HEPATITIS B VACCINATION IN THE COLOMBIAN AMAZON. EFFECTIVENESS AND FACTORS INFLUENCING VACCINATION COVERAGE.

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ABSTRACT

A vaccination coverage survey was carried out in the Colombian Amazon, a former high endemic area for hepatitis B, involving 3573 children less than 11 years old. It was carried out in Leticia, Puerto Nariño, and Araracuara, both urban and rural areas. Children were selected using a one stage cluster sampling, randomly selecting clusters in urban and rural areas where all children under 11 were surveyed. At the same time blood samples were taken from all children with known vaccination status (n=1603), and from their mother, when she was available (n=812). These samples were processed for hepatitis B surface antigen (HBsAg), antibodies to hepatitis B core antigen (Anti-HBc) and antibodies to HBsAg (Anti-HBs). A sample of children without vaccination data available was also bled to compare their results with those of children with vaccination data.

Full vaccination coverage was found to range between 39% and 69% among different areas while hepatitis B vaccination ranged between 73% and 95%. Factors which improve the likelihood of being fully vaccinated in this study were: Age above one year, living in Leticia, being affiliated to the social security, mother's years of schooling. Health worker's knowledge on vaccine contraindications and perceptions of logistical barriers against vaccination or importance of hepatitis B as a public health problem were also related to full vaccine coverage.

Prevalence of hepatitis B infection reached 5% among those who were bled (82/1603) while HBsAg positive status was 1.6% (26/1603). Since the introduction of the vaccine prevalence of hepatitis B infection has fallen from 40%, an 85% reduction, while carrier prevalence has fallen from 5%, a 68% reduction. Age above 7 years, living in a rural area, birth delivery supervised by other than a MD or nurse, and being born from an Anti-HBc+ mother were the most important general factors related to being infected with HBV. Having an incomplete schedule for hepatitis B vaccine was associated with an increase in the risk of being Anti-HBc or HBsAg+. However, some characteristics of the vaccination process were related to being HBsAg+/Anti-HBc+. Delays in receiving the first dose of hepatitis B after birth and delays to receiving the second dose after the first dose were associated with an increased risk of being HBsAg+/Anti-HBc+. None of these characteristics were related to being Anti-HBc+ alone.

In conclusion, the introduction of a recombinant Cuban manufactured hepatitis B vaccine has produced a marked decline in the high infection prevalence of children in the Colombian Amazon area. A higher coverage has been achieved from the beginning of the program though intervals from birth to first dose and between doses are too long leading to new infections that could have been avoided.

There is still room to make improvements in the control program, including the implementation of a surveillance system of the HBV serological status for pregnant women, in order to ensure better vaccination schemes for those born to infected or HBsAg+ mothers.

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Chapter 1: Introduction

Hepatitis B virus can lead to acute and chronic infection. Infection is transmitted by blood exposure, sexual intercourse, perinatally from mother to child and horizontally during early childhood. It is estimated that more than 300 million people are chronically infected with hepatitis B virus (HBV) around the world. Asia and Africa contain most of the carriers but there are also places in South America where highly endemic transmission occurs. (Hadler S and Margolis H 1993; Hall A 1994; Kane M 1995)

Despite a great number of studies the available data on prevalence of hepatitis B virus infection in Latin America are still incomplete. It is estimated that there are 6 million chronic carriers of whom 20% will die as a direct result of HBV infection consequences. In addition 400,000 new HBV infections occur in Latin America each year of which 10-25% could end in hepatocellular carcinoma. Between 25 to 67% of the infections in Latin America become chronic hepatitis, and it is thought that 440-1000 cases of fulminant hepatitis each year are caused by HBV (Tanaka J 2000; Fay O et al 1990; Silveira T et al 1999).

In Colombia there are 5 well-delimited areas where more than 70% of the population have been infected with HBV. These places are located on the Caribbean Coast, the Pacific Coast, the Amazon basin and the Catatumbo River on the border with Venezuela. A serological study made in 1980 using a representative sample covering about 60% of the population found that HBsAg positivity ranged from 3 to 8% through all age groups. Based on these findings there are 600.000 HBV carriers and at least 4.000.000 people that have been infected with hepatitis B virus in Colombia. Co infection and super infection with hepatitis Delta virus (HDV) are common in HBV carriers living in these highly endemic areas (Gast Galvis A 1955;Buitrago B et al 1986; Buitrago B et al 1986; Martinez M 1991; Ljungreen K et al 1985; Juliao O 1991).

The Amazon department in Colombia has one of the highest rates of hepatitis B infection in the world. More than eighty percent of people living in some rural areas are infected with HBV and more than 8% carry HBsAg. Prevalence of infection in urban areas is less well known. Infection with HDV was also common in this region (Martinez M 1991; De la Hoz F et al. 1992; Gayotto LC 1991; Buitrago B et al 1991)

Despite the availability since the early 1980's of a highly efficacious vaccine against HBV virus, control of this infectious disease remains a serious public health problem in many developing and developed countries around the world. In 1992 WHO recommended that

hepatitis B vaccination should be integrated into national immunization programs (EPI) in all countries by 1997. However many barriers have been found to the global application of this vaccine. The relatively high cost of the biological is one of the most important impediments to its universal implementation. (Kane M 1995; Kane M 1993; Hilleman M 1993).

Colombia started a vaccination program against hepatitis B in the Amazon basin in 1992. Children under five years of age and new-borns were targeted to receive three doses of a Cuban recombinant hepatitis B vaccine using a 0,1,2 months schedule (MINSALUD-INS 1992). The objectives of this program were to decrease the prevalence and incidence of hepatitis B infection in the Amazon and to reach and maintain coverage above 90% in children under five years old. No comprehensive evaluation of the vaccination process has been done since implementation of this measure. Small coverage studies have found lower coverage with hepatitis B vaccine than with other EPI vaccines but factors influencing vaccine coverage have not yet been explored (Revelo D 1995; MINSALUD-INS 1996). These studies were carried out in places where hepatitis B is not recognised as a public health problem.

The Colombian control program does not include Hepatitis B Immunoglobulin (HBIG) at birth for several reasons. One is that Colombia does not have a program of HBsAg screening during pregnancy so the prevalence of HBeAg in childbearing age women is unknown. Another more important reason is that in developing countries inclusion of HBIG would make the control program too expensive to be supported by local funds. This absence of HBIG might reduce the effectiveness of the program in preventing the HBsAg carrier state. Conflicting results have come from studies evaluating the efficacy of hepatitis B vaccine alone to prevent the development of HBsAg carrier status in children born to HBeAg positive mothers. Efficacy using plasma derived vaccine ranged between 60-70% while one study with recombinant vaccine has shown an efficacy of more than 90%. It is important to evaluate in children born to HBsAg positive mothers whether vaccination given under field conditions in Colombia has an acceptable impact on HBsAg carrier rates. Previous studies have been done using controlled conditions to deliver vaccine and most used plasma vaccines. No studies have evaluated the effectiveness of recombinant hepatitis B vaccines against perinatal transmission under field conditions. (Chen H et al 1996; Lee Ch et al 1997; Lee P et al 1995; Lee P et al 1995; Wong V et al 1984; Whittle H et al 1991; Greenberg D 1993). If an approach without HBIG is adequate to control perinatal transmission in a normal EPI program it would encourage development of other control programs in the world without HBIG.

Evaluation is critical for all health promotion and control programs. There are two main reasons for evaluating a vaccination program: to improve it and to determine its effectiveness. Additional reasons are: to demonstrate the worth of a program, to compare different types of programs, to meet the requirements of the funding source, and to provide information about the program. One of the major tasks of an evaluation is to judge a program's merit. A meritorious program has worthy goals, achieves its standard of effectiveness, provides benefits to its participants, fully informs its participants of the potential risks of participation and does no harm. We want to evaluate the hepatitis B vaccination control program in Colombia in all of these terms. (Fink A 1993)

The high costs of hepatitis B vaccine compared with other EPI vaccines has been one of the most important barriers to its implementation in developing countries. This raises the issue that whenever this vaccine is implemented in a national EPI it should be evaluated at least in two aspects, impact and process. The impact of vaccine introduction is generally measured through the evaluation of changes in disease trends. The process should be evaluated studying the patterns of vaccine delivery, by measuring coverage, its trends and whether recommendations from national or local health authorities are followed in the vaccination program. Process evaluation is a very important component of program evaluation since effectiveness of an intervention really depends on how the intervention is implemented and how wide is the coverage in the target population.

The evaluation of a program tries to provide data on the extent to which a program's objectives are achieved. It also answers questions about a program's activities and offers insight into a program's implementation and management. Evaluation generally uses one of two sets of evaluation terms. Some authors use the terms **process, impact, and outcome** to identify types of evaluation used to determine the value of a program. Others authors use the terms **formative and summative evaluation** to describe the evaluation that occurs during the program and after the program, respectively. **Process evaluation** provides documentation during program implementation to make adjustments for improvements of the program. There are no published studies on vaccine coverage with hepatitis B in other Latin American countries despite Cuba, Brazil and Peru having introduced the vaccine in their Expanded Program of Immunisation. Even around the world studies on vaccination coverage are limited and most have been done in developed countries where prevalence of infection is low (Freed G et al 1994; Dobson S et al 1995; Walter E et al 1994; Wong W and Tsang K 1994). Studies of vaccination coverage in Colombia have found that hepatitis B coverage ranges between 20-80% in different populations. Some studies have found a lower coverage against hepatitis B compared to other EPI vaccines (Revelo D 1997; Minsalud

1992). Factors influencing this specific lower coverage with hepatitis B vaccines have not been explored in Colombia. A study from Taiwan reported that coverage against hepatitis B was higher at the beginning of the program but has decreased by 30% due to unknown factors (Chen H 1996). Also in Indonesia coverage with three doses of hepatitis B vaccine is under 60% for unknown reasons. (Milne A 1993).

Impact evaluation assesses the overall effectiveness of a program in producing favourable knowledge, attitudes, behaviour, and health status. Many studies around the world have found that hepatitis B vaccine has very high efficacy, high immunogenicity and is relatively safe. Both experimental and observational epidemiological studies have been used in the evaluation of the vaccine. Most observational approaches used in hepatitis B vaccine evaluations consist of follow up studies where cohorts of vaccinated children and adults have been followed for up to twelve years. These studies have demonstrated that the protective levels of antibodies (> 10 IU/ml) remain for more than 7 years in a high proportion of children (more than 60%). Also it has been shown that high protective efficacy against infection and the HBsAg carrier status last for 10 years or more. (Fortuin M et al 1993; Marion S et al 1994; Mahoney F et al 1993; Chen H et al 1996; Chotard J et al 1992; Lee Ch et al 1997; Wainwright R et al 1997; Lee P et al 1995 page 1685; Lee P et al 1995 page 716; Hadler S et al 1986; Wainwright R 1989; Stevens C et al 1992; Wong V et al 1984; Coursaget P et al 1986; Whittle H et al 1991; Greenberg D 1993).

Despite these encouraging findings, more evaluations are needed. Some questions arise around the efficacy of this vaccine. One of the most important is how long the protection lasts and when a booster is needed. Another important point is whether incomplete schedules provide any protection against infection or the HBsAg carrier status or if delays in dose delivery can affect the effectiveness against these outcomes (Mc Mahon B et al 1993; Hibberd P et al 1993). This last aspect is particularly important considering that under field conditions vaccines are delivered when children come to the vaccination clinics and not when indicated by the vaccination program. Some studies have evaluated immunogenicity of hepatitis B vaccine under different schedules found in the field. They have found that immunogenicity is not affected by delays in vaccine application, however one study in Indonesia showed that delays to receive the first dose after birth can increase the risk of being HBsAg+ (Inskip H et al 1991 page 765; Hadler S et al 1989; Inskip P et al 1991 page 770; Ruff T et al 1995).

Outcome evaluation determines whether the program met the stated long-term goals and objectives, such as reduction in morbidity or mortality rates of the target population. Most

studies on effectiveness of hepatitis B are based on children who have received a full course of hepatitis B vaccine. There are few, if any studies of the effectiveness of the incomplete schedules that are frequently found when evaluations of vaccination coverage (proportion of vaccinated people in a population) are done in developing countries (Cutts F et al 1989; Revelo D 1997). This point is very important since in many developing countries many children do not complete the vaccination schedule. If hepatitis B vaccine provides significant protection against the carrier state even if the schedule is not completed, the likelihood of effective control of the spread of the HBV is increased. On the other hand, if only complete schedules are able to protect against carriage then local health services should make greater efforts to ensure adequate coverage with complete schedules. Cohort studies, one of the most common designs found in hepatitis B evaluation, are very expensive and are threatened by loss of a significant amount of people when the length of follow up is long. Many developing countries are unable to undertake this kind of study to evaluate vaccination programs. Therefore alternatives methods are needed for evaluating effectiveness of hepatitis B vaccine. Case control studies, which have been used to evaluate effectiveness in other vaccines, are an inexpensive and rapid method for continuous evaluation of hepatitis B vaccine. In this particular disease no case control study has been done until now. (Smith P et al 1984; Rodriguez L and Kirkwood B 1990; Comstock G 1994) One problem in designing case control studies to evaluate hepatitis B vaccine effectiveness in children is that cases of infection will be detected mostly by serological methods since in most infections there is no clinical manifestation. Therefore only cumulative incidence ratios (Risk Ratios), not incidence density ratios (IDR), would be estimated from the OR's. However since the expected prevalence of surface antigen is very low the OR closely estimates both measures.

Formative evaluation provides immediate feedback during program planning and implementation to improve and refine the program. It is more comprehensive than process evaluation, since information is collected from a variety of sources. **Summative evaluation** is conducted at the end of the program. It determines if outcomes or aims of the program were met. Outcome and impact evaluation are considered forms of summative evaluation. Our evaluation of the Colombian hepatitis B vaccination involved process, impact and outcomes thus being summative and formative. (McKenzie J. and Smeltzer J. 1997; Fink A 1993)

One important neglected issue in evaluating hepatitis B vaccine in the field is the absence of information regarding children's exposure to hepatitis B in most post licensure studies. Only a few studies have considered the mother's serological status in the design and just one has considered other variables such as time at first dose, country of birth, the mother's age at

child's birth and other socio-economic variables. Many of these factors have been associated with hepatitis B infection in the pre vaccine era studies (Marion S et al 1994; Hadler S and Margolis H 1993).

In order to evaluate the vaccination process in the Amazon department we designed a coverage survey in rural and urban areas aimed at measuring vaccine coverage with hepatitis B vaccine and other EPI vaccines such as measles, yellow fever and DPT. In addition to coverage we wanted to evaluate if the vaccination process was following the recommendations issued by the Ministry of Health. We also collected data on factors thought to influence vaccine coverage from parents and health workers. I will compare coverage with hepatitis B vaccine with that of other EPI vaccines and try to identify barriers against timely, complete vaccination with hepatitis B.

In addition a sero-epidemiological survey in children less than 10 years old living in areas endemic for HBV was done. This study measured prevalence of infection with HBV and prevalence of HBsAg positives in children and their mother allowing us to stratify the vaccine's effectiveness by serological status of mothers. Factors related to being HBV infected or HBsAg+ were also assessed. These variables included vaccination, individual, and mothers characteristics.

Chapter 2: Literature review.

I. Hepatitis B vaccines.

I.1. Vaccine development: In 1971, Krugman reported that a crude HBV- containing serum that had been diluted 1:10 and heated at 98 degrees for one minute induced measurable antibody in a majority of human recipients following one or more injections of the preparation. Additionally challenge studies showed that 59% of children previously vaccinated with inactivated serum were completely protected against infection after having been challenged with infectious unheated serum. This was the first step in the development of plasma derived hepatitis B vaccines and the most important development after Blumberg's discovery of the "Australian Antigen." (Hilleman M 1993)

A short time after Krugman's experiment it was possible to develop a standardized technique of purification to produce plasma-derived vaccine on an industrial scale. Hepatitis B vaccine should contain only purified subunits of the HBV surface envelope. However, since the plasma of persons with chronic HBV infection contains both virulent HBV particles and non-infective HBsAg particles, protocols were developed to eliminate HBV or any other virus during the purification of HBsAg. The plasma-derived vaccine was produced by ultracentrifugation of sera from HBsAg carriers. The ultracentrifugation concentrated the 22 nanometre (nm) HBsAg particles, which consist of excess, non-infectious surface antigen protein. The particles were then heated and treated with one or more chemicals including 5M urea, pepsin at low pH, and formalin to inactivate any infectious material in the preparation.. Aluminium hydroxide is added as an adjuvant. (Mac Mahon B and Wainwright R 1993)

In 1975 the vaccine was considered sufficiently well developed to justify first trials in humans. The first clinical efficacy trial was initiated in 1978 by Szmuness et al. in a study conducted among male homosexuals in New York. A second study was performed by Francis et al. from the CDC also in homosexual males using a multi-centre design. (Szmuness W et al 1981; Francis D et al 1981; West D 1993)

Although the production process for this vaccine was state-of-the-art and unique to any vaccine then, the acceptance of the vaccine was very slow, partly because the biological source material was highly infectious for hepatitis B. In addition there were many concerns about the availability of sufficient suitable HBsAg donors. The production process was time consuming and the manufacturing cycle was as long as one year. It was recognized that a second generation of hepatitis B vaccines were needed. There were unsuccessful attempts to

produce enough hepatitis B surface antigen from E. coli. In 1981 it was possible to transfer the portion of the HBV genome coding for HBsAg to an appropriate plasmid that is then inserted in *Saccharomyces cerevisiae*, the common baker's yeast. In 1986 a recombinant DNA (rDNA) vaccine was licensed for human use even though plasma derived vaccine remained available around the world. Most licensed recombinant vaccines consist of the 226 aminoacid S gene product (major surface protein) of HBV. (Sitrin R et al 1993).

Although the HBsAg in both types of vaccine consist of 20 to 22 nm subvirion particles composed of 226 aminoacid there are subtle differences between the plasma derived and recombinant vaccines: 1) the type of lipids present in the HBsAg differ; and 2) approximately 25% of the plasma derived HBsAg is glycosylated whereas the recombinant HBsAg is non-glycosylated. The HBsAg in both vaccines is adsorbed to aluminium hydroxide, and thimerosal is added as a preservative. Both kinds of vaccine have been demonstrated to be safe and immunogenic even though plasma derived vaccines produce higher geometric levels of anti-HBs. (Greenberg D 1993; Dandalos E et al 1985; Hilleman A 1987; Papaevangelou G et al 1985; Mc Aleer W et al 1984).

The decision to select yeast rather than mammalian cells as the target system in which to produce HBsAg was prompted by several important considerations. The yeast cell is considered a less fastidious and expensive medium requirement. Indeed, yeast can be grown in completely synthetic media, thereby offering greater advantages with vaccine purity since no product of biological origin need be employed during the production process. The use of yeast cell technology results in higher productivity and operating cost as compared with mammalian cell systems. Moreover animal cell cultures are more prone to contamination than yeast cultures and require more stringent operating procedures. Finally, the yeast system can be easily scaled up to several cubic metres such that the yield of HBsAg antigen per litre of fermentation broth is greater by a factor of 10 than that achieved in well-established mammalian cell lines. (Stephanne J 1990)

The Colombian EPI uses a Cuban recombinant hepatitis B vaccine that is also produced in yeast cells. The vaccine is manufactured in the Centro de Ingenieria Genetica y Biotecnologia de la Havana (Cuba). Before being introduced in Colombia the vaccine was tested for immunogenicity and safety using workers from hospitals and the Colombian Ministry of Health. In these studies (open clinical trials) it was demonstrated that the Cuban vaccine was as safe and immunogenic as a Belgian manufactured recombinant HBV vaccine (Hoyos A et al 1991; Juliao O et al 1991)

I.2. Efficacy and safety of hepatitis B vaccines: In 1975 the first efficacy study was carry out in a haemodialysis centre. Three doses of vaccine were administered monthly to 46 haemodialysis staff. 66% developed anti-HBs after the complete course of vaccination and none developed hepatitis B. Then 217 people were vaccinated and followed to evaluate efficacy. 5% were infected among the vaccinated while among an unvaccinated control group more than 50% become infected with HBV. $p<0.001$ (Tuoisi C et al 1993).

In 1978 Szmuness conducted the first large-scale study on efficacy of hepatitis B vaccine. A randomised placebo controlled double blind trial was done among homosexuals males. A 40 ug dose was administered intramuscularly at 0, 1, 6 months to 549 individuals. A similar group received only placebo. After 26 months of follow up 158 episodes of HBV infection were recorded. 80% of them occurred in the placebo group and most cases among the vaccinated occurred before day 105 after randomisation, indicating that their infection probably had taken place near the time of initial vaccination.($p<0.001$) (Szmuness W et al 1981).

CDC conducted another randomised double blind trial in homosexual males ($n=1400$) but using a reduced dose of vaccine (20ug). Efficacy results were similar to those obtained by Szmuness et al. After 15 months 2% of the vaccinated ($n=712$) had evidence of infection compared to 11% of the placebo group ($n=688$) ($p<0.001$). In other high risk subgroups of the population studies showed a similar high efficacy. In haemodialysis patients and staff, Guesry, Szmuness and Stevens found high seroconversion rates, more than 90%, while efficacy ranged from 53-85%. (Szmuness W et al 1982; Dienstag J et al 1984; Desmyter J et al 1983; Coutinho R et al 1983; Stevens C et al 1984). Table 2.1 shows a summary of results in studies of hepatitis B vaccination in adults.

In 1983 Beasley and co-workers randomised 243 infants to receive one of three schedules using HBIG and plasma derived HB vaccines or placebo. Only 5.7% of children receiving both vaccine and HBIG developed the carrier state while among controls 88% became HBsAg carrier. In 1984 a randomised placebo controlled trial involving 189 infants of HBsAg/HBeAg positive mothers was made in Hong Kong. The randomised groups were allocated to receive plasma derived vaccine + HBIG, or plasma derived vaccine alone, and placebo. Protective efficacy was 90.7% in the combined group that received only one dose of HBIG, 96% in those who received 7 doses of HBIG and 71.3% among those who receive vaccine alone. ($p<0.005$.) (Beasley R et al 1983; Tuoisi C et al 1993). Table 2.2 displays a summary of results in some studies on efficacy of hepatitis B vaccine in children.

Other studies using only vaccine have shown a protective efficacy ranging from 45.5% to 75.3% indicating that protection from vaccine is lower than protection afforded when HBIG is used together with vaccine. However these studies have been done in countries where the amounts of HBV DNA amongst carrier mothers vary leading to different probabilities for perinatal transmission. Therefore generalizations from these results could lead to erroneous interpretations. (How H et al 1980; Xu Z et al 1985; Poovorawan Y et al 1989; Stevens C et al 1987).

There are relatively few studies on infants of HBsAg + and HBeAg negative mothers. In a study from China, 193 children born to HBsAg + mothers were randomly allocated to receive 20 ug of two brands of plasma vaccine. One of them was made in the Beijing Institute of Vaccines and Serum (BIVS) and the other at the National Institute of Allergy and Infectious Disease (NIAID). Two groups received just vaccine while another received vaccine + HBIG and the last one placebo. In the group of HBeAg + mothers, 20% of children developed chronic carrier status among those who received vaccine alone compared with 6% among those who received vaccine + HBIG. Sixty six percent become HBsAg positive among those who received placebo. In the groups of HBeAg negative mothers there were no differences in the proportion of children who became carriers. Five percent developed the carrier status among those receiving vaccine alone, 15% among those receiving vaccine plus HBIG and 6% among the placebo group.($p>0.05$.) Poovorawan et al. in Thailand found that just 4% of children born to HBeAg+ mothers developed the chronic carrier state after receiving 10 ug recombinant plasma vaccine alone. Poovorawan used four doses given at birth, 1, 2 and 12 months while in China three doses were used at birth, 1 and 6 months of age. More studies comparing schedules at birth are needed on this point. (Poovorawan Y et al 1990; Tong M et al 1984; Schalm S et al 1989; Xu Z et al 1995).

Long-term studies on the effectiveness of hepatitis B vaccines have been done in infants who were vaccinated under one year. Most of them show high protection against the carrier status even after a follow up of between five and ten years. In Senegal 143 children were followed for 6 years after vaccination and 4 children were HBsAg positive among vaccinees. In Hong Kong 183 infants were followed for 5 years and only one became HBsAg positive after one year of age. In The Gambia after three years of follow up, less than 1% of children vaccinated before one year had become carriers and just 5% were infected. The main predictor of infection was the serological status of the mother. The probability of being infected was higher among those whose mothers were HBeAg positive. After nine years of follow up in The Gambia, 8% of vaccinated children have developed infection measured by positivity for anti-HBc, and 1% have become carriers. Compared to a control group taken

from non vaccinated villages in The Gambia, HBV vaccine has a cumulative protective efficacy of 75% against infection and 90% against carriage (Hall A et al 2000; Yeoh E et al 1988; Delage G et al 1988; Lo K et al 1988; Whittle H et al 1991).

The peak of hepatitis B antibody reached after the first course of vaccine is strongly correlated to protection. In The Gambia, Jack et al. have found that there is a significant linear trend relationship between the peak after three doses of plasma derived vaccine and protection against infection. Those who reached titres between 10 and 99 mIU/ml have almost three times less risk of becoming core antibody positive than children with less than 10 IU after primary vaccination. Those with titres 100 to 999 mIU/ml had 10 times less chance to becoming infected and those with titres above 1000 mIU/ml had 20 times less probability of infection. An interesting finding from this study is that children with titres of anti-HBs above 1000 mIU/ml can become infected but most of them lost the marker of infection. This study also shows that there is no absolute protection for any titre of antibody. (Jack A et al 1998)

Studies in infants suggest that while HB vaccine provides excellent long term protection for 4-6 years, HBsAg positive breakthrough infections in vaccine responders may have occurred. Most of these studies show that most children who became HBsAg + during follow up did not respond adequately to the vaccine. It is unknown how many of these HBsAg breakthrough infections are due to mutants strains of the virus and what is the potential transmissibility of these mutants in the vaccinated population. (Hall A 1994; Chotard J et al 1992; Coursaget P et al 1986).

In addition to its high efficacy the plasma-derived vaccine was well tolerated. In most healthy adults vaccinated the most common reaction was mild transient discomfort at the injection site. With the emergence of AIDS there was concern that hepatitis B vaccines might be contaminated with HIV. This concern was quickly discarded since several follow up studies in homosexual and health care workers showed no evidence of HIV sero-conversion among vaccine recipients. In a surveillance system created by CDC to monitor rare neurological events associated with the vaccine the occurrence of Guillian Barre Syndrome was slightly more frequent than expected (9 cases observed vs 4 expected. p=0.01 using Poisson distribution). However this association was not consistent throughout the analysis and there was no conclusive evidence for a causal role for the vaccine. Two reports link hepatitis B vaccination with anaphylaxis and there is a case report linking the recombinant vaccine to Multiple Evanescent White Dot Syndrome, a rare retinal condition. However benefits from hepatitis B vaccination overwhelmed potential dangerous side

effects. (West D 1993; Shaw F et al 1988; CDC 1996; Stratton K et al 1994; Mc Mahon B et al 1992; Baglino E et al 1996).

Table 2.1. Summary of studies of hepatitis B vaccine efficacy in populations other than newborns.

Author	Place	Population	Age	# Vaccinated	Type of vaccine	Dose	Vaccine schedule months	Control Group Y/N	Length of follow (y)	Infected # (%) ***	Carrier # (%)	Protective Efficacy
Goudeau A 1980	France	Health Workers	μ=30 y	482	plasma	10 μg	0-1-2-12	N	1	7 (1.5)	0	-
Goudeau A 1980	France	Hemodyalisis patients	μ=46	167	plasma	10 μg	0-1-2-12	N	1	22 (14)	5 (3)	-
Szmuness W 1981	USA	Homosexuals	μ=30 y	549	plasma	40 μg	0-1-6	Y	2	32(6)	12 (2.6)	81% infection 89% carrier
Szmuness W 1981	USA	Health Workers	Adults	442	plasma	20 μg	0-2-6	Y	2	9 (2.2)	0	77% infection 91% disease
Guesry P 1982	France	Hemodyalisis staff	μ=30 y	184	plasma	5 μg	0-1-2	Y	1	6 (3.6)	0	71% infection 100% carrier.
Guesry P 1982	France	Hemodyalisis patients	μ=48 y	48	plasma	5 μg	0-1-2	Y	1	10 (21)	8 (17)	53% infection 54% carrier
Coutinho R 1983	Nether lands	Homosexuals	16-50 y	397	plasma	3 μg	0-1-2	Y	2	11 (3.1)	9 (2.7)	84% carrier 80% infection
Desmyter J 1983	Belgium	Hemodyalisis patients	μ=53 y	201	plasma	3 μg	0-1-2-5	Y	1.5	7 (4)	6 (3)	86% infection

Author	Place	Population	Age	# Vaccinated	Type of vaccine	Dose	Vaccine schedule months	Control Group Y/N	Length of follow (y)	Infected # (%) ***	Carrier # (rate)	Protective Efficacy
Desmyter J 1983	Belgium	Hemodyalisis staff	μ=30 y	76	plasma	3 μg	0-1-2	Y	1.5	2 (1.8)	0	100% carrier**
Stevens C1984	USA	Hemodyalisis patients	Median 50 y	562	plasma	40 μg	0-1-6	Y	2	35 (4)	8 (1.3)	0%
Hadler S. 1986	USA	Homosexuals	Adults	751	plasma	20 μg	0-2-6	N	5	55 (7)	2 (0.2)	-
Wainwright R 1989.	Alaska	Eskimos	All age groups	1630	plasma	10 μg 20 μg	0-2-6	N	5	4 (0.2)	0	88% carrier. *
Wainwright R 1997.	Alaska	Eskimos	All age groups	959	plasma	10 μg 20 μg	0-2-6	N	10	22 (0.2)	0	-

*** Historical prevalence ** No significant *** prevalence among vaccinated. μ=means**

Table 2.2. Summary of studies on hepatitis B vaccine efficacy in children or newborns.

Author	Place	Age	# Vaccinated	Type of vaccine	Dose µg	Vaccine schedule months	Control Group. Y/N	Length of follow (y)	Infected # (%)	Carrier # (%)	Efficacy
Goudeau A 1980	Senegal	0-2 y	149	plasma	10	0-1-2-12	Y	2	3 (3)	2 (2)	83% carrier 85% infection.
Wong V 1984	Hong Kong	newborn ε	216	plasma + HBIG	10	1-2-6 0-1-2-6	Y	2 y		(3)λ (7)λλ (21)φ (73)φφ	96% carrier 90% carrier 70% carrier
Chung W 1985	Korea	newborn ε	36	plasma + HBIG	10	1-2-6	Y*	1 y		5 (14%)	83% carrier**
Stevens C 1992	USA	newborn ε	679	plasma	20	0-1-6 0-2-6	N	9 y	(15) **	(8.2) **	88% carrier**
					10	0-1-6 0-2-6					
				recombinant	5	0-1-6 1-2-9					
Coursaget P 1986	Senegal	0-2 y	135	plasma	10	0-1-2-12	Y	6 y	13 (13)	5 (4)	77% infection.

Author	Place	Age	# Vaccinated	Type of vaccine.	Dose µg	Vaccine schedule months	Control Group. Y/N	Length of follow (y)	Infected # (%)	Carrier # (%)	Efficacy
Whittle H 1991	Gambia	< 5 y	358	plasma	2 10 20	0-2-6 1-2-4-9 0-2-6 *** 0-2-6δ	Yι	4 y	(5.3)	2 (0.6)	97% carrier** 88% infection 75%ιι
Inskip H 1991	Gambia	newborn	1173	plasma	10	0-2-4-9	N	1 y	7 (0.6)	7 (0.6)	-
Chotard J 1992	Gambia	newborn	704	plasma	10	0-2-4-9	N	3 y	12 (1,7)	4 (0.5)	95% carrier. *
May A 1998	Gambia	9 y	677	plasma	10	0-2-4-9	Y	9 y	56 (8)	4 (0.5)	74% infection 90% carrier
Lee P 1995	Taiwan	newborn	224ε 105″	plasma + recombinant +HBIG	20 10	0-1-2-12 P,R,R,R P,P,R,R P,P,P,P	N	14 mths		20 (8.9)ε*	89-93% carrier *
Lee P 1995	Taiwan	newborε	171	recombinant + HBIG	20 10	0-1-2-12 0-1-6 0-1-2-12	N	5 y	19 (12)*	6 (4)**	96% carrier. *
Lee P 1997	Taiwan	newborn	103″ 58ε	recombinant + HBIG	20	1week-6 week-5.5month	N	9 mths	0″	0″ 4 (7)ε	92% carrier*

Author	Place	Age	# Vaccinated	Type of vaccine.	Dose µg	Vaccine schedule months	Control Group. Y/N	Length of follow (y)	Infected # (%)	Carrier # (%)	Efficacy
Chen H 1996	Taiwan	1-10 y	1515	plasma recombinant	5 20	0-1-2-12 0-1-6	N	cross-section	(4)	(1.2)	-
Fortuin M 1993	Gambia	newborn	720	plasma	10	0-2-4-9	Y	4 y	33 (5)	4 (0,6)	85% infection ιι 97% carrier
Lo K 1988	Taiwan	newborε	199	plasma	5 2.5	0-1-2-12	N	5 y	0	0	
Del Canho R 1992	Nether-lands	newborε	114	plasma +HBIG	5-10	0-1-2-11 3-4-5-11 0-1-6 0-1-6	N	8 y		8 (7%)*	92% carrier**
				recombinant +HBIG	20	3-4-5-11					

****. No statistical differences between groups. *. using historical controls ***. the first two doses IM and the third one ID. δ . all doses IM. ι. Not randomised. ιι. Using controls selected for the study . λ. 7 dose of HBIG + vaccine. λλ. 1 dose of HBIG+ vaccine. φ. Only vaccine φφ. Placebo**

″ Mothers negative ε Mothers HBeAg+

I.3. Dose Schedule:

The standard adult regimen for plasma derived hepatitis B vaccine is 20 ug (40 ug for dialysis patients) administered by intramuscular injection at intervals of 0,1,6 months. For healthy neonates it is 10 ug given in the first seven days after delivery followed by two doses at the second and sixth months. Also schedules using 0,1,2 months as intervals have become widely used in endemic areas or among high-risk groups where a rapid protection is needed. In the latter scheme titres of antibodies are lower than in the first one and a booster at 12 months has been recommended. However recommendations on the need of booster are not widely accepted because the role of immune memory. (Safary A and André F 2000;West D 1993; Mc Lean A 1986; Prozesky O et al 1983).

One important question with hepatitis B vaccine is whether time between doses could affect the effectiveness of the vaccine. Data available from field studies in The Gambia and Venezuela show that variations in vaccination schedule do not influence the protective level of antibodies. (Inskip H et al 1991; Hadler S et al 1989). However these studies were done focusing on antibody level and not on effectiveness against infection or carriage status.

Studies of other vaccines such as DPT show that efficacy depends on the vaccination scheme used. DPT efficacy is lower when only 1 or two doses are applied instead of the three recommended doses. Also the interval between doses has been demonstrated to influence the quality of the immune response to this vaccine. (Fine P and Clarkson J 1987; Halsey N and Galazka A 1985)

After 5-7 years of follow up in most studies, 50-80% of the vaccinated had titres above 10 IU/L.. After nine years of follow up, 75% of vaccinated children in Gambia still have antibodies above 10 IU/L and the GMT was 19 IU/L. It is possible to calculate the mean duration of antibodies in a vaccinated population using these data from Gambia. In the first year after vaccination the GMT reached 2068 IU/L and 98% of children had titres above 10 IU/L. Using exponential models it is possible to predict that after 15 years of the primary vaccination less than 50% of children will have titres above 10 IU/L and GMT will fall to less than 5 IU/L after 13 years of being completed the scheme. Given this assumption it is necessary to keep an ongoing system of monitoring HBV vaccine efficacy to assess if protection remains when most people in the population have lost their antibodies. Since the age when these individuals lose their antibodies is probably above 15 years the chance of clinical illness with HBV infection increases and those studies that attempt to monitor the vaccine efficacy should include this effect in their main outcomes. (Viviani S et al 2000). However most of these studies have been

done on plasma derived vaccine and there are few data above five years for recombinant vaccine, which induces a lower response after the primary course of vaccination. Also a large loss of people to follow up has been observed in homosexuals and population based studies

A booster dose of the vaccine clearly induces antibody response in 90-100% of healthy adults and children who received a primary vaccine series several years earlier. Even those whose titres have decayed below 10 mIU/ml when the booster was given respond adequately. However titres reached after this booster, are not higher than those reached with the preliminary scheme. (Krugman S and Davidson M 1987; Moyes C et al 1990).

There is no agreement about the need for providing a booster dose 5-10 years after the primary course of vaccination in those who reach titres above 100 mIU/ml.. The first indication for a booster is to augment an inadequate or non-response to the basic immunization series. Clemens et al. used boosters of 20 ug of a recombinant vaccine every two months in 79 low responders and 83 non-responders to a previous complete course of hepatitis B vaccine. All of them produced serological titres of anti-HBs above 100 mIU/ml after the second and third booster. Goldwater compared the effectiveness of two doses 40 and 20 ug used as booster in previous non-responders to a complete scheme of HBV vaccine. He found that there were no differences between the two doses and after a second booster half of the people in each group had titres above 10 mIU/ml but the rate of non-responsiveness was high. So, evidence about benefits of more doses in non-responders is inconclusive . Other authors considered that "non-responders" are actually "slow-responders" with different kinetics of humoral response and that most of them do not need boosters. (Clemens R et al 1997; Goldwater P 1997; Safary A and André F 2000; Weissman Y et al 1988)

Most discussion concerns the question whether a booster is needed to raise declining anti-HBs levels after an adequate response to the vaccine has been achieved. Since immunological response differs by age and other characteristics, this question should be addressed looking at the particular risk group concerned. There are enough data showing that the at risk population will be protected against clinical infection by a natural anamnestic response even if their antibodies decline to less than 10 mIU/ml. Resti et al. compare the response to a booster after 10 years following the primary vaccination in two groups of children born to HBsAg + mothers. One group had received a booster at 5 years of age and the other did not. Serological response after the 10-year booster was similar between the two groups suggesting that boosters do not enhance immunological memory before 10 years of age. More studies are needed to determine whether and when to give a booster (Davidson M and Krugman S 1986; Resti M et al 1997; Stevens C et al 1992; Wainwright R et al 1997).

Low doses of hepatitis B vaccine have been proposed as a means to reduce costs of hepatitis B control programs in endemic countries. In some studies schedules using reduced doses were successfully delivered. Goldfarb et al. carried out two studies in healthy infants and children from 0 to 6 years comparing the immunogenicity of 10 ug of a recombinant vaccine against 5 ug of the same vaccine. They used an interval of 0-1-6 months between doses for children above 2 years and a scheme of 2-4-6 months among newborns. They found that GMTs for those receiving 10 ug were 8062 among children from 2-6 years old and 1641 among the newborn. Children who receive 5 ug had the same proportion with more than 10 IU/L (98%) as those receiving 10 IU/L but GMTs were significant lower (3732 and 880 IU/L). (Goldfarb J et al 1996 page 768; Goldfarb J et al 1996 page 764). In other studies low doses also elicit lower titres of antibodies and although it is accepted that more than 10 IU/ml are protective, breakthrough infections are associated in some studies with lower titres. In other words higher titres could reduce the chance of breakthrough infections. However breakthrough infections are not necessarily an important outcome to measure in hepatitis B vaccine effectiveness since the most important outcome of infection is the carrier status. Since lower doses are an attractive alternative to a high cost vaccine it is very useful to study the protection conferred by low doses against carriage in groups such as IVD users, new-borns to HBeAg + mothers and health workers. (Moyes C et al 1987; Milne A et al 1989).

I.4. Vaccine types: It has been demonstrated that the proportion of people who reach protective titres after using recombinant vaccine is similar to the proportion when plasma vaccines are used. They are also as safe as plasma-derived vaccines. (Andre F 1989; West D 1989; Zajac B et al 1986)

Most studies done with recombinant vaccines have had an open design, since high efficacy of the vaccine makes it unethical to use placebo control groups. Based on the well-controlled studies of plasma derived hepatitis B vaccines plus the studies of yeast derived vaccines using historical controls, the induction of anti-HBs titres is now generally viewed as an acceptable surrogate measure of efficacy for s antigen vaccines.

A study in Army recruits in 1984 confirmed that recombinant vaccine was safe and immunogenic even though low titres were reached with recombinant vaccine than with plasma derived vaccine. (Wiederman G et al 1987)

Research on improved immunogenicity of hepatitis B vaccines is focusing on the pre S products of the S gene. It is thought that a vaccine containing both pre S and S antigen might provide a

broader base of protection against hepatitis B infection. Since available hepatitis B vaccines have a high protective efficacy, it is unlikely that vaccines containing pre S antigen could demonstrate a higher efficacy. Probably its role would be limited to those who do not respond well to traditional vaccines containing only S antigen. Leroux Roels et al. compared the effect of a preS1 and preS2 vaccine and a recombinant one in poor responders to a previous course of three doses with a recombinant vaccine. No differences were observed in the proportion with sero-protection nor in GMT. (Leroux Roels G et al 1997).

Some open trials have been done in newborns of HBsAg +/HBeAg + mothers comparing plasma derived vaccines and recombinant vaccines. No statistical differences have been found between these regarding efficacy.

I.5. Immunogenicity: Factors that influence immunogenicity include factors related to the vaccine such as dosage, number and timing of inoculations, storage of the vaccine and the use of adjuvants. The most important host factors are weight, age, antecedent smoking, and presence or absence of chronic diseases such as diabetes, renal failure, HIV, and others.

When different dosages have been compared, those people receiving 40 ugs have shown the highest antibody titres. After 6 months these differences compared to those who received 20 ugs disappeared, and no differences were seen between dosage groups utilising the same schedule. (Hollinger F et al 1981).

Sites of inoculation other than intramuscular in the deltoid region showed inconsistent data. Intradermal inoculation with plasma or recombinant vaccine is safe but has not led to equivalent levels of Anti-HBs titres in most studies. Gluteal administration induces poor response and it is not used. (Bryan J et al 1990; Fessard C et al 1988).

