>
</tr>
<tr>
<td>Mother's age (quartiles)</td>
<td>0.002^2</td>
<td></td>
<td>younger mothers</td>
</tr>
<tr>
<td>Mother's birth country (vertical/horizontal transmission)</td>
<td>0.75^3</td>
<td>1.68^4</td>
<td>vertical</td>
</tr>
<tr>
<td>Mother's birth country (quartiles)</td>
<td>0.78^2</td>
<td></td>
<td>longer time</td>
</tr>
<tr>
<td>Father's birth country (vertical/horizontal transmission)</td>
<td>0.38^3</td>
<td>2.36^4</td>
<td>vertical</td>
</tr>
<tr>
<td>Father's years in birth country (quartiles)</td>
<td>0.86^2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Language (by country of vertical/horizontal transmission)</td>
<td>0.07^1</td>
<td>3.56</td>
<td>vertical</td>
</tr>
<tr>
<td>Mother's HBeAg status (positive/negative)</td>
<td>&lt;0.0001^3</td>
<td>30.36^4</td>
<td>positive</td>
</tr>
<tr>
<td>Number of doses HB vaccine</td>
<td>0.19^2</td>
<td></td>
<td>fewer doses</td>
</tr>
<tr>
<td>Dose 1 (age received, 4 groups)</td>
<td>0.04^2</td>
<td></td>
<td>&gt; 7 days</td>
</tr>
<tr>
<td>Dose 2 (received outside 2 months of dose 1)</td>
<td>1.00^3</td>
<td></td>
<td>within 2 months</td>
</tr>
<tr>
<td>Dose 3 (no/yes)</td>
<td>0.44^3</td>
<td>1.84</td>
<td>no 3rd dose</td>
</tr>
<tr>
<td>Dose 4 (no/yes)</td>
<td>1.00^3</td>
<td>infinite^4</td>
<td></td>
</tr>
<tr>
<td>Dose 5 (no/yes)</td>
<td>1.00^3</td>
<td>infinite^4</td>
<td></td>
</tr>
<tr>
<td>HBIG (not given/given)</td>
<td>0.18^3</td>
<td>5.95^4</td>
<td>not given</td>
</tr>
<tr>
<td>(given/unknown/not given)</td>
<td>0.03^2</td>
<td>0.13, 49.58</td>
<td></td>
</tr>
<tr>
<td>Variable</td>
<td>P value</td>
<td>RR &amp; CI</td>
<td>Direction of effect</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>Moved since child’s birth (yes/no)</td>
<td>0.34(^1)</td>
<td>1.72, 0.56, 5.27</td>
<td>moved</td>
</tr>
<tr>
<td>Jaundice after first month (yes/no)</td>
<td>1.00(^3)</td>
<td>0.04, 0, 108.58</td>
<td></td>
</tr>
<tr>
<td>Breast fed (yes/no)</td>
<td>0.55(^1)</td>
<td>0.75, 0.28, 1.98</td>
<td>no breast feeding</td>
</tr>
<tr>
<td>Ears pierced (yes/no)</td>
<td>0.50(^3)</td>
<td>1.59, 0.37, 5.36</td>
<td>ears pierced</td>
</tr>
<tr>
<td>Acupuncture (yes/no)</td>
<td>1.00(^3)</td>
<td>0.04, 0, 108.58</td>
<td></td>
</tr>
<tr>
<td>Surgery (yes/no)</td>
<td>0.33(^3)</td>
<td>0.32, 0.01, 2.11</td>
<td>no surgery</td>
</tr>
<tr>
<td>Blood transfusion (yes/no)</td>
<td>1.00(^3)</td>
<td>0.04, 0, 68.77</td>
<td></td>
</tr>
<tr>
<td>Daycare (yes/no)</td>
<td>0.09(^3)</td>
<td>0.04, 0, 1.16</td>
<td>no daycare</td>
</tr>
<tr>
<td>Number household contacts (ungrouped)</td>
<td>&lt;0.0001(^2)</td>
<td>0.0003(^2)</td>
<td>&gt; number contacts 7+</td>
</tr>
<tr>
<td>(grouped 1,2,3,4,5,6,7+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalization (yes/no)</td>
<td>0.49(^3)</td>
<td>0.35, 0.01, 2.30</td>
<td>no hospitalization</td>
</tr>
<tr>
<td>Travel (yes/no)</td>
<td>0.02(^3)</td>
<td>0.04, 0, 0.76</td>
<td>no travel</td>
</tr>
<tr>
<td>Months abroad (no months/ &lt;3months/ &gt;3months)</td>
<td>0.03(^2)</td>
<td></td>
<td>no time abroad</td>
</tr>
</tbody>
</table>

1. Pearson’s chi-square  
2. Mantel-Haenszel test for trend  
3. Fisher’s exact test  
4. Odds ratio and exact confidence interval  
5. Exact test for trend
Table 6.18. Association of covariates with HBsAg (Anti-HBc positive cases only)

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>RR &amp; CI</th>
<th>Direction of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Child’s sex</td>
<td>0.70</td>
<td>1.16, 0.54, 2.46</td>
<td>females</td>
</tr>
<tr>
<td>Child’s age in yrs (2-8)</td>
<td>0.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child birth year (84-89)</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s age in quartiles</td>
<td>0.18</td>
<td></td>
<td>older mothers</td>
</tr>
<tr>
<td>Mother’s birth country (vert/horiz transm)</td>
<td>0.56</td>
<td>0.33, 0.01, 7.18</td>
<td></td>
</tr>
<tr>
<td>Mother’s years in birth country</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father’s birth country (vert/horiz transm)</td>
<td>1.00</td>
<td>1.11, 0.11, 14.86</td>
<td></td>
</tr>
<tr>
<td>Father’s years in birth country</td>
<td>0.17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of doses (ungrouped)</td>
<td>0.94</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose one (age rec’d)</td>
<td>0.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose two (rec’d outside 2 months of dose 1, yes/no)</td>
<td>1.00</td>
<td>O.R. 0, 0, 58.50</td>
<td></td>
</tr>
<tr>
<td>Dose 3 (Yes/No)</td>
<td>1.00</td>
<td>1.50, 0.07, 94.31</td>
<td></td>
</tr>
<tr>
<td>HBIG (not given/given)</td>
<td>1.00</td>
<td>1.36, 0.02, 112.06</td>
<td></td>
</tr>
<tr>
<td>(given/unk/not given)</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s HBeAg status (positive/negative)</td>
<td>0.10</td>
<td>6.50, 0.61, 321.49</td>
<td>if mother positive</td>
</tr>
<tr>
<td>Language (for countries of vert/horiz transm.)</td>
<td>1.00</td>
<td>1.56, 0.19, 19.33</td>
<td></td>
</tr>
<tr>
<td>Moved since child’s birth (yes/no)</td>
<td>1.00</td>
<td>1.13, 0.21, 6.68</td>
<td></td>
</tr>
<tr>
<td>Breast-fed (yes/no)</td>
<td>0.86</td>
<td>1.07, 0.51, 2.26</td>
<td></td>
</tr>
<tr>
<td>Earpiercing (yes/no)</td>
<td>1.00</td>
<td>1.13, 0.18, 6.55</td>
<td></td>
</tr>
<tr>
<td>Surgery or stitches (yes/no)</td>
<td>0.37</td>
<td>0.44, 0.07, 2.64</td>
<td>no surgery</td>
</tr>
</tbody>
</table>
### Table 6.18. Association of covariates with HBsAg (Anti-HBc positive cases only)

<table>
<thead>
<tr>
<th>Variable</th>
<th>P value</th>
<th>RR &amp; CI</th>
<th>Direction of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daycare (yes/no)</td>
<td>0.503</td>
<td>O.R. 0.0^ \text{4} , 0, 7.32</td>
<td>no daycare</td>
</tr>
<tr>
<td>Number of contacts</td>
<td>0.032</td>
<td></td>
<td>&gt; number</td>
</tr>
<tr>
<td>Hospitalization (yes/no)</td>
<td>0.623</td>
<td>0.42^ \text{4} , 0.01, 6.01</td>
<td>no hosp’n</td>
</tr>
<tr>
<td>Travel (yes/no)</td>
<td>0.013</td>
<td>O.R. 0.0^ \text{4} , 0, 0.80</td>
<td>no travel</td>
</tr>
<tr>
<td>Total months of travel</td>
<td>0.052</td>
<td></td>
<td>0 months</td>
</tr>
<tr>
<td>Months abroad (grouped)</td>
<td>0.022</td>
<td></td>
<td>0 months</td>
</tr>
</tbody>
</table>

1. Pearson’s chi-square
2. Mantel-Haenszel test for trend
3. Fisher’s exact test
4. Odds ratio and exact 95% confidence interval
5. Exact test for trend

#### 8.3. Multiple logistic regression analysis for HBsAg outcomes - denominator all cases

**Model one**

Models were determined in the same fashion as for the anti-HBc outcome. Variables included in the analysis to determine the first model (without 'Mother’s HBeAg status' included) were: 'Child’s age', 'Child’s sex' 'Mother’s age', 'Mother’s country of birth', 'Mother’s years in birth country', 'Language', 'Number of doses', 'HBIG', 'Number of contacts', histories of 'Moving', 'Breast feeding', 'Ear piercing', 'Surgery', 'Day care', 'Hospitalizations' and 'Travel'. The statistically significant variables selected by the stepwise procedure which best explained the variation in HBsAg outcomes were: 'Number of contacts' (P=0.001), 'Mother’s age'
(P=0.002), 'Language' (P=0.02) and 'Travel' (P=0.006). In the process of building the model it was revealed that there was confounding between 'Number of contacts', the strongest predictor, and 'Mother’s age', 'Language', 'Daycare' and 'Travel'. However, the variable 'Number of contacts' could not explain all of the variation due to 'Mother’s age' resulting in 'Mother’s age' being entered into the model as well. Negative confounding between 'Mother’s age' and 'Language' resulted in the increased significance of 'Language', causing it to be selected into the model. 'HBIG' was significant initially (P=0.02) but gradually lost significance as other variables were added, ending up at P=0.07. 'Travel', on the other hand, could not be explained further by 'Mother’s age' or 'Language' and thus retained its statistical significance.