Regarding duration of protection it has been widely demonstrated that antibody levels wane after vaccination. The rate of decline is independent of initial post vaccination titre, but vaccinees with a high starting titre will remain above some bench mark level (e.g., 10 mIU/ml) longer than those with a lower titter.Protection against clinical hepatitis B or antigenaemia lasts longer than do antibody titres. This long protection has been observed in studies on homosexual men as well as in new-borns. However more studies are necessary to determine accurately how long the immunological memory lasts.

There are few studies of efficacy using Cuban recombinant vaccine. In Colombia two studies of immunogenicity were done before licensure. The first was an open trial comparing Cuban and

Belgian recombinant vaccines. Participants were selected from healthy adults working in the Colombian Ministry of Health or the Colombian National Institute of Health. Two hundred and fifty-seven people agreed to participate in the study and were randomly allocated to receive one of the two vaccines. There were two schemes to deliver the vaccine. Those allocated to the first received Cuban or Belgian vaccines in a 0-1-2 months scheme while those in the second scheme were vaccinated at 0-1-6 months. After three doses 100% of individuals vaccinated with the Cuban vaccine had anti-HBs titres above 10 mIU/ml compared with 84% among those receiving the Belgian vaccine. Ninety-eight percent of recipients of Cuban vaccine had titres above 100 mIU/ml compared with 70% among recipes of the Belgian vaccine. There were no statistically significant differences between different schedules with respect to the amount of antibodies elicited. No important side reactions were detected among the vaccinated. (Juliao O et al 1991)

In the other study 32 health workers from a hospital in Bogota received one dose of the Cuban vaccine. All of them had received full schemes of another brand of HBV vaccine before but were unable to mount an appropriate antibody response. After receiving 20 ug of the Cuban vaccine 75% reached titres above 10 IU. (Hoyos A et al 1991)

Table 2.3. Immunogenicity of hepatitis B vaccine in different trials.

Author	Place	Population	Age	# Vaccinated	Type of vaccine.	Dose µg	Vaccine schedule	Length of follow (months)	GMT IU/ml	% with titres > 10 mIU/ml
Guesry P 1982	France	Hemodyalisis staff	µ=30 y	158	plasma	5	0-1-2-12	4	2,433	90%
								12	904	86%
								16	74,473	
Guesry P 1982	France	Hemodyalisis Patients	µ=48 y	48	plasma	5	0-1-2-12	4	121	54%
Lo K 1988	Taiwan	Children	Newborne	199	plasma	5	0-1-2-12	2		98.5%
						2.5		3		98%
								4		98%
								5		97%
Kanai K 1982	Japan	Children	Newborne	56	plasma	15	3-4-5	3	60	
								6	60	
								12	40	
							6-7-8	3	181	
								6	81	
								12	60	

Author	Place	Population	Age	# Vaccinated	Type of vaccine.	Dose µg	Vaccine schedule	Length of follow (months)	GMT IU/ml	% with titres > 10 mIU/ml
Del Canho R 1992	Nether lands	Children	Newborn	631	plasma +HBIG	5-10	0-1-2-11	12	8,730	96%
								24	737	95%
								36	353	93%
								48	207	93%
								60	137	88%
							3-4-5-11	12	15,739	96%
								24	1,728	95%
								36	820	95%
								48	484	97%
								60	331	97%
							0-1-6Φ	12	1,142	95%
								24	331	92%
								36	202	86%
								48	138	83%
								60	100	75%
					recombinant +HBIG	20	3-4-5-11	12	9,699	99%
								24	1125	97%

Author	Place	Population	Age	# Vaccinated	Type of vaccine.	Dose µg	Vaccine schedule	Length of follow (months)	GMT IU/ml	% with titres > 10 mIU/ml
Wainwright R 1989	USA	Eskimos	All ages	1581	plasma	10	1-2-6	60	49	81%
						20				
			0-19 y					60	-	84%
			20-49 y					60	-	73%
			50 + y					60	-	63%
			< 1 y					Initial	208	61%
								60	25	
			1-9 y					Initial	273	84%
								60	60	
			10-19 y					Initial	260	85%
								60	67	
Stevens C 1992	USA	Children	Newborn	NC	plasma	20	0-1-6	Initial	1821	
								60	96	95%
						10		Initial	697	
								60	41	95%
					recombinant	5		Initial	377	
								60	19	
			1 month		plasma	20		Initial	2747	
						10		60	-	96%
								Initial	1132	

Author	Place	Population	Age	# Vaccinated	Type of vaccine.	Dose µg	Vaccine schedule	Length of follow (months)	GMT IU/ml	% with titres > 10 mIU/ml
Stevens C 1992	USA	Homosexuals	-	NC	plasma	20	0-1-6	120		91%
Wong V 1984	Taiwan	Children	Newborn	189	plasma	3	0-1-2-6	12	90	96%
Whittle H 1991	Gambia	Children	1-9 month	233	plasma	10	1-2-4-9	2	5431	97%
			0-4 y	219	plasma	2	0-2-6	2	270	85%
								50	50	77%
						20-2-2	0-2-6	2	555	95%
								50	50	80%
							0-2-6	2	926	100%
						20		50	110	95%
Hall A 1998	Gambia	Children	9 y	677	plasma	10	0-2-4-9	12	2068	98%
								108	19	68%
Xu Z 1995	China	Children	5 y	55	plasma 1	16	0-1-6	12	1000	94%
								60	125	86%
				56	plasma 2	20	0-1-6	12	500	95%
								60	158	81%
				37	plasma 2 + HBIG	20	0-1-6	12	1000	77%
								60	8	60%

Marion S	Canada	Children	μ=5.1 y	717	plasma	10	0-1-6	μ=5.1 y	79.7	88%
1994			2 y						271	
			3 y						238	99%
			4 y						79	99%
			5 y						58	89%
			6 y						37	88%
			7 y						22	80%
			8 y						106	70%
			days at							90%
			first dose							
			1-3						77.7	
			4-7						126	
			8-61						94	
			>61						590	
Goldfarb J	USA	Children	Newborn	76	recombinant	10	2-4-6	2	1641	98.5 %
1996										
				77		5		2	880	98.5%
Goldfarb J	USA	Children	2-6 y	93	recombinant		0-1-6	2	8062	98.3%
1996										
								2	3732	98.4%

Φ Children who received 5 μg of vaccine had less titters than those receiving 10 μg until 24 months, then differences vanished. NC. No clear.

I.6 Perspectives: There are concerns about the feasibility of eradicating hepatitis B despite the availability of this highly efficacious vaccine. Some authors have stated that eradication is not possible since there is a large mass of carriers and the existence of non-responders to the vaccine. Intrauterine infection is another barrier to eradication as a possible goal. Lack of knowledge about how long protection remains when given by a complete schedule of hepatitis B is another important barrier for control. However recent analysis of dynamics of hepatitis B infection using mathematical deterministic models has shown that eradication is theoretically conceivable (Edmunds W et al 1996; Anderson R and May R 1991; Anderson R and May R 1990; Anderson R 1992). Even using less complicated models we can assume that vaccine coverage above 70% could eradicate hepatitis B if coverage levels are preserved for sufficient time. See Table2.4.

Table 2.4. Ro values for hepatitis B in Colombia and proportion of people to be vaccinated to reach eradication.

L (y)	A (y)	D (y)	Ro	po
65	40	0.8	1.65	30%
55	25	0.8	2.27	50%
45	15	0.8	3.16	70%
45	10	0.8	4.9	80%

L= Average life expectancy
A= Average age of infection
D= Duration of maternal antibody
Ro= Basic Reproductive Number= L/A-D
po= Proportion to be vaccinated = 1-1/Ro

Edmunds et al. developed a dynamic transmission model of HBV to investigate some of the implications of losing vaccine-induced immunity on effectiveness of mass HBV vaccination in high endemicity countries. After running the model for 150 years of continuing vaccination, the prevalence of carriers falls to less than 2% even if the effect of vaccine lasts for only 3 years. However the paper does not state how long it will take to reach 0 prevalence of carriers if vaccine last for longer periods of time. The model also analyses the effect of vaccination on prevalence of acute disease. An increase in prevalence of acute disease, in the long term, appears unlikely regardless of coverage of vaccination or duration of vaccine induced-protection. In the short term, some assumptions in the model such as a low coverage (<60%) and non-permanent protection yielded an increase in the prevalence of acute disease. They

compare a control program that gives a booster 5 years after the primary vaccination against one in which no booster is given. The proportion of carrier projected for 80 years decrease in the same proportion in both programs. Just a little marginal difference is observed in the program that used booster. (Edmunds W et al 1996)

In the Colombian program only recombinant vaccine is being used by the EPI. Other programs in Africa use hepatitis B vaccine without HBIG and they have reported high effectiveness in control of HBV in the first year after delivery even though vaccine is not applied at birth. The impact of a program using vaccine alone depends on whether the prevalence of HBeAg/HBsAg positive mothers is high or not in the general population and this data is unknown in Colombia. Also we do not know if field workers are delivering the vaccine soon after birth. If perinatal transmission is important in the Colombian Amazon and vaccine is applied late after birth it is unlikely to prevent as many HBsAg carriers as we would expect.

In children from endemic countries in Asia and Africa horizontal transmission of HBV is more frequent than perinatal. Epidemiological studies in the prevaccination era showed that at least 50% of persons who became chronic carriers are infected after birth. In Senegal 50% of children infected under the age of 2 years become chronic carrier before vaccine was available. In Alaska about 30% of those infected under five years become HBsAg carriers. (Coursaget P et al 1987; Mac Mahon B et al 1985; Beasley R et al 1982; Maupas P et al 1981)

Hepatitis B vaccine can be administered in conjunction with other EPI vaccines, except measles. In fact one of the strategies used to improve vaccination coverage is to combine it with other vaccines such as DTP and Haemophilus influenzae b (Safary A 2000).

I.7 Barriers against hepatitis B vaccination: Extensive studies on barriers to completion of vaccine schedules have been carried out in developing and developed countries, especially on measles, oral polio and DPT vaccines. Cutts et al. (Cutts F et al 1992), have grouped the causes for low immunisation coverage in the USA in those associated with consumer demand and the supply of immunisation services.

Consumer demand for immunisation services is affected by the following factors:

- **Health beliefs**: This has four components, "perceived susceptibility, "perceived severity, "perceived benefits" and, "perceived barriers". The last component has the most important impact on the acceptance of vaccines.

- **Socio-economic status:** Economic and demographic measures of socio-economic status (parental education, income, family size and race) have been found repeatedly to be strong predictors of vaccine coverage.

The supply of immunisation services is affected by barriers to utilisation of immunisation services, the frequency of missed immunisation opportunities, and the existence of follow up systems.

Multiple methods have been used to assess performance of immunisation program around the world. These evaluation techniques include qualitative and quantitative approaches such as key informants, focus groups and cluster surveys. It is concluded that no one study methodology is ideal and it is recommended that evaluation methods should be combined to obtain more reliable results. Qualitative methods have the general advantage that they are quicker and simpler and frequently yield the same information as more complicated quantitative methods. Qualitative methods are specially suited for evaluating knowledge, attitudes and practices. Quantitative methods such as cluster surveys have the advantage that they allow a better control of sampling errors for those outputs where this is important such as coverage by vaccination. (Cutts F et al 1990 page 769; Cutts F et al 1990 page 199; Cutts F et al 1991; Cutts F et al 1989)

There are few studies concerning how the vaccine has been integrated into the EPI. Most of these studies have been done in Africa and Taiwan. They showed that integration of the vaccine is possible but in other countries this integration has been less successful due to unknown factors. (Schoub B et al 1991). Some evaluations made at the beginning of the program showed that coverage with hepatitis B vaccines were as high as those reached with other EPI biologicals. However in countries such as Taiwan coverage with hepatitis B vaccine has decreased from 83% to 67%. Factors associated in Taiwan with poor compliance to hepatitis B vaccine schedules were: younger age and lower education and career of the parents. The order of the children in the family was also an indicator of vaccination status and the higher order was linked to lower rates of full immunisation schedule. Children born in winter or autumn also have lower coverage than those born in spring or summer and more urbanised families had lower coverage. Parents' attitudes and knowledge about vaccines were also related with achieving full immunisation. Attitudes and knowledge about hepatitis B infection did not influence uptake of HBV vaccine while many missed opportunities were found. (Wong W and Tsang K 1994).

In other countries such as Indonesia, coverage with three doses of HBV vaccine was lower at 60%. In South Africa an assessment in 1991 showed that coverage with hepatitis B was only 39% while coverage with poliomyelitis vaccine was above 90% (Schoub B et al 1991). Reasons

invoked for this include a separate system for HBV vaccine distribution that resulted in a shortage of vaccine in vaccination points. Also there were complaints from mothers and health workers about the need for extra injections for hepatitis B. However no efforts were made to understand specific reasons for the sharp drop in coverage. Also in some high and low endemic areas in Colombia, coverage with hepatitis B vaccine is lower than with the other standard EPI vaccines (Revelo D 1997; Minsalud 1996). An evaluation is necessary to determine the factors leading to this dissimilarity between hepatitis B vaccine and the others.

In developed countries coverage with HBV also varies widely. In British Columbia a universal school based hepatitis B vaccination program shows high coverage with three doses of vaccine, above 90%. However school based programs are not very useful in highly endemic countries were heavy transmission occurs before children go to the school. Studies from USA show that following recommendations from CDC about universal immunisation coverage increased from 1% in 1989 to 32% in 1993. Despite this significant increase universal immunisation has been not achieved in the USA. (Dobson S et al 1995; Woodruff B et al 1996).

Some evaluations of vaccination barriers have been done in other developed countries (mainly USA). These studies focused on attitudes and beliefs of paediatricians and family doctors about the need to implement universal vaccination against HBV. It has been shown that paediatricians and family doctors have an aversion to multiple injections that is reflected in a low coverage with hepatitis B vaccine in children attending their clinical practice. In one of these studies only 53% of paediatricians and less than 30% of family physicians had adopted universal immunisation into practice. Fragmentation of health care provision has also been identified as a major barrier in developed countries where the first dose of vaccine is applied by hospital teams and following doses are delivered by others. Other barriers identified are related to infant's health conditions, age and education of the mother, patient failure to return for second and third dose and parental refuse of immunisation. (Freed G et al 1994; Walter E et al 1994; Woodruff B et al 1996; Bertolino J 1996)

II. Epidemiological aspects of hepatitis B virus infection in Colombia.

Colombia has some recognised zones where transmission of hepatitis B virus is highly endemic. These areas have been identified through serological and histopathological studies and are located in different geographical areas of the country. The main characteristics of these places are described below.

II.1. North part of the country (Zone bananera de Santa Marta): around the Sierra Nevada de Santa Marta and close to the Caribbean Sea, outbreaks of fulminant hepatitis due to coinfection by delta virus and HBV have been identified as early as in 1920. These coinfections were diagnosed in 1985 using a large collection of liver specimens collected by Doctor Augusto Gast Galvis, a former pathologist from the CNIH. People who live in these areas are a mixture of black, Indian and Spanish people. (De la Hoz F et al 1991; De la Hoz F et al 1996; Bauer J and Kerr J 1933; Aguilera A et al 1987; Gast Galvis A 1955; Buitrago B et al 1986; Buitrago B et al 1986; Ljungreen K et al 1985)

II.2. Serrania de los Motilones: close to the Catatumbo river, in an area shared with Venezuela delta and hepatitis B viruses has been detected through serological studies conducted together by Colombian, Venezuelan and CDC scientists. The most affected people in the area are Motilones and Yucpas, two of the most important Indian families in Colombia. At the beginning it was supposed that only aboriginal people were being affected by these viruses in the area, however serological studies in zones around the Indian reserve have shown a high prevalence of hepatitis B virus infection in people living there who are not Indians. Chronologically this endemic focus has been recognised later than the first described above. Oral history from the oldest people in the tribes estimated the first epidemic of fulminant hepatitis around the mid years of 1960. (Buitrago B 1991; Hadler S et al 1991)

II.3.The central region of the country: in the heart of the department of Antioquia where gold mining activity was carried out at the beginning of this century. Fulminant hepatitis was discovered at the same time as in the Sierra Nevada de Santa Marta. This focus has decreased its activity for unknown reasons, but probably because this department has improved its economic development since the early 1950's with an important development in public services such as running water, excreta disposal, etc. Most people living here are descendants from Hispanic people. (Buitrago B 1991)

II.4. The Amazon Basin: in the border area with Peru and Brazil outbreaks of fulminant hepatitis have been detected here since around the 1950's. Most outbreaks in Colombia have occurred on the banks of the Putumayo River, a tributary of the Amazon river and the most affected people have been those from Tucanos, an aboriginal ethnic group in the area. (De la Hoz F et al 1992; Gayotto L 1991)

II.5. The Uraba Gulf: Is an area placed near to the border with Panama, on the Atlantic Ocean. This area is shared by departments of Choco and Antioquia and mainly black people live here.

The first reports of high endemic transmission of hepatitis B in this area were produced around 1980. (Buitrago B 1991; Padilla J 1993; Arboleda N 1987).

Figure 2.1 shows the location of these areas.

Figure 2.1. Hepatitis B endemicity by geographical area

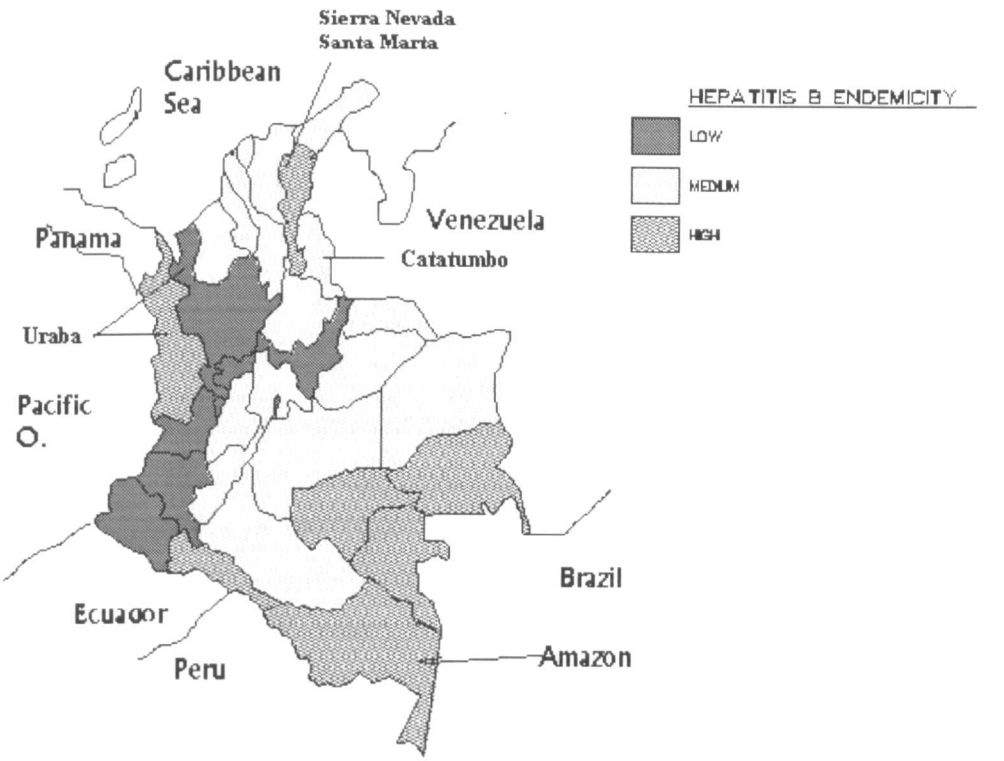


Figure 2.2. Geographical position of Colombia in the Americas.

II.6. Epidemiological studies in the Zone Bananera de Santa Marta: As early as 1906 sporadic outbreaks of fulminant hepatitis had been reported in the Zone bananera de Santa Marta, most of them with a familial pattern of occurrence and with a high case fatality rate. Between 1975 and 1982 64 cases of fulminant hepatitis were reported and 35 of them died. This yields a cumulative incidence of 30/ 10.000 per year. A higher incidence and mortality was observed among males under 30 years. Co infection and super infection with hepatitis D among carriers of surface antigen of hepatitis B virus has been largely identified as the cause of these outbreaks. (Bauer J and Kerr J 1933; Aguilera A et al 1987; Buitrago B 1991; Gayotto L 1991; Gast Galvis A 1955; Buitrago B et al 1986 page 1292; Buitrago B et al 1986 page 1285; Ljungreen K et al 1985)

In 1988 seroepidemiological studies for hepatitis B markers were done in four of the most endemic villages of the zone (Varela, Santa Rosalia, Cerro Azul y Julio Zawady). After this a vaccination campaign was started in order to immunise all people who were susceptible to hepatitis B virus infection. All people living in these villages and who agreed to participate in the study were bled and serum samples were examined for HBsAg, anti-HBc and anti-HBs. Antibodies against delta antigen were tested in those people who were carriers of HBsAg. (Buitrago B et al 1991 page 115; De la Hoz F et al 1996)

About 70% of the population living in these areas agreed to participate in the study. A prevalence of HBV infection of 55% (CI 95%: 53-57%) was found among 2332 people participating in the study. Prevalence ratios did not differ among villages except for Varela that showed the lowest prevalence of infection 29% (CI 95% 25.5-32.5%). For the whole population a prevalence of surface antigen of 7.6% was found and it varied from 2.2% in Varela to 10.5% in Cerro Azul. Prevalence of delta antibodies among HBV carriers was as high as 30% in Cerro Azul while in Varela no carriers were positive for this marker. (De la Hoz F et al 1991; De la Hoz F et al 1996)

A study of risk factors for infection was done in Varela and Cerro Azul. In Varela factors associated with infection were age above 15 years, a history of jaundice, a history of a relative dying of a cause related to hepatitis B, a history of blood transfusion in the local hospital and a history of more than 4 parenteral injections (for medical reasons) during the last year. None of the measures of sexual activity was associated with HBV infection. (De la Hoz F et al 1991)

In Cerro Azul, HBV infection was statistically associated with living in a house with poor sanitation and a history of a relative dying of a cause related to hepatitis B. (De la Hoz F et al 1991)

Cumulative incidence of infection with HBV between the first and second dose of vaccine was recorded for 167 peoples. Thirty-eight (23%) became seropositive for anticore in this period. Incidence was highest for those older than 22 years (55%) and lowest for those under 10 years (7%) No differences were observed by gender.

Over four years 124 carriers of HBsAg were followed for sero-conversion to Anti-HBs. Only 10 became anti-HBs positive (8% CI95% 3-13%) and 3 died from causes related to their carrier state. Causes of death in carriers were hepatocellular carcinoma, cirrhosis and fulminant hepatitis. The incidence of hepatocellular carcinoma in these people was 20 times higher that in the general population. (De la Hoz F et al 1996)

In 1997 a serological study was done in 154 children less than 15 years in Julio Zawady. All of them were fully vaccinated and had lived in the area for more than one year. A prevalence of infection of 10% was observed among them while prevalence of infection in 1988 in a similar age group was 60% (Vaccine efficacy: 83% IC 73-89%). None of the children surveyed in 1997 were found HBsAg positive but in 1987 10% in a similar age were found HBsAg positive (31/296) $p< 0.001$. A large reduction in the number of people with clinical jaundice seeking medical care has been observed in all health centres in the area. This decrease coincided with

the year when mass vaccination of people under 10 was initiated in this area in 1989. Between 1987 and 1989 more than 60 people with jaundice sought medical care in local health centres per year while between 1990 and 1994 this number has been reduced to less than 20 people per year. No other measure that could reduce the incidence of hepatitis A or B has been undertaken in the area, e.g., increase of coverage in running water or excreta's disposal or a decrease of levels of poverty in the area. Instead they have become poorer since an increase in political violence has been observed in the last few years causing migration people from other areas. (De la Hoz F et al 1991; Gamboa M et al 1997)

II.7. Epidemiological studies in the Colombian Amazon Basin: Most studies in Colombia have been carried out on the banks of the Putumayo River, one of the larger tributaries of the Amazon River in Colombia. Despite early reports of Labrea Hepatitis or "Black fever" made by Dr Jorge Boshell, a former Colombian epidemiologist around 1960, there was no published serological studies on HBV infection, in the Colombian Amazon basin before 1990. In 1989 an outbreak of fulminant hepatitis killed 5 children aged 7 to 15 years attending a remote primary school in the Amazons department on the Caraparana River a tributary of the Putumayo. A serological study was done in 404 people, between 1 and 20 years, attending the school. A blood sample was obtained from both staff and students and sera were processed for anticore (anti-HBc), surface antigen (HBsAg) and antibody against surface antigen (anti-HBs) using Abbott ELISA methods. Only 119 were found to be sero-negative for all markers (34%) and 66% were found infected by HBV. Prevalence of HBsAg positives was 27% while 12% showed serological signs of early infection. It was not possible to study these samples for delta antigen or antibody. A similar or higher prevalence of HBV infection was found in other people living in 5 villages around this area. (Gayotto L 1991; De la Hoz F et al 1992; Martinez M et al 1991)

These high endemic patterns of HBV transmission have been observed in others parts of the Colombian Amazon such as in the departments of Putumayo, Vaupes, Vichada, and Caqueta where more than 1000.000 people could be at risk for hepatocellular carcinoma and fulminant hepatitis. (Buitrago B et al 1991 page 115)

Eighteen months later, in 1991, a vaccination program was started in this area using plasma-derived vaccines. Before delivering the first dose serological tests were done in those people new to the area and those found to be sero-negative in 1989. Incidence was measured in 45 children who were sero-negative in 1989. Thirty of them were found to be infected so the cumulative incidence was 67% (53-81%). We estimate a risk of 7% of developing fulminant hepatitis in those positive for HBsAg, and a global incidence of 12/1000 of fulminant hepatitis for all people living in the school (Martinez M et al 1991). Factors found to be associated with

positivity for HBV markers were age above 12 years, living with a relative with a history of jaundice and previous skin lesions. (De la Hoz F 1992)

In 1996 serum samples were obtained from 75 people who were vaccinated against hepatitis B in 1991 and 1992. They were known to be sero-negative for HBV markers at the start of the vaccination campaign. They were aged between 1 and 35 years while 39 (52%) were males. HBV infection was found in 11 of them (14.8%) while 4 people were HBsAg positive (5%). Anti-delta was found in two children less than 10 years (2.7%). Geometric mean titres of anti-HBs were 512 IU/L (CI95% 331-776) among those uninfected. Most people, 53 (85,5%), had titres above 100 IU/L while 32% had titres above 1000 IU/L. Only 4.8% had less than 10 IU/L.

There was high variability of dose intervals while the length of the interval between first and third dose was related to serological response. The GMTs of anti-HBs by time between first and third dose were: 3162 IU/L among those with less than 92 days, 537 IU/L in those with interval between 92-314 days and less than 400 IU/L in those with more than 314 days. (P=0.02.) Prevalence of infection among those with less than 315 days between first and third dose was 4% (1/23) while in those with more than 314 days it was 20% (PR= 0.21 CI 95% 0.03-1.54).

These vaccinated people had a reduction of 78% in prevalence of HBV infection compared with the prevalence observed in those of a similar age group before vaccination was introduced (PR= 0.22 CI95% 0.12-0.37). HBsAg prevalence had been reduced by 67% (PR=0.33 CI95% 0.12-0.82). (De la Hoz F, et al. Unpublished data)

II.8. Epidemiological studies in health workers: Vaccination against hepatitis B among health workers became available in Colombia in 1992 when the Ministry of Health started a campaign to vaccinate all the staff at risk in public hospitals. At least 70% of people targeted for this intervention received one dose of a Cuban recombinant hepatitis B vaccine. However results of this initiative have not yet been evaluated therefore the proportion of people who received a complete schedule of immunisation is unknown.

Some serological studies have been carried out in Colombia in health workers. Seven have been carried out before 1990 and 8 have been done in that year or after. Prevalence of HBV infection in these studies varied between 7.6% and 44.3%. Prevalence of HBsAg varied from 0.4% to 2.1%. Infection rates vary across cities probably reflecting differences in HBV prevalence in the general population. Studies in health workers in high endemic populations (Amazonas y Magdalena) have shown that health workers born in these areas have similar prevalence to the

general population (above 50%) while those who came from less endemic areas have prevalence under 10%. It is unknown if these differences are explained by occupational factors or by the same factors that account for infection in the general population. Age above 25 years, more than 10 years working in a hospital, and not being vaccinated against HBV have been found as factors associated with an increased likelihood of infection. (De la Hoz F et al 1996; Fajardo H and Gomez A 1994; Arroyave A et al 1994; Arroyave M 1985; Juliao O et al 1991; Plata G 1992; Urbina D 1987)

A slight decrease in prevalence of infection is observed between studies made before 1990 and those made after that year. Overall prevalence in studies before 1990 is 16% (630/3972 CI 95% 14.9-17.1%) while it is 14% (CI 95% 13.6-14.4%) in those made in the 1990s. Prevalence of HBsAg has diminished from 2.7% (CI 2.2-3.2%) before 1990 to 1.04% (CI 95% 0.88-1.16) in the 1990. These differences could be due to differences in methodology of the studies such as different range of age, sex and percentage of people in each job category, e.g., nurses, doctors, etc. This reduction also coincides with the start of the vaccination campaign and could be an effect of it. A recent study in more than 2000 health workers in 9 cities has found that blood exposure through percutaneous injuries is frequent. Furthermore, among those health workers who suffered exposure to blood there is a poor understanding of the need for close surveillance and treatment. While more than 50% of people included in one study suffered a needle injury, less than 10% of them reported the accident to the occupational health office that is in charge of the management of these injuries. (De la Hoz F et al 1996)

II.9. Other epidemiological studies of HBV infection: Studies in different populations in Colombia have shown a wide range of prevalence. On the Caribbean coast there are some high endemic populations as described before. Studies in other departments of the same area have shown prevalence of infection from 15% to 58% while prevalence of HBsAg ranged from 3 to 11% (excluding studies in aboriginals). In 268 patients with acute jaundice and hepatitis studied in Barranquilla, the largest city in this area, prevalence of markers for HBV was 9% in children and 21% in adults. (Falsl Borda O et al 1986)

Prevalence of HBV infection in the Andes has been found to be lower than those on the Caribbean Coast. From 3 to 58% of people have been infected with HBV and prevalence of HBsAg ranged from 0 to 9.5%. In 53 patients with acute hepatitis, prevalence of markers for HBV was 26% and 19% were HBsAg positive. (Ochoa L 1989; Botero R 1991; De la Hoz F et al 1995)

On the Pacific Coast prevalence of markers for HBV infection ranked from 35% to 83% and prevalence of HBsAg has been found between 0.6 and 17%. (Buitrago B et al 1991; Padilla J 1993; Buitrago B 1991 page 5)

In women of childbearing age prevalence of HBV infection has been between 3 and 34%. Most of these studies have been carried out in areas where HBV is not endemic. Prevalence of HBsAg in these studies ranged from 0 to 5%. In areas with high endemicity for HBV women have a prevalence of 15% or more. No study for HBeAg prevalence in women has been carried out in Colombia. Some factors have been associated with HBV infection in these studies namely having more than two previous sex partners; tattoos and a history of a relative with an HBV related chronic disease. (Velandia M et al 1997; Sierra F 1988)

In 1980 a nation wide serological study on hepatitis B was carried out in the framework of a National Health Study, using multistage random sampling. ELISA was used for the first time in Colombia to examine the prevalence of HBsAg. Only three geographical areas were included (Central, East and Pacific). Territories in the rain forest as well as the Caribbean coast were excluded from the sample. Researchers investigated a sample of 10,968 people from 0 to 70 years. Overall prevalence for surface antigen was 6% for those 0-9 years, 5.4 % in those 10-14 years, and 4.7% among those above 15 years. Prevalence was higher in the central region (8%) than in the Oriental (3%) or Pacific (3.5%). (Juliao O 1991 page 56)

No study has been done in Colombia on hepatocellular carcinoma and HBV or HDV.

II.10. Needs for more evaluation on effectiveness of hepatitis B vaccine: Considering these findings and based on the WHO guidelines, most areas in Colombia would have a medium endemicity level of HBsAg (2-5% of carriers) and some areas a high endemicity level (>5% of carriers). Considering this the Ministry of Health has started a universal program of immunisation against HBV using the Cuban recombinant hepatitis B vaccine. This program was implemented across the country in 1992. People targeted for the program are new-borns and children less than 5 years. The Colombian EPI recommends that the first dose of hepatitis B vaccine should be administered at birth together with BCG. Second and third doses should be administered at 2 and 6 months with DPT. No boosters have been recommended by the EPI. More than 10 millions' dollars have been expended by the Ministry in acquiring the vaccine. Some surveys on vaccine coverage have found that in highly endemic area's coverage is above 70% while in lower endemic areas coverage is under 50% despite availability of the vaccine (Revelo D 1997; Minsalud 1996; Minsalud 1992)

Although many studies on prevalence of hepatitis B have been carried out in Colombia in different areas of the country, there are some aspects of the epidemiology of HBV that remain unknown. One of the most important is the role of perinatal transmission in the observed prevalence of carriage. It is unknown what the prevalence of HBeAg in women of childbearing age is. Vaccination policies against hepatitis B in Colombia have not taken into account this aspect of the epidemiology of the virus. In endemic zones, it is supposed that children are vaccinated shortly after delivery but it is unknown if this is done systematically. Ignoring this factor could lead to an important shortfall in vaccination objectives especially if women have a high prevalence of HBeAg. In Lombok (Indonesia), those children vaccinated more than 7 days after the birth had a prevalence of 3% of HBsAg compared with 1.4% in children vaccinated in the first 7 days from the birth ($p<0.001$) (Ruff T et al 1995).

Another point of concern is persistence of antibodies and its relationship with protective effectiveness of vaccine. This aspect has not been extensively studied in populations under conditions of heavy transmission. It is known that a high proportion of children are protected for ten years or more but the duration of protection could depend on the force on infection in each place. A study in The Gambia shows that protection could be lower in those places where horizontal transmission is predominant. Also little is known about the characteristics of those children who were infected before ten years. Most villages in the Amazon Basin are in remote areas where accessibility is difficult and hepatitis B transmission is frequent. These access barriers could lead to very long intervals between doses that could reduce the effectiveness of HBV vaccine.

One study in the Amazon Basin found an association between antibody response to the vaccine and number of days between the first and third dose. This finding is against previous evidence from studies in other countries where dose interval was not associated with significant differences in dose response. Further evaluation using larger sample sizes in these areas is needed to resolve the question if large intervals between first and third doses adversely affects vaccine effectiveness. It would be necessary to study factors which lead to difficult access to local health services and possible solutions that guarantee an adequate vaccination schedule to children in high endemic areas.

In addition to the geographical barrier it is possible that social, cultural and economical characteristics of target populations could hamper coverage with hepatitis B vaccine. Also health worker attitudes may provide obstacles to delivery of HBV vaccine. This is a relatively new vaccine and the disease is relatively infrequent among children as many cases are clinically silent. Therefore local health workers could see the utilisation of this vaccine as a less important

measure and/or as an additional work. Barriers arising from health workers need to be assessed quickly as they could be removed by retraining.

A study in China has found difference in antibody titres among people of different ethnic groups (Hsu L et al 1996). Colombia has a rich diversity in ethnic groups and some of them have been affected by HBV and HDV infection. It is important to evaluate if response to vaccine varies among them. If it does then some populations may need different dose schedules. A trial could be implemented in two or three populations with different ethnic composition and assess if titres in new-borns reach the same geometric mean.

Also hepatitis delta has been shown to occur in some vaccinated children but it is unknown if this infection has clinical consequences for children since most of them are not carriers of HBsAg. A follow up using serological markers of HBV and HDV as well as tests for hepatic function would be useful to determine the real probability of being infected with HDV after HBV vaccination. Risk factors and the clinical meaning of these infections also need to be determined.

The Colombian Ministry of Health carried out a vaccination among health workers 4 years ago. This measure also needs evaluation since an important amount of resources were invested in the vaccination process. Studies in health workers are not conclusive about what factors affect inmunologic response to the HBV vaccine. In endemic countries this is very important as health workers are exposed to a larger number of carriers than in developed countries. Booster effect in high risk health workers also remains to be fully studied. Most studies on occupational exposure among health workers have been done in low endemic countries (Europe and USA). Few have been done and published on HBV exposure and consequences among health workers in developing countries where the general population has higher rates of HBV infection. Therefore exposure to infectious blood is likely to be greater and management of these accidents in Colombia is inadequate in most hospitals. A surveillance system for occupational injuries and management of exposures to HBV in hospitals of developing countries could improve our understanding of occupational risk for hepatitis B infection in our country. Vaccine effectiveness in health workers has been studied using prevalence studies and there are no conclusive findings on infection in those with low antibody response when exposed. Studies in Colombia show that prevalence of HBsAg in pregnant women seeking care at general hospitals could be as high as 10%.

Studies on effectiveness of smaller doses of hepatitis B vaccines are always welcome in countries with scarce resources. This could be implemented in new-borns and in high-risk adults such as health workers.

Although other countries in Latin America have a similar pattern of hepatitis B epidemiology to Colombia few have ongoing vaccination program against this disease. In a recent search on hepatitis B reports from Latin American countries few articles on vaccination results were found. Most of these studies were done using small sample sizes in high-risk adult populations such as health workers. They focused on antibody response rather than effectiveness against infection. Therefore evaluation of the Colombian experience could be useful for other countries that have implemented hepatitis B vaccination or are about to implement it.

III. Methodological issues in postlicensure evaluation of hepatitis B vaccine effectiveness:

Post licensure evaluation of vaccines is a very important task in delivering health services for populations. Although vaccines are extensively evaluated before release to the public, most evaluations are conducted under conditions that do not permit policy makers to take decisions about its introduction into public health programs. In the specific case of hepatitis B vaccine many evaluations were done using controlled trials to assess efficacy and immunogenicity. Very few have been done on effectiveness under normal conditions in the EPI programs and most of them have focused only on fully and timely vaccinated people. In most vaccination program, children are immunised when they are able to go to the point of delivery of the vaccine and not when they are supposed to receive it. This aspect has not been assessed in trials and in most post licensure studies. Also pre-licensure trials do not look for the effect of incomplete schedules or poor vaccine storage practices on the efficacy of the vaccine. All these aspects need to be considered in post licensure studies to show the real impact of the vaccine under normal field conditions. (Clemens J et al 1996; Hall A and Aaby P 1990).

Different approaches have been used in evaluating hepatitis B vaccines in the field. However some of them are inappropriate to the main question concerning the true direct and indirect impact of vaccination against hepatitis B spread. Most studies have assessed both direct and indirect effects of vaccination since they have compared prevalence of infection between vaccinated and historical controls. To clarify the approaches that could be used for evaluating hepatitis B vaccines in the field we have reviewed those used to evaluate other vaccines.

In evaluating field effectiveness of hepatitis B vaccine it is important to take into account the following aspects: What is the main outcome to be evaluated, and what are the most suitable methods for these outcomes.

III.1. Outcomes: The following outcomes could be used to evaluate hepatitis B vaccine effectiveness:

Clinical illness: The earliest studies in hepatitis B vaccine, using clinical controlled trials, demonstrated a high efficacy of HBV vaccine against severe or moderate clinical illness. However most of these studies were carried out in adults where hepatitis B infection frequently results in clinical illness. Conversely most studies in the post licensure era of hepatitis B vaccines have focused on the protective effect of the vaccine among children where clinical illness is rare. This biological characteristic of the HBV makes it difficult to select clinical illness as an endpoint to evaluate HBV vaccine effective, particularly in young populations. Another point that makes clinical illness unattractive for HBV vaccine evaluation is that it probably does not represent an important step towards chronic disease. Clinical illness could be considered as an aspect to evaluate just in those countries where universal immunisation against hepatitis B has been implemented more than 15 years ago. At this stage the first cohort of vaccinated people have reached adolescence where sexual risk factors could increase rates of HBV infection and a higher proportion of infected may have clinical symptoms.

Chronic carriage: This is the most suitable end point to be evaluated as effectiveness of hepatitis B vaccine. This status represents an early step in the development of cirrhosis and hepatocellular carcinoma. So if vaccine could prevent it, the later complications of HBV infection could be avoided. Studies using this as outcome should be cautious in the case definition since people positive for surface antigen should be retested in six months.

Fulminant hepatitis: This is an outcome of particular interest in those populations where coinfection with HDV is frequent among carriers of HBsAg. Fulminant hepatitis often has a high fatality rate especially in these isolated populations where it is frequent in South America. There is no large evaluation of HBV vaccines using this result as end point probably because it is not a frequent event in Asia or Africa where most evaluations have been carried out. However populations of intravenous drug users and aborigines' populations in Latin America could be used to monitor the impact of HBV vaccine on this syndrome.

Table 2.5. Serological results of different Hepatitis B surveys done in Colombia by region.

Region	Sample size	Prevalence of infection	Carrier prevalence
Atlantica			
Guajira			
General population	1045	-	4.4%
Indigens	170	1.2%	0%
B/quilla			
Patients with jaundice	268	21.7% adults. 9% child.	3%
Risk groups	434	31.7%	7.8%
Healthy adults	486	14.6%	5.5%
Cesar			
Indigens	864	21.1%	2.8%
Healthy adults	133	58%	11%
Cundinamarca			
General population	264	-	0.3%
Pregnant women	68	-	4.4%
Pregnant women	200	-	1%
Pregnant women	175	-	9.5%
Pregnant women	1000	3.1%	0.1%
Patients with jaundice	53	26%	19%
Healthy individuals	366	7.1%	1.6%
Antioquia			
Uraba	492	83%	17%
Sn Vicente	129	17.4%	10%
Occidente (women)	1690	8.5%	1.1%
Indigens (women)	830	34.3%	4.2%
Manzanares	197	58.4%	2.5%
Pacifico	254	82.3%	9.4%
Indigens Jardin	61	-	9.8%
Pacific			
Tumaco- Imbili	3500	35%	-
Choco-Riosucio	912	76%	-
Tolima			
Pregnant women	246	13%	4%
Prisoners	103	39%	22%
Prison staff	31	12%	0%
Guaviare			
General population	59	45.7%	8%
Guainia			
Indigens	105	67%	
Valle			
Blood donors	20458	-	0.55%

Infection: Since infection does not itself represent a risk for hepatocellular cancer it has been little considered in post licensure studies of HBV vaccine. However this should be considered when an effectiveness study is done, because those who are infected by hepatitis B virus could contribute, even for a short time, to the spread of infection.