Model two
Model two was run with the same variables as model one with the addition of 'Mother’s HBeAg status' and 'Dose one'. Only two variables qualified for selection for the final model. These were 'Mother’s HBeAg status' (P<0.001) and 'Dose one' (P=0.03). Positive confounding was apparent between 'Mother’s HBeAg status' and the variables 'Language', 'Mother’s age', 'Number of contacts' and 'Travel'. Variation due to 'HBIG' and the 'Number of doses' was largely explained by the inclusion of 'Dose one'. Beta coefficients were determined for 'Mother’s age' (P=0.07), 'Number of contacts' (P=0.06) and 'Travel' (P=0.07), as these variables tended toward significance but the evidence was not sufficient to qualify them for entry into the final model. When these variables
Table 6.19. Prediction models for the HBeAg outcome in children age 2 to 8

<table>
<thead>
<tr>
<th>Denominator-all cases</th>
<th>P value</th>
<th>Coef.</th>
<th>RR</th>
<th>CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variables in model 1</strong> (Mother’s HBeAg status not included)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=703</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number contacts</td>
<td>0.0011</td>
<td>0.48</td>
<td>1.62</td>
<td>1.21, 2.18</td>
</tr>
<tr>
<td>Mother’s age</td>
<td>0.0021</td>
<td>-0.18</td>
<td>0.83</td>
<td>0.74, 0.94</td>
</tr>
<tr>
<td>Language</td>
<td>0.021</td>
<td>1.66</td>
<td>5.24</td>
<td>1.04, 26.49</td>
</tr>
<tr>
<td>Travel</td>
<td>0.0061</td>
<td>-7.36</td>
<td>0.0006</td>
<td>1.3E-19, 3.2E+12</td>
</tr>
<tr>
<td>Constant</td>
<td></td>
<td>-1.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Variables in model 2** (Mother’s HBeAg status included) |          |       |    |              |
| N=525                |          |       |    |              |
| Mother’s HBeAg status| <0.0001  | 3.62  | 37.33 | 4.46, 312.33 |
| Dose one (age rec’d) | 0.031    | 1.18  | 3.25 | 1.29, 8.16  |
| Constant              |          | -7.39 |      |              |

| **Variables not in model 2** |          |       |    |              |
| Number of contacts       | 0.0983   | 0.26  | 1.29 | 0.95, 1.76  |
| Mother’s age             | 0.093    | -0.11 | 0.90 | 0.79, 1.02  |
| Travel abroad            | 0.733    | -7.05 | 0.0009 | 1.6E-21, 4.6E+14 |

| **Interaction term (not in the model)** |          |       |    |              |
| Mother HBeAg*Dose one    | 0.42     |       |    |              |

| **Denominator Anti-HBc positive cases only** | N=32      |       |    |              |
| **Variables in model 1** (Mother’s HBeAg status included) |          |       |    |              |
| Travel abroad            | 0.011    | -9.28 | 0.001 | 1.1E-42, 7.9E+33 |
| Constant                 |          | 0.07  |      |              |

| **Variables in model 2** (Mother’s HBeAg status forced in) |          |       |    |              |
| Mother’s HBeAg status   | 0.061    | 1.87  | 6.50 | 0.68, 62.09 |
| Constant                |          | -1.79 |      |              |

1 - 2 Log likelihood Ratio  
2 Score test  
3 Wald test
were forced into the model, the instability of the variable 'Travel', was revealed by a P value of 0.73 (relative risk, 0.0009) and a very wide 95 percent confidence interval, (1.6E-21, 4.6E+14). A summary of the statistics for this model may be seen in table 6.19.

The proportion of variation in the null model accounted for by the final prediction model ('Mother’s HBeAg status' and 'Dose one') was: 

\[ R^2 = \frac{129.105 - 100.324}{129.105} = 0.22. \]

8.4. Multiple logistic regression analysis for the HBsAg outcome - denominator restricted to anti-HBc positive cases

By limiting the analysis to only those cases that were anti-HBc positive, it was hoped to determine more specifically the study variables which may be associated with the progression of infection to the carrier state. The only variable that turned out to be significant in the first procedure (model one) was 'Travel' (P=0.01). When 'Mother’s HBeAg status' was forced into the model for model two, no variables reached statistical significance. P values were as follows: 'Travel' (P=0.07, score test); 'Mother’s years in birth country' (P=0.06, score test); 'Mother’s HBeAg status' (P=0.06; relative risk 6.50; 95 percent confidence interval, 0.68 to 62.09; N=32). Summary statistics related to this model may be seen in Table 6.19. above. None of the children who became HBsAg positive had a history of travel.
CHAPTER SEVEN
DISCUSSION

1. INTERNAL VALIDITY

Because of the design of this study, two of the main concerns with respect to its internal validity were the possibilities of selection and information bias. Bias due to confounding was reduced by adjustment in the multiple regression analyses and is discussed later.

1.1. Considerations with respect to possible selection bias

In order to retain sufficient power for analysis with the small number of events expected, an attempt was made to include the entire population as the sample. The 67% response rate opened the possibility for systematic error which could have resulted in differences between the group that participated and the group that did not participate in the study. Error created in selecting a nonrepresentative sample could lead to the results correlating with participation rather than with the study variables. In this study, analysis of the information collected on variables which were common to both participants and nonparticipants suggested that the sample was representative of the population being investigated. There were no significant differences found in the children’s ages and sex or in the mothers’ ages and HBeAg status. The similarity in the mothers’ ages and HBeAg status between the two groups was particularly important as it was found that the variables mother’s age and HBeAg status were predictors of infection.
Information on language and a history of moving was collected to provide a surrogate measure of confounding due to socioeconomic factors. It was thought this surrogate measure might differentiate between those infected and those not infected. No significant difference was found in language or history of moving. Nor was there a difference in effort to contact children who were found to be infected, versus not infected, as measured by the number of phone calls made in an effort to enrol participants. Similarity in known factors which may be confounders for the outcomes does not rule out selection bias but it would support the likelihood of similarity in unknown factors which may influence study outcomes.

1.2. Considerations with respect to information bias

The three information biases that could affect study outcomes were interviewer bias, recall bias and other sources of misclassification. Since interviewers did not know the results of blood tests before completing questionnaires, prior knowledge of outcomes could not have led to systematic bias. Furthermore, there were no results for which interviewer bias appeared to be an explanation. The only unexplainable result, the association of time abroad with anti-HBs titres, was also not readily explained by interviewer bias. As described in Chapter Four on the questionnaire, a concerted effort was made to prevent bias due to improper interview technique.

Recall bias could have resulted in misclassification of exposures, but the factual nature of the questions may have reduced this threat to validity. Misclassification of timing and number of HB
vaccine doses was possible, but using written records rather than relying on parents' recall likely minimized this source of error. The grouping of some variables into broad categories likely reduced error due to inability to recall lengths of time, (e.g., in the variable 'Months abroad'). However, error due to confusion with respect to which child travelled, or was hospitalized or was breast fed in the case of larger families could still have occurred.

Misclassification may have occurred in the HBsAg and anti-HBc outcomes. An over estimate was possible, but unlikely, in the HBsAg outcomes since confirmation of the carrier state was not carried out within the study period. An over estimate in the anti-HBc outcome was possible due to circulating maternal antibodies in the two year old group. Since maternal anti-HBc usually disappears by two years of age this source of error is likely to be minimal. Some misclassification due to laboratory error in outcome results was also possible, but this was likely limited because of the good posttest likelihoods of the blood sample assays. If misclassification was the case it would have led to an under estimation of the magnitude of associations between outcomes and prediction variables making our results conservative.

1.3. Considerations with respect to consistency of results

There were several factors which support internal validity in this study. Firstly, there was consistency in the anti-HBc and HBsAg outcome results, between those obtained when 'Mother's HBeAg status' was excluded and those obtained when it was included in the modelling procedure. The model for the anti-HBc outcome was the
same for both model building procedures except for the addition of mother’s HBeAg status when this variable was included. In the HBsAg outcome model building procedure it was determined that the mother’s HBeAg status was a confounder for the variables that appeared in the model without mother’s HBeAg status, and therefore these variables were simply surrogate measures for the effect of mother’s HBeAg status.

Secondly, the appearance of the same variables, 'Mother’s HBeAg status' and age at 'Dose one', in both the anti-HBc and HBsAg outcome prediction models, would tend to support the validity of the results.

Thirdly, evidence for internal validity was found by applying the prediction formulas for the anti-HBc and anti-HBs. The parameter estimates provided predictions of risk for infection which are supported by similar neonatal studies in the literature; 0.7% and 7% for mothers with negative and positive HBeAg status respectively. The estimate of geometric mean anti-HBs level derived from the parameter estimates was close to that found in the current study (77 versus 79.7 mIU/ml).