Cancer hepatocellular and cirrhosis: Controlling hepatocellular cancer is the most important aim in most countries where a hepatitis B vaccination program has been implemented. However since the induction time between infection and cancer development is so long it may be difficult to gather information about vaccination status if a case control study is used for evaluation. If a cohort study were done instead, there would be a potential for biased estimates of efficacy due to losses during the follow up. Evaluations using cancer as an end point should be supported by the availability of large databases containing the vaccination status of the population. A recent study from Taiwan has shown that the incidence of hepatocellular carcinoma is decreasing among children 6 to 14 years of age. Investigators collected data from the Taiwan's National Cancer Registry from 1981 to 1994. They observed that annual incidence of hepatocarcinoma has declined from 0.7/ 100.000 children in 1981 to 0.36/100.000 in 1990-1994. After controlling by date of birth, those born among 1984 and 1986 had an incidence of 0.13/ 100.000 compared with 0.52 among those born among 1974 and 1984. Surprisingly all cases of hepatocarcinoma occurred among children who have received 3 doses of hepatitis B vaccine. This fact supports the hypothesis that those who fail to respond to the vaccine are still at risk of hepatocarcinoma. (Chang M et al 1997)

III.2. Methods for post licensure vaccine evaluation: Different methods are available for evaluating post licensure vaccine effectiveness (Orenstein W et al 1985; Orenstein W et al 1988).

Screening Method: This simple method allows us to estimate quickly if vaccine effectiveness is within expected limits. It uses the following formula:

$$PCV = \frac{PPV - (PPV * VE)}{1 - (PPV * VE)}$$

Where,

PPV= proportion of population vaccinated
PCV= proportion of cases vaccinated
VE=vaccine efficacy

Cases are identified through regular surveillance, their state of vaccination is ascertained and vaccine coverage in the population where cases come from is obtained from local statistics from

the public health authorities. Probably this is not a suitable method for hepatitis B in childhood when most cases are asymptomatic.

Outbreak investigation: It has been proposed that this is probably the best situation to evaluate vaccine effectiveness. Unfortunately clusters of hepatitis B infections are rarely detected, as it does not result in clinical illness. This approach could be useful for hepatitis B vaccine in some special settings such as haemodialysis units where closed monitoring of patients and staff could lead to a detection of these outbreaks.

Secondary attack rates in families: This approach has been suggested to avoid biased estimates of vaccine efficacy arising from differences in exposure between vaccinated and unvaccinated participants. In them secondary attack rates are compared between those groups. However as discussed before, secondary attack rates for hepatitis B are very hard to estimate because few people develop clinical illness.

Serological studies: These studies are useful if there is a serological correlate for clinical infection. This approach has been used extensively in hepatitis B vaccine evaluations since it has been found that titres above 10 IU/ml are protective against carrier status. However the answers we can obtain from serology are limited. We can solve questions about how long this level of antibody lasts but we cannot obtain direct or indirect estimates of effectiveness with cases prevented for example.

Most studies have looked for infection and carrier status in vaccinees and doing so have failed to estimate indirect effects of vaccination. This is a very important point in disease control and design of studies that permit estimations of indirect effect of vaccine are still needed. Most effectiveness studies on hepatitis B vaccine compared the prevalence among vaccinees against it in historical controls. This is not particularly wrong but it does not allow evaluation of changes in the dynamic of the infection produced by herd immunity effects.

Cohort studies: These studies have been carried out in some countries such as The Gambia, Senegal, Alaska and Taiwan. They have focused mainly on the long effectiveness of HBV vaccine. Most of them have observed just a cohort of vaccinated people and results have been compared with historical levels of prevalence. This approach does not allow estimation of direct effects of vaccination, it estimates the combined impact of direct and indirect effects of vaccine (Struchiner C 1990). Loss of participants could bias the results.

Case-Control Studies: In the last few years case control studies have been widely used in evaluating vaccine effectiveness for different infectious diseases. Most have been done in BCG, Haemophilus influenzae and measles (Smith P 1987). There are no published studies on effectiveness of HBV vaccine using case control methods. Most discussions on assessment of vaccine effectiveness using case control methods have focused on diseases that result in clinical manifestations. There are few if any which discuss an infection without a distinctive clinical picture such as hepatitis B.

Many advantages have been attributed to cases controls studies in this field. One of the most important is that they save money and time. Also evaluation using the case control approach is more realistic since controlled trials usually are conducted under ideal field conditions and this is not found frequently in public health services like EPI. Ethical considerations can also be a barrier for designing controlled trials when beliefs about high efficacy of an intervention are strong.

Other additional advantage of case control studies is that we can perform evaluation of effectiveness for subgroups of patients not included in randomised trials and for different schemes of treatment or dose delivery. This is a particularly important aspect in hepatitis B since most field evaluations have focused on children who had a complete scheme of vaccination. However effectiveness of incomplete schemes remains unevaluated. For example most endemic areas in Colombia are placed in remote settlements where incomplete schedules of vaccination are common.

Critical points in case control studies on hepatitis B vaccines are:

Case definition: As we discussed before the most useful end points in hepatitis B are hepatocellular carcinoma and the carrier status. Cases of hepatocellular carcinoma must be selected only from those who have a well documented disease including the use of ultrasound and biochemical markers of hepatocellular cancer. Carrier status could be ascertained using less complicated technology and is the outcome of election in most studies.

Case finding: Cases can be detected from a hospital or health centre or from community based surveys. Cases from health facilities are easier to obtain if clinical illness is the outcome of interest. When the main outcome is carrier status, they could be obtained from community based serological surveys. These have some methodological advantages. One of the most important is that we can select controls at random from the population, avoiding one of the most important sources of bias in case control study that is that cases and controls could be

different in many ways aside exposure factor. In that way areas with high and non-high risk could be included in the sample. Another approach to avoid this confounding effect is to restrict the study to those zones with high endemicity where vaccine should have been delivered in a better way. The main disadvantage for community-based surveys is a poor rate of participation. Carriers also can be selected using hospital-based methods.

Control selection: It is well known that odds ratios obtained from a case control study could estimate different parameters of association (incidence rates, risk ratios, or odds) depending on the frequency of disease but also on the way controls are selected. It has been proposed that for vaccine evaluation using the case control method controls should be selected based on the proposed model of action of the vaccine. Smith (Smith P et al 1984) proposed two models of action for vaccines. Vaccines under model 1 are supposed to produce an overall decrease in the incidence of the disease, however its effects tend to become weak when time pass. Under model 2 vaccines would yield a protection of "all or nothing." Hepatitis B vaccine could be classified as having a model 2 action since most people, especially children, are completely protected for at least ten years after vaccination.

For this kind of assumption Smith and Rodriguez (Smith P et al 1984; Rodrigues L and Kirkwood B 1990) proposed a scheme of control sampling selecting people from the population regardless of their disease status. As they have demonstrated theoretically this sampling method leads to an estimation of the relative risk that is an unbiased estimator of the effectiveness of the vaccine. In hepatitis B we use serological tools to determine if anybody has been exposed to hepatitis B before, and including seropositive controls in the study could reduce the estimated effectiveness of the vaccine. On the other hand, as authors have remarked, a traditional approach to select controls could overestimate vaccine effectiveness. An intermediate solution would be selecting two groups of controls and compare the effectiveness in each assessment. Another approach would be restricting cases to those with recent infection and selecting controls from all the population if proportion of recent infectious would be very low. In this case most people selected as controls would be either sero-negative or sero-positive with an old infection. Since the sample size for case control study is low this approach could have many methodological advantages. An additional advantage of HBV infection is that it could have low rates of infection after mass campaigns of vaccination, so both methods of selecting controls could yield similar results.

Table 2.6 showed the effect of these assumptions on evaluating HBV vaccine effectiveness. For this exercise we assume that incidence of disease is 0.04 per person/year.

Table 2.6. Expected effectiveness of HBV vaccine against carrier status under model 2 of efficacy .

Year	N1	C1	Y1	N0	C0	Y0	VEf	VEr	VEor1	VEor2
1	1000	40	980	1000	8	996	0.80	0.80	0.81	0.80
2	960	37	940	992	7	987	0.82	0.80	0.82	0.80
3	922	36	903	984	7	982	0.82	0.80	0.82	0.80
4	885	35	868	979	7	974	0.82	0.80	0.83	0.80
5	850	34	833	971	7	968	0.83	0.80	0.83	0.80
6	816	32	800	965	6	961	0.83	0.80	0.84	0.80
7	784	31	768	958	6	955	0.84	0.80	0.84	0.80
8	753	30	738	952	6	950	0.85	0.80	0.85	0.80

N1= Number unvaccinated. N0=Number vaccinated. C1= Number of cases among unvaccinated. C0= Number of cases among vaccinated. Y1= Number of persons years at risk among unvaccinated at the end of every year. Y0= Number of persons years at risk among vaccinated at the end of every year. VEf= Vaccine efficacy measured using incidence rate of disease. VEr= Vaccine efficacy using risk ratios. VEor1= Vaccine efficacy using odds ratios calculated by selecting as controls those who remain negative at the end of each period. Veor2= Vaccine efficacy using odds ratios calculated by selecting as controls all at the start of the study (regardless serological status)

As we can see above, Veor2 and Ver yield an unbiased estimate of the vaccine effectiveness across the years of follow up. However, differences between the different approaches are narrow probably because yearly incidence of carrier status is low.

Vaccination status ascertainment. One potential source of bias in case-control studies is "recall bias." It can be avoided by determining vaccination status before performing any serological assessment. Since in hepatitis B it is possible to use serological tests to classify cases and potential controls, previous knowledge about vaccination status cannot influence diagnosis of infection. Also all vaccination status should be confirmed by reviewing vaccination cards or records from the local health workers. This will make it unlikely that knowledge about infection of the subject under study could influence classification of vaccination status.

Comparability of vaccinees and non-vaccinees. The principal disadvantage of the case control approach is that the likelihood of being vaccinated is never truly random in a non-experimental situations since 'confounding by indication can arise when vaccine is being delivered in the field. This confounding is unavoidable in the moment of vaccine application but it could be avoided in designing a case control study if cases and controls are randomly selected from the population.

Factors associated with the development of disease should be similar for cases and controls. Perinatal and horizontal transmission of hepatitis B account for most infections in endemic areas. Most cases of horizontal transmission occur among those who have siblings or parents infected with hepatitis B. This potential source of bias could be controlled by performing serological tests on relatives of both cases and controls. Then in the analysis we will stratify association between vaccination and presence of HBV marker in study's subjects by presence or absence of active or past infection in the family. Using this approach we can detect any effect of modification or control any confounding bias introducing by serological status of the family. A problem in this method is that prevalence of mother and siblings could not be assumed as independent since prevalence in siblings could also be explained by mothers' infection. Another approach is assuming that random selection of people under study from the population would lead to these potential confounders being distributed equally among cases and controls.

Using community based surveys to detect those infected means they are prevalent cases that could differ from incident cases in several ways. One of the most important aspects is severity of disease. If prevalent cases have had a less severe disease than acute cases we will be unable to assess vaccine impact against a severe form of disease.

Comstock (Comstock G 1994) discusses infections that are called by him "inapparent infections with subsequent immunity" and "inapparent infections with subsequent disease risk." However none of these categories is suitable for hepatitis B. It is true that hepatitis B produces subclinical and inapparent infection in most cases but clinical efficacy of hepatitis B vaccine may be better than efficacy against infection.

Comstok assumes that if infected people are included in vaccinated and unvaccinated groups in the same proportion the overall protective effect will estimate true efficacy adequately if infected survivors have zero risk of subsequent disease. This is similar to evaluations of effectiveness in hepatitis B, since those infected with hepatitis B have no risk of re-infection.

Table 2.7 shows the expected effects of including in the study people who were infected before vaccine was available in the population selected for the study. We can see that if coverage of vaccination is about 50% and proportion of prevalent cases is similar (2%), VEor2 yields the closest estimate of the true VE. However if previous prevalence of infection increases, VEor1 become a better estimator of TVE. Even if the coverage of vaccination increase, Veor1 remains as a better estimator of TVE. These results support the idea that two groups of controls would help researchers to estimate the range of TVE.

Table 2.7. Effect on vaccine efficacy of different assumptions about prevalent cases before vaccine implementation.

N0	CP0	C0	N1	CP1	C1	OR1	VEor1	OR2	VEor2	TVE
1000	20	167	1000	20	510	0.20	0.80	0.33	0.67	0.70
1000	80	218	1000	80	540	0.24	0.76	0.40	0.60	0.70
1000	80	126	1000	80	460	0.15	0.85	0.24	0.76	0.90
1400	112	176	600	48	324	0.12	0.88	0.23	0.77	0.90

N1= Number unvaccinated. N0=Number vaccinated. C1= Number of cases among unvaccinated. C0= Total number of cases among vaccinated. CP1= Prevalent cases among vaccinated before vaccine was available. CP0= Prevalent cases among non vaccinated before vaccine was available. VEor1= Vaccine efficacy using odds ratios calculated by selecting as controls those who remain negative at the end of each period. Veor2= Vaccine efficacy using odds ratios calculated by selecting as controls all at the start of the study (regardless serological status). TVE= true vaccine efficacy

III.3. Variables: Most effectiveness studies have focused just on vaccination as the exposure of interest. It has been forgotten that there are other variables that are closely related with the risk of infection and carrier status. The most useful of these variables are those related with socio-economic status and in most studies in Colombia they were found associated with infection. Those people living in poor conditions are more likely to be affected by HB. Availability of excreta disposal, crowding, infected people in the same household, antecedent jaundice in relatives, serological status of the mother, among others; have been found consistently associated with infection. However, they have been omitted in most observational studies of hepatitis B vaccine. We propose that all of them should be included in any observational study of HBV vaccine, especially when cross sectional and case control approaches are used. They can help investigators to control the effectiveness of vaccines by the probability of exposure to HBV. Just two observational studies, one in Canada and other in The Gambia, looked for relationship between infection and some socio-economic variables apart from the vaccination status of children. However Canada is a developed country, and the main source of infection for these children was a carrier mother. Horizontal transmission of hepatitis B is low in households with adequate conditions of sanitation while in developing countries this risk is as high as the risk of perinatal transmission. Therefore these kinds of variables are more useful in studies of vaccine effectiveness in developing countries with high endemicity levels of HBV infection.

Chapter 3: Methods.

3.1. Proposed Study:

1) To assess the effectiveness under field conditions of a Cuban recombinant hepatitis B vaccine used in Colombia by the EPI for individual protection against HBV infection and carriage.

2) To evaluate barriers to adequate delivery of hepatitis B vaccine in high endemicity areas in Colombia.

3.2. Objectives:

1) To measure coverage with hepatitis B vaccine among a random sample of Colombian children living in highly endemic areas.

2) To compare the prevalence of infection with hepatitis B and proportion of HBsAg carriers among those children receiving a full course of hepatitis B vaccine against prevalence in those unvaccinated or with an incomplete schedule of hepatitis B vaccine, in highly endemic areas of Colombia.

3) To examine the influence of dose interval (vaccine scheme) on protective efficacy of a recombinant hepatitis B vaccine.

4) To compare prevalence of infection among those who have received hepatitis B vaccine and whose mother are HBsAg negative against prevalence among those vaccinated or unvaccinated whose mother is HBsAg positive.

Objectives 3, 4, and 5 will permit us to evaluate effectiveness, under field conditions, of the recombinant Cuban manufactured hepatitis B vaccine used in Colombia by the EPI programme.

5) To measure factors and barriers related to incomplete vaccination with hepatitis B vaccine.

6) To compare the prevalence of complete coverage with hepatitis B vaccine against coverage reached by other vaccines of the EPI programme. This will allow us to evaluate if there are specific constraints to delivery of hepatitis B vaccine.

3.3. Type of study: A cross sectional survey using one stage cluster sampling was carried out in the rural and urban population of Leticia, Puerto Nariño, Puerto Santander, and Araracuara. The first two areas were included in the study because they are the most populated areas of the department while the latter two had been identified in previous studies as having the highest prevalence of HBV infection in the department (Cristancho LM 1991). Data were analysed as a case control study with cumulative sampling for the main results of the study: vaccination status and factors related with it as well as serological status (HBsAg prevalence) and its relationship with vaccination and other characteristics believed to be important.

3.4 Localisation of the study: Leticia is placed on the left bank of the Amazon River and is the most southern town in Colombia sharing borders with Brazil and Peru. It has a population of 22400 inhabitants, 15400 are urban in urban Leticia and around 7000 live in rural settlements along the Amazon River. It is the capital of the Amazon department. In urban areas about half of its population have an ethnic origin from aboriginal tribes such as the Ticunas and Huitotos. Socio-economic level in the urban area is low. Access to running water is estimated at 85% by the municipal planning office while piped domestic sewage disposal would hardly reach 50% of the urban population.

In rural settlements of Leticia most people live below the poverty line. There is no running water available and most people collect it directly from the Amazon River. Excreta disposal is mostly by latrines or pits. The main economic activities are fishing and vegetable cultivation (cassava, maize, etc.)

Puerto Nariño is also located on the Amazon River to the west of Leticia and shares borders with Peru. It has a population of 3800 inhabitants, 1400 urban in the settlement called Puerto Nariño and 2400 scattered in small villages along the Amazon and Loretoyaco rivers. In Puerto Nariño most people have running water but the water is not treated and goes directly from the river to the houses. There is no municipal sewage disposal system and most people have latrines or pits to dispose of excreta. In rural settlements conditions are similar to those in Leticia's rural areas. Most people in both the urban and scattered areas belong to the Ticunas tribe, one of the two most important ethnic groups in the Amazon.

Araracuara and Puerto Santander are villages located on the banks of the Caquetá River. Combined they have around 1400 inhabitants. Socio-economic conditions are similar to rural areas in Leticia and Puerto Nariño and most of its habitants belong to the Huitotos tribe, the second most important ethnic group in the department.

Tables 3.2 and 3.3 show the population distribution in rural and urban areas in Leticia and Puerto Nariño. Table 3.4 shows the population distribution in urban Leticia by neighbourhood.

3.5 Target population: Children above 1 year old and less than 12 years living in Leticia, Puerto Nariño, Araracuara and Puerto Santander.

3.6 Sample size and selection: We estimated that a sample of 1088 children between one and eleven year old would be required to estimate a prevalence of vaccine coverage of 85% with intervals between 82 and 88% which was similar to the coverage reported by the Amazon EPI in the year before the start of the study. This estimate was calculated with a 95% confidence level and a design effect of 2.0. To calculate this sample size we used the formula provided by Kish & Leslie 1965, which is available in EPIINFO 6.04 c (Dean et al 1994):

Where;

Sample size = n/(1-(n/population)).

N=Z*Z(P(1-P))/(D*D)

However since we had to estimate from the same survey other measures such as the prevalence of infection with hepatitis B virus (HBV), the prevalence of surface antigen carriage (HBsAg), and risk factors for infection we needed a larger sample size due to the low frequency of carriage expected in vaccinated children. Therefore we estimate that a sample of 2239 children would be needed to fulfil the different objectives of the study. See Table 3.4.

This sample was selected proportional to population size. Thus in Leticia we planned to survey 1350 children (59% of the sample), in rural Leticia 407 children (18%) and in Puerto Nariño 538 children (23%). In Araracuara and Puerto Santander, given the small size of the

population but the undoubted importance of including them for the study, it was decided to recruit all children less than 12 years old living in the main settlements

In urban Leticia we selected 60 clusters (blocks) for the study. To select them we divided the city into 163 clusters (blocks) and every cluster was numbered. Then at random we chose 60 numbers.

In rural Leticia villages were listed and numbered. As before a random number list was generated in EPIINFO and villages were arranged and visited in the same order provided by the list. We stopped visiting villages when the sample size for rural Leticia was completed. A similar procedure was used to fill the sample size in rural Puerto Nariño.

In the urban area of Puerto Nariño we divided the population into 22 clusters (blocks) and we surveyed every one because there were a large number of children without accurate vaccination status. A similar procedure was done in Puerto Santander and Araracuara where every household was visited.

3.7 Population survey and logistical aspects: A team of two health promoter was assembled to visit households in rural and urban areas. They were trained by the principal investigator concerning the procedures to carry out the census, taking blood in the field, obtaining parental consent, and applying the mother's questionnaire. Direct observation and assistance in the field was provided by the main investigator and a field co-ordinator, a very skilled field epidemiologist nurse who is in charge of the control of communicable diseases in the local health department. They reviewed the forms filled every day in order to detect missing values or mistakes. They also reviewed blood samples to ensure that they were handled in an appropriate way and that they were correctly identified. Some of the study's villages, especially those located on the Loretoyaco river, were accessible only by river and for a few months of the year, so the trip schedule had to be adjusted to those periods when the Loretoyaco river had sufficient water enough to ensure access. Those located on the Amazon river were accessible by boat all the year and therefore they were visited first.

In every selected cluster or village this team visited every household. First they filled a household census form where we asked the number of people living in the household, number of children less than 11 years and the socio-economic conditions of the family (crowding, running water, social security). We recorded the names and ages of every person living in the house. If at least one child less than one was found living in the household the interviewer asked the child's parents for the vaccination card. If it was available the

interviewer recorded the number of doses of hepatitis B, DPT, BCG, measles or MMR and dates when every dose was given. After that the field workers obtained informed consent to obtain a blood sample from every children living in the household and from their mothers. They also questioned the mothers about general risk factors for hepatitis B infection such as antecedent clinical hepatitis in the household, antecedent death by fulminant hepatitis in the family, and a family history of cirrhosis or hepatocarcinoma. This questionnaire (see appendix 2) also recorded parents' level of education, breastfeeding, mother's age at first birth, mother's age at birth of the child, child's number of siblings, ethnic group, and the site where the child was born.

3.8 Definitions for vaccination status: We defined as a **fully vaccinated children** any one aged between one and eleven years old who, at the moment of the survey, had received at least the following vaccination scheme:

Three doses of hepatitis B.
Three doses of DPT.
Three doses of polio.
One dose of yellow fever.
One dose of measles or MMR.
One dose of BCG

Those failing to fulfil these criteria were defined as **not fully vaccinated** and were used as the control group for the fully vaccinated when risk factors for vaccination were explored.

Only children holding a vaccination card were included in these definitions. We did not consider in the analysis those doses or vaccines that were reported by mothers without written support.

Table 3.1 below show the vaccination schedule recommended by the Ministry of Health and the Amazon Health Service.

Table 3.1. Vaccination schedules recommended by the Colombian Ministry of Health and by the Amazon.

Vaccine	Number of doses	Age	Interval	Booster
BCG	1	At birth	-	-
Polio	4	Birth, 2,4, 6 months.	Four weeks	18 months and 5 years.
Hepatitis B	3	Birth, 2, 6 months.	Four weeks	-
DPT	3	2, 4, 6 months.	Four weeks	18 months and 5 years
Measles.	1	1 year old	-	10 years
Yellow fever	1	1 year old	-	Every 10 years.

As we can see children should receive at birth one dose of BCG, one dose of polio and the first dose of hepatitis B. Then at two months of life they should receive the second dose of hepatitis B, the first dose of DPT and polio. Second doses of polio and DPT should be applied at four month of life. At six month children should have completed the basic scheme for DPT, polio and Hepatitis B (three doses) and at the age of twelve they should receive yellow fever and measles or MMR vaccines.

We considered as **completely vaccinated against hepatitis B** those aged between one and 11 years who had received three doses of hepatitis B vaccine. Those who failed to fulfil these criteria were considered as **not completely vaccinated against hepatitis B**. As before, I only considered in the analysis those children with a vaccination card. Those without written evidence of vaccination were excluded.

3.9 Blood sample collection and handling: Participants were bled using a disposable syringe and needle preferably from the left arm. We tried to obtain ten centilitres from mothers and children above 5 years, while five centilitres were drawn from children under five. A code was assigned to every children participating in the study and was written on the syringe using non-erasable ink. This code was formed by adding the number of the cluster, number of the household, and the number of the child in the household. For mother's sample we used the same code of the first of their children who was bled adding a letter **M**. Sera was obtained from blood samples by centrifugation in the field and kept refrigerated until they were sent to the National Virology Laboratory in the Colombian National Institute of Health in Bogotá. There, samples were stored frozen until the moment that they were analysed for hepatitis B virus markers.

Photo 1. Field workers in Araracuara

Photo 2. Field workers travelling by boat to Puerto Santander on the Caquetá River

3.10 Serological markers: Children's sera were processed in the CNIH's Virology lab for the following markers:

Hepatitis B surface antigen (HBsAg).
Antibody to core antigen total (Anti-HBc).
Antibody against core antigen IgM (IgM Anti-HBc).
Antibodies against surface antigen (Anti-HBs).
Delta virus antibody (IgG).

All sera were processed initially for HBsAg and anti-HBc. Those who were found positive for HBsAg were then tested for Delta antibody and IgM anti-HBc while those anti-HBc positive but HBsAg negative were processed only for IgM anti-HBc. A sample of those who were negative for HBsAg and anti-HBc were processed for measuring quantitative titres of anti-HBs.

Mother's sera were processed for:

Hepatitis B surface antigen (HBsAg).
Antibody against core antigen total (Anti-HBc).
Antibody against core antigen IgM (IgM Anti-HBc).
Hepatitis B "e" Antigen (HBeAg).
Delta virus antibody (IgG).

First mother's samples were processed for HBsAg and anti-HBc. Samples from mothers found positive for HBsAg were also processed for HbeAg, Delta antibody and IgM anti-HBc.

Initial testing was done using ELISA. Samples positives for HBsAg or anti-HBc were confirmed using neutralization methods. For delta virus we repeated all those who tested positive in order to confirm them.

3.11 Definitions for serological study: Children were divided in the following categories regarding their status for hepatitis B infection:

Infected children were any children aged between one and eleven years who was positive for anti-HBc or HBsAg.

HBsAg positive children were any children aged between one and eleven years who was positive for HBsAg and anti-HBc, both by ELISA and neutralisation techniques.
Seronegative children were all those aged between one and eleven years who were negative for both HBsAg and anti-HBc .

For the quantitative analysis of anti-HBs titres we divided children in two categories: Children with more than 10 IU were classified as **protected** while those with titres under that level were classified as **negative for anti-HBs**.

Mothers were classified as follows:

Infected mothers were those positive for HBsAg or anti-HBc.
HBsAg positive were those who were repeatedly positive for HBsAg, both by ELISA and neutralization techniques.
Highly infective mothers were those who were positive for HBsAg and HBeAg.
Low infective mothers were those who were positive for HBsAg but negative for HBeAg.
Seronegative mothers were those who were negative for HBsAg and Anti-HBc

All serological markers were processed using ELISA techniques and commercial available kits (ABBOTT). These kits have in general more than 99% of sensitivity and more than 99% of specificity. However, we carried out some additional procedures to ensure the quality of the results. First, we used a high absorbance ratio (observed absorbance/cut-off point), to classify samples as positives for HBsAg or Anti-HBc. The selected value for the absorbance ratio was 2.0. Second, all positives samples for HBsAg were tested twice using the same technique and those repeatedly positive were confirmed by neutralization. Samples positives for Anti-HBc were processed twice with the same technique and only those found repeatedly positive were included in the analysis as positive. Third, we only included as HBsAg + those who tested positive for both HBsAg and Anti-HBc, which reduced even further the likelihood of having included negative children as positives. No attempts were done to retest negative samples given the high costs that it had imposed on the study budget. However given a prevalence of 5% and a test sensitivity of 99% we should expect only a maximum of 2 false negatives over 2000 samples processed. Therefore the impact of false negatives on our estimates, odds ratios (OR) and prevalence, should not be important.

3.12 Data collection from health workers on vaccination knowledge and practices: We interviewed 24 health workers in 19 towns in the Amazon department. We used a questionnaire combining structured and open questions to measure their knowledge in the following areas: name of the vaccine preventable diseases, contraindications for the vaccine most commonly used in the EPI (polio, DPT and hepatitis B), site of application of hepatitis B vaccine, age when a child should have completed the basic vaccine schedule, and vaccination coverage in the area where they worked. We also collected information on administrative aspects and operational characteristics of each health centre included in the study e.g. the number of health workers, the number of medical doctors, daily working hours in the centre, availability of physical structure to carry out vaccination activities (freezer and cold chain). We tried to assess the attitudes of these health workers towards children who do not attend the vaccination centre and the reasons (logistical, administrative, cultural or health worker related) some children are not vaccinated according to the government schedule.

For the interview we selected only health workers directly involved in vaccination activities (administrative and operational) in the area, regardless of their time in the job or their professional level. We found that health workers in three professional categories: nurses, auxiliary nurses and health promoters were involved in vaccination. According to Colombian regulations nurses spend 6 years in a university in order to get their degree, auxiliary nurses should have a technical training of 2 years in a non-university institution and health promoters should be trained for one year in the same kind of institutions as auxiliary nurses. Most auxiliary nurses and health promoters have not completed the basic school scheme available in Colombia. Since the interviews were carried out some weeks after the vaccination coverage survey not all the health centres in the area could be included, because in rural areas a few health workers were not available at the time the interview was done.

One trained auxiliary nurse applied the questionnaires in rural and urban health centres. He was trained over two days by the principal investigator in Leticia. We performed a pilot interview on three health workers at the departmental level who were formerly involved in vaccination activities. First the principal investigator showed the questionnaire to the interviewer teaching him the correct manner to ask the questions and to record the answers and encouraging him to make suggestions concerning the phrases used in the questionnaire, or to ask questions if anything was unclear for him. Then he carried out one interview in the presence of the auxiliary nurse that was followed by doing one interview in the presence of the principal investigator. After these initial procedures the interviewer performed two other interviews alone that were reviewed by the principal investigator in order to ensure that no question was left blank due to mistakes or misunderstanding. Special emphasis was made

concerning open questions where the interviewer was instructed to write down all the ideas given by the interviewee.

3.13 Data handling and analysis:

The census and questionnaires were entered in several databases using EPIINFO 6.04. One had data concerning child's vaccination status, the second one environmental and socio-economic characteristics of the household, the third general risk factors for hepatitis B infection, a fourth mother's serological status, and the last vaccination knowledge and practices. Files containing data about children and mothers shared a common identification number that was constructed from the cluster household numbers.

3.13.1. Analysis of health worker data:

Variables obtained from health workers were divided in four broad categories: general characteristics of health centre, general knowledge on vaccines, general knowledge on hepatitis B vaccine, and health worker's perception of barriers for adequate vaccination coverage. The last category was divided into subcategories: logistical barriers, parent related barriers, geographical barriers and health worker related barriers.

First we describe the frequency of every variable using percentages for nominal and median for continuous variables. Then we performed an ecological analysis aiming to identify those health worker or health centre characteristics related statistically with higher or lower levels of fully vaccination coverage and hepatitis B vaccine coverage. The ecological unit of analysis was every village or town. The dependent variable was the proportion of children fully vaccinated or completely immunised against hepatitis B treated as continuous variables. The bivariate approach in the ecological analysis was done comparing the median of vaccination coverage between categories of the independent variables and median differences were tested using the Kruskall Wallis test.

Variables found to be associated with vaccination ($p<0.2$ or differences in coverage above 15%) were included in multivariable models. We also included some health worker or health centre characteristics believed to be theoretically important even if in the bivariate analysis they were not strongly related to vaccination. Models were built using linear regression to assess which variables were more important for the determination of vaccination coverage, as well as to assess the presence of confounding. We ran models using the option "robust" and as analytical weights the number of children under 10 years in every village. Vaccination

coverage was included in different models both in its original scale as a proportion and using a base 10 logarithmic transformation. But we found that log transformation did not improve the fit of the model so we decided to use coverage in its original scale. The "robust" options in Stata use the Huber/White/sandwich estimator of variance instead of the traditional calculation that allowed us to calculate linear regression coefficients even if linear assumptions were not completely filled. (Stata 1999).

To select the best set of predictors for vaccination coverage and the most parsimonious model we used a stepwise procedure (backward). The decision whether to keep a determined variable in the model or not was taken on the basis of the partial F test (Fisher L and Van Belle G 1993) comparing the square sum of regression of the model without the independent variable under study to the square sum of residuals of the complete model. To detect correlation between independent variables and to avoid its effects on coefficients and standard errors we built a correlation matrix including independent variables. Those variables that were correlated at more than 0.5 were not included together in the same model.

3.13.2. Cross sectional survey data:

A) Analysis of vaccination status and related factors: As dependent variables in the analysis we considered several outcomes: 1) **being fully vaccinated** and 2) **being completely vaccinated against hepatitis B**.

Vaccination coverage was described by categories of place, person and time variables. Percents of fully vaccinated children and its 95% confidence intervals were calculated for rural and urban areas stratified by age. The number and proportions of vaccinated children were calculated taking into account the complex design of the sample and using the following formulae provided by Stata:

$$Y = \sum_{h=1}^{L} \sum_{i=1}^{n_h} \sum_{j=1}^{m_{hi}} w_{hij}\, y_{hij}$$

Where Y is the number of children vaccinated in the total population, y is the number of children (j) vaccinated in the h.. strata (L= total number of strata) and i... primary sampling unit, and w_{hij} are the user-specified sampling weights. *Y* might also be another measure such as a proportion or a mean.

The estimate of the variance for Y is obtained as follow:

$$V(Y) = \sum_{h=1}^{L}(1-f_h)\frac{n_h}{n_h-1}\sum_{i=1}^{n_h}(z_{yhi}-\bar{z}_{yh})^2$$

Where,

$$z_{yhi} = \sum_{j=1}^{m_{hi}} w_{hij}\, y_{hij} \quad \text{and} \quad \bar{z}_{yh} = \frac{1}{n_h}\sum_{i=1}^{n_h} z_{yhi}$$

and $(1\text{-}f_h)$ is the finite population correction.

To calculate confidence intervals the design effect (deff) was taken in account therefore they are more conservative than those that might be obtained using a simple random sampling approach as seen in the following formula:

$$deff = \frac{V(\theta)}{V_{srswor}(\theta_{srs})}$$

Where $V(\theta)$ is the design- based variance for a parameter θ, and $V_{srswor}(\theta_{srs})$ is an estimate of the variance for an estimator θ_{srs} that would be obtained from a similar hypothetical survey conducted using simple random sampling (srs) without replacement (wor) with the same number of sample elements as in the actual survey. (Stata 1999, volume 4:68-70)

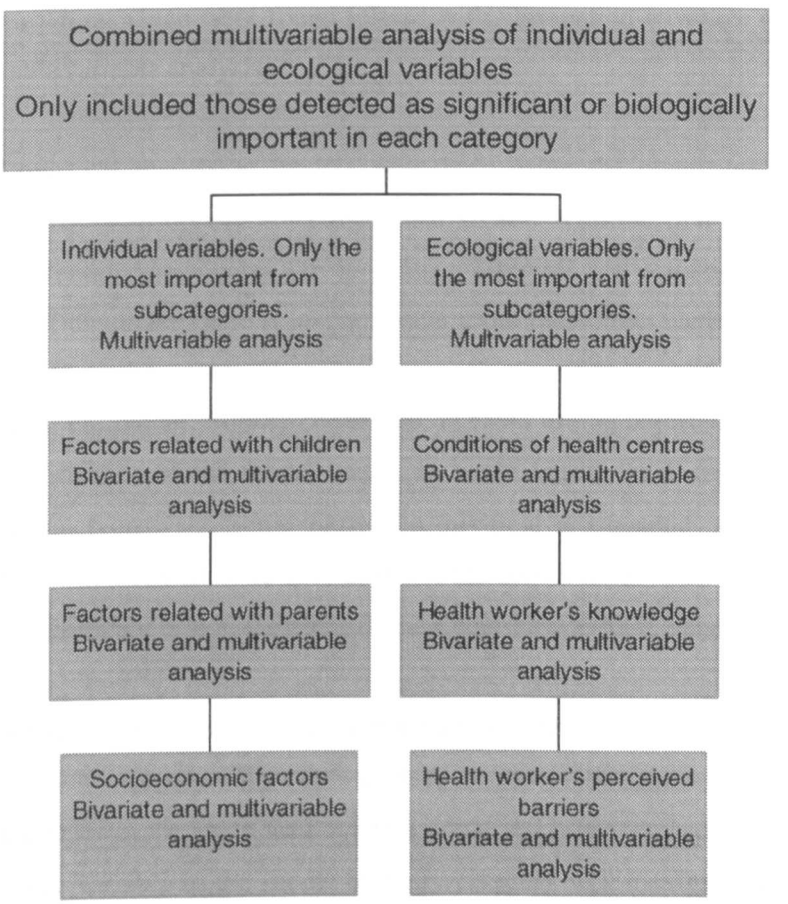
We also described the lag of time before starting hepatitis B vaccination, to complete hepatitis B vaccination and to complete the full vaccination scheme. To carry out this we calculated medians of the number of days between doses or between date of birth and doses then I described differences by area and age. Bar graphs and line graphs were used to visualise differences.

Independent variables considered in the analysis were divided in two broad categories: 1) **individual variables** which were also divided in individual factors **related to children**, **related to parents** and **related to socioeconomic conditions**. 2) **Ecological variables** which were also divided in those describing **general conditions of health centre**, those

related with **health worker knowledge on vaccines**, and variables related with **health worker perceptions about barriers** for vaccination

The aim of this part of the analysis was to identify all those variables that were statistically related with vaccination status in each category using bivariate and multivariable analysis. Within each category we used a multivariable technique (logistic regression) to identify the most important variables and after that they were included in models that combined the most important individual and ecological variables. First we analysed and identified the most important individual variables, then the ecological, and finally we combined them and identified those which were more strongly related to full and hepatitis B vaccination coverage. Figure 2.1 showed the scheme of the analysis by categories and subcategories.

Figure 3.1. Organization of the statistical analysis for vaccination coverage and related factors .



In the bivariate analysis vaccination coverage was analysed separately with the independent variables within each category. The first step was to calculate Odds Ratios and 95% confidence intervals for every association (OR and 95%CI). These measures were calculated using univariable logistic regression since Stata did not allow calculation of OR in tables when the complex design was taken into account. Nominal variables with more than two categories, such as ethnic group, were analysed as dummies. Numerical variables were transformed to logarithms when analysed as continuous variables but none of them showed a linear trend. Therefore I included only the results analysing them as categorical variables. To collapse continuous variables in categories I first took into account evidence from previous studies about the existence of a significant cut-off point. Where this evidence existed I used it but the distribution by percentiles (25%, 50%, and 75%) was also used and results of both approaches were compared. In fact most of the numerical variables used did not have a consistent and known method of collapsing them therefore results using my approach are presented. These categories were also treated as dummies. When no differences were found between contiguous categories they were joined to simplify models and interpretations. All variables which were found related with vaccination coverage ($p<0.2$) were included in the multivariable analysis.

Logistical regression models were built using the command **svylogit** and the command **logistic** with options for **cluster** and **strata**. With the first approach we obtained the most conservative estimates for confidence intervals and statistical test for individual variables coefficients but there is no consensus about the correct methods to assess the significance of whole models and to compare the contribution of individual variables when they are dropped from the model. Survey commands in Stata use an adjusted Wald test to assess the overall significance of the model that is an extension of the F test used in linear regression and variance analysis. Some authors in this field recommend using a more classical approach (Hosmer Lemeshow test) to assess if the contribution of an individual variable to the model is significant or not. (Hosmer D and Lemeshow S 2000, page 211-222).

Multiple logistic regression was used to examine which variables were going to be selected in each category. Then it was used to assess the combined effect of the most relevant variables in the ecological and individual level on vaccination coverage. The contribution of every variable to the model in every category was measured using the Hosmer Lemeshow test with a cutoff point of $p=0.1$. With the results from the logistic regression we were able to identify which variables were more statistically related with vaccination status in each of the categories and in a second step we carried out another multivariable analysis where the most important variables from each category were evaluated together.

When the ecological variables were analysed especial efforts were made to detect collinearity between covariates and to avoid the influence of this correlation on the estimates. Although some authors have claimed that only correlations coefficients above 80% influence variances and coefficients we decided to follow a more conservative approach and when two variables showed a correlation above 50% they were not included together in the same model. (Katz M 1999, page 55-59). When a higher value was found we ran models containing the correlated variables separately. If one of the variables remained statistically associated with vaccination coverage it was kept for further analysis and the other was dropped. When both remained associated the one with the highest OR was used in further models though that with the lower value was also tested in subsequent models. Some correlated ecological variables were kept until the last step of the analysis, that with individual characteristics, and they remained associated with vaccination coverage so more than one final model had to be fitted in the combined step.

B) Analysis of Hepatitis B infection and related factors: Being HBsAg positive was considered as the main outcome**. Being infected with hepatitis B** was also considered in the analysis but only in the descriptive analysis.

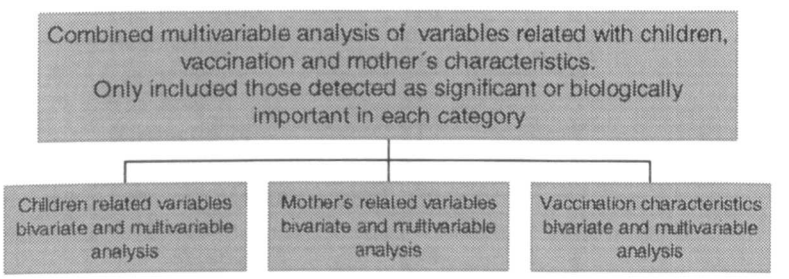
Prevalence of HBsAg positivity was calculated using the same approach for complex surveys that was described above in vaccination coverage. It was calculated for urban and rural areas stratified by age groups and by gender. Prevalence of infection with HBV was described by the same variables. Bar graphs were used at this step to show trends and differences by categories.

We compared prevalence of HBV infection and HBsAg positivity found in our study with prevalence from former studies (Cristancho LM 1993). This comparative analysis was stratified by age, sex, and place of the study. We calculated percentage differences, proportion of reduction, and 95% confidence interval. Prevalence before vaccination were obtained from the study of Cristancho 1995 who surveyed a number of rural populations in the Amazon including Puerto Nariño, Araracuara and Puerto Santander. Only results from rural areas were included to calculate the prevalence after vaccination because Cristancho did not include an urban sample of Leticia in her study. Specific results from Araracuara and Puerto Santander were compared since they were the areas with the highest prevalence before vaccine introduction.

Then we attempted to identify explanatory variables for HBsAg positivity and independent variables were divided into the following categories: 1) **Child related variables**, among these we considered age, sex, gender, birth order, qualification of the person delivering the child, and ethnic group. 2) **Vaccination characteristics**, here we considered time in days between birth and the first dose of hepatitis B, time between first and second dose, and time between second and third dose. 3) **Mother related variables**, which were basically the serological status of the mothers regarding hepatitis B infection, place where mother was born, and mother's history of clinical hepatitis.

As before each category was analysed separately, using bivariate (OR and 95%CI) and multivariable analysis (logistic regression), and the most important variables in each category were considered for a final analysis using multivariable logistic regression techniques. As for vaccination coverage I built logistic models using the **svy** and the **logistic** command with **cluster** and **strata** option. Criteria to introduce or to drop variables were similar to those described above.

Figure 3.2. Organization of the statistical analysis for HBV prevalence of infection and related factors.



C) **Analysis of Anti-HBs titres:** Anti-HBs titres were considered as the dependent variable but in the analysis we treated it in two ways. First we divided it into two categories, **being seroprotected or not** and in the second as **a continuous variable**.

In the analysis with titres as categories we tried to identify variables related with not being protected, i.e.having undetectable levels of anti-HBs. As independent variables in these analyses we considered children's age, gender, ethnic group, breastfeeding, time in days between doses of vaccine, and time in days between last dose and the date when the sample was taken. Bivariable analysis was done calculating OR and 95% CI as a measure of the degree of the association. Those variables found related ($p<0.1$) in the bivariable analysis

were included in a logistical model where the contribution of each variable to the model was assessed as described before.

The same independent variables were considered when anti-HBs titres were treated as a continuous variable. In this case geometric means and medians of titres were calculated for every category of the independent variable. Means or medians differences were tested with non-parametric techniques such as the Kruskall Wallis test. A multivariable model was constructed using lineal regression techniques in order to include those variables that showed important differences in mean anti-HBs ($p<0.1$).

Table 3.2. Population distribution in urban and rural areas of the municipality of Leticia

Village	# of habitants	Selected for study
Leticia	15400	Y
Kilómetro 18	81	Y
Huacarí	42	Y
Vergel	120	Y
Mocagua	175	Y
Arara + Sta. Rosa	364	Y
Bora	6	N
San José Km. 6	635	N
Palmeras	126	N
Santa Sofía	245	N
Los Escobedo	158	Y
San Pedro	17	Y
Zaragoza	347	N
San José	24	Y
Kilometro 11	81	Y
Isla Mocagua	57	N
San Martín de Amocayacu	396	N
San Sebastián	194	Y
San Miguel	110	Y
Loma Linda	112	N
San Juan de los Parentes	72	N
Huanganayo	224	N
Macedonia	637	N
Kilometro 7	46	Y
Nazareth	581	N
La Milagrosa	144	N
Multietnica	150	N
Yaguas	140	N
San Antonio	168	N
Monillamena	42	N
Total rural area	**5494**	

Y= Included in the study N= Not included

Table 3.3. Population distribution in urban and rural areas of the municipality of Puerto Nariño.

Village	# of habitants	Selected for study
Puerto Nariño (urban)	1433	Y
Tipisca	77	Y
Villa Castillito	25	Y
Nuevo Porvenir	22	Y
Pozo Redondo	168	Y
Santa Teresa	50	Y
Villa Andrea	59	Y
Quebrada Nonten	15	Y
Lago Tarapoto	35	Y
Santarén	47	Y
San Juan del Soco	193	Y
Isla Patrullero	73	N
Boyahuasu	334	Y
Siete de Agosto y Bocas de Atacuari	539	Y
Naranjales	349	Y
20 de Julio	191	N
Nuevo Paraíso	54	N
San Francisco	430	N
Hacienda San Francisco	30	N
Total rural population	**2691**	

Y= Included in the study N= Not included in the study

Table 3.4. Population distribution in Leticia by neighbourhoods and blocks

Neighbourhood	# of blocks n (%)	Population n (%)	Children under 10 y. n (%)	# of blocks selected for study
Colombia	13 (8)	980 (6)	244 (8)	7 (12)
Simón Bolivar	18 (11)	1650 (11)	209 (7)	8 (13)
Esperanza	9 (6)	890 (6)	212 (7)	0 (0)
Victoria Regia	8 (5)	360 (0.4)	139 (5)	2 (3)
Porvenir	33 (20)	3400 (22)	671 (23)	6 (10)
San Martín	5 (3)	665 (4)	106 (4)	3 (5)
11 de Nv/bre	9 (5)	950 (6)	196 (7)	3 (5)
Gaitán	9 (5)	843 (6)	181 (6)	6 (10)
Centro A	20 (12)	1675 (11)	261 (9)	6 (10)
IANE	6 (4)	602 (4)	98 (3)	4 (7)
Aguila	1 (0.6)	300 (2)	61 (2)	0 (0)
Centro B	27 (16)	2690 (17)	390 (14)	12 (20)
Humarizal	5 (3)	395 (2)	90 (3)	3 (5)
Total	**163 (100)**	**15,400 (100)**	**2,858 (100)**	**60 (100)**

Table 3.5. Expected results of the serological survey. n=2500.

Variable	Expected %	Expected #
Prevalence of infection	15 (10-20)	375(250-500)
Prevalence of HBsAg	5 (3-7)	125(75-175)
Expected # of mothers	1.5-3.0 chd/m	833-1667
Expected infected mothers	50-70	416-1167
Expected HBsAg + mothers	15 (10-20)	250(83-333)
Expected HBeAg+ mothers	10% of HBsAg+ (5-15)	25(4-50)
Children with complete immunization	40-70	1000-1750
Children with 2 and 3 dose later than recommended	30	300-525
Children with interval between 1 and 3 dose later than 6 months	50	500-875
Children with timely and complete schedule	50-70% of those with complete schedule	500-1225
Infected among those timely and completely immunized	4-8	20-98
Children with incomplete immunization	60-30	750-1500
Infected among incompleted immunised children	8-16	60-240
Infected among those with untimely schedule	6-10	30-122
Children born from HBsAg + mothers	1.5-3.0 children/mother	375(124-1000)
Children born from HBsAg+ mothers and unvaccinated	30-60	37-600
Children born from HBsAg+ mothers vaccinated	40-70	50-700
Children born from HBeAg + mothers	1.5-3.0 children/mother	37(6-150)
Children born from HBeAg+ mothers unvaccinated	30-60	22(2-90)
Children born from HBeAg+ mothers vaccinated	40-70	26(2-105)
Infected in unvaccinated children from mother HBsAg+	20	7-120
Infected in vaccinated children from mother HBsAg+	5	2-35
Infected in unvaccinated children from HBeAg+	90	20(2-80)
Infected in vaccinated children from HBeAg+	10	3(0-10)

Chapter 4: Results on vaccination coverage

Summary: We surveyed 3044 children between one and 11 years old. Vaccine coverage was highest for yellow fever (96%), followed by measles (94%), BCG (91%), DPT (90%) and hepatitis B (88%). Children in rural areas had to wait for longer periods to receive HBV vaccine dose than children in urban areas. The median age to complete the HBV scheme was 4 months in urban areas while it was 8 months in rural. Factors related to vaccination were divided broadly into individual and ecological variables and they were analysed separately for HBV vaccination and for full vaccination.

The following individual variables were related to not being fully vaccinated: "living in Puerto Nariño" (OR=4.3 95%CI 2.4-7.6) and "not being affiliated to the social security" (OR=1.7 95%CI 1.1-2.6). In urban areas "living in a house roofed with palm tree leaf" was also associated with a lower chance of full vaccination (OR=3.5 95%CI 1.6-7.8). Belonging to a non Indian group was protective against no vaccination (OR=0.4 95%CI 0.2-0.7). The individual variables related with not being completely vaccinated against hepatitis B were: "number of siblings above 3" (OR=3.2 95%CI 1.0-11.0) and "living in Puerto Nariño" (OR=2.3 95%CI 1.3-4.2). Living in Araracuara increased the chance of being completely vaccinated (OR=0.2 95%CI 0.1-0.7). In urban areas, "living in a house roofed with palm tree leaf" was again related with less chance of HBV vaccination (OR=3.1 95%CI 1.1-8.2).

The most important ecological variables analysed were the number of contraindications that health workers mentioned for every vaccine (polio, DPT and hepatitis B), the length of time working in the community, and the perception about the severity of hepatitis B disease. After controlling for the most important individual variables we found that the ecological variables related with lower full vaccination were: "lack of supplies" (OR=3.0 95%CI 1.5-6.0), perceiving "parents' fear of vaccine side effects" as a barrier (OR=2.2 95% CI 1.3-3.9), "number of contraindications against polio" (OR=1.4 95%CI 0.8-2.3). "Working for more than 14 years in the health centre" was protective against lower levels of full vaccination (OR=0.4 95%CI=0.3-0.6). The same variables were related with hepatitis B vaccination except for "contraindications against polio" that was replaced by "contraindication against hepatitis B vaccine" (OR=2.3 95%CI 1.1-5.1). The length of time working in the health centre was associated again in a protective way with hepatitis B vaccination.

Vaccination coverage and characteristics:

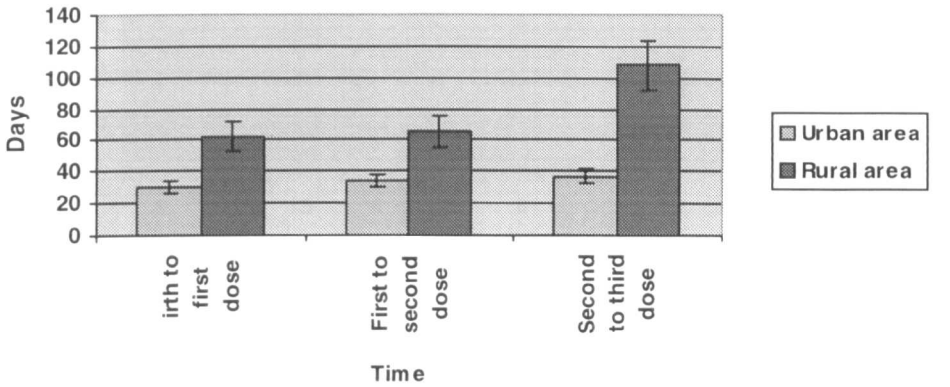
General description: We surveyed 104 clusters, 60 in urban Leticia, 12 in rural Leticia, 19 in the urban area of Puerto Nariño, 9 in rural Puerto Nariño and 4 in other rural areas of Amazons, namely Puerto Santander and Araracuara.

The census recorded 3573 people and 3044 of them were one year old or older. Most of them, 1621, lived in Leticia's urban area (56%), 341 in Puerto Nariño's urban area (7%), 765 in rural areas of Leticia (21%), 508 in the rural areas of Puerto Nariño (9.6%), and 331 (4%) in Araracuara and Puerto Santander. Among the 3475 children of whom data on age was available, the range was from 0 to eleven years while the median was 5.0 years. Twenty five percent of the children were less than 2 years old while 75% were under 7. Among 3548 children with gender data available, 1890 (53%) were males and 1658 females (47%). Most children (82%) were living in their place of birth.

Vaccine information was not available for every one. It was more frequently found for hepatitis B with information available for 2242 children, followed by DPT with 2158, BCG with 2005, yellow fever with 1839, and measles with 1791. Vaccine coverage was higher for yellow fever (96%), followed by measles (94%), BCG (91%), DPT (90%), and Hepatitis B (88%). Coverage for hepatitis B was similar for rural and urban areas; only among children aged one there were differences by area. In rural areas, hepatitis B coverage in this age group was 67% (67/100) while in urban areas it was 83% (106/127).

Fifty percent of the children under 8 years old (born after vaccine was introduced), received the first dose of hepatitis B vaccine within the first 45 days after birth, while 25% received it in the first 3 days and 75% before day 342 after birth. The median interval between the first and second dose was 41 days and between second and third was 72 days. In general, intervals in rural areas were longer compared to urban areas. Figure 4.1.

Figure 4.1. Time in days between hepatitis B doses by area.



The median age to complete hepatitis B schedule was 8 months for children between one and 8 years of age and 75% percent completed it before 16 months of life. Here again differences were greater between urban and rural areas. In urban areas the median time to complete the schedule was 143 days and 75% percent of urban children completed the schedule before 362 days. In rural settlements the median time to complete hepatitis B schedule was 334 days and only 25% of children completed it before 6 months after birth. In older children, five years and above, there were no important differences, but among the youngest rural children, intervals were longer. Figure 4.2 shows intervals by age and area.

Figure 4.2. Median time between birth and third dose of hepatitis B by area and age

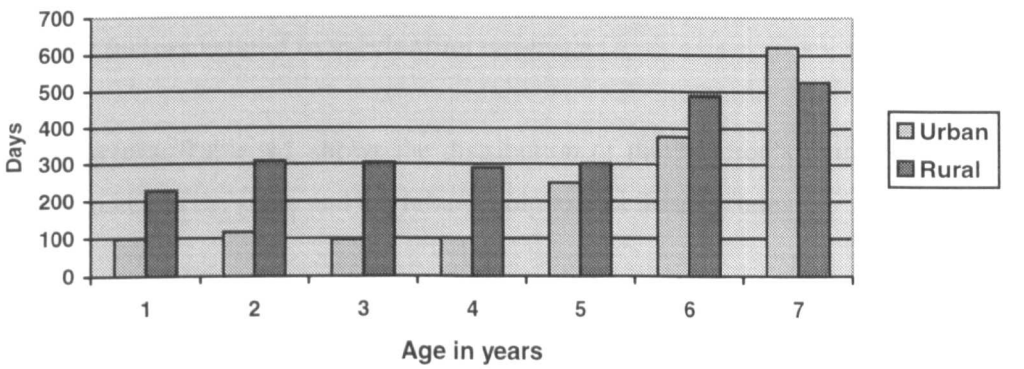
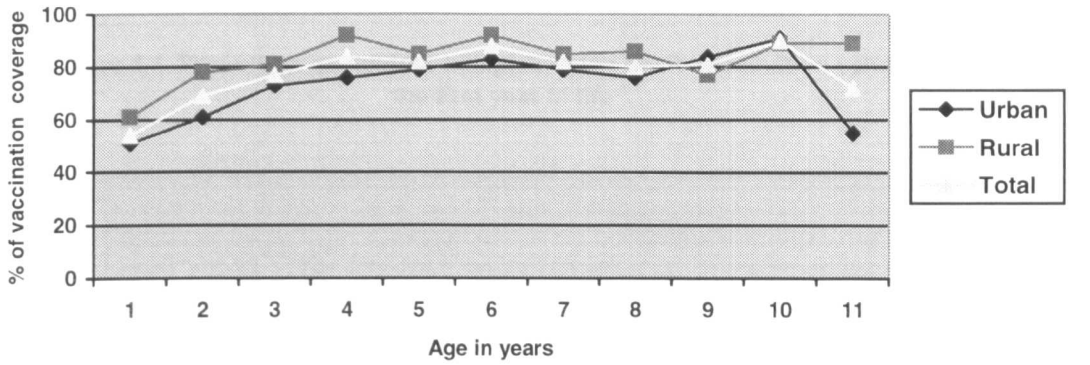


Figure 4.3. Full vaccination coverage by age and area



For 1380 children there was enough information to evaluate their full vaccination status. Full immunisation coverage was 78% (CI95% 74%-83%), but there were differences by area. Surprisingly, in urban areas full vaccination coverage was 73%, while in rural areas it was 84%, p=0.01. Figure 4.3 shows coverage by age and area. A slight difference in coverage in every age group was observed, especially in the first four years of life and in the last category, though in this there are very few observations (9 in each area). None of these differences were statistically significant, p>0.05.

Time between birth and yellow fever vaccine was used as a proxy to evaluate the age at which children completed the vaccination schedule. The reason to do so was that yellow fever is the last vaccine applied in Colombia's scheme. Only 38% of children completed the vaccination scheme in the first year of age, 26% in the second year, 14% in the third year, and 22% completed it in the fourth year or beyond. Figure 4.4 shows the proportion of children that completed the vaccination scheme in the first year of life, by age and area.

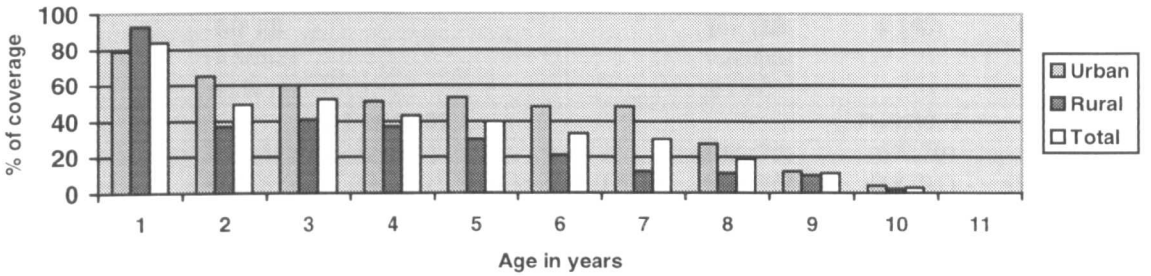
II. Individual factors related to vaccination coverage.

II.1. Child factors: Table 4.1 shows the distribution of the children's main demographic variables evaluated in the study and the relationship to vaccination status.

In bi-variable analysis, age was strongly related to being fully vaccinated and being completely vaccinated against hepatitis B. Children less than 2 years old had the highest probability of not being completely vaccinated against hepatitis B as well as against other

diseases. The peak of full vaccination is reached at the age of 6 while the peak of hepatitis B vaccination is reached at the age of five. At older ages coverage remains stable.

Figure 4.4. Proportion of children completing the basic vaccination scheme in the first year of life



As regards geographical characteristics, the urban area of Puerto Nariño had the lowest coverage of full vaccination and hepatitis B vaccine. The highest coverage was found in rural areas of Leticia and Puerto Nariño followed by urban Leticia and Araracuara. The highest hepatitis B coverage was found in Araracuara, though it was also high in rural and urban Leticia. In rural areas of Puerto Nariño vaccine coverage was higher than in urban areas.

"Birth order" was related to full vaccination showing a U shaped relation. The first born had a higher probability of being fully vaccinated compared with those born second through fifth. Those born sixth or later had again a higher chance of being fully vaccinated. A similar finding was observed for hepatitis B, though differences were less significant.

"Huitotos" showed the lowest full vaccination while "Mestizos" had the highest, followed by members of Indians groups other than "Huitotos" or "Ticunas", the biggest Indian ethnic groups in the area. Hepatitis B coverage was higher in "Mestizos" and Huitotos while it was lower among "Ticunas" and "non Indian populations" but the overall differences between groups was not significant (p=0.2).

Being affiliated to social security was protective against not being fully vaccinated. Unaffiliated people had 76% more chance of not being fully vaccinated compared with those affiliated. Interestingly, no difference was found regarding hepatitis B vaccination.

Table 4.1. Vaccination coverage by variables related to children.

Variables	Children with information for all vaccines # (%)*	Fully vaccinated # (%)	OR (CI95%) Incomplete schedule	Children with information for HB vaccine # (%)*	Completely vaccinated with HB # (%)	OR (CI95%) Incomplete HB vaccination
Age		**P<0.0001**			**P=0.0001**	
1 year	158 (57)	65 (41)	1.0	203 (73)	167 (79)	1.0
2/3 years	351 (52)	235 (67)	0.34 (0.2-0.5)	514 (76)	468 (91)	0.37 (0.2-0.6)
4/5 years	354 (55)	269 (76)	0.22 (0.1-0.3)	402 (63)	374 (93)	0.26 (0.1-0.5)
6/7 years	315 (49)	236 (75)	0.22 (0.1-0.3)	359 (56)	316 (88)	0.48 (0.3-0.9)
8/11 years	351 (43)	249 (71)	0.28 (0.2-0.4)	430 (53)	379 (88)	0.47 (0.3-0.8)
Area		**P=0.02**			**P=0.05**	
Urban Leticia	723 (49)	514 (71)	1.0	837 (56)	745 (89)	1.0
Rural Leticia	408 (60)	282 (69)	1.1 (0.7-1.8)	533 (79)	480 (90)	0.98 (0.5-2.0)
Puerto Nariño	120 (39)	47 (39)	3.8 (2.4-6.1)	136 (44)	99 (73)	3.13 (2.1-4.8)
Rural Puerto Nariño	192 (29)	131 (68)	1.15 (0.5-2.7)	240 (76)	202 (84)	1.41 (0.7-3.0)
Araracuara	125 (48)	80 (64)	1.36 (0.8-2.4)	185 (71)	178 (96)	0.37 (0.1-0.9)
Ethnic group		**P=0.009**			**P=0.025**	
No Indians	618 (43)	408(66)	1.0	708 (49)	616 (87)	1.0
Mestizos	196 (58)	163 (83)	0.4 (0.2-0.6)	283 (84)	269 (95)	0.32 (0.2-0.6)
Ticunas	453 (58)	290 (64)	1.1 (0.65-1.8)	563 (72)	484 (86)	1.11 (0.6-2.1)
Huitotos	72 (48)	43 (60)	1.3 (0.7-2.2)	97 (65)	89 (92)	0.54 (0.2-1.4)
Other groups	203 (60)	150 (74)	0.7 (0.4-1.1)	267 (78)	246 (92)	0.59 (0.3-1.0)
Birth order		**P=0.04**			P=0.16	
1	277 (51)	205 (74)	1.0	368 (68)	335 (91)	1.0
2/3	500 (54)	350 (70)	1.2 (0.8-1.8)	628 (67)	553 (88)	1.61 (1.0-2.6)
4/5	321 (61)	209 (65)	1.5 (1.0-2.2)	389 (75)	342 (88)	1.87 (1.1-3.1)
6/20	175 (56)	133 (76)	0.9 (0.6-1.4)	239 (77)	220 (92)	1.10 (0.6-2.2)
Number of siblings		P=0.2			P=0.2	
1	46 (54)	27 (61)	1.0	77 (90)	72 (94)	1.0
2/3	511 (55)	357 (72)	0.6 (0.3-1.2)	632 (68)	562 (89)	1.8 (0.6-6.0)
4/5	424 (57)	283 (67)	0.8 (0.4-1.6)	510 (68)	442 (87)	2.4 (0.8-7.1)
6/20	303 (58)	221 (74)	0.5 (0.2-1.2)	393 (75)	360 (91)	1.5 (0.5-4.5)
Health security		**P=0.008**			P=0.30	
Affiliated	836 (51)	602 (72)	1.0	1133 (69)	1020 (90)	1.0
Not affiliated	188 (48)	109 (58)	1.8 (1.2-2.2)	251 (64)	216 (86)	1.67 (0.7-4.0)

* Percentage calculated on the total number of children of every category identified in the survey.

All variables associated with vaccination and showing a p value of 0.2 or less were included in one step. In multivariable models "ethnic group", area, and "being affiliated to social security" remained significantly associated to not being fully vaccinated. Besides age, the strongest association was observed for "area", Puerto Nariño being the place where the risk of not being vaccinated was the highest. As concerns "ethnic group", the only statistical difference was observed for "other groups", who were better vaccinated than the others. "Number of siblings" and "birth order" were not related to full vaccination. Age and area

were also associated to not being completely vaccinated against hepatitis B, but "ethnic group" and "being affiliated to the social security" were not. Instead a larger number of siblings was related to incomplete hepatitis B vaccination. "Birth order" was not related to hepatitis B vaccination but it was kept in the final model to control the effect of "number of siblings". Tables 4.2 and 4.3.

Table 4.2 Children's characteristics and not being fully vaccinated. Final model

Variables	OR (CI95%)	P
Age		
1 year	1.0	
2/3 years	0.20 (0.1-0.3)	<0.001
4/5 years	0.11 (0.1-0.2)	<0.001
6/7 years	0.12 (0.1-0.2)	<0.001
8/11 years	0.20 (0.1-0.3)	<0.001
Ethnic group		
No Indians	1.0	
Mestizos	0.64 (0.3-1.2)	0.17
Huitotos	1.32 (0.7-2.6)	0.42
Ticunas	0.91 (0.5-1.6)	0.73
Other groups	0.53 (0.3-0.99)	0.04
Area		
Urban Leticia	1.0	
Rural Leticia	1.34 (0.8-2.2)	0.23
Puerto Nariño	4.3 (2.4-7.6)	<0.001
Rural Puerto Nariño	1.47 (0.6-3.6)	0.40
Araracuara	1.45 (0.8-2.8)	0.24
Affiliated to health security		
Not affiliated	1.69 (1.1-2.6)	0.02

II.2. Parent factors: In bi-variable analysis, "mother's age at survey time" was associated with being fully vaccinated but not with hepatitis B vaccination. A child whose mother was less than 21 years old at the time of the survey had less chance of being fully vaccinated than one whose mother was older. "Mother's age at child's birth" was not associated with being fully vaccinated or hepatitis B vaccination.

"Mother's years of schooling" showed some relation to being fully vaccinated in the multivariable model. Children whose mothers never went to school or who did not complete primary level had the lowest coverage (70%) of full vaccination. Full vaccination coverage tended to be higher for children whose mothers attended school for more than 6 years.

Table 4.3. Children's characteristics and not being vaccinated against hepatitis B. Final model

Variables	OR (CI95%)	P
Age		
1 year	1.0	
2/3 years	0.24 (0.1-0.4)	<0.001
4/5 years	0.16 (0.1-0.3)	<0.001
6/7 years	0.29 (0.1-0.6)	<0.001
8/11 years	0.36 (0.2-0.6)	<0.001
Number of siblings		
1	1.0	
2/3	2.5 (0.8-7.5)	0.092
4/5	3.1 (1.0-10.2)	0.05
6/20	2.4 (0.7-8.2)	0.15
Birth order		
1	1.0	
2/3	1.1 (0.7-1.8)	0.70
4/5	1.0 (0.5-2.1)	0.94
6/20	0.8 (0.3-2.3)	0.67
Area		
Urban Leticia	1.0	
Rural Leticia	0.5 (0.2-1.0)	0.06
Puerto Nariño	1.5 (0.8-2.7)	0.19
Rural Puerto Nariño	0.8 (0.3-2.1)	0.60
Araracuara	0.3 (0.1-0.9)	0.03

In the multivariable model, factors related to parents were not strongly associated with not being fully vaccinated. Only one category of "mother's schooling" was statistically related, at the borderline of significance, to not being vaccinated and the same with mother's age at survey time: only those mothers aged 20/24 showed a difference for their children

No variable in this category was associated to not being vaccinated against hepatitis B. All of them were included in a logistic model and discarded based on the likelihood ratio test ($p>0.1$).

II.3. Socio-economic factors: In this category there are variables related to house characteristics (roof, floor, walls, piped water, excretal disposal, and crowding), and variables related to economic affluence of family (owning things like freezer, TV, radio, and outboard motor). Full vaccination was associated with both kinds of variables but almost all variables in the second group were statistically related while in the first group only "roof

material" (p=0.003) and "floor material" (p=0.07) showed a relation to full vaccination. In the second group the strongest association was observed with "owning a freezer" (p=0.001). Children living in a house without a freezer had an 80% decrease in the chance of being completely vaccinated. In general, all characteristics that could be related to a better standard of living were protective against not being fully vaccinated, and the strongest relation was to roof material. In the multivariable model only "roof material" and "owning a freezer" remained associated with not being fully vaccinated. Tables 4.6 and 4.7.

Table 4.4. Vaccination coverage according to parents' characteristics.

Variable	Children with information for all vaccines # (%)*	Fully vaccinated # (%)	OR (CI95%) Incomplete schedule	Children with information for HB vaccine # (%)*	Completely vaccinated with HB # (%)	OR (CI95%) Incomplete HB vaccination
Mother's age at survey		**P=0.04**			P=0.43	
16/19	58 (55)	32 (55)	1.0	94 (89)	86 (91)	1.0
20/24	267 (57)	200 (75)	0.4 (0.2-0.8)	352 (75)	313 (89)	1.19 (0.5-2.6)
25/30	334 (54)	246 (73)	0.45 (0.2-0.9)	426 (69)	388 (91)	0.96 (0.4-2.4)
31/35	289 (59)	191 (66)	0.6 (0.3-1.27)	376 (76)	323 (86)	1.52 (0.6-3.9)
36/51	300 (55)	219 (73)	0.4 (0.2-0.8)	372 (69)	339 (91)	1.0 (0.4-2.4)
Father's age at survey		P = 0.18			P =0.65	
16-25 y	95 (56)	57 (60)	1.0	132 (77)	115 (87)	1.0
26-30 y	190 (55)	135 (71)	0.59 (0.3-1.0)	256 (74)	228 (89)	0.83 (0.4-1.5)
31-35 y	228 (59)	164 (72)	0.56 (0.3-1.0)	273 (70)	243 (89)	0.80 (0.4-1.7)
36-57 y	501 (57)	361 (72)	0.56 (0.3-0.9)	631 (71)	568 (90)	0.71 (0.4-1.2)
58-76 y	28 (56)	17 (61)	0.92 (0.3-2.7)	37 (74)	30 (81)	1.54 (0.5-4.8)
Mothers education level (years in school)		P=0.22			P=0.69	
0/4	426 (55)	298 (70)	1.0	593 (77)	534 (90)	1.0
5	304 (59)	222 (73)	0.8 (0.6-1.3)	396 (76)	356 (90)	1.0 (0.6-1.6)
6/8	136 (48)	102 (75)	0.7 (0.4-1.2)	196 (70)	184 (94)	0.60 (0.3-1.2)
9/10	63 (55)	51 (81)	0.5 (0.2-1.4)	74 (65)	68 (92)	0.82 (0.3-2.6)
11/17	182 (57)	135 (74)	0.8 (0.5-1.4)	227 (71)	204 (90)	1.0 (0.5-2.1)

* Percentage calculated on the total number of children of every category identified in the survey.

Table 4.5. Parents' characteristics and not being fully vaccinated. Final model

Variable	OR (CI95%)	P
Children's age (years)		
1	1.0	
2/3	0.31 (0.2-0.5)	<0.001
4/5	0.21 (0.1-0.3)	<0.001
6/7	0.19 (0.1-0.3)	<0.001
8/11	0.25 (0.2-0.4)	<0.001
Mother's schooling years		
0/4	1.0	
5	0.81 (0.5-1.2)	0.305
6/8	0.59 (0.3-1.0)	0.069
9/10	0.48 (0.2-1.4)	0.165
11/17	0.67 (0.4-1.1)	0.139
Mother's age at survey (years)		
16/19	1.0	
20/24	0.46 (0.2-0.9)	0.029
25/30	0.62 (0.3-1.2)	0.174
31/35	0.89 (0.4-1.8)	0.760
36/51	0.60 (0.3-1.2)	0.155

Hepatitis B vaccination was associated with fewer variables. "Roof materials" and "owning an outboard motor" were related to hepatitis B vaccination and this association remained in the multivariable analysis. Table 4.8.

II.4. Models combining individual variables:

II.4.1 Not being fully vaccinated: At this stage the following variables were included:

Children's age by categories.

Mother's years of schooling.

Kind of material of roof.

Mother's age at survey.

Ethnic group.

Being affiliated to the Health Security System.

Owning a freezer.

Three basic sets of models were constructed. In the first all participants were included, in the second only children form urban areas and in the last children from rural areas. Table 4.9 shows the final model when the whole population was analysed. "Age", "area", "ethnic

group" and "not being affiliated to the social security" were associated with not being fully vaccinated.

Table 4.6. Vaccination coverage by socio-economic characteristics.

Variables	Children with information for all vaccines # (%)*	Fully vaccinated # (%)	OR (CI95%) Incomplete schedule	Children with information for HB vaccine # (%)*	Completely vaccinated with HB # (%)	OR (CI95%) Incomplete HB vaccination
Roof made with:		**P=0.003**			**P=0.01**	
Tile	1284 (50)	899 (70)	1.0	1672 (65)	1488 (89)	1.0
Palm tree leaf	129 (34)	67 (52)	2.0 (1.4-3.0)	223 (58)	183 (82)	1.91 (1.1-3.2)
Floor made with:		**P=0.07**			P=0.58	
Cement	540 (51)	389 (72)	1.0	612 (58)	551 (90)	1.0
Wood	972 (53)	622 (64)	1.4 (1.0-2.0)	1232 (67)	1085 (88)	1.20 (0.8-1.8)
Soil	43 (43)	32 (74)	0.9 (0.4-1.9)	57 (58)	51 (89)	1.02 (0.4-2.6)
Crowding: # of people by room		P=0.11			P=0.78	
1/3	697 (49)	495 (71)	1.0	827 (58)	736 (89)	1.0
4/6	484 (54)	305 (63)	1.4 (1.1-1.9)	614 (68)	540 (88)	1.03 (0.7-1.6)
7/9	197 (58)	134 (68)	1.1 (0.7-1.8)	251 (74)	223 (89)	0.95 (0.6-1.4)
10/15	110 (45)	74 (67)	1.2 (0.8-1.8)	155 (64)	141 (91)	0.76 (0.4-1.6)
Freezer at home		**P=0.001**			**P=0.09**	
Y	345	266 (77)	1.0	461	424 (92)	1.0
N	789	513 (65)	1.8 (1.2-2.6)	1037	913 (88)	1.55 (0.9-2.6)
TV at home		**P=0.06**			P=0.36	
Y	493	360 (73)	1.0	644	586 (91)	1.0
N	645	421 (65)	1.4 (1.0-2.2)	859	756 (88)	1.27 (0.8-2.2)
Radio at home		**P=0.05**			P=0.22	
Y	512	374 (73)	1.0	775	628 (81)	1.0
N	617	407 (66)	1.39 (1.0-2.0)	811	714 (88)	1.34 (0.8-2.2)
Outboard motor		P=0.11			**P=0.06**	
Y	96	73 (76)	1.0	140	132 (94)	1.0
N	1020	704 (69)	1.48 (0.9-2.4)	1360	1210 (89)	1.78 (1.0-3.3)

Table 4.7. Socio-economic characteristics and not being fully vaccinated. Final model

Variable	OR (CI95%)	P
Children's Age		
1	1.0	
2/3	0.24 (0.2-0.4)	<0.001
4/5	0.13 (0.1-0.2)	<0.001
6/7	0.14 (0.1-0.2)	<0.001
8/11	0.21 (0.1-0.4)	<0.001
Roof made with		
Palm tree leaf vs. Tile	1.91 (1.2-3.0)	0.006
Freezer		
N	1.71 (1.2-2.4)	0.004

Table 4.8. Socio-economic characteristics and not being vaccinated against hepatitis B. Final model

Variable	OR (CI95%)	P
Children's Age		
1	1.0	
2/3	0.22 (0.1-0.4)	<0.001
4/5	0.13 (0.1-0.3)	<0.001
6/7	0.20 (0.1-0.4)	<0.001
8/11	0.32 (0.2-0.6)	<0.001
Roof made with		
Palm tree leaf vs. tile	2.04 (1.1-3.9)	0.031
Owning an outboard motor		
N	1.85 (0.9-3.8)	0.09

Tables 4.10 and 4.11 shows the final results when the analysis was stratified by area. "Ethnic group" has a different effect when area is taken into account. In urban areas, "Mestizos" were better vaccinated than other groups while "Ticunas" had the lowest chance of being fully vaccinated. In rural areas, "ethnic group" was not associated with full vaccination. The socioeconomic characteristics were also related to full vaccination but in different ways for rural and urban areas. In urban areas, "living in a house with a palm tree leaf roof" is associated with a decrease in the chance of being fully vaccinated, but ownership is not. In rural areas, "palm tree leaf roof" is also associated with a lower chance of vaccination, but the relation is not as strong as it is in urban areas. On the other hand, "owning a freezer" is not associated with full vaccination in urban areas, but it is in rural areas where not owning one was associated with a lower chance of being fully vaccinated. Interestingly, not being affiliated to the social security was associated with a lower likelihood of full vaccination in rural areas but not in urban. Children lacking a social security card have twice the chance of not being fully vaccinated than children holding one.

II.4.2. Not being vaccinated against hepatitis B:

The variables included at this stage were:

Children's age.

Number of siblings.

Birth order.

Study area.

Roof's material.

Owning an outboard motor.

Ethnic group.

Table 4.9. Selected individual variables and not being fully vaccinated. All children. Final model

Variable	OR (CI95%)	P
Age		
1	1.0	
2/3	0.20 (0.1-0.3)	<0.001
4/5	0.11 (0.1-0.2)	<0.001
6/7	0.12 (0.1-0.2)	<0.001
8/11	0.20 (0.1-0.3)	<0.001
Area		
Urban Leticia	1.0	
Rural Leticia	1.3 (0.8-2.2)	0.232
Urban Puerto Nariño	4.3 (2.4-7.6)	<0.001
Rural Puerto Nariño	1.47 (0.6-3.6)	0.397
Araracuara	1.45 (0.8-2.7)	0.245
Ethnic group		
No Indians	1.0	
Mestizos	0.53 (0.3-1.0)	0.048
Huitotos	1.32 (0.7-2.6)	0.422
Ticunas	0.91 (0.5-1.5)	0.731
Other groups	0.64 (0.3-1.2)	0.174
Affiliated to social security		
N	1.69 (1.1-2.6)	0.02

Table 4.10. Selected individual variables and not being fully vaccinated. Urban area. Final model

Variable	OR (CI95%)	P
Age		
1	1.0	
2/3	0.44 (0.2-0.8)	0.005
4/5	0.30 (0.2-0.5)	<0.001
6/7	0.35 (0.2-0.6)	0.001
8/11	0.43 (0.2-0.8)	0.007
Ethnic group		
No Indians	1.0	
Mestizos	0.42 (0.2-0.7)	0.002
Huitotos	1.06 (0.4-2.8)	0.900
Ticunas	1.65 (1.0-2.8)	0.069
Others groups	0.99 (0.4-2.3)	0.99
Roof made with		
Palm tree leaf vs. tile	3.48 (1.6-7.8)	0.003

Table 4.11. Selected individual variables and not being fully vaccinated. Rural area. Final model

Variable	OR (CI95%)	P
Age		
1	1.0	
2/3	0.16 (0.1-0.3)	<0.001
4/5	0.07 (0.03-0.2)	<0.001
6/7	0.09 (0.04-0.2)	<0.001
8/11	0.13 (0.1-0.3)	<0.001
Roof made with		
Palm tree leaf vs. tile	1.7 (1.0-3.0)	0.06
Freezer		
N	1.8 (1.0-3.0)	0.031
Affiliated to social security		
N	2.2 (1.3-3.6)	0.005

Again, three basic models were created. One for the whole population, another for the rural population and one for urban children. In the model including the whole population, four variables (children's age, number of siblings, study area, and roof's material) remained statistically associated with not being vaccinated against hepatitis B. "Owning an outboard motor" and "ethnic group" were dropped from the model (p=0.75 and p=0.3 respectively). An attempt to reintroduce "mother's schooling" and "mother's age at children birth" in the final model did not produce any change in the results showed in Table 4.12

In urban areas, "children's age", "number of siblings" and "roof's materials" were found associated with complete vaccination against hepatitis B. "Number of siblings" showed an especially strong relation to not being completely vaccinated against hepatitis B. Compared to children without siblings those who have two or more had a very high risk of not being completely vaccinated. A clear trend was observed for categories of this variable, and the risk of not being vaccinated against hepatitis B was as high as 6.5 when number of siblings was over 5. Children living in a house with a palm tree leaf roof had a higher chance of not being completely vaccinated against hepatitis B, an association that was stronger in urban areas than in rural areas or when the whole population was considered. Table 4.13

In rural areas "children's age" and "number of siblings" were statistically associated with hepatitis B vaccination. The association of "number of siblings" with hepatitis b vaccination was weaker in rural areas compared to urban ones. Having 4 or more 3 siblings was associated with a 3 fold increase in the risk of not being vaccinated against hepatitis B

(OR=3.3 CI95% 0.9-13.1, p=0.08). "Palm tree leaf roof" showed some relation but without statistical significance (OR=1.8 CI95% 0.8-3.9 p=0.13)

III. Ecological variables related to individual vaccination data.

III.1 Results of health worker questionnaire

III.1.1. General characteristics of health centres: We interviewed 4 nurses, 5 auxiliary nurses and 15 health promoters. The median time working in their profession was 7 years ranging from 0 to 32. "Time working in the health centre" also ranged from 0 to 32 years with a median of 6 years. Most of the health centres included in the study had only one health worker (15/19), three had between 6 and 17 health workers and 2 had more than 100 employees. Medical doctors were available in four centres and nurses in three.

Table 4.12. Selected individual variables and not being vaccinated against hepatitis B. Final model

Variables	OR (CI95%)	P
Age		
1 year	1.0	
2/3 years	0.23 (0.1-0.4)	<0.001
4/5 years	0.16 (0.1-0.3)	<0.001
6/7 years	0.30 (0.1-0.5)	<0.001
8/11 years	0.33 (0.2-0.6)	<0.001
Number of siblings		
1	1.0	
2/3	2.2 (0.7-7.0)	0.092
4/5	3.2 (1.0-11.0)	0.05
6/20	2.7 (0.8-9.1)	0.11
Birth order		
1	1.0	
2/3	1.0 (0.6-1.7)	0.91
4/5	0.9 (0.4-1.8)	0.76
6/20	0.7 (0.2-2.0)	0.50
Area		
Urban Leticia	1.0	
Rural Leticia	0.7 (0.3-1.7)	0.44
Puerto Nariño	2.3 (1.3-4.2)	0.005
Rural Puerto Nariño	1.2 (0.4-3.5)	0.67
Araracuara	0.2 (0.1-0.7)	0.01
Roof made with		
Palm tree leaf vs. tile	2.0 (0.8-4.7)	0.13

Table 4.13. Selected individual variables and not being vaccinated against hepatitis B. Urban area. Final model

Variables	OR (CI95%)	P
Age		
1 year	1.0	
2/3 years	0.44 (0.2-0.9)	0.031
4/5 years	0.29 (0.1-0.8)	0.017
6/7 years	0.52 (0.2-1.3)	0.152
8/11 years	0.72 (0.3-1.8)	0.471
Number of siblings		
1	1.0	
2/3	5.0 (0.5-45.5)	0.113
4/5	5.7 (0.5-62.7)	0.15
6/20	6.5 (0.7-60.5)	0.09
Birth order		
1	1.0	
2/3	1.2 (0.6-2.5)	0.58
4/5	1.1 (0.4-2.9)	0.85
6/20	0.7 (0.2-2.7)	0.58
Palm tree leaf vs. tile	3.07 (1.1-8.2)	0.027

The number of children less than 5 years covered by these health centres varied from 10 to 5000, and the index children less than five years/health worker ranged from 10 to 1000 with a median of 74 children/health worker. All health centres had some kind of educational material on vaccines available for public information. Most of these materials had been made by the health worker (18/24) and just one centre had a videotape on vaccines available for public education. Only two health centres (hospitals in Leticia and Puerto Nariño) had vaccines in storage at the time of the visit. Vaccine temperature records of the previous week were reviewed in Leticia and Puerto Nariño. They ranged between 2 and 5° centigrade with a median of 3° centigrade in both hospitals.