Ideally, one would want to include measurements on all possible confounders which may affect the outcomes. For this study, all variables that were thought to have plausible associations with the outcomes and that could feasibly be measured were included in the model-building procedures. Nevertheless, all the variation due to the outcomes was not accounted for by the prediction models.
Therefore, the variables in the final models cannot be considered
the only explanations for the outcome variation. Only 24% of the
variation for anti-HBc outcome and 22% for the HBsAg outcome was
explained by the variables in the prediction models. The majority
of variation was due to unknown factors, among which could be
virulence and dose of the HB virus as well as the genetic
predisposition of the child.

Thus far the internal validity of this study has been discussed.
Internal validity is important in terms of the conclusions drawn
from the results of the present study and forms the basis for
external validity which is discussed next.

2. EXTERNAL VALIDITY
The advantage of this study of hepatitis B vaccine, within the
context of a Program, is that it will be useful for generalization
to similar programs. However, to apply the results of this study
to other programs, those factors that were not measured by the
current study, but that may account for variation in outcomes
should be similar to this Program. Variables measured in this
study need not be the same as in the comparison population. For
the variables that were measured and found to be significant,
adjustment can be made. The adjustment is made by using the
coefficient for that variable in the prediction formula. For those
variables measured but not found significant, adjustment is not
necessary. For example, given similarity in unmeasured factors
which may account for variation in outcomes, these results could be
applied to a program that gave the second dose of HB vaccine at or
close to two months of age, since the coefficient for "Dose two timing" was zero. In addition, using the prediction formulas to extrapolate at the extremes of continuous variables in the prediction formula (relative to what occurred in these study data), may not be valid.

3. INFECTION AND CARRIER RATES
The infection rate found in this study was 5.1% in children ranging from two to eight years of age. This is close to the long-term weighted average of 5.5% estimated from the literature reviewed prior to this research. Both higher and lower rates have been found in long-term studies of immunized neonates born to HBsAg positive mothers. Ip et al. (1989) found a 38% rate of infection after three years in infants born to HBeAg positive mothers. These children had received four doses of 3 ug plasma derived HB vaccine with or without HBIG. This 38% rate is not consistent with other findings. An 11.9% rate of HBV events was found by Tajiri et al. (1989) after 4 years in infants who had a successful response to a preventive schedule including two doses of HBIG and three doses of 10 ug vaccine. Lo et al. (1988) in Taiwan, found an even lower rate in infants who initially responded with >=10 mIU/ml anti-HBs. No children were anti-HBc positive at three years of age and none became HBsAg positive by five years of age. All of the mothers were HBeAg positive as well as HBsAg positive and a four dose schedule of 5 or 2.5 ug plasma-derived vaccine with or without HBIG was used.

The carrier rate (HBsAg positive) in this study was 2.3%. This is
similar to a rate of 2.8% by five years of age found by Resti et al. (1990) in Italy. These infants were given HBIG at birth and a three dose series of plasma-derived vaccine with doses at 20 days, two months later and in the twelfth month of life. Zhu et al. (1992) found a 13.7% carrier rate after 6 years using 3 different schedules of vaccination, two with vaccine only. The HBeAg status of the mothers in this study was not stated, but the research was carried out in Shanghai, an area of high e antigen prevalence.

Attack rates after immunization have also been studied in other high prevalence populations and have generally been shown to be lower than in populations of neonatally immunized infants whose mothers are carriers. After five years of follow up only four (0.36%) persons became anti-HBc positive in a follow-up of 1114 Yupik Eskimos of all ages in Southwest Alaska (Wainwright et al. 1989). After three years, a two percent attack rate and a 0.7% carrier rate has been reported in the Gambian hepatitis intervention study (Chotard et al. 1992). HB vaccine in this program was given to all infants, not just infants of carrier mothers. Coursaget (1986) noted a 1.8% attack rate three to four years after a booster dose given 12 months after the first dose. This rate increased to 8% after five to six years. This increase over time may imply waning immunity or time to exposure to an infective dose of virus. Because an 8% vaccine failure rate is similar to other neonatal studies, it is likely the latter explanation.
4. THE OCCURRENCE OF INFECTION AND THE CHILD'S AGE

In this present study no significant association was found between the overall attack rate and the child's age from two years to eight after adjustment for covariates. Also, it was found, using logistic regression, that the higher attack rate in children immunized in the earlier years of the Program could be accounted for by 'Dose one' timing. The change of attack rates with time since immunization reported in the literature for similar neonatal research are consistent with this result. Lo et al. (1988) and Resti et al. (1990) do not show an increase over five years.

The infection rates in this present study varied between 2.6 and 7% for ages less than eight with no consistent trend. At age eight, however, the rate increased to 23% (3/13). Of the anti-HBc positive eight year old children, one child had only one dose of HB vaccine at 126 days of age, the second child had three doses of vaccine but the first one was at 158 days of age and the third child had three doses of vaccine but no HBIG at birth. Also, for this third child the age at the first dose was not known. Since these children did not receive vaccines according to Program guidelines the high rate of infection at age eight is not evidence of lack of vaccine efficacy or waning immunity.

5. PREDICTORS FOUND FOR ANTI-HBc AND HBsAG POSITIVE OUTCOMES

According to the logistic regression analysis, the best group of independent predictors of HB infection consisted of the following factors: the mother's HBeAg status, the child's age at the first dose of HB vaccine, the number of years the mother spent in her
country of birth and the mother’s age. The carrier state was best predicted by only two variables: the mother’s HBeAg status and the age of the child when the first dose of vaccine was administered.

The statistical models used to determine which predictors were most strongly related with the outcomes were association models, and therefore do not necessarily imply causation. Rothman (1986) states that:

"To an extent, every variable measured in an epidemiologic study can be considered only a surrogate variable for some more appropriate measure of the underlying phenomena."

A causal explanation may be true in some cases but this judgement would depend on other factors such as biological plausibility and consistency of results. There are four other possible reasons for certain variables to be chosen in a prediction model. These are: reverse causation, confounding, bias or chance. How one might view the variables that were selected to be in the prediction models is discussed in the following.

**Mother’s HBeAg status**

That the 'Mother’s HBeAg status' is a predictor of infection and carrier status has previously been shown by many other researchers (Beasley et al. 1977; Theppisai et al. 1990; Stevens et al. 1979; Assateerawatt et al. 1991). Also, biological plausibility is supported by evidence of viral replication in mothers who are HBeAg positive. In this study there was a strong association between 'Mother’s HBeAg status', 'Mother’s country of birth' and 'Language', and the association of infection with 'Mother’s country
of birth' and 'Language' was accounted for by 'Mother's HBeAg status'.

Age at Dose one

The importance, as seen in this study, of the age of the child when the first dose of HB vaccine is administered is not so clearly stated in the literature. Several researchers have done studies with a delayed first dose of vaccine, on the theory of physiological immunosuppression of newborns producing a less effective response to vaccination (Resti et al. 1990). After one dose of HBIG at birth, Resti et al. (1990) delayed the first dose of HB vaccine to 20 days and found a 2.8% (2/72) HBsAg positive rate after five years. Only 3.9% of the mothers were HBeAg positive and most were anti-HBe positive. Monna et al. (1988) observed after one year, an 8% (5/65) rate of HBsAg positivity when the first vaccine dose was given at one, two or three months of age. All of the mothers were HBeAg positive and HBIG was given at two months of age as well as at birth. In a randomized study, Schalm et al. (1989) found a four percent (4/90) rate of infection after a four dose schedule which included vaccine at birth but only a one percent (1/90) rate after a four dose series starting at three months of age. The group receiving the first dose of vaccine at three months also received a second dose of HBIG at three months of age. The difference in rates between early and late vaccine start groups was not statistically significant. The results of these studies imply that the rate of infection is not necessarily greater when the first dose of HB vaccine is delayed. However, our findings indicated that a delay in the first dose of vaccine was
more likely to result in infection. Furthermore, the early timing of the first dose of HB vaccine was more important than HBIG in preventing infections.

During the analysis, it was apparent from the stepwise regression that 'Dose one' was highly confounded with 'HBIG'. In one model building procedure which did not include the 'Mother's HBeAg status', 'HBIG' was selected over 'Dose one'. However, when 'mother’s HBeAg status' was included, 'Dose one' was selected over 'HBIG' even when HBIG was forced into the model. Thus it would appear, from our data, that getting 'Dose one' on time was a more important factor than getting the HBIG. However, the importance of HBIG cannot be ruled out by this finding since three of the four children who had a late 'Dose one', and became infected (4 of 39 anti-HBc positive cases), also did not have HBIG at birth or it was unknown if HBIG was given at birth.

Furthermore, how soon after birth 'Dose one' must be given to prevent infection is not clear from these data. The association between delay in 'Dose one' and infection, in the current study, arose because of the high rate of infection in children who received 'Dose one' after seven days. Because there were only 12 children who received 'Dose one' between four and seven days of age (none of whom became infected), it is not possible to say whether children who receive the vaccine between four and seven days of age are at higher risk of infection than those who receive it before four days of age.
The use of HB vaccine alone at birth, without concomitant administration of HBIG, has also been studied by other researchers but with varied results. Poovarawan et al. (1990) found no infections when both treatments were given (0/48), but a 3.4% (2/58) carriage rate when only vaccine was administered. In a later randomized controlled study by Poovarawan et al. (1992) no statistical difference was observed in long-term protective efficacy (carrier) between vaccine alone (3/60) or vaccine and HBIG at birth (1/61). Sehgal et al. (1992) found a trend toward greater efficacy when HBIG was not given but the power of the study was inadequate.

**Mother's years in birth country**

The variable 'Mother's years in birth country' would appear to be a surrogate measure for other risk factors. These factors may be cultural or social practices that put children at greater risk of infection and thus may be a marker for horizontal transmission. One such practice may be the care of children in the home and by extended family members who are also infected. This would be supported by the stepwise regression results when the variable 'Daycare' appeared, on the basis of change in score statistics, to be confounded with 'Mother's years in birth country'. Mothers who spent more years in their country of birth were less likely to use 'Daycare' (P=0.0002) and there was a univariate trend for 'Daycare' attendance to be protective of infection (P=0.09). Use of daycare may also be a marker for socio-economic conditions such as less crowding at home.
Mother’s age

'Mother’s age' was a predictor of infection, resulting in a higher rate of infection for younger mothers even after adjusting for 'Mother’s HBeAg status'. This variable may be a surrogate for other factors related to vertical transmission which are not being measured directly, such as the mother’s anti-HBe status. Information on anti-HBe status for the mothers was not collected. Apart from being more infectious with respect to vertical transmission, younger mothers may also have been more likely to infect their children by horizontal transmission. This variable, therefore, also raises the possibility of cultural or social factors. Younger mothers may adhere to practices which may through themselves or through other contacts increase the child’s risk for infection. It could also be, that the mothers of children who became infected (who were more likely to be older), were younger just by chance.

Breast feeding in this study was not associated with risk of infection. This is compatible with the findings of Beasley (1975) and Woo (1974).

Of the variables studied, the 'Mother’s HBeAg status' and the child’s age at 'Dose one' were the only variables significantly associated with becoming a carrier, after adjustment in the multiple logistic regression model. These are infection related variables and they accounted for only 22% of the variation in HBsAg outcomes. Of more importance to the persistence of viral infection may be host related factors, some of which were not measured in
this study. Some authors have suggested that the age of initial infection, race and other genetic factors may influence the ability to suppress viral replication (Evans ed. 1989).

6. SEROPOSITIVITY RATES
The overall seropositivity rate in this study was 87.9% (>=10 mIU/ml). As this was a one-time follow-up study, this overall rate may not be comparable to other long term study rates where the same children are tracked over time. In this study each age group constitutes a separate cohort of individuals. Thus each rate, as calculated, applies to only its age cohort. The seropositivity rates by age group were as follows: two years 98.6%; three years, 99.4%; four years, 90%; five years, 88%; six years, 80%; seven years, 70.4%; and eight years, 90%. These rates are compared to other study results in the following discussion.

After two years, Poovorawan et al. (1992) found a seropositivity rate of 94.9% (37/39) using 10 ug vaccine. This compares to a seropositivity rate of 98.6% for the age two group for the current study using a 20 ug vaccine. In other respects the immunization schedules were the same.

The current study’s age three seropositivity rate was 99.4% compared to 100% (47/47) at three years found by Poovorawan et al. (1992) (using a four dose schedule of 10 ug vaccine and HBIG). In contrast, Ip et al. (1989) in Hong Kong, reported a 73% seropositivity rate after three years. The treatment schedule for Ip’s study used four doses of 3 ug plasma-derived vaccine whereas
the current study used three doses of 20 ug yeast-derived vaccine for this age cohort. Ninety-five percent of infants immunized in The Gambia Program using a four dose series of 10 ug vaccine had protective titres of antibody after a period of three years (Chotard et al. 1992).

The age four seropositivity rate for the current study was 90% while the rate for Poovorawan et al. (1992) after four years was 100% (44/44) in children immunized with four doses of 10 ug vaccine and no HBIG.

While the current study found a seropositivity rate of 88% for the age five group, Resti et al. (1991) reported a seropositive rate of 95.8% after five years for infants immunized with plasma-derived vaccine: the first dose at 20 days, the second two months later and the third in the twelfth month of life. Infants in the Yupik Eskimo population study had a more rapid loss of antibody. Only 11 (61%) of 18 had anti-HBs titres of 10 SRU’s (protective level) or greater after five years (Wainwright et al. 1989). They had received three 10 ug doses of plasma-derived vaccine in the first year of life. In a subsequent follow-up of this Yupik Eskimo population, Wainwright et al. (1991) found that in the age group 19 years and under, 87% had anti-HBs titres of 10 or more mIU/ml after seven years (rate not stated for infants alone). The median anti-HBs level at seven years for infants immunized in their first year of life was only 21 mIU/ml. In the current study 70.4% in the age seven group had anti-HBs titres above 10 mIU/ml and the median anti-HBs level for the age seven group was 27.1 mIU/ml.
Seropositivity rates appear to remain higher in children who initially respond with at least 10 mIU/ml of anti-HBs. Lo et al. (1988) found rates of 94% (60/64), 91% (31/34) and 97% (98/101) for ages 3, 4 and 5, respectively, in a cohort of 199 infants. These children had an initial response of >=10mIU/ml anti-HBs following HBIG at birth and a four dose series of 5 or 2.5 ug of plasma-derived vaccine. Using a three dose series and 20 ug, Hwang et al. (1990) found, in infants who initially responded, that 95% had persistence of anti-HBs after five years. These rates at ages 4 and 5 were slightly higher than those for the current study (at 90% and 88% respectively).

7. PREDICTORS FOR ANTI-HBs OUTCOMES

We have seen that seropositivity rates vary considerably between studies. This may be related to the population immunized, vaccine type, dosage and schedule and other possible factors. There is, however, generally a decline in rate over time. In this study it was found that the 'Child's age' was the strongest predictor for anti-HBs titres. 'Number of doses', age at 'Dose one' and 'Months abroad' were the other significant predictors.

Child's age and Anti-HBs titres

The anti-HBs geometric mean titre at three years of age, which in this study was 238 mIU/ml, can be compared to two other long-term studies which also gave estimates at three years of age. Ip et al. (1989) found a geometric mean of 30 mIU/ml using a 3 ug dose of hepatitis B vaccine. Schalm et al. (1989) found between 400-800 mIU/ml using a four dose schedule and 10 ug of vaccine.
In the present study, the geometric mean anti-HBs titres differed most dramatically between age groups three and four: 238 mIU/ml compared to 79 mIU/ml, respectively. The difference in titres, between age groups, then dropped more slowly to 23 mIU/ml for the age seven group. Other authors have found a decline of anti-HBs titres associated with age but the greatest decline is generally described to occur within the first two years after immunization (Hadler et al. 1988; Jilg et al. 1988). We did not have anti-HBs data in mIU/ml units for children under two years of age.

Wainwright et al. (1989) also found age to be associated with anti-HBs titres, but they found that the initial antibody level is a stronger predictor of the persistence of protective antibody titres. This finding is supported by other authors (Chotard et al. 1992; Jilg et al. 1988) and by the current research as well since study anti-HBs titres were highly associated with titres at or above 10 mIU/ml at 6 to 18 months of age (P<0.0002).

**Age at Dose one and Anti-HBs titres**

An interesting phenomena occurred in this study in that there was a geometric mean titre increase of 83 mIU/ml anti-HBs in the age eight group over the age seven group level. This increase to 106 mIU/ml at age eight is explained by late initiation of the vaccine series in some children around the time the vaccine was first made available and thus may be attributed to a program effect. Four out of the ten eight year old children had the first dose of HB vaccine after five days of age and these children also had anti-HBs titres well over 100 mIU/ml. Resti et al. (1990) found after a delayed
first dose at 20 days that only 3 of 72 children at five years of age had less than 10 mIU/ml anti-HBs: four percent as compared to 12% in this study. Schalm et al. (1989) found statistically significantly higher titres of anti-HBs at 11 and 24 months in children who received a delayed vaccine series compared to vaccine starting at birth.

Several authors (Chotard et al. 1992; Coursaget et al. 1986; Lo et al. 1988) have noted boosting of anti-HBs titres in the absence of immunization. An explanation suggested for this 'boosting' was viral exposure without cell invasion and replication. This is not a likely explanation for the increase in titres for eight year old children in this study. If it were, one would have expected to have seen this effect in the seven year old group as well.

**Number of doses and Anti-HBs titres**

Evidence from these data supports the effect of 'Number of doses' on antibody titres. Children with fewer than three doses were more likely to be non-responders or have less than 100 mIU/ml of anti-HBs and those with three or more doses were more likely to have over 100 mIU/ml of anti-HBs. Chotard et al. (1992) also found the proportion of children with high antibody titres increased with the number of doses received, though only a slight increase occurred between three and four doses.

**Months abroad and Anti-HBs titres**

Children who spent time abroad had higher anti-HBs titres but the interpretation of this is not clear and the association requires
further study.

8. RELATIONSHIP BETWEEN ANTI-HBs TITRES AND INFECTIONS

The effect of anti-HBs titres on subsequent infection rates cannot be determined directly in this study. Because the two were measured simultaneously the effect of anti-HBs on infection risk cannot be separated from the effect of infection on anti-HBs titres. For 183 (24%) children, 6 to 18 month results were available but this number is small and the analyses based on this sample lacks power.

That infections may not be related to anti-HBs titres was suggested in this study in several indirect ways. First of all, anti-HBc outcomes were not statistically significantly associated with 6 to 18 month anti-HBs outcomes (P=0.12; relative risk 0.30; 95% confidence interval, 0.06, 2.03). This suggests that infections occurred even in children who had responded to the initial series of HB vaccine. As well, initial non-responders did not necessarily become infected. Since the power of this test was low and the relative risk of 0.30 suggests protection (seropositivity) the argument that infections are not related to anti-HBs titres on this basis is weak.