III.1.2. General knowledge on vaccines: Participants correctly identified a median of 8 diseases preventable by vaccination (range 0 to 13), most of them included in the schedule delivered by the Amazon Health Service. Measles, hepatitis and tetanus were the most frequently recalled, while Haemophilus influenza, chicken pox and meningococcal diseases were mentioned the least. They were also asked to recall the age when a child should have been fully vaccinated. About half of them (14/24) identified it as 12 months of age. Table 4.14 shows in more detail how HW performed on these questions.

Table 4.14. Description of variables related to general knowledge of vaccines among HW.

Variable	Number	%
Name of the vaccine preventable disease mentioned by HW		
Measles	23	13
Hepatitis	21	12
Tetanus	21	12
Pertussis	19	11
Polio	18	10
TB	17	10
Difteria	16	9
Yellow fever	15	8
Mumps	10	6
Rubella	9	5
Chicken pox	3	2
Meningococcal disease	2	1
Haemophilus influenza	2	1
Age when a child should have been fully vaccinated		
6 months	1	4
12 months	14	58
24 months	4	17
48 months	3	12
60 months	1	4
U	1	4

Table 4.15 shows the contraindications mentioned by health workers (HW) for polio, DPT and HB. They identified as major contraindications body temperature above 38.5°, a history of febrile convulsions, being born prematurely, and previous reactions to the specific vaccine. Cough was the only contraindication that varied between vaccines, being identified as a contraindication for polio vaccine but not for DPT or hepatitis B. All other contraindications were identified in similar proportion for the three vaccines.

III.1.3. Knowledge on hepatitis B vaccine: All HW knew the number of doses needed to immunise a child against hepatitis B, but few of them were able to recall the right part of the body to administer the vaccine (6/24). All of them knew that the third dose of hepatitis B vaccine should be given regardless of the length of time since the second dose (more than 28 days). However, most of them believed that there were contraindications for hepatitis B vaccine (19/24) and 18 pathological conditions were mentioned. Fever was the most frequent contraindication followed by diarrhoea and malnutrition. Table 4.16 shows the frequencies for these variables.

For HW the most important diseases in the area were: ARI (mentioned 23 times), diarrhoea (19 times), dermatitis (7 times) and fever (6 times). Hepatitis B was mentioned just once but 22 HW agreed, when asked, that it was an important cause of disease in their area, mentioning reasons such as frequency of the disease, severity, infectiousness, preventability and curability.

III.1.4. Perception of barriers for adequate vaccination coverage by health workers: Health workers were encouraged to give their own point of view concerning possible reasons why children in their communities were not completely vaccinated. Some structured questions asking specific points were used but also two open questions were included. Barriers perceived by health workers were classified in the following categories: 1) Logistical or administrative, if HW identified problems concerning inadequate supplies or shortage of human resources in their area as a cause of no immunisation; 2) Parent related barriers, such as beliefs about vaccine effectiveness or side effects; 3) Geographical barriers, if they believed that there were children in their area living too far away to be reached during vaccination activities; 4) Health worker barriers. This point included barriers arising from deficiencies in HW performance, such as parent's lack of knowledge about when, how, and why vaccination activities are carried out in the community.

In the structured questionnaire the most common barriers identified were those related to parents and geographical barriers. Curiously, most health workers believe that they have enough supplies to deliver vaccination and they do not perceive lack of cold chain as a barrier to better vaccination coverage. Instead, in logistical causes they remarked on the lack of health workers despite the fact that most of these communities were relatively small. In the unstructured questionnaire, parent's beliefs about vaccines were again the most important barrier identified. Specifically parent's fear about vaccines collateral effects was the most important cause of non-vaccination. Tables 4.17 and 4.18

III.2. Relationship between individual vaccination and characteristics of health workers/centres.

III.2.1. Health worker's perception: In this category the same variables were associated with not being fully vaccinated or not being completely vaccinated against hepatitis B. However the relation with hepatitis B coverage was stronger.

A perception that parent's education or parent's fear were a barrier to vaccination was one of the strongest predictors of low coverage. In areas where HWs were aware of these barriers, the chance of being fully vaccinated decreased almost twofold and for HB, fourfold. Perceiving hepatitis B as an important disease because of its severity, as opposed to infectiousness, was related to both full vaccination and HB. In those areas where HW did not perceive hepatitis B as a severe disease, the probability of not being fully vaccinated was 90% higher than in places where it was. The relation to incomplete hepatitis B vaccination was even larger (OR=3.2 CI95% 1.7-6.0). Both associations remained significantly associated through the multivariable analysis. Tables 4.19, 4.20, and 4.21

The presence of another health provider was not associated with either full vaccination or HB, in the bivariate analysis (p=0.18 and p=0.17), but it was identified as an important protective factor against no vaccination in the multivariable model. Tables 4.20 and 4.21.

Other variables related to poor vaccination were perception concerning children who do not come to the health centre, lack of time in the health centre for vaccination activities and parent's lack of time to take children to the health centre. The last two variables did not remain associated in the multivariable analysis.

Table 4.15. Symptoms or diseases contraindicating the application of polio, DPT and hepatitis B vaccines as indicated by the health workers in the study.

Symptom or disease	Polio vaccine	DPT vaccine	Hepatitis B vaccine
Is temperature < 38.5° a contraindication?			
Y	2	4	4
N	21	19	19
Is temperature >38.5° a contraindication?			
Y	22	23	23
N	1	0	0
Is being prematurely born a contraindication?			
Y	20	20	20
N	3	3	3
Is a history of febrile convulsion a contraindication?			
Y	20	22	21
N	3	1	2
Is a history of non-febrile convulsion a contraindication?			
Y	8	9	8
N	15	14	15
Are familiar antecedents of epilepsy or convulsion a contraindication?			
Y	3	2	2
N	20	21	21
Is a previous reaction to vaccines a contraindication?			
Y	20	22	21
N	3	1	2
Is cough a contraindication?			
Y	15	6	5
N	8	17	18
Is leukaemia a contraindication?			
Y	15	15	14
N	8	8	9
Is HIV infection a contraindication?			
Y	15	14	14
N	8	9	9
Is diarrhoea a contraindication?			
Y	14	14	14
N	9	9	9

Table 4.16. Description of responses given to questions on knowledge about hepatitis B vaccine.

Question	Number	%
Number of doses of hepatitis B needed to immunise a child		
3 doses	24	100
Body's area where hepatitis B vaccine should be given.		
Arm	1	4
Shoulder	3	12
Buttock	17	72
Tight	3	12
Time between first and second dose of HB vaccine		
1 month	24	100
Time between second and third dose		
1-6 months	19	79
More than 6 months	4	17
U	1	4
Is there any contraindication for hepatitis B vaccine?		
Y	19	79
N	4	17
U	1	4
How many contraindications for hepatitis B vaccine do you know?		
0	1	4
1	3	12
2	3	12
3	4	17
4-high	11	46
U	2	8
Name of contraindications		
Fever	16	23
Diarrhoea	11	16
Malnutrition	9	13
ARI	8	11
Dermatitis	5	7
Others	21	30

Table 4.17. Barriers against vaccination perceived by health workers. Structured questionnaire.

Barriers	N
1) Logistical	
Are there enough supplies to deliver vaccines in this health centre?	
Y	2
N	22
Is there a shortage of health personnel for vaccine delivery in this health centre?	
Y	13
N	9
2) Parents related causes	
Do you think that parents do not spare enough time to get children vaccinated?	
Y	21
N	2
3) Geographical	
Do you think that in your area some people live too far to take children to be vaccinated in the health centre?	
Y	14
N	9
4) Health worker related	
Do you think that people in your community do not have enough information on vaccination activities?	
Y	9
N	14

Table 4.18. Barriers against vaccination perceived by health workers. Unstructured questionnaire.

Barriers	Number
1) Logistical	
Lack of resources for outreach vaccination activities	2
2) Parents related causes	
Lack of interest on getting children vaccinated	5
Fear of vaccine side effects s	7
Lack of money	4
Lack of confidence on vaccine effectiveness	2
3) Geographical	
Population mobility	2
4) Health worker related	
Lack of information about vaccination activities	1
Parent's lack of information on vaccination benefits	4

For multivariable models a correlation between "lack of time in health centre" and " having right to be vaccinated by other health providers" (0.56) was found. To avoid co linearity they were not included at the same time in the same model. For both dependent variables, the "presence of other health providers" performed better. The best model for full vaccination is showed in table 4.20. The final model when "lack of time" was included had lower F and Log likelihood values. "Lack of time in the health centre" (p=0.399), and "parent's time" (p= 0.642) were not related to not being fully vaccinated and therefore were dropped.

Table 4.21 shows the best model for not being vaccinated with hepatitis B. Perception concerning the importance of hepatitis B could not be dropped from the model despite its high Wald's p value. (Log likelihood ratio test =21.3 p=0.000).

Table 4.19. Health workers' perceptions and individual vaccination.

Variable	Fully vaccinated N (%)	OR (CI95%) Incomplete schedule	Completely vaccinated with HB N (%)	OR (CI95%) Incomplete HB vaccination
Are there children in your community that do not come to the health centre for vaccination?	**P=0.002**		**P=0.000**	
Y	892 (64)	1.9 (1.3-2.9)	1451 (88)	3.2 (2.4-4.2)
N	104 (77)	1.0	179 (96)	1.0
Are there children in your community who have the right to be vaccinated by another health provider?	P=0.18		P=0.17	
Y	515 (66)	0.8 (0.5-1.1)	747 (89)	0.7 (0.4-1.2)
N	411 (60)	1.0	763 (85)	1.0
Why do you believe that hepatitis B is an important disease in your area?	**P=0.001**		**P=0.0003**	
Infectiousness	756 (63)	1.9 (1.3-2.8)	1190 (87)	3.2 (1.7-6.0)
Severity	207 (77)	1.0	366 (95)	1.0
What do you believe is an important reason for children not being vaccinated in your area?	**P=0.007**		**P=0.000**	
Parent education/ Parent fear to vaccine side effects	747 (63)	1.8 (1.2-2.8)	1162 (86)	4.1 (3.1-5.3)
Logistic reasons/ Poverty	120 (76)	1.0	210 (96)	1.0
Do you believe that there is not enough time in the health centre for vaccination activities?	**P=0.02**		**P=0.03**	
Y	701 (63)	1.5 (1.1-2.2)	1128 (87)	1.9(1.04-3.4)
N	295 (72)	1.0	502 (93)	1.0
Do you believe that in your community parents do not spare enough time to take children to health centre?	**P=0.008**		**P=0.000**	
Y	952 (66)	0.5 (0.3-0.8)	1549 (89)	0.3 (0.2-0.5)
N	44 (47)	1.0	81 (71)	1.0

Table 4.20. HW's perceptions and not being fully vaccinated. Final model.

Variable	OR (CI95%)	P
Are there children in your community who have the right to be vaccinated by another health provider?		
Y	0.41(0.3-0.60)	<0.001
Why do you believe that hepatitis B is an important disease in your area?		
Infectiousness	2.65 (0.8-8.5)	0.099
Severity	1.0	
What do you believe is an important reason for children not being vaccinated in your area?		
Parent education/parent's fear	2.55 (1.7-3.9)	<0.001
Logistic/poverty	1.0	
Are there children in your community who do not come to the health centre?		
Y	2.1(1.6-2.8)	0.01
Children's age		
1	1.0	
2/3	0.18 (0.1-0.3)	<0.001
4/5	0.13 (0.1-0.2)	<0.001
6/7	0.13 (0.1-0.2)	<0.001
8/11	0.16 (0.1-0.3)	<0.001

Table 4.21. HW's perception and not being vaccinated against hepatitis B. Final model.

Variable	OR (CI95%)	P
What do you believe is an important reason for children not being vaccinated in your area?		
Logistic reasons/poverty	1.0	
Parent's fear to vaccine side effects/parent's education	11.0 (8.2-15.5)	<0.001
Are there children in your community who have the right to be vaccinated by another health provider?		
Y	0.32 (0.2-0.5)	<0.001
Are there children in your community that do not come to the health centre for vaccination?		
Y	6.90 (4.9-9.6)	<0.001
Why do you believe that hepatitis B is an important disease in your area?		
Infectiousness	3.74 (0.4 -36.6)	0.252
Severity	1.0	
Children's age		
1 year	1.0	
2/3 years	0.35 (0.2-0.6)	<0.001
4/5 years	0.29 (0.2-0.6)	<0.001
6/7 years	0.65 (0.3-1.3)	0.212
8/11 years	0.68 (0.4-1.2)	0.209

III.2.2. Variables related to HW's knowledge on vaccines: Unexpectedly, the "number of correct answers on general knowledge about immune preventable diseases" was inversely related to full vaccination coverage, which decreases when the number of correct answers increase. On the other hand, "number of polio and DPT contraindications" are inversely related to full vaccination coverage, which is higher where HW mentioned less contraindications.

For hepatitis B coverage, fewer knowledge variables were related to coverage. The number of correct answers on general knowledge is again negatively associated with hepatitis B coverage. In places where HW had more correct answers there were few children with complete vaccination against hepatitis B. On the other hand, the effect of contraindications was very specific for hepatitis B. Only contraindications for hepatitis B vaccine were related to its coverage, and a stronger relation was observed for the nominal version of the variable ("Is there any contraindication.")

There was strong correlation between variables in this category. Contraindications against polio, DPT and hepatitis B correlated between them (>0.90), and only "number of contraindications against polio" was included in further analysis. There was also correlation between "number of correct answers on general knowledge" and "polio contraindications" (0.69).

For multivariable modelling of not being vaccinated against hepatitis B, the variables selected were: "general knowledge", "number of contraindications against hepatitis B vaccine" mentioned spontaneously and if the health worker considered that there was any contraindication for hepatitis B (Y/N). The last two variables were strongly correlated (0.82) and were not included together in the same model. The final model including the "number of contra HB" had a larger Log likelihood model (LL= -587.23 vs. LL= -614.24) than the model with the nominal version (Y/N). Table 4.24

Table 4.22. Vaccination coverage in relation to health worker's knowledge.

Variable	Fully vaccinated N (%)	OR (CI95%) Incomplete schedule	Completely vaccinated with HB N (%)	OR (CI95%) Incomplete HB vaccination
Number of correct answers on general knowledge about immune preventable diseases	**P=0.000**		**P=0.000**	
0/2	145 (81)	1.0	227 (95)	1.0
3/4	911 (64)	2.39(1.8-3.1)	1481 (88)	3.0 (2.1-4.2)
Number of correct answers on hepatitis B vaccine	**P=0.07**		P=0.32	
3	60 (84)	1.0	78 (94)	1.0
4/5	787 (65)	2.9 (2.3-3.6)	1219 (88)	2.1 (1.6-2.8)
6/7	209 (64)	3.0 (2.0-4.6)	411 (91)	1.5 (0.7-3.5)
Number of polio vaccine contraindications mentioned.	**P=0.004**		P=0.31	
0	60 (84)	1.0	78 (94)	1.0
1/3	155 (76)	1.7 (1.3-2.2)	253 (92)	1.31 (0.6–2.9)
4/5	146 (59)	3.8 (2.5-5.7)	269 (85)	2.8 (1.3-6.2)
6/9	695 (64)	3.0 (2.4-3.8)	1108 (88)	2.0 (1.6-2.5)
Number of DPT vaccine contraindications mentioned.	**P=0.02**		P=0.57	
0	60 (84)	1.0	78 (94)	1.0
2/5	301 (67)	2.7 (1.8-4.0)	522 (88)	2.1 (1.1-4.0)
6/9	695 (64)	3.0 (2.4-3.8)	1108 (88)	2.0 (1.6-2.6)
Is there any hepatitis B vaccine contraindication ?	P=0.73		**P=0.0001**	
Y	881 (65)	1.1 (0.7-1.6)	1391 (88)	3.5 (1.8-6.7)
N	115 (66)	1.0	239 (96)	1.0
Number of hepatitis B vaccine contraindications mentioned spontaneously	P=0.32		**P=0.07**	
0	80 (64)	1.0	178 (96)	1.0
2/3	193 (58)	1.3 (0.6-2.8)	340 (82)	4.9 (1.6-14.8)
4/5	688 (67)	0.9 (0.5-1.6)	1051 (89)	2.6 (1.1-6.4)
Number of contraindications mentioned for hepatitis B aside polio and DPT	P=0.13		P=0.57	
0	60 (84)	1.0	78 (94)	1.0
2/5	301 (67)	2.7 (1.8-4.0)	522 (88)	2.0 (1.1-4.0)
6/9	695 (64)	3.0 (2.4-3.8)	1108 (88)	2.0 (1.6-2.6)

The multivariable modelling of not being fully vaccinated included the following variables: "children's age", "number of correct answers on hepatitis B", "contraindications for polio", and "general knowledge". The last two variables were not included together in the same model due to their correlation. Table 4.23 shows the results when "contraindications for polio" are included. "Number of right answers" was dropped from the model because its relation to not being fully vaccinated lost significance (p=0.92). In the model including

correct answers on general knowledge, all variables remained significantly associated: "children's age" (p<0.001), "number of correct answers on general knowledge" (p<0.001), and "number of correct answers on hepatitis B vaccine" (p=0.02). Both variables, "contra polio" and "general knowledge", were kept for further multivariable modelling with selected variables from other categories.

Table 4.23 HW knowledge and not being fully vaccinated. Final model when considering polio contraindications.

Variable	OR	P
Number of polio contraindications		
0	1.0	
1/3	1.85 (1.3-2.6)	0.001
4/5	3.80 (2.1-6.8)	<0.001
6/9	2.7 (2.2-3.3)	<0.001
Children's age		
1	1.0	
2/3	0.21 (0.1-0.3)	<0.001
4/5	0.14 (0.1-0.2)	<0.001
6/7	0.15 (0.1-0.2)	<0.001
8/11	0.18 (0.1-0.3)	<0.001

Table 4.24. HW knowledge and incomplete vaccination against hepatitis B.

Variable	OR	P
Number of hepatitis B vaccine contraindications mentioned spontaneously		
0	1.0	
2/3	7.78 (3.1-19.5)	<0.001
4/5	2.69 (1.1-6.7)	0.034
Age (years)		
1	1.0	
2/3	0.34 (0.2-0.6)	<0.001
4/5	0.28 (0.1-0.5)	<0.001
6/7	0.59 (0.3-1.1)	0.102
8/11	0.62 (0.4-1.1)	0.115
Number of correct answers on general knowledge about immune preventable diseases		
0/2	1.0	
3/4	7.03 (5.0-9.9)	<0.001

III.2.3. Variables related to geographical or general health centres characteristics.

Logistical barriers represented by "rejection of children looking for vaccination" showed some relation to not being fully vaccinated (OR=1.6 CI95% 0.9-2.8) and a more strong association with not being hepatitis B vaccinated (OR=2.7 CI95% 1.2-5.8). An interesting relation was observed between "time working in the HC" and vaccination coverage (full or HB). In communities where HW had more than 14 years of continuous work, the likelihood of not being fully or HB vaccinated decreased by a half approximately (OR=0.6 CI95% 0.4-0.8 and OR=0.4 CI95% 0.3-0.7). Table 4.25

Geographical variables influenced the likelihood of not being vaccinated fully or against hepatitis B. The worst coverage was- observed in villages located on the Loretoyaco river shores and the effect was stronger for hepatitis B vaccine. The highest coverage was observed in villages located on the Caquetá River.

Statistical correlation was found between the -following variables:

- "Number of health workers (HW) in the health centre" and "time working as health professional" (0.51).
- "Number of health workers in the health centre (HC)" and "time working in the health centre" (0.64).
- "Time working in the health centre" and "time working as a health professional" (0.87). In further multivariable models only "time in the health centre" was included because its relation to not being fully vaccinated was stronger.

For not being fully vaccinated, "number of HW" and "time in the HC" were not included together in the same model. The best model was obtained with the set of variables including "number of health workers" shown in table 4.26. However, "time in the health centre" remained significantly related to not being fully vaccinated even after controlling by "geographical situation" and "children's age" (OR=0.47 IC95% 0.33-0.68). Therefore it was taken into account for the final models combining all ecological variables

Table 4.25. Vaccination coverage in relation to geographical or health centres characteristics.

Variable	Fully vaccinated N (%)	OR (CI95%) Incomplete vaccination	Complete HB vaccination N (%)	OR (CI95%) Incomplete HB vaccination
Have children looking for vaccination in the last month been rejected due to lack of supplies?	P=0.12		**P=0.01**	
Y	793 (64)	1.6 (0.9-2.8)	1248 (87)	2.7 (1.2-5.8)
N	180 (73)	1.0	347 (95)	1.0
Geographical village's situation	P=0.11		**P=0.0007**	
Amazon river	942 (66)	1.0	1462 (89)	1.0
Caquetá river	47 (68)	0.93 (0.3-2.5)	104 (98)	0.15 (0.1-0.3)
Loretoyaco river	34 (46)	2.2 (0.9-5.5)	68 (72)	3.2 (1.5-7.0)
Number of health workers in the health centre	**P=0.008**		P=0.25	
1	443 (69)	1.0	725 (89)	1.0
14/17	98 (45)	2.8 (1.7-4.6)	236 (82)	1.8 (0.8-4.1)
100	515 (66)	1.2 (0.7-1.8)	747 (89)	0.9 (0.5-1.8)
Number of nurses in the health centre	P=0.23		P=0.84	
0	443 (70)	1.0	725 (89)	1.0
1/3	613 (64)	1.3 (0.8-2.0)	983 (88)	1.1 (0.6-2.0)
Time working as health professional	**P=0.02**		**P=0.03**	
0/14 years	269 (56)	1.0	524 (83)	1.0
15/21 years	787 (68)	0.6 (0.4-0.9)	1184 (90)	0.5 (0.3-0.9)
Time working in the health centre	**P=0.005**		**P=0.001**	
0/14 years	312 (56)	1.0	593 (82)	1.0
15/21 years	744 (69)	0.58 (0.4-0.8)	1115 (91)	0.4 (0.3-0.7)

For hepatitis B vaccination, a correlation was detected between "time in the health centre" and "time as a health worker", and only the first was included in multivariable models. The final model is shown in table 4.27. "Logistic impairments" and "time working in the health centre" remained strongly associated with hepatitis B vaccination while "living on the Loretoyaco River" became not statistically associated.

Table 4.26. Health centres geographical and general characteristics and their relationship to not being fully vaccinated.

Variable	OR (CI95%)	P
Have children looking for vaccination in the last month been rejected due to lack of supplies?		
Y	1.80 (1.0-3.0)	0.036
Children's Age		
1	1.0	
2/3	0.18 (0.1-0.3)	<0.001
4/5	0.12 (0.1-0.2)	<0.001
6/7	0.12 (0.1-0.2)	<0.001
8/11	0.16 (0.1-0.2)	<0.001
Number of health workers in the health centre		
1	1.0	
14/17	3.14 (1.9-5.3)	<0.001
100	0.84 (0.6-1.3)	0.426
Geographical village's situation		
Amazon river	1.0	
Caquetá river	1.06 (0.6-1.9)	0.851
Loretoyaco river	2.31 (1.0-5.6)	0.063

Table 4.27. Health centres geographical and general characteristics and their relation to not being vaccinated with hepatitis B. Final Model.

Variable	OR (CI95%)	P
Have children looking for vaccination in the last month been rejected due to lack of supplies?		
Y	2.71 (1.1-6.5)	0.025
Children's Age		
1	1.0	
2/3	0.33 (0.2-0.6)	<0.001
4/5	0.25 (0.1-0.5)	<0.001
6/7	0.54 (0.3-1.0)	0.061
8/11	0.55 (0.3-1.0)	0.050
Geographical village's situation		
Amazon river	1.0	
Caquetá river	0.11 (0.04-0.3)	<0.001
Loretoyaco river	1.51 (0.6-3.6)	0.352
Time working in the health centre		
0/14 years	1.0	
15/21 years	0.37 (0.2-0.6)	0.001

III.2.4. Models combining significant ecological variables:

Not being fully vaccinated: The following variables were selected for models combining ecological variables:

a) Perceptions: "Having right to be vaccinated by other health providers", "reasons for children not being vaccinated", and "reasons for hepatitis B importance".

b) Knowledge: "Number of polio contraindications".

c) General characteristics of HC: "Geographical situation", "rejecting a child due to lack of supplies" and "number of years working in the health centre".

"Having right to other health providers" was correlated with "number of polio contraindications" (0.66) and with "number of years in the health centre" (0.91). None of these variables were considered together in the same model; instead, two sets of variables were constructed. One set included: "Children's age", "Having right to be vaccinated by other health providers", "reasons for children not being vaccinated", "geographical situation", and "rejecting a child due to lack of supplies". "Geographical situation" was dropped from this model due to lack of significance (p=0.72). Table 4.28 shows the final results of this set.

Table 4.28. Model combining knowledge, perceptions, geographical, and general characteristics of health centres and not being fully vaccinated. First set.

Variable	OR (CI95%)	P
Children's age		
1	1.0	
2/3	0.32 (0.2-0.5)	<0.001
4/5	0.23 (0.1-0.4)	<0.001
6/7	0.23 (0.1-0.4)	<0.001
8/11	0.28 (0.2-0.5)	<0.001
Have children looking for vaccination in the last month been rejected due to lack of supplies?		
Y	2.33 (0.9-5.9)	0.08
Are there children in your community who have the right to be vaccinated by other health providers?		
Y	0.41 (0.3-0.6)	<0.001
What do you believe is an important reason for children not being vaccinated in your area?		
Logistic reasons/poverty	1.0	
Parent's fear to vaccine side effects/parent's education	2.30 (1.5-3.4)	<0.001

The second set included: "Children's age", "number of polio contraindications", "reason for children not being vaccinated", "rejecting a child due to lack of supplies", "number of years working in the health centre", and "reason for hepatitis B importance". "Reason for children not being vaccinated" and "reason for hepatitis B importance" were dropped from the model

(p=0.698 and p=0.702). "Rejecting a child due to lack of supplies" had to be excluded because it introduced some degree of co linearity between the categories of "contra polio". Table 4.29 shows the final result for this group.

Table 4.29. Model combining knowledge, perceptions, geographical, and general characteristics of health centres and not being fully vaccinated. Second set

Variable	OR (CI95%)	P
Children's age		
1	1.0	
2/3	0.33 (0.2-0.5)	<0.001
4/5	0.22 (0.1-0.4)	<0.001
6/7	0.24 (0.2-0.4)	<0.001
8/11	0.27 (0.2-0.4)	<0.001
Number of polio contraindications		
0	1.0	
1/3	1.54 (1.2-2.0)	<0.001
4/5	2.25 (1.6-3.1)	<0.001
6/9	2.10 (1.7-2.6)	<0.001
Time working in the health centre		
0/14 years	1.0	
15/21 years	0.50 (0.4-0.7)	<0.001

Summarizing, variables from every area (HW's perceptions, HW's knowledge and health centres) were related to not being fully vaccinated and the magnitude of their relation was similar. Some variables increased the risk of not being fully vaccinated: Perceiving that parent's fear was a major barrier for children's vaccination (OR=2.3 CI95% 1.5-3.5), logistical shortcomings represented in children's rejection (OR=2.3 CI95% 0.91-5.9), and number of polio vaccine contraindications mentioned by HW (the OR increased above 2.0 when more than 3 contraindications were mentioned). The protective factor was the presence of a health provider other than the Amazon Secretary of Health in the community (OR=0.41 CI95% 0.28-0.61). These ecological variables were selected for further analysis combined with individual variables.

Incomplete Hepatitis B vaccination:

The following variables were selected:

a) **Perceptions**: "Having right to be vaccinated by other health provider", "reasons for children not being vaccinated", and "reasons for hepatitis B importance".

b) **Knowledge**: "Number of hepatitis B vaccine contraindications" (categorical) and "whether or not hepatitis B contraindications existed" (Y/N).

c) **General health centres characteristics:** "Rejecting a child due to lack of supplies" and "number of years working in the health centre".

There was a correlation between the knowledge variables (0.78); the nominal variable was used in the analysis rather than the categorical because the latter correlated strongly with two other variables: "having right to be vaccinated by other health providers" (0.65) and "number of years in the HC" (0.68). The first also correlated with "reason for children not being vaccinated" (0.82). "Rejecting a child due to lack of supplies" correlated to "reasons for hepatitis B importance" (0.80).

Two sets of variables were constructed to avoid co linearity. One contained "children's age", "having right to another health provider", "reason for children not being vaccinated", and "reason for hepatitis B importance". All of them were associated with not being vaccinated against hepatitis B. The higher p value was achieved by "reason for hepatitis B importance", but it could not be removed from the model (Log likelihood ratio test: Chi2=20.07, P=0.000). "Reasons for hepatitis B importance" was replaced by "rejecting a child" and, again, all variables remained associated with not being hepatitis B vaccinated.

The second set of variables contained: "children's age", "having right to another health provider", "considering that there are contra indications for HB", and "reasons for hepatitis B importance". "Having right to another health provider" was replaced by "number of years working in the health centre" and this variable was also significantly related to not being vaccinated against hepatitis B (OR=0.37 CI95% 0.23-0.61). The strongest relation was found with "hepatitis B contraindications" (OR=7.2 CI95% 3.9-13.0) followed by the "perception about the reason for hepatitis B importance" (OR=3.5 CI95% 1.2-10.1). Table 4.30.

Summarizing, there were ecological variables associated with both not being fully vaccinated and with incomplete hepatitis B vaccination. "Rejecting a child looking for vaccination" (logistical), "reasons for children not being vaccinated" (perception), "vaccines

contraindication" (knowledge), and "number of years working in the health centre" were associated with both dependent variables. Reason for hepatitis B importance was only associated with not being completely vaccinated against hepatitis B.

Table 4.30. Model combining knowledge, perceptions, health centres geographical and general characteristics, and not being vaccinated against hepatitis B.

Variable	OR (CI95%)	P
Children's age		
1	1.0	
2/3	0.36 (0.2-0.6)	<0.001
4/5	0.29 (0.2-0.6)	<0.001
6/7	0.65 (0.3-1.3)	0.199
8/11	0.63 (0.4-1.2)	0.134
Why do you believe that hepatitis B is an important disease in your area?		
Infectiousness	3.5 (1.2-10.1)	0.018
Severity	1.0	
Are there children in your community who have the right to be vaccinated by another health provider?		
Y	0.42 (0.2-0.7)	0.002
Is there any hepatitis B contraindication vaccine		
Y	7.16 (3.9-13.0)	<0.001
N	1.0	

IV. Models combining ecological and individual variables and not being fully vaccinated: The following variables were included in these models:

Individual variables:

Children's age by categories.

Study area.

Name of the ethnic group.

Being affiliated to the Health Security System.

Ecological variables:

Number of polio contraindications polio by categories.

Rejecting a children looking for vaccination due to lack of supplies.

Reasons for children not being vaccinated.

Number of years working in the Health Centre.

Having right to be vaccinated by another health provider.

"Study area" correlated with three ecological variables, "polio vaccine contraindications" (0.63), "other health providers in the community" (0.80), and "number of years working in the health centre" (0.79). Therefore, "study area" was kept out of the analysis. Other correlations were found between:

"Number of polio contraindications" with "other health providers in the community" (0.59). "Number of years working in the health centre" with "other health providers in the community" (0.83).

Two sets of variables were constructed in order to avoid co linearity. One set included all individual variables (except study area) plus "rejecting a child", "other health providers", and "reasons for not vaccinating". The other one included the same individual variables plus "contra polio", "number of years working in the health centre", and "rejecting a child due to lack of supplies".

Interestingly, all variables in both categories remained associated in both sets. The strongest association was observed for logistical troubles (children rejection) (OR=3.0 in one set and OR=1.6 in the other one). Another strong association was observed with "number of years working in the health centre" (OR=0.4 CI95% 0.3-0.58) when HW had more than 15 years in post. Both indicators of better health security coverage (ecological and individual) were associated with not being fully vaccinated though the ecological variable shows a stronger relation (OR=0.32 vs. OR=1.55/1.45). Tables 4.31 and 4.32.

An attempt to drop "number of polio contraindications" from the model in table 4.32 was done, but its contribution to the overall model was at the border line of statistical significance (Log likelihood test: Chi2=5.72, p=0.057) and therefore it was kept in the model. There was a large number of missing values mainly due to the influence of the variable "being affiliated to the social security" (~ 400 observations). Therefore, a new category of this variable was created to evaluate whether this loss of information influenced the results in models 4.31 and 4.32. The new variable was introduced in both models but no major changes in the magnitude of the associations was observed for any variable, though p values became lower and CI became narrower (number of observations jumped from 747 to 1203 and from 951 to 1460 respectively).

Table 4.31. Individual and ecological variables and their relationship to not being fully vaccinated. First set. Final model

Variable	OR (CI95%)	P
Age		
1	1.0	
2/3	0.17 (0.1-0.3)	<0.001
4/5	0.11 (0.05-0.2)	<0.001
6/7	0.10 (0.05-0.2)	<0.001
8/11	0.18 (0.1-0.3)	<0.001
Ethnic group		
No Indians	1.0	
Mestizos	0.39 (0.2-0.9)	0.022
Huitotos	2.0 (0.8-5.2)	0.151
Ticunas	0.84 (0.5-1.6)	0.585
Other groups	0.53 (0.3-1.0)	0.062
Affiliated to social security		
N	1.55 (1.0-2.4)	0.053
Are there in your community children who have the right to be vaccinated by other health providers?		
Y	0.32 (0.2-0.6)	<0.001
Have children looking for vaccination in the last month been rejected due to lack of supplies?		
Y	3.0 (1.5-6.0)	0.002
What do you believe is an important reason in your community for children not being fully vaccinated?		
Parent's education/Parent's fear	2.25 (1.3-3.9)	0.004
Logistic	1.0	

Table 4.32. Individual and ecological variables and their relation to not being fully vaccinated. Second set. Final model

Variable	OR (CI95%)	P
Age		
1	1.0	
2/3	0.18 (0.1-0.3)	<0.001
4/5	0.10 (0.1-0.2)	<0.001
6/7	0.10 (0.1-0.2)	<0.001
8/11	0.17 (0.1-0.3)	<0.001
Ethnic group		
No Indians	1.0	
Mestizos	0.51 (0.3-0.9)	0.036
Huitotos	1.7 (0.9-3.1)	0.091
Ticunas	1.04 (0.6-1.8)	0.868
Other groups	0.48 (0.3-0.9)	0.020
Affiliated to social security		
N	1.45 (1.0-2.1)	0.050
Number of polio contraindications		
0	1.0	
1/3	Dropped	
4/5	1.38 (0.8-2.3)	0.198
6/9	1.35 (0.8-2.2)	0.190
Have children looking for vaccination in the last month been rejected due to lack of supplies?		
Y	1.6 (1.0-2.4)	0.040
Number of years working in the health centre		
0/14	1.0	
15/21	0.43 (0.3-0.6)	<0.001

V. Models combining ecological and individual variables and not being hepatitis B vaccinated.

The following variables were included:

Individual:

Children's age.

Number of siblings.

Birth order.

Study area.

Ecological:

Having right to be vaccinated by another health provider.

Reason for children not being vaccinated.

Reason for hepatitis B importance.

Rejecting a child due to lack of supplies.

Contraindications for hepatitis B vaccine (Y/N).

Number of years working in the health centre.

Strong correlation between variables was detected as follows:

"Study area" correlated with "having right to be vaccinated by another provider" (0.83) and with "number of years in the HC" (0.82).
"Having the right to another health provider" correlated with "number of years working in the HC" (0.82).
"Reasons for children not being vaccinated" correlated with "contraindication for hepatitis B vaccine" (0.81).
"Reasons for hepatitis B importance" correlated with "rejecting a child due to lack of supplies". (0.83).

Variables were divided in two sets. One included: "children's age", "number of siblings", "having right to another health provider", "reason for children not being vaccinated" and "reason for hepatitis B importance". The other one included: "children's age", "number of siblings", "number of years working in the HC", "existence of contraindications for hepatitis B" and "rejecting a child due to lack of supplies".

From the first set of variables, "reasons for hepatitis B importance" was dropped (p=0.590) and no variable could be dropped in the second set. Results for both sets are shown in tables 4.33 and 4.34. There were variables related to both inadequate hepatitis B vaccination and with not being fully vaccinated. Variables that increased the risk of not being vaccinated were: "Perceiving parents' education or parents' fear of vaccination side effects" (OR=8.0 CI95% 4.8-13.3), "logistical shortcomings", and "rejecting children", (OR=3.2 CI95%1.8-5.5). The variables that reduced the risk of inadequate vaccination for hepatitis B were: "presence in the community of more health providers" (OR=0.5 CI95% 0.3-0.9) and "time working in the health centre" (>14 years). The only variable related to hepatitis B vaccination that was not associated with full vaccination was "number of siblings" that remained associated in both models though the effect was slightly lower in the second set.

Table 4.33 Ecological and individual variables and their relationship to not being vaccinated against hepatitis B. First set. Final model

Variable	OR (CI95%)	P
Age		
1	1.0	
2/3	0.21 (0.1-0.4)	<0.001
4/5	0.15 (0.1-0.3)	<0.001
6/7	0.29 (0.1-0.6)	0.001
8/11	0.32 (0.2-0.6)	0.003
Number of siblings		
1	1.0	
2/3	2.6 (0.8-8.0)	0.09
4/5	4.2 (1.2-14.6)	0.024
6/20	4.0 (1.1-14.5)	0.037
Birth order		
1	1.0	
2/3	1.0 (0.6-1.7)	0.93
4/5	0.7 (0.3-1.5)	0.41
6/20	0.5 (0.2-1.6)	0.26
Are there in your community children who have the right to be vaccinated by another health provider?		
Y	0.48 (0.3-0.9)	0.014
What do you believe is an important reason in your community for children not being fully vaccinated?		
Parent's education/Parent's fear	8.1 (4.8-13.5)	<0.001
Logistic	1.0	

Table 4.34. Ecological and individual variables and their relation to not being vaccinated against hepatitis B. Second set. Final model

Variable	OR (CI95%)	P
Age		
1	1.0	
2/3	0.23 (0.1-0.4)	<0.001
4/5	0.16 (0.1-0.3)	<0.001
6/7	0.26 (0.1-0.5)	<0.001
8/11	0.33 (0.2-0.6)	<0.001
Number of siblings		
1	1.0	
2/3	2.8 (0.9-8.6)	0.06
4/5	3.3 (1.0-10.5)	0.05
6/20	2.8 (0.8-9.1)	0.09
Birth order		
1	1.0	
2/3	1.1 (0.6-1.9)	0.78
4/5	1.1 (0.5-2.4)	0.15
6/20	0.8 (0.3-2.4)	0.72
Time working in the health centre		
0/14	1.0	
15/21	0.38 (0.2-0.6)	<0.001
Is there any hepatitis B contraindication		
Y	2.3 (1.1-5.1)	0.03
N	1.0	
Have children looking for vaccination in the last month been rejected due to lack of supplies		
Y	3.2 (1.8-5.5)	<0.001
N	1.0	

Chapter 5: Serological results.

Summary. Among 2145 children aged 1 to eleven years examined, the overall prevalence of HBV infection was 6.2% (95%CI 4.7-7.9) while the prevalence of HBsAg+/anti-HBc+ was 1.1% (95%CI 0.4-1.8%). Prevalence of infection and HBsAg+/anti-HBc was higher in rural than urban areas (9.2% and 2.6% versus 2.6% and 0.17%). Infection and prevalence of HBsAg+/anti-HBc+ was also higher in children 8 years and older especially among girls. There has been a reduction in the prevalence of HBV infection and HBsAg+ of between 60% to 75% since the vaccine was introduced, especially in the most endemic areas such as Araracuara.. Factors related to HBV infection and to being HBsAg+/anti-HBc were divided into child-related, mother-related, and vaccine-related (time from birth to first dose and time between doses).

For HBV infection the most important child-related variables were: belonging to an ethnic group different to Ticunas or Huitotos (OR=4.6 95%CI 2.4-8.6), belonging to Ticunas (OR=2.4 95%CI 1.2-4.6), and not being born in a hospital or health centre (OR=2.4 95%CI 1.5-4.1). Among the mother-related variables the most important association was found with being born to an Anti-HBc+ mother (OR=1.7 95%CI 1.1-2.6). None of the vaccine-related variables was found associated with being HBV infected. The most important child-related variables associated with HBsAg+/anti-HBc+ were: not being born in a hospital or health centre (OR=6.5 95%CI 1.5-2.7.6) and living with more than 5 siblings (OR=3.3 95%CI 1.1-10.0). The most important mother-related variable was being born to an Anti-HBc+ mother (OR=3.5 95%CI 1.0-11.8). Time from birth to first dose of HBV vaccine was related to being HBsAg+/anti-HBc+ even after controlling for mother and child-related variables. Receiving the first dose of vaccine two months or later after birth was related with an increase in the risk of being HBsAg+ especially among those who received it after 2 years of life (OR= 12.5 95% CI 1.2-125.7). Time between first and second dose was related with being HBsAg+/antiHBc+ only in rural areas. Receiving the second dose 35 days after the first was associated with a two fold risk of being HBsAg+ (OR=2.3 95%CI 1.4-3.8)

In a sample of 481 children HBsAg-/antiHBc- we quantified levels of anti-HBs. We found that 23% of them did not have detectable anti-HBs while anti-HBs levels ranged from 0 to 10,000 mIU/ml. The GMT and the median of anti-HBs were 66 mIU/ml and 123 mIU/ml respectively. 13% of the children had anti-HBs levels above 1,000 mIU/ml. The variables related to lack of detectable anti-HBs were "time from third dose to sampling" and "time from birth to first dose of HBV". Children who received the first dose within 14 days from birth had lower levels of anti-HBs (GMT=33 mIU/ml vs. 66 to 174 among the other groups)

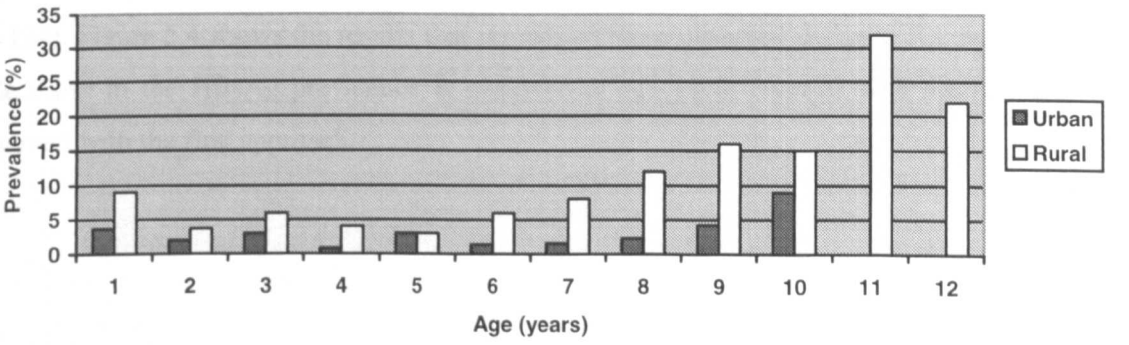
I. General description of HBV infection prevalence.