Study HBsAg positive outcomes were associated with early anti-HBs outcomes (P=0.008; relative risk, 0.05; 95% confidence interval, 0, 0.62). The direction indicated that chronic carriers were more likely to be those who did not initially respond to the HB vaccine. These results would also suggest that if infection does occur after
an initial response, progression to the carrier state is less likely.

Secondly, this study demonstrated little increase in cumulative infection rates from ages two to seven years. This suggests that if infections occur, they are more likely to occur early. For the sub-sample of children tested twice, 7 of 9 (78%) infections found on study results had already occurred by 6 to 18 months (P=0.008). However, 51 of the 58 children who were anti-HBc positive in infancy converted to being negative by study testing. Thus, one might conclude that the 6 to 18 month anti-HBc result does not accurately reflect infection. This persistence of what are most likely maternal anti-HBc has been noted by other authors as well. Chotard et al. (1992) found that of 31 anti-HBc positive children at one year, 27 (87%) were negative at two years. Lo et al. (1988) found that 4 of 137 (2.9%) infants retained the anti-HBc at two years but were negative at three years. The association with infancy HBsAg positivity is stronger for HBsAg study results. Of five HbsAg positive study results four were already HBsAg positive at 6 to 18 months (P<0.0001).

The results of Coursaget et al. (1986) do not agree with the finding that most infections occur early. They found that no children became infected until the third to fourth year period, following which the rate for HBV events jumped from 1.8% (for the 3 to 4 year period) to 8% at the fifth to sixth year period and the carrier rate rose correspondingly from 0% to 4%. On the other hand, some researchers have noted that children who became chronic
carriers are the ones who were already identified as being HBsAg positive at birth or within the first year of life (Resti et al. 1990; Poovarawan et al. 1992). Ip et al. (1989) found both early and later chronic carrier cases, the later cases occurring following loss of anti-HBs titres to below 10 mIU/ml. Anti-HBc positivity also arises in children with greater than 10 mIU/ml of anti-HBs but infections with greater than 100 mIU/ml are rare (Hwang et al. 1990; Schalm et al. 1989; Coursaget et al. 1986; Chotard et al. 1992).

Since cases were not tracked over time in this study, it is not known when the infections occurred and what the prior anti-HBs titres were. However, as the infection rate was fairly consistent over age groups, this would suggest that infection was not due to waning immunity. Therefore it would appear that susceptibility was more likely to arise early rather than later in infected children. This possibility is supported by the 14% of inadequately protected children at the 6 to 18 month testing and the fact that some of these non-responders became infected. Later susceptibility following early protection cannot be ruled out because two of the children tested at 6 to 18 months who were anti-HBc negative and anti-HBs positive initially, later became infected. But perhaps, as Ip et al. (1989) suggest, what appears to be early response is actually evidence of passive antibodies which have not yet waned and the lack of an active response becomes apparent with later susceptibility. This is when other factors such as those which are related to the 'Mother's age', 'Mother's years in birth country' and 'Number of contacts' may possibly take on more importance.
9. PROTECTIVE EFFICACY OF HB VACCINE

Using the formula: \( \frac{\text{expected rate} - \text{observed rate}}{\text{expected rate}} \), the overall protective efficacy\(^1\) of HB vaccine in this British Columbia Neonatal Program in preventing infections was 89.1\%, and in preventing the carrier state was 94.2\%. These rates are similar to those found by other studies after 3 to 5 years except for Ip et al. (1989) who found a vaccine efficacy rate of 80\% for the carrier state after 3 years (for the group on a similar vaccine schedule) and Coursaget et al. (1986) who found a vaccine efficacy rate of 67.2\% after 5 to 6 years. For the current study expected estimates were taken from control groups cited in the literature and were as follows:

1. For infants of HBsAg and HBeAg positive mothers: 96\% for infection and 85\% for the carrier state (Stevens et al., 1979).

2. For infants of HBsAg positive and HBeAg negative mothers data are less adequate because of low numbers but the rates used were 25\% for infection (Zanetti et al. 1982) and 19\% for the carrier state (Theppisai et al. 1989).

Calculations were based on the proportions of children with mothers who were HBeAg positive or HBeAg negative as follows:

Infection vaccine efficacy rate =

\[
\frac{(0.96 \times 182(\text{HBeAg}^+) + 0.25 \times 406(\text{HBeAg}^-))}{588} = 0.47
\]

\[
\frac{(0.47 - 0.051)}{0.47} = 0.891
\]

\(^1\)The term vaccine 'protective efficacy' is commonly used in the literature to compare the outcomes of disease between use of vaccine and no use of vaccine. Although the term 'protective effectiveness' may be more appropriate for this effectiveness study, the conventional term is used.
Carrier vaccine efficacy rate = 
\[
(0.85 \times 182(\text{HBeAg}^+) + 0.19 \times 406(\text{HBeAg}^-))/588 = 0.394
\]
\[
(0.394 - 0.023)/0.394 = 0.942
\]
A sensitivity analysis using lower expected rates indicated that the vaccine efficacy remained high. Using values of 0.65 for HBeAg positive mothers and 0.10 for HBeAg negative/HBsAg positive mothers the vaccine efficacy would be 81%.

10. SUMMARY
The questions this research attempted to answer were:
1. What is the HBV attack rate over time?
2. What are the anti-HBs titres and how do they change over time?
3. Is there an association between attack rates and anti-HBs titres?
4. How are the HBV attack rates and anti-HBs titres affected by various demographic, program intervention, and exposure factors?

The attack rate for HBV infections was 5.1% and the carrier rate was 2.3% over seven years. These rates were not associated with age and were similar to those found in other long-term studies. Anti-HBs seropositivity overall was 87.9% The geometric mean anti-HBs level was 79.7 mIU/ml and declined from 272 mIU/ml to 23 mIU/ml in two year old versus seven year old children. An increase to 106 mIU/ml in eight year old children was seen which can be explained by late initiation of the HB vaccine series and additional doses.

No association was found between the study infection results and infant anti-HBs titres but this was on the basis of only 183 children. The relatively stable infection rate does not suggest waning immunity but rather early susceptibility which may be due to lack of active response to the HB vaccine or some other reason.
Lack of early protection is consistent with the 14% non-response rate in infancy.

The best group of predictors for infection were the mother’s HBeAg status, the timing of dose one, the number of years the mother spent in her birth country and the mother’s age. The last two variables are probably related to cultural or social factors or may be indirectly measuring a factor related to the mother’s anti-HBe status. The mother’s HBeAg status and the timing of dose one were the best predictors for the HBsAg carrier state. The age of the child, number of doses of HB vaccine, timing of the first dose and the number of months the child spent abroad were the factors which were significantly associated with anti-HBs titres.

According to the multivariate results children were more likely to be infected if their mothers were HBeAg positive, the first dose of HB vaccine was delayed, the mother spent a longer time in her birth country and/or if the mother was younger. Children were more likely to become a carrier if their mother was HBeAg positive and/or if they had a delayed first dose of vaccine. Children who were younger, had more doses of vaccine, had a delayed first dose and/or had travelled abroad were more likely to have higher titres of anti-HBs.

The protective efficacy of the HB vaccine found in this study was 89% for infection with the HBV and 94% for the carrier state.
11. RECOMMENDATIONS

1. There is no evidence from these data of the need for additional doses of vaccine up to age eight. Therefore a booster dose for the children studied, after the six month dose, is not recommended at this time.

2. A delay in the first dose resulted in increased infections and it is therefore recommended that efforts are made to assure this dose is given within the first three days after birth.

3. Although the timing of dose one was found to be critical, it could not be concluded from these data that HBIG was not important and therefore it is recommended that HBIG at birth be continued.

4. The timing of dose two of HB vaccine later than two months after dose one was not found to be associated with increased infections; therefore, this dose could be incorporated with the regular immunization schedule at two months of age if administratively more feasible.

5. Identification of non-responders to the HB vaccine within one or two months after the booster dose at six months followed by a supplemental dose is advisable because of the 14% non-response rate found at 6 to 18 months. This may, however, not be a cost effective endeavour.

12. RECOMMENDATIONS FOR FURTHER STUDY

It is recommended that a similar study be undertaken in three to five years from the last collection of blood specimens to look for evidence of infection and to determine whether a booster dose may be needed. Although this study found decreasing anti-HBs titres
over time there was no evidence that this increased susceptibility. However, we cannot be sure that waning immunity will not occur over a longer term and therefore for the protection of these children, who are continuously exposed to the HBV by contact with the mother, further follow-up should be considered. Since infections are in some way related to younger mothers who may be new immigrants, consideration should be made to include in future studies variables such as the mother’s anti-HBe status which may help to explain this association.
BIBLIOGRAPHY


Lo KJ. Lee SD. Tsai YT. et al. Long-Term Immunogenicity and Efficacy of Hepatitis B Vaccine in Infants Born to HBeAg-Positive HBsAg-Carrier Mothers. Hepatology 1988;8(6):1647-50.


APPENDIX 1

STUDY DOCUMENTS

Physician information letter 137
Parent information letter 138
Telephone introduction guide 139
Consent for participation 140-1
Questionnaire 142-5
Questionnaire guide for interviewers 146-8
Laboratory test report form 149
Results of tests - form letters for parents and physicians 150-59
Dear Physician:

Some time ago, your patient born to
received hepatitis B vaccine after birth as part of the Ministry of Health, Neonatal Hepatitis B Vaccine Program. We will soon be sending a letter to his/her parent(s) requesting participation in a study on the effectiveness of hepatitis B vaccine given to neonates.

The goals of the study are to measure the HBV infection rate in children who received hepatitis B vaccine during 1984 through 1989 and to find out how many of these children still have antibodies to HBV. Since very few studies have been done on the long-term effectiveness of hepatitis B vaccine in infants this information will be valuable in terms of assessing the need for boosters.

We will be arranging for a finger-prick blood sample to be taken from those children whose parents agree to their participation in the study. The blood samples will be tested for presence of anti-HBc, anti-HBs and HBsAg. Parents will also be asked to answer a few questions concerning risk factors their child may have for hepatitis B infection.

As well as advising parents of the blood test results, we will request consent from the parents to inform the child’s physician of the results so that the necessary follow-up may be provided.

Enclosed is a copy of the letter we are sending to your patient’s parent(s). We would appreciate hearing from you in the event of an address and/or telephone number change for this child. Also, if circumstances exist with this family which would suggest that contact with these parents is not advisable, we would appreciate hearing from you. If we don’t hear from you within a week of your receipt of this letter, we will assume you have no objection to us contacting these parents.

If you would like to speak with us or have any questions about this study, you may call Dr. Richard Mathias, Principal Investigator, at 822-2772, Marian Tomm-Pastore, Study Coordinator, at 879-7551 local 307 or myself at 660-6063. Please refer any address or telephone number changes to Marian Tomm-Pastore as soon as possible.

Sincerely,

Dr. John Farley
Epidemiologist,
B.C. Centre for Disease Control,
Ministry of Health, Preventive Health Services.
Dear Parent:

You may recall that after being born your child received several doses of hepatitis B vaccine to protect him or her from hepatitis B infection.

The Ministry of Health, Centre for Disease Control, the Canadian Red Cross Society (Vancouver Blood Centre), and the Department of Health Care and Epidemiology at the University of British Columbia are conducting a study to obtain information on how well this vaccine has worked in children who have received it. This information will be important in helping make decisions about whether booster doses will be required in the future.

We would like to enrol your child born in the study. This research will be conducted by Dr. R. Mathias (a Communicable Disease Specialist at U.B.C.), Dr. David Pi (Deputy Medical Director, Canadian Red Cross Society), and Dr. J. Farley (Epidemiologist, Ministry of Health).

Children taking part in the study will have a finger-prick blood sample taken. The parents will be asked to answer a few questions that will help us to better understand hepatitis B infections in children.

A member of the study team will contact you in the near future to offer you more information. Please understand that your participation in this study is entirely voluntary.

If you would like to contact us, you may call Dr. Richard Mathias at 822-2772 or Marian Tomm-Pastore, Study Coordinator, at 879-7551 local 307. We will gladly answer your questions. If you have not heard from us in a week, we would appreciate a call from you at 879-7551 local 307 as we may not have been able to locate a phone number for you.

Sincerely,

Dr. R.O. Mathias
THE UNIVERSITY OF BRITISH COLUMBIA
Faculty of Medicine
Department of Health Care & Epidemiology
James Harper Building
5804 University Avenue
Vancouver, B.C. V6T 1Z3
TEL: (604) 822-2772
FAX: (604) 822-4964

Dr. J. Farley
THE PROVINCE OF BRITISH COLUMBIA
Ministry of Health
British Columbia Centre for Disease Control
529 Wesbrook Mall
Vancouver, B.C. V6T 1Z2
TEL: (604) 860-8083

Ms. Marian Tomm-Pastore
Study Coordinator
TEL: (604) 879-7551
or (604) 877-7805

Dr. John Farley
Epidemiologist
B.C. Centre for Disease Control
Ministry of Health
TELEPHONE INTRODUCTION

LONG-TERM FOLLOW-UP OF NEONATES WHO HAVE BEEN IMMUNIZED WITH HEPATITIS VACCINE

"Hello, my name is ___________.
Is this the ___________ residence?
I am calling about the Hepatitis B vaccine study.
Did you receive a letter in the mail inviting your child _______ to be part of this study?
Do you have any questions about the study?
We would very much like to include _________ in the study.
It will help us to understand how well the hepatitis B vaccine has worked in children who were immunized at birth.
Many short term studies have shown that the vaccine has worked very well over a short term. We would like to find out if this is true for periods three years or longer.
Would you be willing to allow__________ to be a part of this study? It would involve answering a few questions about risk factors for getting hepatitis B and taking a finger blood sample.
Would you be willing to come to _________ with you child for this interview?
(make appointment if agree to participate)
Thank you for agreeing to participate in the study."

Options: clinic appointment
 home visit appointment

Arrange for interpreter to telephone if language is a problem. Also advise Marian if it is expected there will be difficulty with communication at the appointment.

If the parent is hesitant about participating, ask: "Would you like to talk to your doctor about the study and then we’ll call you back?"

Give a name and phone number to contact in case of questions. The Red Cross number for the study is 879-7551 local 307.

If the parent refuses to participate, please ask: "What is the usual language spoken in your home?" Record the language on the printout and forward to Marian (Study Coordinator).
NEONATAL HEPATITIS B VACCINE STUDY CONSENT

STUDY TITLE: LONG-TERM FOLLOW-UP OF NEONATES WHO HAVE BEEN IMMUNIZED WITH HEPATITIS B VACCINE

The purpose of this study is to establish whether the hepatitis B vaccine which your child received at birth has protected him/her from infection. This can be assessed by testing for the presence of certain markers in the blood. Your child does not need to have had symptoms to have experienced an infection.

The test will also determine if antibodies to hepatitis, stimulated by the vaccine, are still circulating in your child's blood.

We are requesting your permission to obtain a finger blood sample from your child. Pain from a finger-prick blood sample is mild and short lived. The blood sample will be tested only for evidence of past or present hepatitis B infection and for the presence of hepatitis B antibodies. The specific markers include anti-HBs, anti-HBc and HBsAg.

Results of the tests on your child's blood will be sent to you with an explanation of their meaning. Some follow-up by your physician may be recommended if your child's blood shows evidence of hepatitis B infection. Information on blood test results will be released to your physician with your consent only.

All information from the questionnaire and on blood test results will be STRICTLY CONFIDENTIAL. The study records will be available only to members of the study team. Your child will not be identified in any public report of the study.

Your participation in this study is COMPLETELY VOLUNTARY. Your refusal will in no way affect your or your child's future medical care.

With your consent, we would also like to contact you in the future for similar follow up.

Investigators:

Dr. R. Mathias, Associate Professor, Department of Health Care and Epidemiology, U.B.C.
Dr. D. Pi, Deputy Medical Director, Canadian Red Cross, Vancouver Blood Center, and Clinical Instructor, Department of Pathology, U.B.C.
Dr. J. Farley, Epidemiologist, B.C. Centre for Disease Control, Clinical Assistant Professor, Department of Health Care and Epidemiology, U.B.C.
Marian Tomm Pastore, M.Sc. student, Department of Health Care and Epidemiology, U.B.C.

If you have any questions about the study, please contact Dr. Mathias, Principal Investigator, at 822-2772, or Marian Tomm Pastore, Study Coordinator, at 879-7551 Loc. 307.
I CONSENT FOR MY CHILD TO PARTICIPATE IN THE HEPATITIS B VACCINE STUDY DESCRIBED ABOVE. THE STUDY HAS BEEN CLEARLY EXPLAINED TO ME AND I UNDERSTAND THE INFORMATION PROVIDED. I ACKNOWLEDGE THAT I HAVE RECEIVED A COPY OF THE CONSENT FORM.

Child’s family name: ___________________________ given name: ___________________________

Parent or Guardian Signature: _______________________________________________________

Please print full name: _____________________________________________________________

Relationship to child: __________________________________ Date: ______________________

Witness Signature: __________________________________ Print full name: ________________

Date: __________________________________________

I CONSENT TO BEING CONTACTED IN THE FUTURE FOR SIMILAR FOLLOW-UP ON MY CHILD. IN THE EVENT OF A CHANGED ADDRESS, MY CHILD’S PERSONAL HEALTH NUMBER MAY BE USED FOR LOCATING PURPOSES ONLY.

Child’s family name: ___________________________ given name: ___________________________

Parent or Guardian Signature: _______________________________________________________

Please print full name: _____________________________________________________________

Relationship to child: __________________________________ Date: ______________________

Witness Signature: __________________________________ Print full name: ________________

Date: __________________________________________

I CONSENT TO THE RELEASE OF MY CHILD’S HEPATITIS B BLOOD TEST RESULTS TO MY CHILD’S PHYSICIAN, DR. __________________________, FOR THE PURPOSE OF FOLLOW-UP.