For serological studies, 2145 children aged 1 to 11 years were bled. The median age was 6 years while 52% were males. Hepatitis B (HBV) infection prevalence was 6.2% (95% CI 4.7-7.9%) which corresponded to 138 children with positive results for either, HBsAg or anti-HBc. For anti-HBc, 124 children were positive (5.8%, 95% CI 3.7-6.9%), for HBsAg, 39 (1.7%, 95% CI 1.0-2.4%), and 25 (1.1%, 95% CI 0.4-1.8%) were positive for both markers. Hepatitis delta antibody was tested on 34 children with positive results for HBsAg or anti-HBc and 4 were positive (12%, 95% CI 3.9-28.4%).

In urban areas 1104 children were studied. For anti-HBc, 29 were positive (2.6%, 95% CI 1.5-4.2%), for HBsAg, 8 (0.8% 95% CI 0.4-1.5%), for both markers, 2 (0.17% 95% CI 0.01-1.2), and 35 for at least one of them (3.3%, 95% CI 2.3-4.7%). In rural areas, 1041 children were bled. Prevalence for all markers was statistically higher in rural than in urban areas. For anti-HBc, 95 were positive (9.2%, 95% CI 7.0-12%), for HBsAg, 31 (2.7%, 95% CI 1.7-4.4%), for both markers, 23 (2.6%, 95% CI 1.4-4.6%), and for at least one of them, 103 (10.3%, 95% CI 8.2-13.0%).

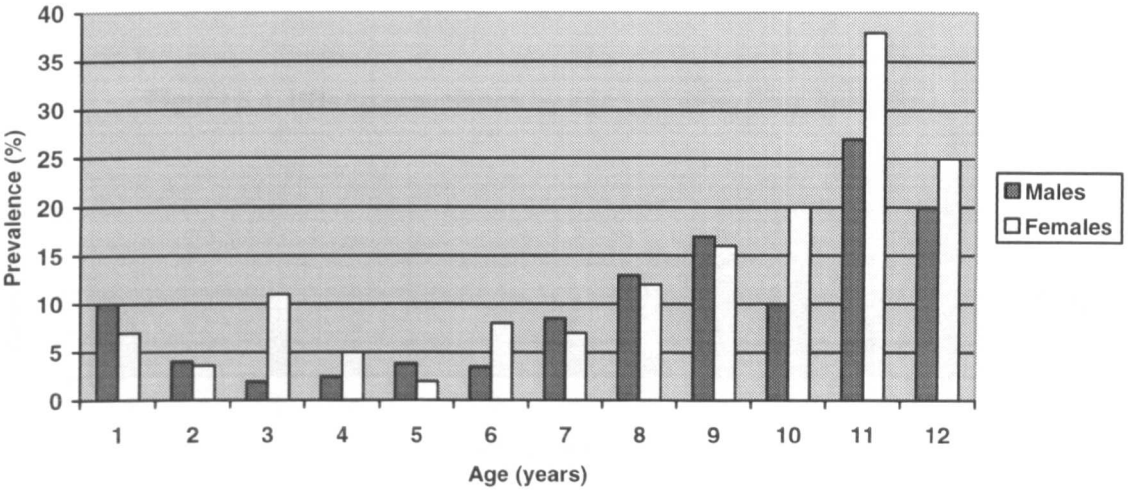
The prevalence of anti-HBc was analysed by area and age. Figure 5.1 showed that this prevalence was higher in rural areas in all categories of age. The increase in prevalence started from six years of age in rural areas and from about 8 years in urban areas. In rural areas there was a considerable increase from 10 years to 11 and 12, as prevalence jumped from around 10% to more than 20%. This increase is less noticeable in urban areas.

Figure 5.1. AntiHBc prevalence by age and area



A difference in anti-HBc prevalence by sex was observed. Overall, 68 out of 1007 girls were positive (6.1%, 95% CI 3.4-6.5%) while among boys there were 56 out of 1105 (4.7%, 95% CI 4.3-8.4%), p=0.056. In rural areas, the statistical difference became wider because girls had a prevalence of 10% (53/498) and boys 7.7% (42/532), p=0.01. On the other hand, no important difference was observed in urban areas (2.8% in girls and 2.4% in boys). Figure 5.2 shows the trend of the anti-HBc prevalence by age and sex in rural areas. Females were more likely to be positive than males, especially after 10 years of age.

Figure 5.2. AntiHBc prevalence in rural areas by age and sex



HBsAg prevalence was analysed by age and area following two approaches. First, all children with available serological data (n=1881) in each area were included in the denominator and HBsAg+/anti-HBc+ children were included in the numerator. Figure 5.3 shows the results using this method. Two peaks of HBsAg prevalence can be observed. The smaller one in children aged 3 to 5 years while the larger is seen in children 9 years old and older. The increase with age is clearly sharper in rural than in urban areas. As for the second approach, only anti-HBc+ children were in the denominators to calculate HBsAg prevalence (n=124). Figure 5.4 shows the results that resembled those observed in figure 5.3; however, a decrease in the HBsAg prevalence is observed at age 11 in contrast with the increase observed with the first approach.

Figure 5.3. HBsAg prevalence by age and area. All children

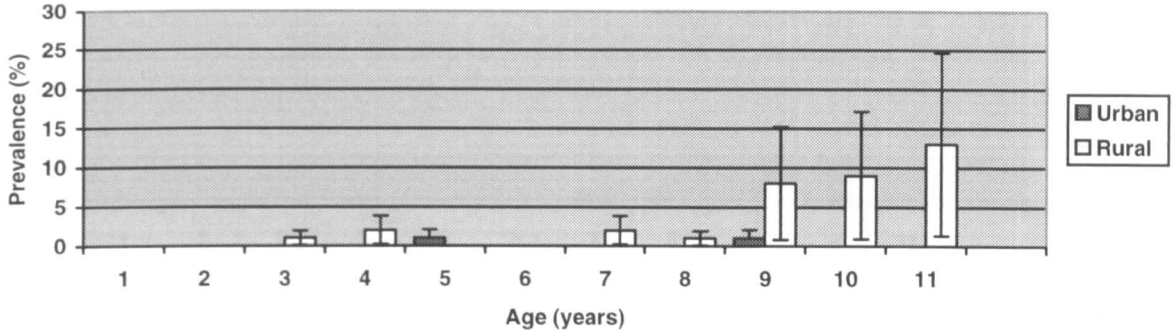
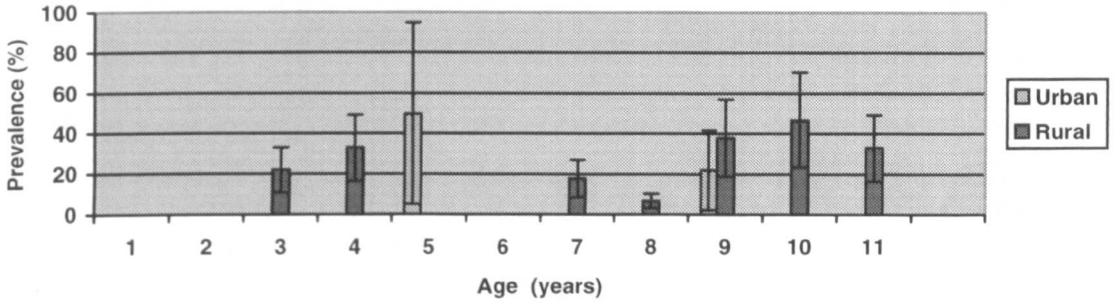


Figura 5.4. HBsAg prevalence by age and area. Only AntiHBc +.



With the first approach no difference was observed in HBsAg prevalence by sex. In both groups the prevalence was 1.1% and this did not vary when the area was taken into the analysis. In rural areas, prevalence was 2.3% for both groups while in urban areas it was 0.2%. When age and rural area were taken into account an interesting pattern arose. The first peak of HBsAg prevalence occurs in males only, while the second and most important rise is in both genders. It is important to note that females tend to have a higher prevalence than males at ages 7, 9, and 11. Figure 5.5.

Figure 5.6 shows the results when only anti-HBc+ children are included in the denominator. Again, a peak in the male HBsAg prevalence was observed at age 3, which coincided with the trend observed in figure 5.5. A higher prevalence in girls at age 11 was observed as well.

Figure 5.5. HBsAg prevalence in rural areas by age and sex. All Children.

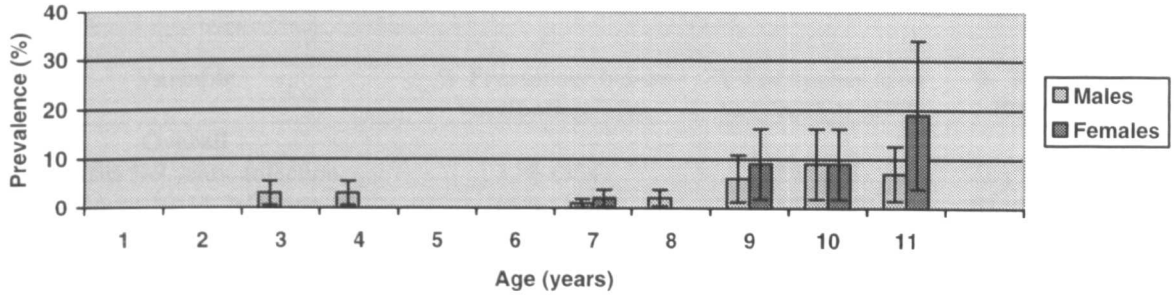
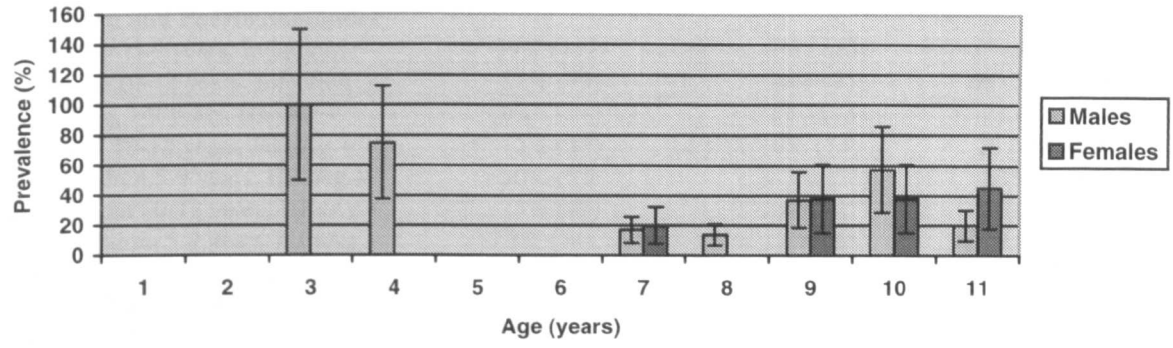


Figure 5.6. HBsAg prevalence in rural areas by age and sex. Only AntiHBc+



II. Overall impact of hepatitis B vaccine in the Amazon:

Compared with data taken from Cristancho 1995, there has been an important reduction in both infection and prevalence of HBsAg. Reduction in infection is greater in children 5-9 than in children 10-14 years. The largest reduction in infection is observed in children 5-9 years old in Araracuara (77%), while, in general, no reduction was observed in HBsAg prevalence for children aged 10-14. This lack of effect is observed for both genders but it is especially marked for females among whom HBsAg prevalence seems to be higher than before vaccine introduction. Table 5.1.

Table 5.1. Prevalence of hepatitis B infection and HBsAg found before and after the introduction of hepatitis B vaccine by age group and place.

Variable	% Prevalence before vaccination * (n)	% Prevalence after vaccination (n)**	% Reduction (95% CI)
Overall			
Children 5-9 years. Infection	32% (334)	9% (493)	72 (59-78)***
Children 10-14. Infection	66% (189)	25% (160)	62 (49-72)***
Male children 5-9 years. Infection	34% (157)	9% (247)	73 (59-83)***
Female children 5-9 years. Infection	30% (177)	10% (246)	67 (48-78)***
Male children 10-14 years. Infection	85% (144)	19% (87)	78 (64-85)***
Female children 10-14 years. Infection	76% (135)	32% (72)	58 (40-70)***
Children 5-9 years. HBsAg +	7% (334)	2% (495)	71 (35-84)***
Male children 5-9 years. HBsAg +	8% (157)	2% (247)	75 (26-90)***
Female children 5-9 years. HBsAg +	6% (177)	2% (248)	67 (-3-85)
Children 10-14 years. HBsAg +	9% (279)	10% (161)	-11 (-58-52)
Male children 10-14 years. HBsAg +	10% (144)	6% (87)	48 (-46-79)
Female children 10-14 years. HBsAg +	7% (135)	15% (73)	-114 (-205-0.8)
Araracuara and Puerto Santander			
Children 5-9 years. Infection	39% (111)	9% (125)	77 (54-86)***
Children 10-12 years. Infection	87% (75)	28% (75)	68 (53-78)***
Children 5-9 years. HBsAg +	9% (111)	2% (125)	73 (6-93) ∈
Children 10-12 years. HBsAg +	12% (75)	9% (74)	25 (-100-69)
Male children 5-9 years. HBsAg +	10% (57)	1.7% (57)	83 (-34-98)
Male children 10-12 years. HBsAg +	15% (40)	2.4% (41)	84 (-29-98)
Female children 5-9 years. HBsAg +	7% (54)	1.5% (68)	78 (-72-98)
Female children 10-12 years. HBsAg +	9% (35)	18% (33)	-100
Puerto Nariño			
Children 5-9 years. Infection	9% (11)	2% (105)	78 (-113-97)
Children 10-14 years. Infection	86% (22)	13% (31)	85 (62-94)

* Year 1992 **Year 1999 (Including only children from rural areas) ***$p<0.001$ ∈ $p<0.05$

III. Prevalence of hepatitis B infection and related factors:

III.1 Child-related variables:

The influence of these factors were analysed separately for anti-HBc and HBsAg prevalence. When anti-HBc was analysed all children positive for this marker were included in the numerator and when the analysis focused on HBsAg, children positive for both HBsAg and anti-HBc went into the numerator

Anti-HBc prevalence: All child characteristics in table 5.2 were associated with being anti-HBc positive. The strongest associations were observed with ethnic group and qualification of the people attending the birth.

Children belonging to Indian groups (Huitotos, Ticunas, and Others) had a higher chance of being infected than those who did not (Non Indians). The highest prevalence was observed among children belonging to the "Other Indian groups" who had a 7 times greater chance of being positive for anti-HBc, while "Ticunas" and "Huitotos" were three times more likely to be positive. In rural areas similar associations were observed while in urban areas only "Other groups" had an increased risk of being anti-HBc positive (OR=3.7 95% CI 1.3-11.0)

When a child's birth was not attended by a physician or a nurse, which also meant that it was not attended in a hospital or health centre, his (her) risk of being anti-HBc positive was 4 times higher than that of a child born in a health facility. For children living in rural areas this relation was stronger than for children living in urban areas (OR=3.1 95% CI 1.4-6.7 in rural and OR= 1.91 95% CI 0.9-4.2 in urban areas).

Living in a household with more than 4 siblings increased by 70% the chance of being anti-HBc positive. This relation disappeared when the data were analysed for rural and urban areas separately (OR=1.2 95% CI 0.8-1.84 in rural and OR=1.3 95% CI 0.4-4.2 for urban areas). On the other hand, "being born to a mother with four or more previous deliveries" increased the risk of infection by 50%. As with number of siblings, no relationship was found when the analysis was stratified by area (OR= 1.4 95% CI 0.8-2.2 for rural and OR=0.7 95% CI 0.2-2.1 for urban areas).

"Birth order" and "number of siblings" were strongly correlated (0.71) and, therefore, it was decided to keep only "number of siblings" for further analysis based on the size of the effect.

Age was also related to infection but the most important difference appeared among children aged 8 to 11 years. The oldest children had twice the risk of the others. This difference was found only in rural areas since in urban places the increase in prevalence among the oldest age group was small and not statistically significant. No relation was observed between breastfeeding and anti-HBc prevalence.

Multivariable models were constructed separately for rural and urban areas. The following variables were included: "age groups", "birth received by..", "ethnic group", and "number of siblings". Table 5.3 shows the results when children from all areas were analysed. Ethnic groups ("Others groups" and Ticunas) showed the most important associations with being

positive for anti-HBc, but "birth received by somebody different to a doctor or nurse" was statistically related as well. "Number of siblings" was dropped due to lack of statistical significance and small effect size (OR=0.95 95% CI 0.6-1.5, p=0.81). The results for rural areas were very similar, see table 5.4. In urban areas only "ethnic group" was statistically related to being anti-HBc positive, specifically, belonging to "Other groups" increased the risk by 4 fold (OR=3.7 95% CI 1.3-11.0). Belonging to Ticunas was associated with a 2 fold increase of the risk, but it was not statistically significant (OR=2.0 95% CI 0.8-4.8)

HBsAg Prevalence: The same variables related to anti-HBc prevalence were associated with being positive for HBsAg and anti-HBc. Table 5.2. As before, children belonging to an ethnic group had a larger chance of being HBsAg positive. "Other Indian groups" and "Huitotos" showed the highest prevalence of HBsAg followed by "Ticunas". All these relations were stronger than those observed with anti-HBc. For example, a child belonging to the Huitoto Indians had seven times greater chance of being HBsAg+/anti-HBc+, but this ratio was less than three when anti-HBc alone was considered. None of the Indian groups had HBsAg positive children in urban areas and therefore the association with HBsAg prevalence was limited to rural settings.

Children whose birth was not attended by medical personnel had a higher prevalence of HBsAg. This was the strongest relationship found with child related variables. As with other variables, this relation was more important in rural than in urban areas. Local midwives, relatives, and the mother themselves attended all births from HBsAg+ children in rural areas (n=23). On the other hand, in urban areas, MD or nurses attended the births of all HBsAg positive children (n=2).

Age was important in rural but not in urban areas. The sharp increase in the HBsAg prevalence among children aged 8 to 11 years did not occur in urban areas where this group had a low prevalence (0.3%). In rural areas this age group had an HBsAg prevalence of 6.6%.

"Living with more six or more siblings" was also associated with a higher chance of being HBsAg positive. As with the other variables, this relationship was important for children living in rural but not in urban settings. In rural areas, the risk of being HBsAg positive was 5 times higher among those with four or more siblings (OR=4.9 95% CI1.5-16.2). In urban places, no child with this characteristic was found HBsAg positive. A similar finding was

observed with birth order. This was related to HBsAg only in rural settlements (OR=2.8 95% CI 1.3-5.9), but no child in the risk group were found HBsAg positive in urban areas.

Table 5.2. Prevalence of HB infection by children-related variables.

Variable	Anti-HBc +			HBsAg+/Anti-HBc+		
	Anti-HBc- N (%)	Anti-HBc+ N (%)	OR (95% CI)	HBsAg - N (%)	HBsAg + N (%)	OR (95% CI)
Age groups (years)		**P<0.0001**			**P<0.0001**	
1-3	485 (95.3)	22 (4.7)	1.0	434 (99.8)	1 (0.2)	1.0
4-5	453 (97.5)	13 (2.5)	0.5 (0.3-1.0)	432 (99.3)	3 (0.7)	2.6 (0.3-27.2)
6-7	470 (96.2)	19 (3.8)	0.8 (0.4-1.6)	454 (99.7)	2 (0.3)	1.1 (0.1-11.5)
8-11	581 (90.8)	70 (9.2)	2.0 (1.1-3.7)	561 (97.0)	19 (3)	10.0 (1.2-90.8)
Birth order		**P=0.05**			**P<0.001.**	
1-3	982 (94.6)	60 (5.4)	1.0	926 (99.8)	9 (0.2)	1.0
4-20	542 (92.0)	51 (8.0)	1.5 (1.0-2.3)	511 (97)	15 (3)	3.4 (1.6-7.2)
Number of siblings		**P=0.02**			**P=0.003**	
1-5	1265 (95.0)	67 (5.0)	1.0	1202 (99.5)	8 (0.5)	1.0
6-20	360 (91.9)	34 (8.1)	1.7 (1.1-2.7)	345 (96.8)	11 (3.2)	6.2 (2.1-18.2)
Birth received by		**P<0.0001**			**P<0.0001**	
MD/Nurse	1139 (97.3)	32 (2.7)	1.0	1089 (99.8)	2 (0.2)	1.0
Other	833 (90.3)	92 (9.7)	3.9 (2.4-6.6)	778 (97.3)	23 (2.7)	13.0 (3.0-57.0)
Ethnic group		**P<0.0001**			**P=0.01**	
Non Indians	640 (98.0)	17 (2.0)	1.0	602 (99.7)	2 (0.3)	1.0
Huitoto	147 (94.0)	11 (6.0)	2.7 (1.1-6.3)	142 (98.0)	3 (2.0)	7.3 (1.0-51.3)
Other Indian groups	235 (86.0)	44 (14.0)	6.9 (3.4-13.8)	229 (97.0)	10 (3.0)	11.3 (2.3-55.9)
Ticunas	648 (92.0)	46 (8.0)	3.5 (1.7-6.9)	603 (98.4)	9 (1.6)	6.0 (1.0-36.4)
Mestizo	319 (98.0)	6 (2.0)	0.8 (0.4-1.9)	305 (99.6)	1 (0.4)	1.2 (0.1-14.0)

Table 5.3. Final model of children-related variables and anti-HBc prevalence. All areas

Variable	OR (95% CI)	P
Age groups (years)		
1-3	1.0	
4-5	0.4 (0.2-0.9)	0.03
6-7	0.8 (0.4-1.6)	0.49
8-11	1.9 (1.0-3.6)	0.04
Birth received by		
MD/Nurse	1.0	
Other	2.4 (1.5-4.1)	0.001
Ethnic group		
Non Indians	1.0	
Huitoto	1.9 (0.9-4.3)	0.10
Other Indian groups	4.6 (2.4-8.6)	0.000
Ticunas	2.4 (1.2-4.6)	0.01
Mestizo	0.8 (0.4-2.0)	0.70

Table 5.4. Final model of children-related variables and Anti-HBc prevalence. Rural area

Variable	OR (95% CI)	P
Age groups (years)		
1-3	1.0	
4-5	0.5 (0.2-1.1)	0.09
6-7	1.0 (0.4-2.6)	0.98
8-11	2.7 (1.2-6.0)	0.02
Birth received by		
MD/Nurse	1.0	
Other	2.5 (1.1-5.6)	0.03
Ethnic group		
Non Indians	1.0	
Huitoto	2.1 (0.7-6.4)	0.17
Other Indian groups	4.4 (1.7-10.9)	0.003
Ticunas	2.5 (1.0-6.4)	0.05
Mestizo	Dropped because no Anti-HBc+ was found in rural areas	

In the multivariable model the following variables were included: "age groups", "birth received by.." , "number of siblings", and "ethnic groups". Table 5.5 shows the final model when all areas were included in the analysis. "Number of siblings" and "birth received by .." were statistically related to HBsAg prevalence, but ethnic group or age were not. Among ethnic groups the highest relation was observed with "Other groups", but the relation was not statistically significant (OR=2.6 95% CI 0.6-11.8, p=0.22). In order to evaluate if the fall in the strength of the association between "ethnic group" and HBsAg was due to a loss in the number of observations, a new category for missing values was created in the variable "number of siblings". The number of observations in the model increased sharply from 1557 to 1892 and the relation between "Other groups" and HBsAg prevalence increased, but still did not reach statistical significance (OR=3.6 95% CI 0.8-16.1, p=0.09).

In rural areas none of the variables reached formal statistical significance in relation to HBsAg positivity but several had a large point estimate of effect; "number of siblings" (OR=3.4 95% CI 0.9-12.4, p=0.07), "Other Indian groups" (OR=4.4 95% CI 0.5-36.6, p=0.16), belonging to Ticunas (OR=4.0 95% CI 0.4-45.4, p=0.24), and "age group 8-11" (OR=6.4 95% CI 0.4-97.7, p=0.17). In urban areas none of the independent variables were related to HBsAg.

Table 5.5. Final model of children-related variables and HBsAg prevalence. All areas

Variable	OR (95% CI)	P
Age groups		
1-3	1.0	
4-5	2.1 (0.2-22.8)	0.54
6-7	0.8 (0.1-10.0)	0.89
8-11	6.0 (0.6-65.4)	0.14
Birth received by		
MD/Nurse	1.0	
Other	6.5 (1.5-27.6)	0.01
Number of siblings		
1-5	1.0	
6-20	3.3 (1.1-10.0)	0.03

III. 2. Mother-related variables:

Anti-HBc prevalence: The strongest relation was observed with "place where mother was born". Prevalence of anti-HBc was the highest among children whose mothers were born in rural Amazon (9%) having almost 4 times greater risk of being positive than children from mothers born in other places (OR=3.6 95% CI 2.1-6.3). This relation was present in rural (OR=4.2 95% CI 1.8-9.8) but not in urban areas (OR=1.1 95% CI 0.4-3.4).

Children born to HBsAg+ mothers had the highest prevalence in this group (10%), followed by those born to HBeAg+ mothers (9.6%), and to anti-HBc+ mothers (8.4%). However, a statistically significant relation was observed only among children born to an anti-HBc+ mother (OR=2.6 95% CI 1.7-4.1). For those born to an HBsAg+ mother, the relation was not significant (OR=2.0 95% CI 0.9-4.4, p=0.09). Being born to an HBeAg+ mother was even less related to anti-HBc prevalence (OR=1.9 95% CI 0.4-8.2, p=0.4). Table 5.6

The effect of "mother's serological status differed by area". In rural areas, children born from an anti-HBc+ mother had an anti-HBc prevalence of 11.5% (73/634), while in urban areas it was 2.2% (7/241). In rural areas, the risk of being anti-HBc+ doubled in children born to an anti-HBc+ mother (OR=2.2 95% CI 1.4-3.6), while no increase in the risk was observed in urban areas. Children born to HBsAg+ mothers and living in rural areas had an anti-HBc prevalence of 14% (6/48), while in urban areas it was 0% (0/16). However, the increase in the risk of being anti-HBc+ for those born to an HBsAg+ mother was not significant in rural areas (OR=1.7 95% CI 0.8-3.8).

The "mother's history of clinical hepatitis" was not related to HBsAg prevalence, though in urban areas it slightly increased the risk of being anti-HBc+ (OR=2.6 95% CI 0.8-8.2, p=0.1).

In the multivariable models "children's age group", "place where the mother was born", "mother's anti-HBc status", and "mother's HBsAg status" were included. "Place where mother was born" (rural Amazon vs. others) and being born from an anti-HBc+ mother remained significantly associated with anti-HBc prevalence (OR=3.4 95% CI 2.0-5.8 and OR=1.7 95% CI 1.2-2.5 respectively). In rural areas, multivariable models yielded similar findings but in urban areas none of the variables were related to anti-HBc prevalence.

Table 5.6. Anti-HBc and HBsAg prevalence by mother-related factors.

Variable	Anti-HBc +			HBsAg+/Anti-HBc+		
	Anti-HBc- N (%)	Anti-HBc+ N (%)	OR (95% CI)	HBsAg - N (%)	HBsAg + N (%)	OR (95% CI)
Place where mother was born		**P<0.0001**			**P<0.001**	
Rural Amazon	910 (91.0)	94 (9.0)	3.6 (2.1-6.3)	857 (97.8)	22 (2.2)	9.1 (1.9-42.9)
Other	935 (97.3)	26 (2.7)	1.0	888 (99.7)	2 (0.3)	1.0
Mother's antecedent of clinical hepatitis		P=0.19			P=0.57	
Y	100 (91.0)	9 (9.0)	1.7 (0.7-3.9)	92 (99.3)	1 (0.7)	0.6 (0.1-4.6)
N	1712 (94.6)	110 (5.4)	1.0	1617 (99)	23 (1.0)	1.0
Born from an HBsAg positive mother		**P=0.09**			P=0.33	
Y	58 (90.0)	6 (10.0)	2.0 (0.9-4.4)	55 (97.1)	2 (2.9)	2.6 (0.4-18.3)
N	1695 (95.0)	104 (5.0)	1.0	1600 (98.9)	21 (1.1)	1.0
Born from an infected mother (Anti-HBc)		**P<0.0001**			**P=0.001**	
Y	802 (91.6)	80 (8.4)	2.6 (1.7-4.1)	761 (97.6)	21 (2.4)	6.6 (1.8-25.1)
N	948 (96.6)	32 (3.4)	1.0	892 (99.6)	3 (0.4)	1.0
Born from an HBeAg positive mother		P=0.4			P=0.76	
Y	25 (90.4)	2 (9.6)	1.9 (0.4-8.2)	24 (100)	0 (0)	Undefined
N	1964 (95.0)	122 (5.0)	1.0	1857 (99)	25 (1)	

Table 5.7. Final model of mother-related variables and anti-HBc prevalence. Rural areas.

Variable	OR (95% CI)	P
Age groups		
1/3	1.0	
4/5	0.4 (0.2-1.1)	0.07
6/7	1.0 (0.4-2.7)	0.93
8/11	2.63 (1.0-6.6)	0.04
Born from an infected mother (Anti-HBc)		
Y	1.7 (1.1-2.6)	0.02
N	1.0	
Place where mother was born		
Rural Amazon	3.6 (1.5-8.3)	0.005
Other	1.0	

HBsAg prevalence: The strongest relation was observed with the variable "place where mother was born". Children whose mothers were born in rural Amazon were 9 times more likely to be HBsAg+ than children whose mothers were born elsewhere. When this association was stratified by areas, the magnitude of the OR and the p values fell sharply and became non statistically significant in both areas (OR=3.5 95% CI 0.4-30.6, p=0.24 in rural areas and OR=4.0 95% CI 0.2-67.0, p=0.33 in urban areas).

Among the variables related to mother's serological status the strongest relationship was found with the "mother's anti-HBc status". The risk of being HBsAg+ was 7 times higher when a child was born from an anti-HBc+ mother and this relation was even stronger in rural areas though it lost precision (OR=8.3 95% CI1.0-69.4, p=0.05). In urban areas, none of the children born to an anti-HBc+ mother was HBsAg positive and, therefore, assessing a relationship was not possible. The association between being born from an HBsAg+ mother and HBsAg prevalence was not significant even after stratifying by area (OR=1.8 95% CI 0.3-13.3 in rural areas and no HBsAg+ children were found in the risk category in urban areas).

A clinical history of hepatitis in mothers was not related to HBsAg prevalence in children.

In the multivariable model the following variables were included: "age group", "mother's anti-HBc status", "mother's HBsAg status", and "place where the mother was born". Table 5.8 shows the results from the final model for all areas. "Mother's HBsAg status" was

dropped from the model because it was no longer related to HBsAg prevalence (OR=1.5 95% CI 0.2-10.9, p=0.69). In rural areas, only "mother's anti-HBc status" was associated with HBsAg prevalence (OR=6.9 95% CI 0.8-61.2, p=0.08)

Table 5.8. Final model of mother-related variables and HBsAg prevalence. All children.

Variable	OR (95% CI)	P
Age groups		
1/3	1.0	
4/5	2.3 (0.2-24.3)	0.48
6/7	1.0 (0.1-10.9)	0.97
8/11	9.5 (1.0-90.4)	0.05
Born from an infected mother (Anti-HBc)		
Y	3.5 (1.0-11.8)	0.04
N	1.0	
Place where mother was born		
Rural Amazon	6.0 (1.5-23.1)	0.01
Other	1.0	

III.3. Vaccination characteristics.

There were 1407 (66%) children with data available on hepatitis B vaccination. According to the vaccination card, 91% (1277) of those children had completed the basic scheme for hepatitis B (3 doses). There were no differences in HBsAg prevalence between vaccinated and unvaccinated children. The prevalence in children completely vaccinated was 1.2% (15/1129) while no HBsAg+ was found among unvaccinated children (0/119), p=0.3. Interestingly, HBsAg prevalence among children without vaccination data was very close to the prevalence in vaccinated children, 1.1% (10/658). Similar results were observed when the dependent variable was anti-HBc prevalence. Anti-HBc prevalence among vaccinated children was 6% (76/1258) while no positive was found among those with an incomplete vaccination series (0/126). Children without vaccine information had an anti-HBc+ prevalence of 5.2% (48/729)

The time lag between hepatitis B doses and its relation to hepatitis B markers was assessed using the following indicators: time in days from birth to first dose, time in days from first to second dose, and time in days from second to third dose. Figure 5.7 shows the distribution

of medians and quartiles (Q_1, Q_3). The median time from birth to first dose was 77 days, the value for Q_1 was 9 days, and for Q_3 was 417 days. The median time from first to second dose was 47 days, Q_1 was 31 days, and Q_3 was 114 days. The median time from second to third dose was 87 days, Q_1 was 33 days, and Q_3 was 87 days. The largest inter quartile range was observed for time from birth to first dose while the shortest was for time from first to second dose.

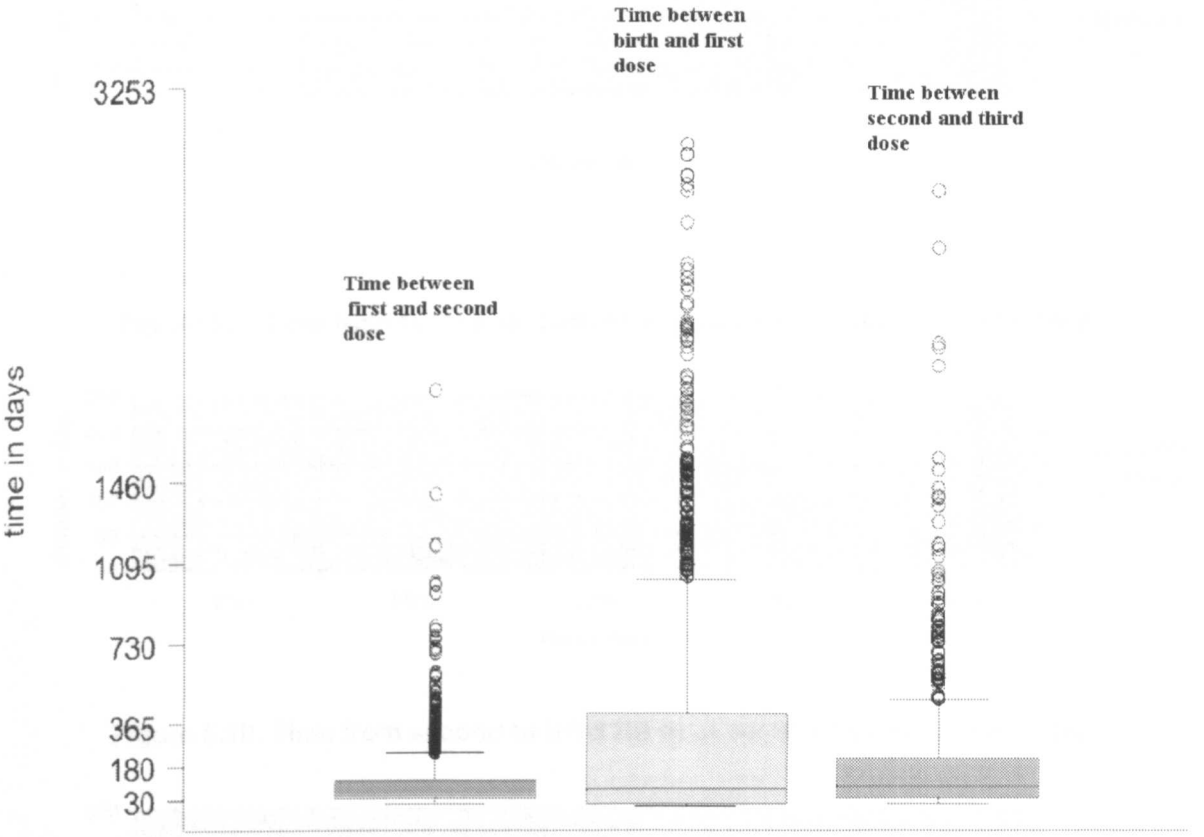


Figure 5.7. Box plot showing time lag distribution between hepatitis B doses.

HBsAg prevalence: The relation between HBsAg prevalence and time between doses was assessed first by comparing the time distribution by HBsAg status categories. Figures 5.8 to 5.10 show how these times were distributed by percentiles between HBsAg+ and HBsAg-. The sharpest difference was observed with "time between birth and first dose". For uninfected people, the Q_1 and median values were 10 and 77 days, respectively, while for infected peoples, they were 161 and 817 days ($p_{Kruskall\text{-}Wallis}$=0.003). "Time from first to

second dose" also differed by HBsAg status. Q_1 and median values for HBsAg- were 31 and 44 days, respectively, but for HBsAg+ they were 47 and 80 days ($p_{Kruskall\text{-}Wallis}$=0.12). "Time from second to third dose" showed smaller, non statistically significant differences. Q_1 was 33 days in HBsAg- and 62 days in HBsAg+ while median was 100 days in HBsAg+ but 86 in HBsAg- ($p_{Kruskall\text{-}Wallis}$=0.54).

Figure 5.8. Time from birth to first HB dose by HBsAg status. Percentiles.

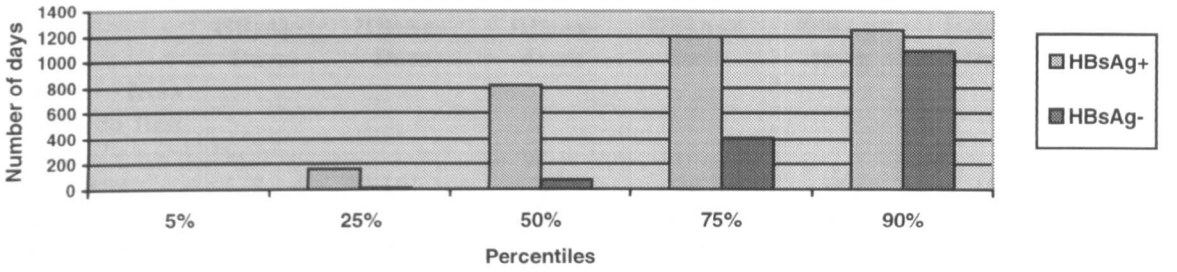


Figure 5.9. Time from first to second HB dose by HBsAg status . Percentiles

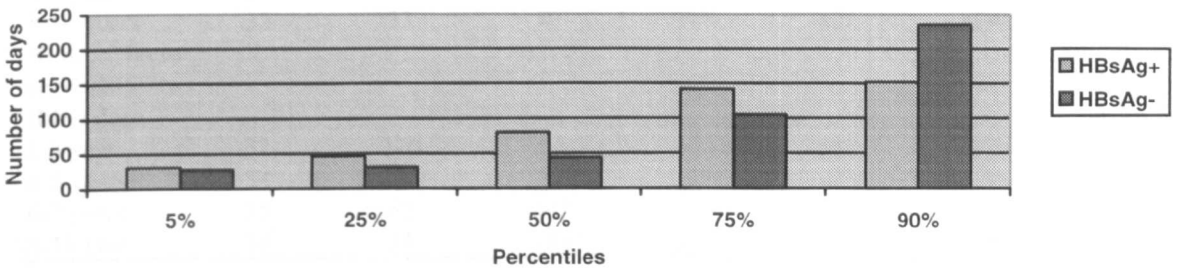
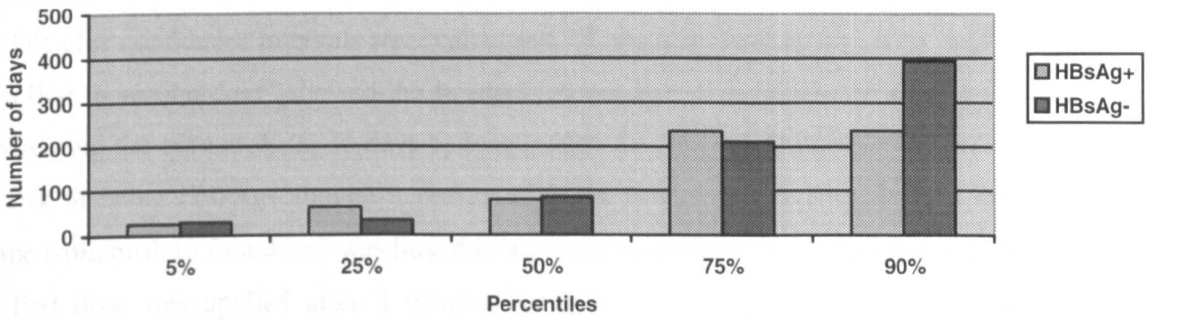


Figure 5.10. Time from second to third HB dose by HBsAg status. Percentiles



Percentiles of time between doses were stratified by age and differences remained for the variables "time between birth to first dose" and for "time between first to second dose". Table 5.9 shows the number of days by age group, time interval, and HBsAg status. Time

between birth and first dose was longer for HBsAg+ through all ages and percentiles, excepting children aged 1-3 years among whom only one HBsAg+ was present. For "time between first to second dose" HBsAg- children had shorter intervals in all age groups, especially for Q_1 and Q_3. When "time between second and third dose" was analysed, only one to three years old HBsAg- children had shorter intervals for Q_1.

Table 5.9. Percentiles of time lag distribution by age and HBsAg status.