Child’s family name: ___________________________ given name: ___________________________

Parent or Guardian Signature: _______________________________________________________

Please print full name: _____________________________________________________________

Relationship to child: __________________________________ Date: ______________________

Witness Signature: __________________________________ Print full name: ________________

Print full name: __________________________________ Date: ______________________

Page 2 of 2
LONG-TERM FOLLOW-UP OF NEONATES WHO HAVE BEEN IMMUNIZED WITH HEPATITIS B VACCINE

INTERVIEW QUESTIONNAIRE FOR PARENTS OR GUARDIANS

SECTION A

Interviewer Instructions:
Complete Date of interview and identifying information in SECTION A.
PLEASE PRINT CLEARLY

Date of interview: [YY MM DD]

Child's given name __________________ family name __________________

Male □ Female □ Date of birth [YY MM DD]

Mother's given name __________________ last _______ D.O.B. [YY MM DD]
(if different from child's)

Father's given name __________________ last _______ D.O.B. [YY MM DD]
(if different from child's)

Address ____________________________ No. ______ street ______ city ______ postal code ______ Ph. No. ______

Country of birth for: [round off years to the nearest year]
Mother __________________ Length of residence in country of birth: ______ years
Father __________________ Length of residence in country of birth: ______ years

Usual language at home __________ Has child moved since birth? Yes □ No □

Physician's name __________________ Ph. No. ______ City ______

COMPLETE THIS SECTION AT THE END OF THE INTERVIEW

Can you give us the name of an alternate contact who could assist us in locating you in the event that:
 a) you move before we can get the results of this study to you or
 b) we would like to reach you for a follow-up to this study in a few years?

Name: last _________________________ first _________________________

Phone number: __________ Relationship: _________________________

Or, may we have your child's Personal Health Number? __________________

Your PHN will be kept confidential and will not be used for any other purposes.

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### SECTION B

**Interviewer instructions:**
Complete SECTION B by asking the parent or guardian the questions as stated, except, using the child's name for "this child". Answer by circling the correct response as indicated in the sample question unless otherwise directed in the question. Interviewer instructions are in square brackets. Refer to the questionnaire guide for more complete instructions.

**Sample question only:**

<table>
<thead>
<tr>
<th>Question</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Has &quot;this child&quot; had chickenpox?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(a) To your knowledge, has &quot;this child&quot; ever been ill with hepatitis?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If answered &quot;Yes&quot; for 1(a):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1(b) What type was it? [circle the number next to the correct response]</td>
<td>1</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>NonA NonB</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Don't know</td>
</tr>
<tr>
<td>2. Has &quot;this child&quot; ever had jaundice starting after the first month of life?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3(a) Was &quot;this child&quot; breast fed? [Must be at least 1 week in duration and at least one feeding per day]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>If answered &quot;Yes&quot; to 3(a):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3(b) What was the duration of breast feeding in months?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>[consider 1 week to equal .25 months]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4. Has &quot;this child&quot; experienced any of the following?</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(a) Ear piercing country if other than Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(b) Acupuncture country if other than Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(c) Tattooing country if other than Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(d) Surgical procedure country if other than Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(e) Blood transfusions country if other than Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(f) Receipt of blood products country if other than Canada</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(g) Day care country if other than Canada</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5(a) Other than the mother, does "this child" live with anyone else in the home? Yes No

[Include only those household members who share the same kitchen as the child and have lived in the home for at least one month.]

[If answered "Yes" to 5(a), then complete the table below with respect to questions 5(b) to 5(j) as illustrated.]

Sample question only:

<table>
<thead>
<tr>
<th>Relationship</th>
<th>5(b)</th>
<th>5(c)</th>
<th>5(d)</th>
<th>5(e)</th>
<th>5(f)</th>
<th>5(g)</th>
<th>5(h)</th>
<th>5(i)</th>
<th>5(j)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>M</td>
<td>F</td>
<td>1960</td>
<td>Y</td>
<td>N</td>
<td>1987</td>
<td>Y</td>
<td>N</td>
<td>1981</td>
</tr>
</tbody>
</table>

5(b) What is their relationship to "this child"?
5(c) What is their sex? [Circle "M" for male and "F" for female]
5(d) What is their year of birth? [If uncertain, approximate the year]
5(e) Have they had hepatitis? [Circle "Y" for yes and "N" for no]
5(f) If so, what kind? [Indicate if A, B, NonA NonB]
5(g) In what year? [If uncertain, approximate the year of diagnosis]
5(h) Was it laboratory confirmed? [Circle "Y" for yes and "N" for no or uncertain]
5(i) Have they had hepatitis B vaccine? [Circle "Y" for yes and "N" for no]
5(j) What year was the hepatitis B vaccine received? [Approximate the year if uncertain]
6(a) Has "this child" ever been hospitalized?  

Yes  No

If answered "Yes" to 6(a):

6(b) How many times has "this child" been hospitalized?_____

6(c) What were the reason(s) for hospitalization?

________________________________________________________________________

________________________________________________________________________

7(a) Has "this child" ever travelled overseas?

Yes  No

[If answered "Yes" to 7(a) complete the table below with respect to questions 7(b) to 7(d):]

7(b) What countries did "this child" visit on each trip?

7(c) How many months was the child abroad?

7(d) What was "the child's" age at the time of leaving on the trip?

<table>
<thead>
<tr>
<th>trip #</th>
<th>country/countries visited</th>
<th># of months (one week=.25 months)</th>
<th>Child's age at time of visit in years</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SECTION C

THIS COMPLETES THE QUESTIONNAIRE. THANK YOU VERY MUCH FOR ANSWERING THESE QUESTIONS. WE APPRECIATE VERY MUCH YOUR ASSISTANCE IN THIS STUDY.

Respondent: [check] Mother ____ Father ____ Other ____

What is their relationship to the child if "other"? ______________________

Interviewer: Name:[print]_________________________ Phone #:____________________

Comments:

REMEMBER TO ASK THE RESPONDENT FOR AN ALTERNATE CONTACT (PAGE 1)
QUESTIONNAIRE GUIDE FOR INTERVIEWERS

LONG-TERM FOLLOW-UP OF NEONATES WHO HAVE BEEN IMMUNIZED WITH HEPATITIS B VACCINE

The purpose of this study is to determine how effective the hepatitis B vaccine has been in preventing infections in those children who were immunized with the hepatitis B vaccine at birth.

The purpose of this questionnaire is to determine if there are factors which explain the occurrence of infection in some children. Children who have had an infection may not have had any signs of illness. A positive blood test for anti-HBc or HBsAg markers may be the only indication that an infection has taken place.

To make sure the results of the questionnaire are as accurate as possible it is important to follow these guidelines:
1. Use the same wording as in the questionnaire.
2. Use simple clarifications if necessary. (Refer to the guide.)
3. Arrange for an interpreter to be available if parents have difficulty with English.
4. Complete every question.

SECTION A

Be sure to complete the identifying information. This is essential to allow us to match records and to assist in locating families for future follow-up.

In the case of Asian children’s names, some children are called by the family name first, followed by given names. We would consider the first name the family name as long as it is common to others in the family, and the rest of the names which are different as the given names.

Parent’s length of residence in country of birth should be rounded off to the nearest number of years. For under one year indicate one year.

With regard to physician, it is sufficient to indicated the city name only unless the physician has recently moved his/her office.

Parents may have concerns about giving alternate contact information. They may be reassured that this information will be kept confidential and giving this information does not constitute giving consent to participate in future studies. Parents would be asked to give consent at the time of contact. The purpose is only to make it easier to locate them if another study is undertaken. If parents are reluctant to give this information do not press them.

PLEASE PRINT CLEARLY IN BLOCK LETTERS
SECTION B

Question 1
"Yes" implies the hepatitis disease was diagnosed by a physician or health care worker.

Question 2
Indicate "Yes" only if the child had jaundice after one month of age. Do not include jaundice or yellow skin caused by breast feeding or physiological jaundice due to birth.

Question 3
3(a) Indicate "Yes" only if the mother continued breast feeding after the first week of the child's life.
3(b) Duration of breast feeding should include the length of time at least one feeding per day of breast milk was fed, even if formula was fed for all other feedings.

Question 4
4(a), (b), (c) (d) (e) (f) (g) For ear piercing, tattooing, acupuncture, surgery, receipt of blood products or blood transfusions and day care, indicate the country or place where this procedure took place if other than Canada, for example, Hong Kong or Taiwan.
4(d) Surgical procedure refers to any procedure that has involved an incision or suturing.
4(f) Blood products include plasma, serum, thrombin, fibrinogen, packed red blood cells and cryoprecipitate. They do not include immune globulin, heat-treated plasma protein fraction, albumin or fibrinolysin.
4(g) Day care includes any out-of-home, formal, paid-for day care in a group setting, for example, group or family day care. It does not include in home baby sitting whether done on a regular or occasional basis.

Question 5
5(b) Include all household contacts who share the same kitchen as the child and have lived in the same house for at least a month. Contacts should include relatives such as the father, grandparents, cousins, aunts, uncles etc. as well as nanny's, live-in baby sitters and boarders. If more than one grandparent lives in the home, indicate if maternal or paternal.
5(d) If the exact date of birth of the contact is not known, indicate the approximate age.
5(f) If the exact year of hepatitis is not known, indicate the approximate year of diagnosis.
5(g) If the hepatitis diagnosis was confirmed by laboratory test, circle "Y" for yes and circle "N" for no or uncertain. If the contact's blood was not tested, and diagnosis was made on the basis of clinical symptoms or contact with someone else who was diagnosed with hepatitis B circle "N".

Add more lines or use the back of the page if there are more than 8 household contacts.
**Question 6**

6(a) "Hospitalization" refers to actual admission for overnight stay, not visits to the Emergency Room which are not followed by admission.

6(b) refers to the number of separate admissions, not the length of stay.

6(c) indicate the reason/s for each separate admission. Add extra lines if necessary or use the back of the page.

**Question 7**

"Overseas" implies travel to another continent such as Asia or Africa. Include Mexico, Central America and South America but do not include the U.S.A. If the child visited three different countries, for example, Taiwan, Thailand and Japan on one trip, indicate these countries on the same line. If one country was revisited on another trip, include that country again on a separate line so that each line id trip specific. Include any extended periods of time the child may have lived abroad. If in doubt whether a country or place qualifies, include it. Length of time should be recorded in months using .25 (one week), .5 and .75 as portions of a month. Record the age of the child on the last birth date at the time of leaving on the trip. Please use the back of the page if there were more than 5 trips.

Under comments, include any special circumstances such as difficulty communicating or concerns about the responses obtained. Also indicate if a satisfactory blood sample was not obtained and why. At least 2 microtainer tubes should be filled.

If you have any questions or concerns about the questionnaire or procedures please call Marian Tomm Pastore at ph.________ or Dr. Rick Mathias at ph. _________

Thank you very much for your assistance with this study.
LAB TEST REPORT FORM

LONG-TERM FOLLOW-UP OF NEONATES WHO HAVE BEEN IMMUNIZED WITH HEPATITIS B VACCINE

SECTION A

I.D. NUMBER: ______________________

Child's given name __________________ family name __________________

Child's D.O.B. YY MM DD

Mother's given name __________________ family name __________________

Mother's D.O.B. YY MM DD

Date blood sample taken YY MM DD by __________________

Location of sample taking __________________ ph# __________________

Section B

Date blood sample received at CRCS YY MM DD

<table>
<thead>
<tr>
<th>Test</th>
<th>Reading</th>
<th>Date DD/MM/YY</th>
<th>Reset</th>
<th>Date DD/MM/YY</th>
<th>Pos/Neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Anti-HBc (IMX)</td>
<td>S/CO</td>
<td></td>
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<tr>
<td>2. Anti-HBs (IMX)</td>
<td>mIU/ml</td>
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<tr>
<td>3. HBsAg (IMX)</td>
<td>S/N</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

Is the sample haemolysed? (circle) Y N

Technologist comments:

Note to person taking blood sample:
The minimum amount of blood required is 1.2 mls. To get this amount, fill 2 microtainers to the top. If blood is flowing freely, fill a third microtainer in case retesting is needed. Write I.D. number on the microtainer, checking with the questionnaire I.D. number. Complete part A of the LAB TEST REPORT FORM. Place form and microtainer into the baggie and then into a delivery container. Deliver to the Red Cross Blood Transfusion Centre by 9 am the next morning or as soon as possible. Areas outside the Lower Mainland will require special arrangements for delivery. Blood samples should be kept at about 4 degrees C until delivered. Do not freeze or allow them to get too hot.
Dear Ms.

We would like to thank you for your child's participation in the hepatitis B vaccine study. Your child, _______ born _______ has the following blood test results:

- Anti-HBc: Negative
- Anti-HBs: Positive
- HBsAg: Negative

These results indicate that your child has an adequate protective response to the hepatitis B vaccine.

A copy of the results will be sent to your physician.

Please keep this record of results in your child's immunization booklet. It may be necessary for reference when your child is offered the hepatitis B vaccine through the grade six program.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Ms.

We would like to thank you for your child's participation in the hepatitis B vaccine study. Your child,_______, born _______ has the following blood test results:

- Anti-HBc: Positive
- Anti-HBs: Positive
- HBsAg: Negative

These results indicate that your child has been infected with the hepatitis B virus in the past, but is not a carrier and now has an adequate protective response. Further immunization is not indicated.

A copy of the results will be sent to your physician.

Please keep this record of results in your child's immunization booklet. It may be necessary for reference when your child is offered the hepatitis B vaccine through the grade six program.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Ms.

We would like to thank you for your child's participation in the hepatitis B vaccine study. Your child, ________, born ________ has the following blood test results:

- **Anti-HBc:** Negative
- **Anti-HBs:** Negative
- **HBsAg:** Negative

These results indicate that your child's response to the hepatitis B vaccine is not considered adequate at present. Please see your physician concerning these results.

A copy of the results will be sent to your physician.

Please keep this record of results in your child's immunization booklet. It may be necessary for reference when your child is offered the hepatitis B vaccine through the grade six program.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Ms.

We would like to thank you for your child's participation in the hepatitis B vaccine study. Your child, __________, born _______ has the following blood test results:

- **Anti-HBc**: Negative
- **Anti-HBs**: Insufficient blood to test
- **HBsAg**: Insufficient blood to test

These results indicate that your child has not been infected with the hepatitis B virus. However, sufficient blood was not obtained to measure response to the vaccine.

A copy of the results will be sent to your physician.

Please keep this record of results in your child's immunization booklet. It may be necessary for reference when your child is offered the hepatitis B vaccine through the grade six program.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Ms.

We would like to thank you for your child's participation in the hepatitis B vaccine study. Your child, ________, born ________ has the following blood test results:

- Anti-HBc: Positive
- Anti-HBs: Negative
- HBsAg: Positive

The blood tests indicate that your child is infected with the hepatitis B virus. We suggest that you arrange to see your physician about these results. Further immunization with hepatitis B vaccine is not indicated.

A copy of the results will be sent to your physician.

Please keep this record of results in your child's immunization booklet. It may be necessary for reference when your child is offered the hepatitis B vaccine through the grade six program.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Dr.

Your patient, , born to has participated in the study on long-term effectiveness of hepatitis B vaccine.

The results of this child's blood tests are as follows:

- Anti-HBc: Negative
- Anti-HBs: 100 mIU/ml
- HBsAg: Negative

We consider this child to have an adequate protective response to the hepatitis B vaccine.

The parents of this child have also been advised of the blood test results. Please feel free to discuss these results with me at 822-2772 if you have any questions.

Yours truly,

Dr. Richard Mathias
Principal Investigator
Dear Dr.,

Your patient, ________, born ________ to __________ has participated in the study on long-term effectiveness of hepatitis B vaccine.

The results of this child's blood tests are as follows:

- Anti-HBc: Positive
- Anti-HBs: 50 mIU/ml
- HBsAg: Negative

We consider that this child has had a past infection with the hepatitis B virus, but is not a carrier and now has an adequate antibody response. Revaccination is not recommended.

The parents of this child have also been advised of the blood test results.

Please feel free to discuss these results with me at 822-2772 if you have any questions.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Dr.

Your patient, __________, born ________ to ________, has participated in the study on long-term effectiveness of hepatitis B vaccine.

The results of this child's blood tests are as follows:

- Anti-HBc: Negative
- Anti-HBs: 6.5 mIU/ml
- HBsAg: Negative

The results indicate that this child has not been infected with the hepatitis B virus. However, we consider this child to have below protective levels of anti-HBs and would recommend a booster dose of hepatitis B vaccine.

The parents of this child have also been advised of the blood test results.

If you wish your patient to receive the booster dose, the vaccine can be obtained by leaving a message at 879-7551 local 307. It will be sent to your office by Marian Tomm Pastore for you to administer.

Please feel free to discuss these results with me at 822-2772 if you have any questions.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Dr. [Name],

Your patient, [Name], born [Date] to [Parents] has participated in the study on long-term effectiveness of hepatitis B vaccine.

The results of this child's blood tests are as follows:

- Anti-HBc: Negative
- Anti-HBs: Insufficient blood to test
- HBsAg: Insufficient blood to test

We consider that this child has not been infected with the hepatitis B virus. However, sufficient blood was not obtained to assess the child's response to the hepatitis B vaccine.

The parents of this child have also been advised of this blood test result.

If you have any questions, please feel free to discuss the outcome of this child's participation in the study with me at 822-2772.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
Dear Dr.

Your patient, ________, born ________ to ________ has participated in the study on long-term effectiveness of hepatitis B vaccine.

The results of this child's blood tests are as follows:

- Anti-HBc: Positive
- Anti-HBs: 0 mIU/ml
- HBsAg: Positive

The results indicate that this child is infected with the hepatitis B virus and is considered a carrier at this time.

The parents of this child have also been advised of the blood test results.

Please feel free to discuss these results with me at 822-2772 if you have any questions.

Yours sincerely,

Dr. Richard Mathias
Principal Investigator
APPENDIX 2

RED CROSS REGISTRY FORM 161
# HBIG Follow-up of Neonates from HBV Infectious Mothers

<table>
<thead>
<tr>
<th>Name of Mother:</th>
<th>D.O.B:</th>
<th>E.D.C:</th>
<th>D.O.B:(Baby)</th>
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<tbody>
<tr>
<td>Address:</td>
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<table>
<thead>
<tr>
<th>HBIG</th>
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<tbody>
<tr>
<td>Lot No:</td>
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<tr>
<th>Sample From:</th>
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<th>Vancouver Test Results</th>
<th>NRL Test Results</th>
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<tbody>
<tr>
<td>Mother</td>
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<td>HBsAg (EIA)</td>
<td>HBsAg (IMX)</td>
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<tr>
<td>Mother</td>
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</table>

| Comments:    |            |

<table>
<thead>
<tr>
<th>Physician(s):</th>
<th>Hospital:</th>
<th>Health Unit:</th>
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</table>
APPENDIX 3

STUDENTIZED RESIDUAL PLOTS

Dose one for the Anti-HBc model  163
Number of doses for the Anti-HBs model  164
Dose one for the Anti-HBs model  165
Studentized Residuals for the Anti-HBc Model

580 cases plotted.
NUMBER OF DOSES OF HS VACCINE

707 cases plotted.

Studentized Residuals for the Anti-HBs Model
PLOT OF SRE_2 WITH DOSEONE

# OF DAYS AFTER BIRTH WHEN DOSE1 GIVEN

707 cases plotted.

Studentized Residuals for the Anti-HBs Model