	Q_1		P50		Q_3	
	HBsAg- Days	**HBsAg+ Days**	**HBsAg- Days**	**HBsAg+ Days**	**HBsAg- Days**	**HBsAg+ Days**
Time from birth to first dose						
1-3 years	2	161	30	161	80	161
4-5 years	3	130	53	130	162	130
6-7 years	34	913	141	1080	503	1247
8-11 years	104	604	486	817	1089	1197
Time from first to second dose						
1-3 years	31	180	42	180	98	180
4-5 years	31	47	49	72	109	98
6-7 years	32	60	47	60	127	61
8-11 years	32	33	41	117	102	143
Time from second to third dose						
1-3 years	32	270	54	270	157	270
4-5 years	34	64	67	67	143	70
6-7 years	35	62	147	92	249	123
8-11 years	38	33	143	166	246	236

Time variables were grouped into four or five categories based on percentile value and/or on the number of HBsAg+ children by categories. Then, prevalence of HBsAg+ by category and OR plus confidence intervals were calculated. "Time from birth to first dose" and "time from first to second dose" showed the strongest association with HBsAg prevalence. Delay in receiving the second dose, 36 days or longer after the first was related to an increase in the risk of being HBsAg+ though it decreased in the final category (148 days or longer). "Time from birth to first dose" was linked to an increase in the risk of being HBsAg+ when the first dose was applied after 2 months. Delay in receiving the third dose tended to increase the chance of being HBsAg+, but the difference did not reach statistical significance. Table 5.10

Table 5.10. Distribution of HBsAg prevalence by time lag between HB doses.

Variable	HBsAg+/ Anti-HBc+			
	HBsAg - N (%)	HBsAg + N (%) P=0.07	OR (95% CI)*	OR (95% CI)* Rural areas
Time from birth to first dose				
0-14 days	294 (99.4)	1 (0.2)	1.0	1.0
15-60 days	190 (100)	0	Undefined	Undefined
61-183 days	196 (99.4)	2 (1.2)	6.6 (0.5 - 90.4)	6.7 (0.4-119.0)
184-665 days	193 (99.4)	1 (0.6)	2.2 (0.1 - 40.4)	1.0 (0.05-22.7)
666-3253	192 (96.5)	7 (3.5)	8.9 (0.9-88.2)	4.1 (0.4-46.1)
Unknown	816 (98.8)	14 (1.2)	4.1 (0.5-35.4)	3.1 (0.4-26.8)
Time from first to second dose		P=0.15		
28-35 days	464 (99.4)	3 (0.6)	1.0	1.0
36-62 days	198 (98.1)	4 (1.9)	3.3 (1.2-8.9)	3.0 (1.7-5.2)
63-147 days	212 (97.9)	4 (1.5)	3.2 (0.9-11.2)	3.3 (0.8-13.3)
148-1877 days	207 (99.0)	3 (1.5)	2.5 (0.7-9.2)	2.0 (0.6-7.0)
Unknown	800 (99)	11 (1.0)	1.3 (0.3-4.7)	1.9 (0.5-6.9)
Time from second to third dose		P=0.5		
28-32 days	237 (99.3)	2 (0.7)	1.0	1.0
33-61 days	190 (99.3)	1 (0.7)	1.1 (0.1-14.1)	2.1 (0.1-44.6)
62-128 days	192 (97.4)	5 (2.0)	2.7 (0.5-12.6)	4.7 (0.8-28.7)
129-235 days	198 (98.8)	2 (1.2)	1.2 (0.1-13.8)	2.7 (0.2-42.8)
236-2787 days	207 (98.8)	4 (1.8)	1.8 (0.3-12.7)	3.6 (0.3-43.7)
Unknown	857 (99)	11 (1.0)	0.9 (0.2-4.2)	2.5 (0.3-17.0)

*** Adjusted by age group**

The same analysis was done for rural areas and the magnitude of the association between HBsAg prevalence and "time from birth to first dose" decreased. For "time from first to second dose", the relation was similar to that observed for the whole population. However, for "time from second to third dose", it seems that there was an increase in the magnitude of the relation between different time categories and HBsAg prevalence, though none reached statistical significance. Table 5.10

Since few unvaccinated children (among those with vaccination card) were found in the study and none were HBsAg positive, it was decided to create a category in each time variable to include children without vaccination data aimed at evaluating if they had a different risk of being infected than children with data, under the supposition that many might be unvaccinated. The largest difference was found for children without data on "time between birth and first dose" (OR=4.1 95% CI 0.5-35.4), though it was not statistically significant (p=0.20). Table 5.10

Combined analysis of vaccine-related and other covariates with HBsAg prevalence: Those factors related with HBsAg prevalence in previous steps were analysed together with time from birth to first dose and time from first to second HB dose using logistic multivariable models to control for potential confounding effects and to evaluate interactions. The following variables were included at this stage:

1) Birth attended by MD/nurse or others.
2) Number of siblings.
3) Mother's anti-HBc status.
4) Mother's HBsAg status
5) Time from birth to first HB dose. In order to improve the efficiency of the multivariable analysis, the first category (0-14 days) was collapsed with the second (15-60 days). This decision was based on the fact that the second category had the lowest prevalence (0 cases) and, therefore, it could form part of the baseline category.
6) Time from first to second dose. This variable was recoded collapsing the last two categories to one, 63 days and more. This decision was taken since these two categories had the same HBsAg prevalence (see table 5.10)
7) Age group.

The multivariable analysis was done separately for "time between birth and first dose" and for "time between first and second dose". These two variables can not be combined since they correlate at 0.70.

Modelling time from birth to first dose: Table 5.11 shows the results when all covariates were included together. Children who received the first dose after the second year of life had 12 times more chance of being HBsAg+ than those who received it within two weeks after birth. Receiving the first dose of the vaccine after 2 months of life was also riskier, but it did not reach statistical significance. Not having accurate data on vaccination dates was also associated with a higher HBsAg carriage risk when compared with children who received the first dose within two weeks of life. It did not reach statistical significance but it might have been only a matter of the number of positives in the baseline category (n=1). Being born from an anti-HBc+ mother increased the risk of carriage by three times.

Area was included and kept in the model to control for its potential confounding effect since living in rural areas was associated with being HBsAg+ and with having longer intervals from birth to first dose. This variable and "birth received by..." correlated at 0.56 but

models run without one of them did not show any important change in the magnitude of the associations or standard errors, and therefore were included together.

Table 5.11. Time from birth to first dose, covariates and their relationship to HBsAg status. (Urban and rural areas)

Variable	OR (95% CI)	P
Age groups (years)		
1-3	1.0	
4-5	1.5 (0.1-19.4)	0.74
6-7	0.6 (0.05-6.0)	0.62
8-11	3.3 (0.3-33.8)	0.30
Time from birth and first dose		
0-60 days	1.0	
61-183 days	7.2 (0.5-115.1)	0.16
184-665 days	2.6 (0.1-50.0)	0.53
666-3253	12.5 (1.2-125.7)	0.03
Unknown	6.6 (0.6-66.4)	0.11
Birth received by		
MD/Nurse	1.0	
Other	2.7 (0.9-8.0)	0.07
Number of siblings		
1-5	1.0	
6-20	2.7 (1.0-7.3)	0.05
Mother Anti-HBc+		
Y	3.4 (1.1-11.2)	0.04
N	1.0	
Area		
Urban	1.0	
Rural	2.2 (0.9-5.6)	0.09

The model was repeated creating a category for missing values on number of siblings (n=391), but results were remarkably similar for all variables included in the model. The model was also fitted excluding those without data on "time from birth to first dose" and, again, results resembled closely to those observed in table 5.11. Fitting an interaction term for "mother's anti-HBc status" and "time from birth to first dose" was attempted but it was impossible due to the small number of cases. However, in bivariable analysis it was observed that the relation between "time from birth to first dose" and HBsAg prevalence varied across mother's serological status. Table 5.12.

An attempt to built a similar model for rural areas was hampered because "number of siblings" had missing data and estimates for other variables (birth attendance and mother's anti-HBc status) became so unstable that they were dropped from the model (cells with 0). It was decided to model them one by one with time from birth to first dose. Table 5.13 shows the results for rural areas when "mother's anti-HBc status" is included in the model. The

magnitude of the associations with HBsAg prevalence was similar to those found when all areas were included in the same model, though there was a loss in statistical significance due to a decrease in the sample size. Other models including "number of siblings" or "birth received by.." showed similar results, but the one in table 5.13 had the highest F and likelihood value.

Table 5.12. Relation between time from birth to first dose, HBsAg prevalence and mother's Anti-HBc status. (Rural areas)

Time from birth to first dose	Mother Anti-HBc+		Mother Anti-HBc-	
	HBsAg- N (%)	HBsAg+ N (%)	HBsAg- N (%)	HBsAg+ N (%)
0-60 days	119 (99.4)	1 (0.6)	65 (100)	0
61-183 days	76 (96.0)	2 (4)	27 (100)	0
184-665 days	81 (98)	1 (2)	35 (100)	0
666-3253	69 (91)	6 (9)	28 (100)	0
Unknown	184 (95.5)	11 (4.5)	103 (99.0)	1 (1.0)

Table 5.13. Model containing time from birth to first dose, covariates, and their relation to HBsAg prevalence. (Rural areas)

Variable	OR (95% CI)	P
Age groups		
1-3	1.0	
4-5	0.9 (0.92)	0.92
6-7	0.7 (0.05-8.0)	0.75
8-11	6.4 (0.8-53.9)	0.08
Time from birth and first dose		
0-60 days	1.0	
61-183 days	10.0 (0.5-203.8)	0.12
184-665 days	2.0 (0.1-48.8)	0.65
666-3253	8.4 (0.7-102.3)	0.09
Unknown	5.9 (0.7-51.1)	0.10
Mother Anti-HBc+		
Y	6.9 /0.8-63.1)	0.08
N	1.0	

Modelling time from first to second dose: When both areas, rural and urban, and other covariates were analysed together, "time from first to second dose" was not statistically related to HBsAg prevalence. These results did not change even after running the model without area or when missing values for the time interval variable were removed from the model. Table 5.14.

It was not possible to fit an interaction between "mother's anti-HBc status" and "time from first to second dose" but in bivariable analysis it could be observed that the relation between HBsAg prevalence and time from first to second dose was different by categories of mother's infection. Table 5.15

Table 5.14. Time from first to second dose, covariates and their relation to HBsAg status. (Urban and rural areas)

Variable	OR (95% CI)	P
Age groups		
1-3	1.0	
4-5	2.1 (0.2-22.2)	0.53
6-7	0.9 (0.08-10.3)	0.96
8-11	6.2 (0.6-70.8)	0.13
Time from first to second dose		
28-35 days	1.0	
36-62 days	1.6 (0.7-3.8)	0.29
63-1877 days	1.2 (0.4-3.6)	0.67
Unknown	0.8 (0.2-3.7)	0.79
Birth received by		
MD/Nurse	1.0	
Other	2.8 (1.1-7.1)	0.02
Number of siblings		
1-5	1.0	
6-20	2.6 (1.0-7.0)	0.05
Mother Anti-HBc+		
Y	3.1 (1.0-9.8)	0.05
N	1.0	
Area		
Urban	1.0	
Rural	1.9 (0.8-4.4)	0.15

Table 5.15. Relation between time from first to second dose, HBsAg prevalence and mother's Anti-HBc status. (Rural areas)

Time from first to second dose	Mother Anti-HBc+		Mother Anti-HBc-	
	HBsAg- N (%)	HBsAg+ N (%)	HBsAg- N (%)	HBsAg+ N (%)
28-35 days	91 (97.7)	2 (2.3)	50 (100)	0
36-62 days	84 (95.0)	4 (5)	31 (100)	0
63-1877 days	186 (96.2)	7 (3.8)	83 (100)	0
Unknown	168 (96.4)	8 (3.6)	94 (98.7)	1 (1.3)

For rural areas, the relation between "time from first to second dose" and HBsAg prevalence was stronger. A delay to receive second dose, between 36 to 62 days from the first, was associated with a twofold increase in the risk of being HBsAg positive. No statistical trend was observed since the risk increase for the next category did not reach statistical

significance (p=0.28). Interestingly, those children without data available on this variable did not have an increase in the risk of being HBsAg positive. Being born from an anti-HBc positive mother and having a larger number of siblings also remained associated with a higher risk of being HBsAg positive. The variable "birth received by.." was not included in the model because it had empty cells in rural areas. Table 5.16

Table 5.16. Time from first to second dose, covariates and their relation to HBsAg status. (Rural areas)

Variable	OR (95% CI)	P
Age groups (years)		
1-3	1.0	
4-5	1.3 (0.1-16.7)	0.83
6-7	0.96 (0.1-11.4)	1.0
8-11	8.4 (0.8-91.6)	0.08
Time from first to second dose		
28-35 days	1.0	
36-62 days	2.3 (1.4-3.8)	0.003
63-1877 days	2.0 (0.6-7.4)	0.27
Unknown	1.4 (0.3-5.3)	0.64
Number of siblings		
1-5	1.0	
6-20	3.2 (1.0-10.5)	0.05
Anti-HBc+ mother		
Y	5.9 (0.7-48.3)	0.09
N	1.0	

Anti-HBc prevalence: "Time from birth to first dose" was statistically significant related to anti-HBc prevalence. The time interval was longer for anti-HBc+ than for anti-HBc- in all percentiles. Figure 5.11. The largest differences were observed for the median (145 days for anti-HBc+ and 77 days for anti-HBc-) and for the 75th percentile (732 days for anti-HBc+ and 405 days for anti-HBc-). p=0.05.

When time between doses was analysed no important differences were found. The median time from first to second dose was larger for anti-HBc+ than negatives but the difference was small (62 days for anti-HBc+ and 46 days for anti-HBc-). p=0.17. The median time from second to third dose was 87 days for anti-HBc+ and 123 days for anti-HBc-. p=20. Figure 5.12 and 5.13

Figure 5.11. Time from birth to first dose by AntiHBc status.

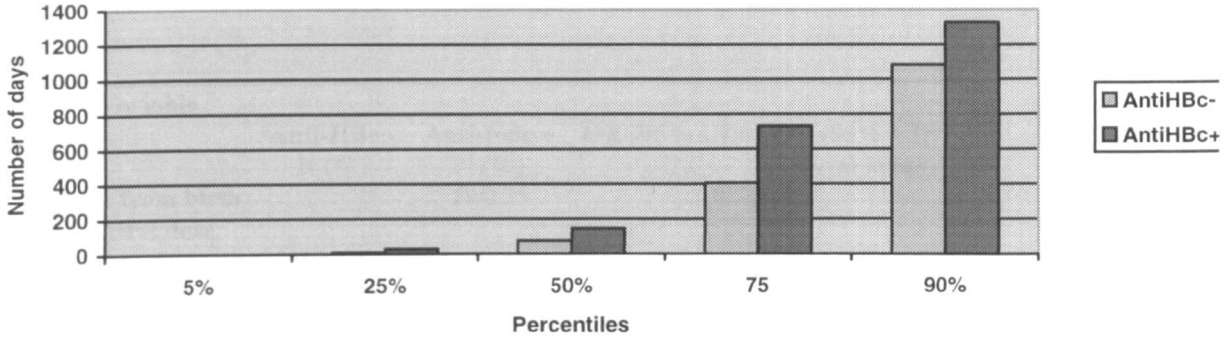


Figure 5.12. Time from first to to second dose by AntiHBc status.

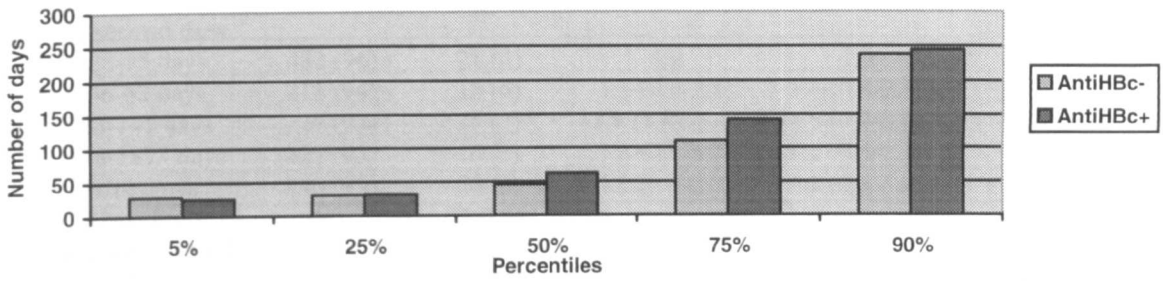


Figure 5.13. Time from second to third dose by AntiHBc status.

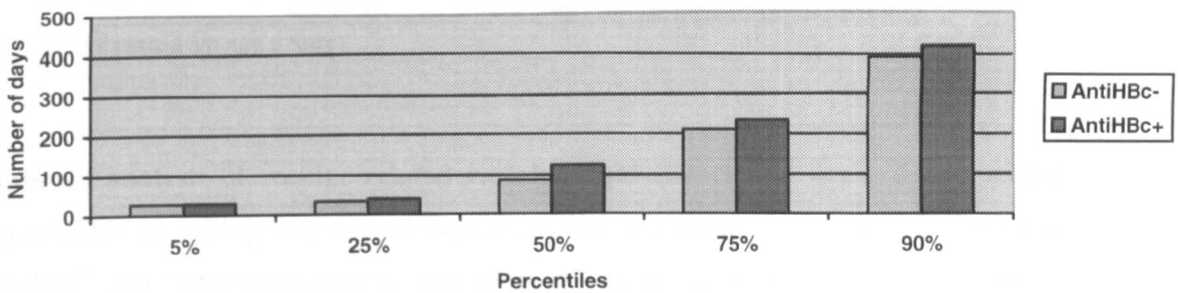


Table 5.17 shows the results when anti-HBc+ prevalence was analysed by categories of "time from birth to first dose" and time between doses. Overall, no statistically significant difference was observed for any of these variables. Only one category in "time from first to second dose" was related with being anti-HBc+. Children receiving the second dose between 63 to 147 days from the first dose had two times more chance of being anti-HBc+. When the analysis was stratified by area, some categories of the variable "time from birth to first dose" showed a stronger relationship with being anti-HBc+ but none reached statistical significance.

Table 5.17. Time lag between Hepatitis B doses and their relationship with being anti-HBc positive.

Variable	Anti-HBc - N (%)	Anti-HBc + N (%)	OR (95% CI)*	OR (95% CI)* Rural areas
Time from birth to first dose		P=0.25		
0-14 days	313 (96)	13 (4)	1.0	1.0
15-60 days	204 (96)	8 (4)	1.2 (0.4-3.1)	1.9 (0.8-4.8)
61-183 days	206 (95)	10 (5)	1.4 (0.6-3.4)	1.9 (0.9-4.1)
184-665 days	206 (96)	10 (4)	0.9 (0.3-2.6)	1.1 (0.3-3.9)
666-3253	201 (92)	17 (8)	1.5 (0.5-4.2)	1.4 (0.3-5.9)
Unknown	859 (94)	66 (6)	1.5 (0.7-2.9)	2.1 (0.8-5.8)
Time from first to second dose		P=0.42		
28-35 days	482 (96)	21 (4)	1.0	1.0
36-62 days	213 (94)	13 (6)	1.5 (0.8-2.8)	1.4 (0.8-2.4)
63-147 days	229 (93)	18 (7)	**1.9 (1.02-3.4)**	1.5 (0.7-3.0)
148-1877 days	225 (93)	16 (7)	1.8 (0.9-3.4)	1.2 (0.5-2.7)
Unknown	840 (95)	56 (5)	1.1 (0.6-2.2)	1.3 (0.5-3.0)
Time from second to third dose		P=0.64		
28-32 days	251 (95)	12 (5)	1.0	1.0
33-61 days	197 (96)	8 (4)	0.9 (0.4-2.3)	1.1 (0.5-2.4)
62-128 days	206 (94)	15 (6)	1.3 (0.5-3.2)	1.5 (0.4-5.8)
129-235 days	217 (93)	15 (7)	1.2 (0.6-2.5)	1.2 (0.5-3.0)
236-2787 days	219 (93)	17 (7)	1.4 (0.7-2.7)	1.9 (0.7-5.1)
Unknown	899 (95)	57 (5)	0.9 (0.4-1.9)	1.4 (0.4-4.6)

*** Adjusted by age group**

Combined analysis of vaccine-related variables and covariates: Those factors related with anti-HBc prevalence in previous steps, were analysed together with "time from birth to first dose" and "time from first to second dose" using logistical multivariable models. Variables included at this stage were:

1) Birth attended by MD/nurse or others.

2) Ethnic group.

3) Mother's anti-HBc status.

5) Time from birth to first dose of hepatitis B vaccine.

6) Time from first to second dose.

7) Age group.

None of the time variables, "time from birth to first dose" or "time from first to second dose", was related with anti-HBc prevalence after controlling for the effect of the covariates. This lack of effect was observed both when all children were analysed and after stratifying by areas. On the other hand "belonging to an ethnic group", "being born from an infected mother", and "birth attended by somebody different to MD/nurse" remained statistically significant in the multivariable analysis. Children belonging to "Other Indians groups" or "Ticunas" had the highest risk of being infected (OR=4.6 95% CI 2.4-8.8 and OR=2.4 95% CI 1.3-4.5 respectively). Being born from an anti-HBc+ mother increased the risk of being anti-HBc+ by 50% (OR=1.45 95% CI 1.1-2.1). Finally, those whose birth was attended by non medical personnel (MD or nurse) had twice the risk of being infected (OR=2.3 95% CI 1.5-3.7).

IV. Analysis of anti-HBs titres:

Only children negatives for anti-HBc and HBsAg were included in this part of the analysis. A randomly selected sample of 481 was studied. Age of participants ranged from 1 to 12 years (median and mean coincided in 5 years) and 51% were female. Fifty six percent came from urban areas (n=272) and 56% belonged to an ethnic group (n=270). Levels of anti-HBs ranged from 0 to more than 10,000 IU/ml but the geometric mean was 66 IU/ml (95% CI 52-83), and the median was 123 IU (95% CI 86-147). Twenty three percent of the population (n=112) did not have detectable antibody (<10 IU/ml), in 23% (n=115 children) anti-HBc levels ranged between 10 to 99 IU/ml, in 40% (n=193) between 100 to 999 IU/ml, and 13% (n=61)1000 IU/ml or higher.

Anti-HBs levels and related variables. Table 5.18 shows the results of the bivariable analysis. "Breastfeeding" and "time between birth and first dose of hepatitis B" were related to having detectable anti-HBs. The proportion of children without antibody among those not breastfed was 44% compared to 22% in children who were (p=0.03). The proportion of children without antibody was also higher among those receiving the first dose of hepatitis B vaccine close to the date of birth. Conversely, those who received the first dose between the second and the sixth month of life had the smallest chance of being anti-HBs negative. Gender, ethnic group, time between doses, and age were not related to not having antibody.

Similar findings were observed when the dependent variable was the quantity of antibody. Time between birth and first dose was the most important predictor of the level of anti-HBs. Those receiving the first dose long after birth had the highest level. Receiving the second

dose closer to the first was also associated with lower quantity of anti-HBs but this was not statistically significant.

Table 5.18. Anti-HBs levels by selected variables.

Variable	# without anti-HBs (%)	# with anti-HBs>10mIU/ml. (%)	# with anti-HBs ≥1000 mIU/ml (%)*	Anti-HBs GMT [Median]
Breastfeeding	**P=0.03**		P=0.13	P=0.13
N	8 (44)	10 (56)	1 (5.5)	22 [60]
Y	104 (22)	359 (77)	60 (13)	69 [120]
Time between birth and first dose	**P=0.02**		**P=0.01**	**P=0.002**
0-14 days	19 (33)	39 (67)	3 (5)	33 [68]
15-60 days	10 (19)	42 (81)	4 (8)	81 [169]
61-183 days	4 (8)	45 (92)	9 (18)	174 [153]
184-665 days	9 (20)	36 (80)	9 (20)	66 [110]
666-3253 days	10 (17)	49 (83)	14 (24)	145 [357]
No data	60 (27)	158 (72)	22 (10)	47 [79]
Time between first and second dose	P=0.24		P=0.14	**P=0.09**
28-35	24 (23)	80 (77)	11 (11)	64 [119]
36-62	8 (17)	38 (83)	4 (9)	85 [148]
63-147	10 (17)	50 (83)	11 (18)	93 [142]
148-1877	9 (18)	41 (82)	11 (22)	126 [223]
No data	61 (28)	160 (72)	24 (11)	50 [82]
Time between second and third dose	P=0.41		P=0.58	P=0.22
28-32 days	12 (29)	29 (71)	2 (5)	47 [111]
33-61 days	8 (22)	29 (78)	3 (8)	56 [86]
62-128 days	7 (17)	34 (83)	6 (14)	83 [150]
129-235 days	12 (20)	47 (80)	7 (12)	87 [147]
236-2787 days	7 (14)	41 (85)	10 (21)	148 [182]
No data	66 (26)	189 (74)	33 (13)	55 [94]

*** This category is included in the total number of those with anti-HBc levels>10 mIU/ml**

Anti-HBs levels and "time to sampling". The minimum period between the third dose and time of sampling was two months while the maximum was 114 months. Fifty percent of the children were bled 47 months or longer after the third dose and 25% were bled after 65 months.

Figure 5.14. Proportion of people without detectable Anti-HBs by time since the third dose.

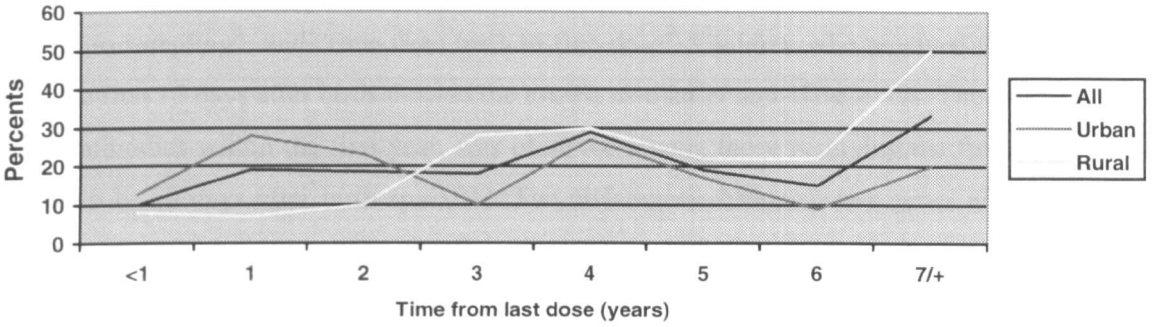


Figure 5.14 shows the proportion of people without detectable surface antibodies. It seems to increase when the time from the last dose increases though no clear pattern is observed because, after a sustained raise, there is a decrease in the proportion of children without antibody at five and six years since the last dose. Differences by area were observed. In the first two years, no change in antibody level is observed for rural areas while in urban areas the proportion of children without antibodies rises from 12% in the first 11 months, to more than 20% during the second and third year. Then, this trend switches from urban to rural areas.

Figure 5.15. Median of AntiHBs titres by time from third dose.

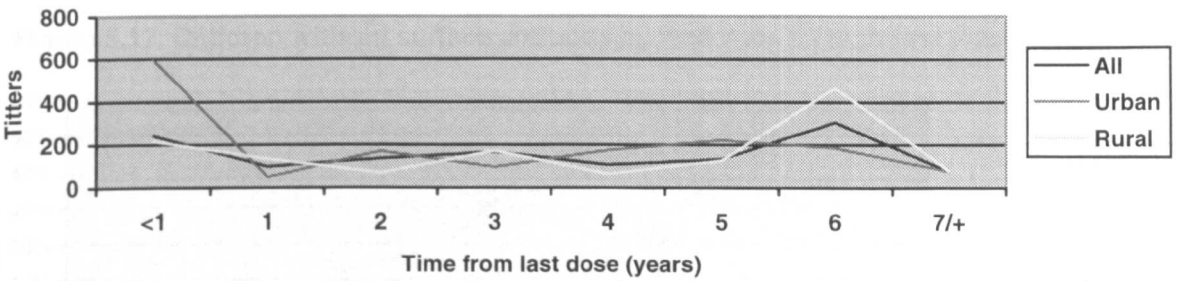


Figure 5.15 shows the median level of antibodies by "time since the third dose of hepatitis" B. In the first eighteen months the median of anti-HBs titres for the whole population reached a peak of 250 IU/ml. Then, a fall is observed and the median of anti-HBc level remained around 180 IU/ml until 7 years, when another fall occurs. The small number of observations (n=9) in this category might be influencing the magnitude of the decrease in anti-HBc levels.. The initial peak is sharper in urban than in rural areas but after eighteen months no important difference was observed.

Anti-HBs levels by "time from birth to first dose" and "time from third dose to sampling". Figure 5.16 shows the distribution of anti-HBs levels (median) by "time since third dose to sampling" and "time from birth to first dose". Children who received the first dose in the first 15 days after birth showed the lowest median of anti-HBc levels. The largest peak of antibodies within the first year, was observed among those receiving the first dose between 15 to 60 days after birth (p=0.01). The differences disappear two years after the third dose, though those receiving the first dose 61 to 180 days from birth showed the highest median anti-HBc level after the 4th year and the largest peak at year 7 (p=0.45).

Figure 5.16. Median of AntiHBs titre by time from birth to first dose.

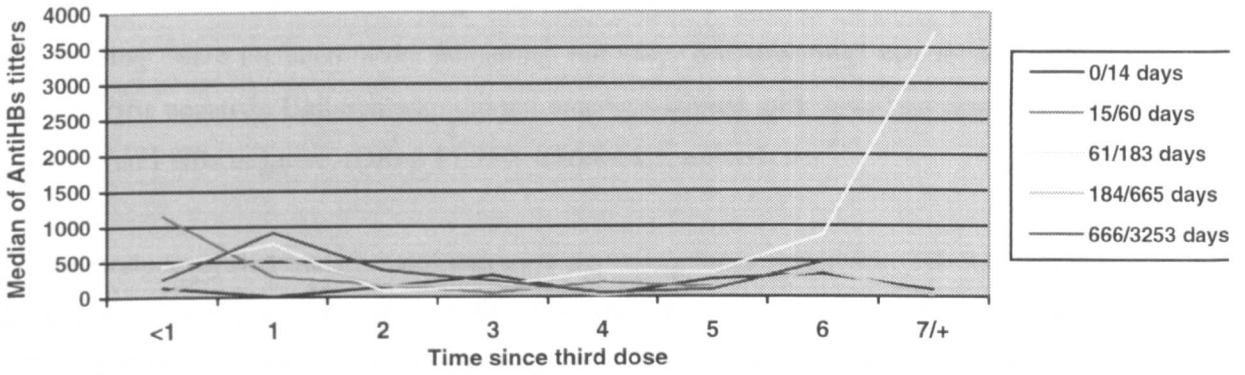


Figure 5.17. Children without surface antibody by time from birth to first dose.

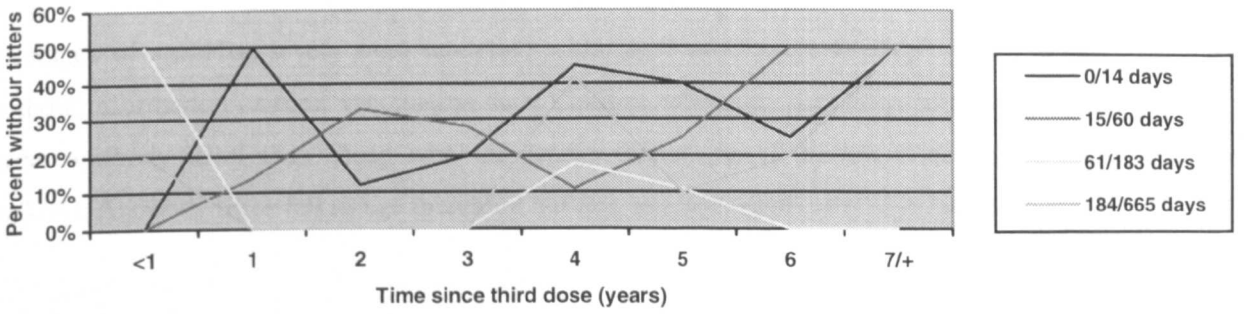


Figure 5.17 shows the percent of people without detectable anti-HBs. Children who received the first dose closer to birth (0/14 days) had the highest proportion without anti-HBc followed by those who received it between 15 to 60 days.

Multivariable analysis. A multivariable analysis, using logistic regression, was used to evaluate factors related to surface antibody sero-negativity. The following independent variables were included: "time since third dose", "time from birth to first dose", "time from first to second dose", and "breastfeeding". Only "time since third dose" and "time from birth to first dose" were related to being negative for anti-HBs. The likelihood of being anti-HBs negative became greater as the time since the third dose increased and the largest ORs were observed at years 4 and 7. On the other hand, children who received the first dose of hepatitis B from day 61 up to day 183 after birth were less likely to be anti-HBs negative. Table 5.19.

Multivariable models were also fitted for children without vaccination data, in order to determine variables related to being anti-HBs negative among them. "Age" and "breastfeeding" were included in the modelling, but only "breastfeeding" was related with being anti-HBs negative. Children who did not receive maternal milk were four times more likely to be anti-HBs negative. (OR=3.97 95% CI 0.8-18.1, p=0.09)

Mean antibody level (log transformed) was modelled using linear regression. The following independent variables were included: "time since third dose", "time from first to second dose", and "breastfeeding". "Time since third dose" and "time from birth to first dose" remained associated with antibody levels. Means were higher among those who received the first dose either, between 61 to 183 days, or after 22 months after birth (666 days or more). Linear models were also run using robust methods, in order to avoid the influence of outliers, but results were similar. Table 5.20

Averages of anti-HBs levels were modelled with "age" and "breastfeeding" for children without information on their vaccination date. Children who were not breastfed had a lower mean antibody level than those who did (mean difference=10 IU/ml 95% CI: 1.0-93, p=0.04). Antibody titres fell as age increased but this was not statistically significant.

Table 5.19. Vaccination characteristics and its relationship with being Anti-HBs negative. Logistic model.

Variable	OR (95% CI)	P
Time since third dose (years)		
<1	1.0	
1	2.0 (0.3-11.6)	0.42
2	1.6 (0.2-10.3)	0.60
3	1.9 (0.3-10.7)	0.45
4	4.1 (0.8-20.9)	0.09
5	2.4 (0.4-13.2)	0.31
6	2.0 (0.3-13.9)	0.49
7/+	5.9 (0.7-49.1)	0.09
Time between birth and first dose		
0-14 days	1.0	
15-60 days	0.6 (0.2-1.5)	0.26
61-183 days	0.18 (0.05-0.6)1	0.005
184-665 days	0.5 (0.17-1.4)	0.18
666-3253 days	0.6 (0.2-1.8)	0.37

Table 5.20. Anti-HBs titres (log transformed) and its relationship with time since third dose and time from birth to first dose. Linear regression model

Variables	β Coefficient (95% CI)	P
Time since third dose (years)		
<1	1.0	
1	-0.48 (-1.1-0.12)	0.12
2	-0.5 (-1.1-0.14)	0.12
3	-0.43 (-1.0-0.16)	0.15
4	-0.7 (-1.3 - -0.15)	0.01
5	-0.4 (-1.0-0.14)	0.13
6	-0.25 (-0.9-0.45)	0.48
7/+	-0.85 (-1.7- 0.03)	0.06
Time between birth and first dose		
0-14 days	1.0	
15-60 days	0.32 (-0.1-0.7)	0.13
61-183 days	0.7 (0.2-1.1)	0.001
184-665 days	0.15 (-0.3-0.6)	0.50
666-3253 days	0.47 (0.01-0.9)	0.04

"Time since third dose" and "time from birth to first dose" were, again, related with having an antibody level ≥ 1000 IU/ml. Interestingly, the association was stronger for years since the third dose than for time from birth to first dose. This finding is opposed to the effects observed when the dependents variables were anti-HBs negative or mean anti-

HBs. Children were less likely to have antibody above 1000 IU/ml at year 1, 2, and 4 after the third dose. On the other hand, children receiving the first dose between 61 and 183 days after birth were almost four times more likely to have an anti-HBs titre ≥1000 IU/ml. Table 5.21

Table 5.21. "Time since third dose" and "time from birth to first dose" and their relationship with having Anti-HBs titres ≥ 1000 IU/ml. Logistic model.

Variable	OR (95% CI)	P
Time since third dose (years)		
<1	1.0	
1	0.2 (0.04-0.98)	0.04
2	0.09 (0.01-0.85)	0.04
3	0.6 (0.16-2.2)	0.43
4	0.2 (0.03-0.77)	0.02
5	0.33 (0.09-1.3)	0.12
6	0.3 (0.07-1.7)	0.19
7/+	0.28 (0.03-3.02)	0.30
Time between birth and first dose		
0-14 days	1.0	
15-60 days	1.3 (0.3-6.6)	0.7
61-183 days	3.9 (0.9-16.2)	0.06
184-665 days	2.3 (0.5-10.9)	0.30
666-3253 days	3.1 (0.7-14.4)	0.14

Chapter 6: Discussion.

This chapter discuss the weaknesses and strengths of this research. It is divided it in two broad parts.-One is about vaccine coverage and factors found to be related to it. The second is on serological findings, vaccine effectiveness, and factors related to with HBV infection among children (including vaccination characteristics). In each part I start by discussing those methodological issues that could influence the interpretation or extrapolation of our results as well as the limitations and strengths of the research. Potential sources of biases, both selection and misclassification, are analysed and discussed. This methodological discussion is followed by an attempt to put the results in an international or national context and to explain the causes and relevance of our findings.

I. Vaccine coverage and related factors:

I.1 Methodological concerns: The objective of this study was to measure the success of the introduction of a new vaccine into the Amazon EPI in terms of coverage. In addition, we attempted to measure those factors that could be influencing vaccine intake.. In order to accomplish these objectives a population survey was carried out followed by a case control analysis.

As with all cross-sectional surveys, a potential weakness in assessing causality is that effect variables and some exposures were measured at a single time point. However, the most important relations found in the study consisted of fixed variables such as HW's knowledge or perceptions and belonging to an ethnic group, which means that the temporal criteria still hold. (Elwood M 1998, page 20)

The potential sources of selection bias in this study are due to sampling, non-response, and differential survival. Non-response to some variables was the most frequent problem. There was no vaccine information available for a large number of children, and this lack of information was related to some of the independent variables assessed. However it is unlikely that this potential source of information bias causes the differences observed. Let's take, for example, the differences shown in table 6.1 and estimate whether lack of vaccination information could have led to these findings. Vaccination coverage differed by 24% between urban Leticia and urban Puerto Nariño (71% vs. 39%), but the difference in the proportion of children without information is 10% (49% vs. 39%). If the difference in information were the cause of the difference in coverage at the lowest level observed, 39%, then only 8% of children without information in Leticia would have to have been already

vaccinated, that is only 61 out of 752 children without information. This is equivalent to 9 times less chance of actually being vaccinated when compared with the coverage among children with a vaccination card. At the other extreme if the true coverage were equal in the two areas at a level of 71%, then 171 children out of 187 without information in Puerto Nariño would have to have been vaccinated. Clearly it is highly implausible that these differences exist between children with and without vaccination records.

Table 6.1. Simulation of changes that should occur in order to vanish the observed differences for full vaccination.

	Urban Leticia	Urban Puerto Nariño
Number of children	1475	307
Number with information	723 (49%)	120 (39%)
Observed coverage (full vaccination)	514 (71%)	47 (39%)
Children that should be vaccinated among those without information to equate coverage at 39%.	61 (8%)	73 (39%)
Children that should be vaccinated among those without information to equate coverage at 71%.	533 (71%)	171 (91%)

We believe that the true prevalence of vaccination coverage in the area of study should lie very close to the values described in chapter 4, despite a proportion of children not having a vaccination card. Some evidence from the study seems to support this. First, we found that when vaccines were considered separately, more than 60% of the children in the census had accurate information on them. Sixty three percent (63%) of the children had written information for hepatitis B, 61% for DPT, and 60% for measles and yellow fever. The proportion of children with information was even higher for children under five years old (73-76%). This proportion of children with accurate information on vaccine status is higher than that found in other studies on vaccination coverage in South America, and other developing countries, where this proportion barely reached 50%(Cassio de Moraes et al 2000, Cutts F 1989, Cutts F 1990, Da Silva L 1997.). As we have seen in table 6.1, children without information should have extreme differences in vaccination coverage compared to children with an information card, in order to change the overall coverage in a significant way. Second, serological results did not support the idea that children with and without information on hepatitis B vaccination have significant differences in vaccination coverage. Children without information on hepatitis B did not have a higher prevalence of Anti-HBc or HBsAg than children with information. HBsAg and Anti-HBc prevalence were 1.2% and 5%

respectively among vaccinated children while they were 1.1% and 6% among those without information and these differences did not change even after controlling for mothers' infection status or children's age.

Some potential sources of information bias have been avoided in this study. "Recall bias" is not possible since people who answered the questionnaire were unaware of their children vaccination status. "Interviewer bias" is also unlikely since interviewers did not know the criteria to classify a child as fully vaccinated.

The likelihood of misclassification of vaccination status cannot be completely ruled out. An important proportion of the children (around 20%) did not have dates of vaccination in their vaccination cards, only the number of doses they had received. Some others (3-12%) had dates that we could not use because we were unable to interpret whether the numbers stood for months or days. This bias would tend to decrease the true magnitude of an association and therefore would not negate positive findings. On the other hand, some variables related to vaccination found in other studies may not have been found in this study due to misclassification. (Rothman & Greenland 1998; Rothman 1986; Kristensen et al 2000; Elwood M 1998)

Some factors found in other studies to be vaccine coverage predictors did not show an effect in this study. Mother's years of schooling is one of the most important. The possible reasons for not having found an association in this study include non-differential information bias; where exposure is not adequately measured leading to exposed and non-exposed participants being confused. (Rothman & Greenland, 1998). Information on number of years of schooling was collected in oral interviews with mothers, and it may have been difficult to recall the exact number of years that they spent studying, especially amongst the older ones. Unfortunately, we had no way of measuring the quality of this information during the field survey. Another potential explanation could be that the level of variation in education was less than in other studies. Actually this may be one of the plausible causes since most mothers in our study (67%), were classified in only one category of analysis, one or more years of primary level Other studies in Latin America have found that low maternal education level is related to lower coverage in their children, but as a weak association (Moura da Silva 1999). Kutty has pointed out that maternal education is not a predictor of child vaccination when the program is conducted in a proactive way. (Kutty V 1989)

I.2. Importance of the results: This is the first field evaluation of the process and impact of a hepatitis B vaccine in the EPI in an endemic area in Latin America. By 2000, hepatitis B

vaccine had been introduced in most Latin American countries, but using different vaccination policies. Cuba, Colombia and Brazil were the first countries in the region to introduce universal child hepatitis B vaccination in the early 90's. Others have introduced the vaccine more recently, but vaccination is limited to high endemic areas. In a thorough search of the most important medical literature data bases we were unable to identify similar population-based studies on hepatitis B vaccine evaluation. (Tambini et al, 1998; Slusarsky & Magdzikw, 2000; Cabezas C et al 2000; Cabezas C et al 1995).

One of the strength of the present study was that not only hepatitis B vaccination was analysed, but also full vaccination coverage, and reasons for incomplete coverage. These factors were studied at two levels, individual and ecological. In the latter category, the variables of interest were related to health workers knowledge and attitudes. This is not a common approach in the literature where most studies on vaccination and coverage have focused mostly on individual factors such as socio-economic differences or on maternal factors.

Results on coverage are encouraging since a new vaccine has reached a high coverage similar to others with similar schedules such as DPT. This is especially remarkable because this is an area where geographical and logistic barriers can easily hamper the efforts of health services to provide vaccination to the population living in the forest. According to health workers' perception, hepatitis B is considered an important public health risk and this has played an important role in attaining this high coverage.

Despite the high coverage found for hepatitis B and other vaccines it is important to highlight the lack of adherence to the Ministry of Health schedule. The time interval between hepatitis B doses is longer than recommended which appears to influence vaccine effectiveness. As we will see later, these delays had many causes and some of them could be corrected by direct action of the health authorities. This delay in completing the schedule is more frequent in rural areas where resources for vaccine storage and health worker transportation are scarce. In these areas no public system of transportation is available and even villages close to Leticia can be difficult to reach and, what is even more important for health workers, to leave. Therefore, vaccination activities in remotes areas can only be started when a round trip is ensured for the HW.

Our results will help health authorities to become aware of the characteristics that vaccination activities have in remote and/or isolated areas. First, there were differences between the vaccine coverage reported to the central level by the Health Secretary (HS) and

those actually found in the field. For hepatitis B, the coverage found was slightly higher than the one reported by the Health Secretary. According to this last source, children born in 1995 reached 74% coverage with three hepatitis B doses, in 1996 it was 79%, in 1997, 88%, and in 1998, 59%. For the same years, we found 93%, 91%, 91% and 79% coverage. One reason for these differences could be the size of denominators used by the HS in calculating coverage, which might be inflated since regional authorities use them to demand resources from the central level. The two sources agree, however, in the fact that coverage with other vaccines is slightly higher than hepatitis B. (PAI-MINSALUD 2001)

Another reason for the differences in 1998 could be the changes in the national health care organisation. According to the central EPI office, vaccine coverage seems to be decreasing around the country since 1997, after a health care reform was implemented in Colombia (PAI-MINSALUD 2001). This reform consisted in allowing private enterprises to have an active role in health care, health promotion and prevention while the State paid them a fixed amount of money for every person reported to be covered by them. This has been followed by governmental attempts to reduce bureaucracy in the public health sector which has often left regional health services without enough personnel to carry out their monitoring and supervision work adequately.

Official vaccine coverage in Colombia has decreased by 30 to 50% for polio, hepatitis B and DPT from 1994 to 1999. Hepatitis B vaccine has shown one of the sharpest decreases, from 95% in 1996 to about 50% in 1999. This fall may not be completely true but influenced by loss of information at the local level since new actors, other than state-run health centres, are delivering vaccines and some of them have not received adequate training in how to report vaccination activities. In fact, the decrease in HB coverage observed in our study was less, in percentage terms, than that reported by the HS (14% in our study, 93% to 79%; and 29% in the HS, 88% to 59%). Other studies from developing countries showed that the quality of routine information could be influenced by changes in procedures or by lack of supervision and monitoring at the local level, though in most reported cases coverage overestimation is the main concern (Onta SR et al 1998; Streefland P 1995 page 49; da Silva et al 1997)

I.3. Factors related to coverage: The study found that the proportion of fully vaccinated children is lower than the coverage reached by individual vaccines, and it is especially low among children under two years, which coincides with other vaccine coverage evaluations in South America (Moura da Silva 1999). This suggests that children in the Amazon did not complete the basic scheme of immunisation in their first year of life, but in their second or even in their third year, especially in rural areas. A fact that is confirmed when the length of

time to complete hepatitis B scheme is analysed. Fifty percent of children in urban areas completed hepatitis B vaccination before six months, but in rural areas fifty percent of children completed this vaccine over 10 months after birth and it is worse in children older than 5 years. This lag is also observed for yellow fever vaccine since most children received this vaccine in their third year of life.

Different factors underlie delays in completing the basic scheme, among them the lack of cold chain for vaccine storage and the calendar of agricultural activities in these communities. When harvest time arrives, villagers leave their houses and stay several weeks or months in the jungle, away from public health services. So, if a vaccination team arrives at the village while people are away, children must wait for several months to receive vaccination doses. This delay to complete schedules has been observed even in urban areas in South America. Cassio da Moraes carried out a vaccination survey in Sao Paulo and he found that the basic DPT scheme was not completed in the first year for all children. Between 15% and 50% of children in that study completed the first three doses of DPT during the second year of life. (Cassio da Moraes J 2000)

Individual features related to not being vaccinated were age, area where children lived, ethnic group, being affiliated to the social security and some socio-economic characteristics. For health services evaluation the finding that not being affiliated to the social security is related to less coverage is important. In Colombia no evaluation has been carried out on the impact that the health reform has had on health care and prevention programs. It is interesting that the main effect of this variable has been found in rural areas where vaccination is provided by public health services alone. The explanation for this association is not that people without a security social card are rejected from vaccination centres, but rather that people without this document tend to exclude themselves and their children from the vaccination service in the belief that health workers might reject them. The concept of wide social security coverage has been recently introduced in Colombia and its significance may not yet be well understood by people, especially among those with low levels of education or living in isolated areas where information on people's rights is scarce. This finding is usual in developed countries like the USA, where private health care system are predominant, but for us it is new. This relationship has been less studied in developing countries where social security is weaker. For example, in a recent study in Brazil, no differences were found in vaccine coverage by social security status (Moura da Silva 1999)

Other variables closely related to socio-economic disadvantage were found to be associated with not being vaccinated. Living in a house with palm tree leaf roof was associated with

lower full vaccination which is a reflection of socio-economic differences since the poorest people in urban and rural areas tend to live in houses with roofs made of this material, which is considerably cheaper and easier to find than tile or corrugated. Economic and educational differences are commonly reported as associated with low vaccination coverage. This might be due not only to discriminatory programs but also to differences in the way that the more educated people look for vaccination services. It has been found that the poorest and less educated people have a passive acceptance of vaccination activities while active demand for vaccination is a more common attitude among those with a higher educational level.

Another important individual variable was the place where children live. Living in urban Puerto Nariño was associated with low coverage for hepatitis B and other vaccines. This is shocking because Puerto Nariño is one of the few places in the Amazon which have conditions to store vaccines; in fact, enough doses of all vaccines were found stored in the hospital of that town at the time of the study. Therefore, we believe that factors related to health workers' attitudes or knowledge is probably more important than logistic constraints to explain this low coverage. In fact, nurses who have been appointed only for a one year term carry out vaccination in the hospital and have little experience as they are newly graduated. At the time of the survey, the nurse in charge had been less than a year in the hospital and her knowledge of vaccines was deficient to say the least. She mentioned four false contraindications for hepatitis B vaccination: children with fever above 38 degrees, children born prematurely, malnourished children, and antecedents of hospitalisation. For polio and DPT she also mentioned false contraindications such as fever above 38 degrees, febrile or non febrile convulsion and previous reaction to the vaccine. Regarding attitudes toward the job, she felt that there was little time for vaccination activities and more personnel were needed. All these HW´s characteristics could lead to low vaccination among the population cared for by such a HW.

Some indigenous groups in Mexico have showed low vaccination coverage explained by health worker's hostile attitudes towards traditional health beliefs and knowledge in these communities. Such attitudes may raise a barrier between health providers and communities that could lead to a decrease in the demand for health services including vaccination. More educated health workers, especially those with little experience in community work with ethnic groups, might show more hostility to these traditional beliefs among indigenous communities, which could well be the case in Puerto Nariño. (Nigenda-López G et al, 1997)

There were differences by ethnic group that could be only partially explained since we did not collect information about knowledge or attitude towards vaccination from mothers, and

these are the main factors that might help us to explain the reason for this difference. Differential exclusion of people belonging to a specific ethnic group is not plausible because in this region there are no marked social differences between them as is the case in other developing countries such as India where the caste system interferes with accessibility to vaccination (Streefland P et al 1999).

Ticunas had the lowest coverage when full vaccination was analysed for urban areas. The existence of more barriers in urban areas to vaccination, e.g. command of Spanish, is a potential explanation, though "Huitotos" and "Others Indians groups" had a greater coverage in urban areas. Therefore, it is plausible that maternal beliefs in vaccine's good or bad effects contributed to this difference. Another factor that can help to explain differences in vaccination coverage by ethnic groups is education level that could be regarded as a proxy for mother's knowledge or beliefs on vaccination. Only 12% of Ticunas' mothers had more than 5 years of education while among Huitotos mothers it was 19% and among "Other groups", 18%. This proportion was even higher among non-Indians and Mestizos (62% and 70% respectively). It is plausible that the lower the mother's education level the higher the chance that she has incorrect knowledge or beliefs about vaccination.

Less individual variables were related to hepatitis B compared to full vaccination. An explanation for this phenomenon is that people and health workers identify hepatitis B as an important menace since the fulminant hepatitis clinical picture is impressive and it frequently produce familial cluster of deaths and disease. Another potential explanation is that hepatitis B vaccine has been more available than other vaccines in this region since the Ministry of Health identified hepatitis B as a priority in these areas. Other vaccines recently introduced in the Colombian EPI have not reached as good coverage as hepatitis B. *Haemophilus influenzae*, for example, has reached coverage of no more than 50% in the first two years after introduction (Agudelo C et al, 2000; Higuera B et al 2001; PAI-MINSALUD 2000).

"Place where children live" is related to hepatitis B in a similar way as to full vaccination. Puerto Nariño had the lowest coverage but the highest is observed in Araracuara. In this town there are people still affected by chronic hepatitis and cirrhosis that must be periodically evacuated for treatment, and fulminant hepatitis had the highest occurrence in the region. Therefore people are aware of the severity of the disease and the need to have children vaccinated. In a recent study about factors related to hepatitis B vaccination in adolescents, those who believed that hepatitis B was a severe disease with no easy treatment had a higher chance of completing the hepatitis B schedule (O'Rourke 2001).

Among the variables associated with socio-economic disadvantage, roof material showed the strongest relation to hepatitis B vaccination. Other socio-economic factors had weaker associations confirming that hepatitis B vaccine has been widely available in the Amazon. The fact that being affiliated to the social security is not related to hepatitis B vaccine is another reason to suggest that hepatitis B vaccine has been delivered in a fairer way or that people feel that this vaccine is more important that others.

Another individual characteristic related to hepatitis B was size of family that, on the other hand, is not related to being fully vaccinated. We did not collect evidence that could help to explain this relation but it could be strongly related to poverty and probably to mothers' lack of time to attend vaccination activities. Similar findings have been reported in Brazil where living in a house with more than three children reduced vaccine coverage by 20 to 30%, though that difference disappeared in the multivariable analysis (Moura da Silva 1999)

Gender has been found associated with coverage in other developing countries, but it was not so in our study. Other studies in Latin America have not found differences in vaccine coverage between males and females. On the other hand, studies from India showed that boys tended to be more vaccinated than girls, especially in some rural areas (Moura da Silva A, 1999; Cassio da Moraes J, 2000; Greenough P, 1995; Streefland P, 1995)

I.4. Health workers' (HW) knowledge and perceptions and their relationship to vaccination coverage: Knowledge of vaccines and perceptions about barriers to vaccination were measured among health workers who were in charge of the vaccination activities in the area under study. Hepatitis B was the vaccine second most commonly mentioned spontaneously by health workers, thus confirming our previous statement about the high degree of awareness among health workers in the area of the importance of hepatitis B as a public health problem. On the other hand, it is clear that other recently introduced vaccines such as *Haemophilus influenzae* or meningococal vaccines are hardly recalled by health workers since only one mentioned them. Regarding general knowledge on vaccines, only half of the health workers were able to respond correctly to a simple question about the age when children should complete the basic scheme of vaccination, while almost the same contraindications were identified for several different vaccines such as polio, hepatitis B and DPT.

Another deficiency in knowledge was detected in regard to hepatitis B vaccine. Most HW identified the buttock as the place for hepatitis vaccine application and this explain the lower antibody titres observed among children studied for anti-HBs. A stronger concern arises

when contraindications for hepatitis B vaccine are examined. Fever, diarrhoea, and malnutrition were identified as contraindications against hepatitis B and this might partially explain why some children are not completely vaccinated as is demonstrated when further analysis is conducted on the relation of this variable and vaccine coverage. Among Amazon children these three conditions have a high incidence especially in rural areas; therefore false contraindications could also contribute to longer periods between doses and to delays in completing the schedule.

Health worker's perceptions and knowledge influenced level of vaccination coverage. Perceptions of why children were not vaccinated explained low full vaccination as well as low hepatitis B vaccination, but the association was stronger with hepatitis B vaccination. A good example is parental fear of vaccine side effects. Children living in communities where HW perceived this as a barrier had about three times less chance of being fully vaccinated, while for hepatitis B the decrease in that probability was 11 times. In this study there was no survey on parental knowledge or attitude to vaccines and that lowered our ability to evaluate if this perception was justified or not. The correlation between this perception and other HW characteristics was assessed in order to try to better explain the relation. More contraindications against polio and hepatitis B were mentioned by the HW who perceived parental fear, but at the same time they had more correct answers on hepatitis B and other vaccines. Mothers had more years of schooling in areas where parental fear was perceived (median 5 years vs. 3 years) and poverty indicators were lower (47% owning a freezer vs. 7% and 6% having a palm's made roof vs. 16%). These findings stress the need to provide more information on vaccines and side effects to these communities.

Parental fear of side effects should be understood under the premise that people from different areas could assign different values to the act of vaccination based on previous experiences that include perception of health worker efficiency and honesty (Greenough P 1995). Those HW who perceived parental fear might be at the same time identified by the community as inefficient or as having bad vaccination practices. Paying more attention to mobilisation of political will than to public attention when introducing immunisation campaigns may be an additional explanation to the low vaccination coverage reached in those areas where parental fear is prevalent. In most developing countries, for health services vaccination only means reaching a target number of doses applied and this is particularly critical at local level. Prevention or public health notions are not as important as the number of doses delivered. It is well known that vaccines are not free from side effects, and in the case of DPT or BCG parents are less likely to return to complete schedules when their children have been affected by one of their collateral problems (Wright P 1995; Nichter M

1995; Streefland P 1995 page 648). Other perceptions related to coverage, such as perceiving that hepatitis B was a serious disease, were found as well.

Parental vaccination acceptance could be influenced by issues such as parent's occupation, time available to attend a vaccination session, distance that should be covered and questions that should be answered during the session. In the Amazon, mothers have to work in farming activities which take them between 5 to 6 hours daily. This makes it difficult for them to take children to the health centre except when a vaccination date is stipulated in advance or when vaccination is delivered every day and all through an entire day.

Streefland et al stress that there are three modes of non-acceptance of vaccination. In the first, *mothers are willing to go but unable to do so.* In our conditions heavy workload and long distances are important barriers for mother's attendance at vaccination activities. People living in Amazon rural areas need to harvest their agricultural products at different periods of the year. This kind of activity usually involves moving all the family some hours away into the jungle in order to increase the manpower in the field. Moving has been related to low or incomplete vaccination coverage even in developed countries (Findley S et al, 1999)

In the second mode, *mothers refuse to go*, it has been pointed out that malfunctioning or inadequacy of the vaccination services could be the leading reason for this rejection. We did not measure the proportion of mothers rejecting vaccination nor the reasons underlying that rejection. However, there were some health workers who perceived parental rejection of vaccination activities in their communities and in some stages of the analysis this variable looked an important predictor of lower coverage for full vaccination.

Another mode of non-acceptance is *questions about the need for vaccination.* This collective non-acceptance may go beyond the sum of individual refusals and become organised resistance. Religious objections, doubts about the role that the state should play in the control of individual risks and even conspiracy-type theories have been involved as causes for this mode of non acceptance. The latter aspect, we believed, is the most relevant among populations with low levels of education. Nichter (1995) mentions that in India and the Philippines people strongly believed that Tetanus Toxoid (TT) was being used in women for family planning purposes. This does not seem to be the case in the Amazon, at least in the areas where the survey was done, since most people seem to trust in vaccine's efficacy. Furthermore, mothers welcome vaccination activities with great enthusiasm in remote villages.

False contraindications were strongly correlated to low vaccination coverage for both full vaccination and hepatitis B vaccines. The number of contraindications against polio was related to the chance of not being fully vaccinated while contraindications mentioned for hepatitis B vaccine were specifically related to lower coverage for this vaccine. This finding is important because it reinforces the point that our questionnaire was able to discriminate specific associations related to HW´s knowledge. The lack of a continuous education process would be the most probable cause of this failure and periodic training should help to improve vaccination coverage in some areas. Some authors have called attention to this aspect emphasizing that educating health workers on contraindications would not necessarily guarantee higher vaccination coverage. In health sectors, especially in rural areas, responsibility for death is avoided at all costs. So, health workers cannot be expected to vaccinate ill children if they are accountable for children's health by a community that deems vaccinating during illness a sign of disregard. (Nichter M 1995)

Time working in the health centre was also related to vaccination coverage, the longer the time the higher the coverage. At this point, it is important to note that health promoters have stayed for longer periods in their communities than professional nurses and this could be one reason underlying differences in coverage. Professional nurses are requested by law to spend a year working in rural communities which is seen by some of them as hard and boring duty. Therefore, they may be less tolerant to the community's perceptions about need or risk of vaccination and so be ruder towards mothers which would lead to rejection or to low quality of family care. Longer time working in the same community could be related to a sense of trust in the competence of health providers. Giddens (cited in Streefland et al, 1999) defines trust as "confidence in the reliability of a person or system, regarding a given set of outcomes or events, where that confidence expresses a faith in the probity or love of another, or in the correctness of abstract principles (technical knowledge)". Health workers in small villages usually live in the same health centre and share every day events with the community, so it is plausible that the longer the time living in the community the stronger the confidence and trust the people will put in them.

Curiously, when HW were asked to identify barriers against vaccination they did not identify any that could be related to their performance as health providers in their communities. The most important barriers identified by them were those related to parents attitudes towards vaccination that, on the other hand, should not be perceived as important if continuous education activities were provided by them to the community. Continuous health education in the communities is one of the most important roles for HW at the local level. However, sometimes they forget this and see themselves as providers of clinical services only. In most

of the village's health centres inspected by us there was no public information displayed on vaccine activities. This could be due to the high proportion of illiterate mothers in rural communities, but also to lack of interest of the health worker. Whether it could be argued that not necessarily providing more information to mothers could lead to higher coverage it is true that better informed parents could seek for vaccination activities more actively.

In a review of the literature on vaccination and reasons for low coverage, these were analysed separately for developed and developing countries. The authors reviewed 42 articles, 16 from developing and 12 from developed countries. Three factors they thought affected immunisation coverage: immunisation policy, psychological aspects, and **the role of health workers as providers of information for the community**. Common people in developing and developed countries could share false beliefs and myths about vaccine efficacy and vaccination risks. People in developed countries have more access to vaccine information than in developing countries, and many vaccine rejections have been made by parents based on information about vaccine safety published in journals, newspapers or given by health providers. In 1984, an editorial in the Lancet blamed for low measles vaccine coverage the attitudes of physicians who do not provide enough information to parents about vaccination benefits. Regarding psychological aspects they remarked that parent's fear of vaccine side effects and lack of motivation were important barriers to reaching high immunisation coverage. (Nigenda-López G et al 1997).

Logistic barriers were not identified spontaneously as a problem. This is probably due to the fact that most HW in rural areas are used to working in these areas and probably believe that not having vaccines in the health centre or travelling long distances in order to vaccinate children is natural. However, a question asking for a specific logistical barrier, "children rejected due to lack of supplies", was related to not being fully vaccinated. Teams based in Leticia or Puerto Nariño periodically go out to the river and stay for several weeks visiting every village, vaccinating and carrying out other medical activities along the Amazon River. These trips, so called "correrias", should be done three times a year, but they are extremely costly and delays in the schedule are common. Lack of cold chain is the main reason for delivering vaccines in rural areas using "correrias"; however, it seems that other approaches could be cheaper and more effective to keep good vaccination levels in rural populations than this outreach vaccination strategy. Providing rural health centres with gas or petroleum refrigerators appears to be a more comprehensive strategy for adequate vaccination activities in these hard to reach areas.

Viability of vaccination programs is determined by coverage, quality of vaccination service delivery, acceptance of vaccination and the way it is provided, and prospects of long term sustainability. In most countries, under WHO and UNICEF pressure, emphasis in national vaccination programs is put on reaching high coverage, developing logistic arrangements, and management procedures. But once the "take off "period is over, in what form and under what conditions can vaccination programs be expected to continue? A possibility in developing countries is that vaccination programs stagnate after starting due to political or economic constraints on resources (Streefland P 1995 page: 647). Until now the Colombian government has maintained the political will to buy hepatitis B vaccines partly because Cuban manufacturers have been able to keep prices at a low level affordable to us. Financial and managerial restrictions are seen as a threat for further immunisation developments in Colombia. These restrictions emerge mainly from the fact that our external debt has grown sharply in the last 8-10 years, thus worsening a trend to reduce State expenditure. Political instability and health workers security can also contribute to low coverage in some areas of the country. Another threat to vaccination program sustainability comes from changes in the parents' perception of the severity of vaccine preventable diseases. It is possible that after some more years of continuous vaccination and after a large reduction in the number of cases of fulminant hepatitis, parental perception of hepatitis B related diseases could change and acceptance of this vaccine could decrease. But it has not been seen so far, despite the fact that there have been no cases of fulminant hepatitis in the last two years in these communities (Spier R. 1999; Greenough P 1995). A technological advance that could help to maintain hepatitis B vaccine sustainability is the introduction of combined vaccines. Colombia is about to add to the EPI schedule a combined vaccine containing hepatitis B, *Haemophilus influenzae* and DPT. A drastic reduction in the number of injections needed to immunise a child might increase parental support of vaccination. (André F, 2001; Nolan T et al 2001; Kurstak E 2001)

The way people look for immunisation could also be a good predictor of vaccination sustainability. Nichter made a distinction between active demand and passive demand for vaccination. Active means that the public is informed about benefits and need for specific vaccination. Passive acceptance denotes compliance, the public yields to the recommendations and social pressure, if not prodding, of health workers and community leaders (Nichter M 1995). The role of the HW is prominent in promoting active demand. Education activities could enhance mothers' knowledge of vaccines leading them to seek more actively for vaccination for their children and decreasing their anxiety about side effects. It is probable that most of the fears about vaccine side effects found in the communities in this study came from more educated mothers, as has been pointed out before.

Resistance to vaccination activities may be an important barrier to achieving adequate coverage. This resistance may arise from different sources; one from the public and also from health workers themselves. How and why vaccination acceptance becomes a prevailing pattern in a community is the result of various factors. In rural Amazon the same staff in the health centres are involved in curative and preventive health care and in most villages under study, contact between primary health workers and patients usually includes a range of activities which lead communities to perceive health workers as important members of the community. On the other hand, delays in the schedule of "correrias" and, therefore, delays in completing the vaccination schedule may lead communities to reject vaccination or to become resistant to these activities. In Ethiopia's rural areas it has been documented that late arrival at villages due to transport difficulties for vaccination teams have led to communities' rejection of vaccination activities. This is reinforced by the HWs attitude of being in a hurry and by poor vaccination practices when HWs are under time pressure in remote areas. (Streefland P et al, 1999).

It has been reported that when national or regional health measures are introduced from above, resistance among health workers may be induced. This is especially true if high rank health officers use intimidation and/or coercion against local HWs in order to increase vaccination coverage. This can lead to HWs negative attitudes and low motivation for vaccination activities that jeopardises the vaccination program's sustainability. (Greenough P, 1995 page 633). While health workers interviewed in our study showed some deficiencies in their knowledge and perception about vaccination it is clear to me that most of them are deeply identified with their role as health providers in these communities and with the success of vaccination activities if they have adequate logistic support. This would assure that the sustainability of the vaccination program will not be hampered by health workers' resistance or by communication barriers between local people and the HW.

It is very encouraging to find that more than 90% of children had received BCG vaccine in such poor areas. Besides the benefits that BCG has in counteracting tuberculosis infection, it has been suggested in recent studies that children receiving BCG have a reduced chance of dying in the first six months of life (Kristensen 2000; Fine 2000).

II. Serological results and vaccine effectiveness:

II.1. Methodological issues: Selection bias could arise in studies where the population is requested to donate blood samples. It is not an uncommon feeling among ethnic groups in the Amazon that these samples are used for copying genetic characteristics of their populations. The field team was asked by old Huitoto leaders in some communities if one objective of drawing blood samples was cloning them. Another potential source of selection bias in community-based surveys is self-selection bias, where people who referred themselves to participate are different from those who are theoretically eligible for the study. This was avoided in our study because participants were engaged in the study by random cluster selection followed by a house to house search in selected clusters only and because those people who had survived a fulminant hepatitis episode and who referred themselves to participate, were bled but their results were excluded from the analysis.

We found that 35% of all non-infected children lacked vaccination data but 52% of the HBsAg+/Anti-HBc+ did not have vaccination card as well. . This differential proportion in missing data might be seen as a potential source of selection bias, but as Schlesselman has pointed out, different proportions of missing data between cases and controls does not introduce selection bias in itself. If the exposure proportion were equal between cases with and without data and the same is true for controls then, the OR based only on the respondents would be equal to the OR in the entire population. We try to test this fact indirectly using the distribution of another important risk factor, mothers' Anti-HBc status. The prevalence of this variable across the levels of cases and controls, with and without data, is showed in table 6.2.

Table 6.2. Proportion of Anti-HBc+ mothers by cases and controls and across levels of vaccine information. Rural areas.

Cases	**Mother Anti-HBc+**
Cases with vaccination data	10/10 (100%)
Cases without vaccination data	11/12 (92%)
Controls	
Controls with vaccination data	345/500 (69%)
Controls without vaccination data	184/287 (64%)

We can see that the distribution of the exposure to Anti-HBc+ mothers is similar between cases with and without data, and between controls with and without data. This suggests that

lack of information on vaccine status is not an important source of selection bias in our study and that results on children with information can be extrapolated to the whole population from which they were selected. (Schlesselman J 1985 page 132)

Comparing infection data from time periods before and after vaccination may be a subject for concern because different study methods and laboratories procedures were used. The serological survey made by Cristancho before vaccination started was done by gathering people in health centres and taking samples from all who agreed to participate in the study. Therefore, selection bias was more probable in her study. However, public awareness about hepatitis B infection in these communities was high at the time of Cristancho's study and this was the first time that a vaccine was offered, so high rates of participation were likely. Less validity should be assigned to Cristancho's results in Puerto Nariño because she recognised in her report that due to logistic problems, the response of the community was lower than expected, and few children were included in the sample. On the other hand, our survey was done house to house and we tried to motivate reluctant people to participate. In fact, the proportion of people who refused to participate was very low - less than 5% of the target population, and the main reason for not being included in the study was absence from the village due to farming or fishing. This high rate of participation is certainly due to the level of awareness about hepatitis B in these communities.

Potential concerns in the interpretation of the results arise from the type of the study itself. Causal relationship may be hard to assess for some factors when a cross-sectional survey design is used. Mothers' anti-HBc status is one such factor where the causal relationship may be difficult to interpret, because both the factor and the outcome were measured at the same time. Theoretically, it is not possible for us to determine accurately if mother infection actually preceded children's infection especially in urban areas, where prevalence is low, or in the youngest mothers. However, for this relation other causal criteria hold and we can mention: strength of the association, consistency, plausibility, and coherence. (Rothman K and Greenland S 1998 pages 24-28; Mahoney F 1999 pages 360-62; Hilleman M 2001). Other variables, namely time from birth to first dose or time between doses, filled the temporality criteria more clearly because time is a fixed variable and, when controlling by age, we are controlling for age at infection. In table 5.9 we can observe that HBsAg+ children probably received the first two doses of hepatitis B after becoming infected. For example, one child was HBsAg+ in the age group 1-3 years and he received the first dose more than 6 months after birth, while the second dose was given to him 6 months after the first one. This meant that he completed two doses of hepatitis B, which had a protective effect of 70-80%, when he was one year old well after the intended EPI schedule.

Furthermore, this child received the third dose 9 months after the second one, i.e. 21 months after birth. During such a long interval, a child living in rural areas and born from an infected mother has many opportunities to be in contact with HBV and to become a carrier.

"Recall bias" or "interviewer bias" were avoided by us because both the interviewed and interviewer were blind regarded case status. Case and controls were classified after the laboratory results were obtained, and this happened after the field data collection. Additionally, exposure to vaccine was confirmed only when a vaccination card was available. Other potential source for misclassification bias was the absence of information on vaccine status in a large proportion of the target population. In fact, we assessed the direction of this potential problem by collecting blood samples on children without vaccine information and processing it for HBV markers. It seems as if most children without information were actually vaccinated as they had a similar risk of hepatitis B infection than children with vaccination card and furthermore, they had similar Anti-HBs levels than children with vaccination card (70% had levels >10 IU/ml).

Another source of concern was the study's lack of power to detect associations that have been identified as important in other studies. The most relevant was the association between HBsAg mothers' status and their children's HBV infection. Only 3% of the HBV negative children were exposed to that risk factor and it yielded statistical power of only 30%. This means that we had a higher probability, ≅ 70%, of overlooking an association that truly existed. The same is true for evaluating the role of being born to an HBeAg positive mother. Few children were found to be born to such a mothers and lack of power limited our ability to find a statistical relation. It should be born in mind that in a cross sectional study it might be hard to assess the relationship between surface antigen, e antigen, and child infection because those markers may have been present when children were born but not at the time when the study is carried out, several years later. In Alaska, it has been found that up to 70% of people HBeAg + can lose this marker after 6 to 10 years of follow up (Harpaz R et al 2000). Table 6.3 shows that prevalence of HBsAg varies across age groups in mothers from the Amazon. There are two peaks of HBsAg prevalence, at age groups 20 to 24 years and 35 to 39 years, but, at mid age groups or in the older, prevalence decreases. This reinforces the idea about cross sectional studies being a weak tool to assess the role of HBsAg+ mothers when evaluating vaccine effectiveness especially in areas where horizontal, and not perinatal transmission, is the main source of infection for children.

Table 6.3. Distribution of hepatitis B infection and HBsAg carriage by age, among mothers.

Age groups	Number tested. Total population. (% Anti-HBc+)	Number tested. Rural. (% Anti-HBc+)	Number tested. Total population. (% HBsAg+)	Number tested. Rural. (% HBsAg+)	Number tested. Rural area. (# HBeAg+)
15-19 y	49 (33)	29 (52)	49 (0)	29 (0)	-
20-24 y	228 (38)	114 (53)	229 (3.9)	115 (7.0)	4 (2)
25-29 y	285 (36)	116 (60)	287 (3.5)	115 (4.3)	5 (2)
30-34 y	254 (47)	109 (72)	254 (3.9)	109 (3.7)	3 (2)
35-39 y	190 (47)	91 (76)	190 (5.3)	90 (9.0)	5 (4)
40-44 y	103 (51)	33 (70)	103 (2.9)	33 (3.0)	1 (1)
45-59 y	65 (75)	40 (90)	65 (3.0)	40 (5.0)	-

Case misclassification is another potential source of concern for studies that rely on serological tests. When factors associated with HBsAg prevalence were analysed, HBsAg+ cases were restricted to those who were HBsAg+/anti-HBc+, and those who were positive for HBsAg only were not included in further analysis. This allowed us to decrease the probability of including false HBsAg+ positives as cases which would lead to an underestimation in the magnitude of the association. In fact an assessment was done to evaluate the potential effect of including children HBsAg+/anti-HBc- among the cases. The absorbance ratio, observed absorbance/cut-off point, was compared between those samples HBsAg+/anti-HBc+ and HBsAg+/anti-HBc-. The median of the absorbance ratio in the first groups was 42 while it was 4.7 in the second group. The 25th percentile was 26 in the first group and 2.5 in the second while the 75th percentile in the first group was 66 and 20 in the second. These findings confirmed that many samples HBsAg+/Anti-HBc- were false positives and, therefore, should have been excluded from further analysis, as we did. On the other hand, this approach could decrease the sensitivity of the case definition but it has been demonstrated that a lower sensitivity would not bias the RR if the non-detected cases are not included in the denominator. Therefore, children HBsAg+/Anti-HBc- were exclude from denominators as well (Rothman and Greenland 1998, pages 128-132).

II.2. Hepatitis B infection and vaccine effectiveness: Compared with results from studies done before HBV vaccine introduction in the region the prevalence of hepatitis B infection and HBsAg positivity has decreased. Both markers have fallen between 60% to 80% depending on the age group and gender. In five to nine-years old children the reduction is greater than for older ages (72% for infection and 71% for HBsAg positivity). When stratified by area, reduction is greater among children living in Araracuara and Puerto Santander (77% for infection and 73% for HBsAg positivity) while there is no difference by

gender in this age group. At the oldest ages (above 9 years) there is a statistical reduction in the prevalence of infection (62%, 95% CI 49-72%), but not for HBsAg positivity (-11%). Additionally, in this age group HBsAg prevalence was higher in females than in males.

This decrease in effectiveness might be an effect of the age of children when vaccination campaigns were started. Children under 8 years were born after vaccination started so they have more chance to have received HBV doses early in life and, therefore, of the vaccine preventing development of HBsAg positivity. Children who are now older than 9 years were three years or older when vaccine was introduced and had at least two factors which could potentially lead to decrease the vaccine efficacy. First, they may have been more difficult to reach by vaccination teams, either for starting the scheme or for completing it, since they are more likely to refuse vaccine injections than younger children. This is supported by the fact that older children were less likely to have a vaccination card. In 36% of the children in our survey it was not possible to establish what their real vaccination status was, and this absence of information increased with age. A second factor might be that they were more likely to have been infected before the vaccine campaign started. Before vaccine was introduced in Araracuara five year old children had a 14% prevalence of HBV infection (95% CI 4-37%) and a similar prevalence of HBsAg. (Cristancho LM 1992). It means that, in Araracuara and other rural areas, around 10% (4-20%) of children aged 1 to 4 years where already infected at the time of being vaccinated. In Thailand, Poovorawan et al. (2001) found a trend to increasing HBsAg prevalence with age because in children aged 1 to 2 years it was 4% but jumped to 9% in those aged 11 to 12 years.

Despite the large number of hepatitis B vaccine effectiveness evaluations published over the last 15 years there are still relatively few population based reports on the reduction in HBV infections after vaccine introduction. This information gap is especially important in Latin American where Brazil, Peru, Cuba, and Colombia have introduced hepatitis B vaccination but there has not yet been a comprehensive evaluation of its effectiveness. Only Cuba and Colombia have introduced vaccine using a universal vaccination strategy; other countries in Latin America use hepatitis B vaccination only in endemic areas (Tambini et al, 1998; Slusarsky & Magdzikw, 2000). Recently, a Peruvian group of researchers has made an assessment of the impact of the introduction of hepatitis B vaccine in the Huanta Valley, an endemic area of Peru located on the highest places of the Andes. There the vaccine was introduced in 1994 using a 0-2-4 months schedule and coverage in newborn children was 98%, while it was 84% among those children aged one to 4 years and born before the vaccine introduction. They measured the whole prevalence of hepatitis B infection and found a reduction from 83 to 92% in the prevalence of all markers of infection. However, they did

not evaluate if vaccination characteristics such as length of time between doses could influence effectiveness or the impact of other risk factors for HBV infection. Besides almost all the vaccination process was supervised directly by researchers since this was a pilot study area before the introduction of HB vaccine in Peruvian endemic areas (Cabezas C et al 2000; Cabezas C et al 1995).

In a recent report from Taiwan the authors showed the effectiveness of a recombinant Hepatitis B vaccine. The study involved two year old children and results are similar to those we are presenting from the Amazon. A prevalence of HBsAg of 2.5% was reported which resembles the prevalence of the same marker in our children of the same age living in rural areas (2.7%). When global infection (any marker positive) is considered, a prevalence of 6.5% was found in Taiwan while it was 10.3% in children from rural areas in our study. Children born from HBsAg negative mothers had a similar HBsAg positivity proportion to ours (0.6% to 1%). (Hsu Mei 2001)

We were unable to determine what the real prevalence of HBsAg carriers was in this study since only one sample was collected from every participant though the high absorbance ratio showed by those HBsAg+/anti-HBc+ might indicate that many of them are carriers. Other studies have found that effectiveness against chronic carrier status is higher than against HBsAg positivity as measured in cross sectional studies. In China a median effectiveness of 78% against HBsAg prevalence was found after 15 years of follow up in a cohort of children vaccinated when they were aged 3 to 36 months. However, protection against hepatitis B chronic carrier status was 96% compared with 78% against HBsAg positivity, an important quantitative and qualitative difference (Liao Su 1999).Effectiveness against infection was 84% .HBsAg prevalence in vaccinated children ranged from 0 to almost 6% (median 2%) during the study period, while anti-HBc prevalence ranged from 1 to 11% (median 4%)

In the Pacific islands, a known high endemic area, a plasma derived vaccine was introduced at the beginning of the 90's. Wilson et al (2000) carried out a cross sectional serological study in five Pacific countries, in order to determine the vaccine's ability to reduce hepatitis B infection. The sample included children aged 12 to 24 months (mother's were bled as well) and as a control group 10 to 13 year old children born before vaccine introduction. In vaccinated children, they found a prevalence of infection that ranged from 5 to 12% with a median of 9%, while HBsAg prevalence ranged from 0.7% to 3.8%.. In the control group, the HBV infection prevalence ranged from 47% to 77% while HBsAg prevalence was 7% to 27% (median=13%). Among mothers, infection prevalence ranged from 78% to 94% while

HBsAg was found between 7% and 19%. As expected, higher prevalence in mothers correlated with higher prevalence in vaccinated and unvaccinated children.

The HBeAg prevalence found by Wilson among HBsAg positive mothers (52%) is higher than the level we found in the urban Amazon but similar to levels in mothers from rural settlements. They found that 27% of children born from an HBeAg positive mother were HBsAg +(13/48) while none was found positive among children with the same risk factor in our study (0/24). It has been estimated that children born from an HBeAg positive mother have a 70-90% chance of becoming HBsAg positive while it is only 5 to 10% when mothers are HBsAg positive but HBeAg negative (Mahoney F and Kane M. 1999). The risk of HBsAg antigenaemia in children born from HBsAg positive mothers was higher in children from the Pacific Islands than in our study (OR=15.0 vs. none).It has been described that there are differences in the chance of perinatal transmission between different areas because HBeAg prevalence and HBV-DNA levels vary among HBsAg+ mothers across regions. (Mahoney F 1999, Shapiro C and Margolis H 1992, Botha J et al 1984) . Mothers in Asian countries are more infectious to their children than mothers in Africa, and our results showed that perinatal transmission risk is even lower for Amerindian children. This finding has been repeatedly observed in other studies in Amerindian populations living in the Amazon. In a recent study in the Brazilian Amazon area, Miranda Braga et al found an HBeAg prevalence of only 6% among 70 HBsAg+ Indians examined. All of them were children under 10 years old from the same family, data that supports the idea that perinatal transmission in the Amazon areas has only a marginal importance. Reasons for these differences in perinatal transmission rates are still unclear, but ethnic and therefore genetic characteristics could well be involved. (Hino K et al 2001; Tsebe K et al 2001; Miranda Braga W et al 2001). Poovorawan Y et al (2001) remarked that 35 to 40% of all HBV infections around the world are caused by perinatal transmission which is less than the proportion of perinatal transmission we found in our sample population.

In our study the proportion of HBsAg positive children born from an HBsAg mother was 10% compared to 68% in the Pacific countries and around 50% in Taiwan (Wilson et al 200; Hsu et al 2001). These differences reinforce that perinatal transmission in the Amazon is less important than in other endemic countries. Another point in favour of this statement is the higher effectiveness we found with the vaccine alone compared with the findings in Taiwan (Hsu et al 2001; Beasley et al 1983). Beasley found that protection against hepatitis B positivity was 95% when using HBIG plus vaccine but only 75% when using vaccine alone.

There are differences in vaccination policies between Colombia and other countries. HBIG is used in Taiwan for children born from HBeAg positive mothers, which represents almost 20% of their children, while in most endemic countries, including Colombia, vaccine is used alone because the proportion of children born from HBsAg positive mothers is lower and because HBIG is expensive.. In Liao's study Chinese children received hepatitis B vaccine in a 0-1-6 months schedule while in the Pacific Islands a 0-2-5 months schedule was applied. Another difference that is important to remember is the type of vaccine evaluated since there are not many studies published evaluating the performance on the field of a recombinant vaccine, which was the focus in our results. Even now, 15 years after the licensing of the yeast-derived vaccines, there are more published studies evaluating plasma-derived vaccines. The important decrease in HBsAg prevalence among children born from HBsAg positive mothers suggest that vaccination is adequate to control HBV infection even without HBIG. . A simple maternal screening plan focusing on mothers who live in rural zones would help to reach elimination quicker. (Andre F and Zuckerman AJ 1994). In a recent study from Gambia, Viviani et al showed one of the highest protective efficacies against carrier status (95%) using only hepatitis B vaccine, similar to those found in Taiwan where HBIG is incorporated in the scheme for children born from HBeAg positive mothers. One important aspect that distinguishes this study is that children received four doses of plasma derived vaccine (at birth, 2, 4, and 9 months of age). By nine years of age, 8% of vaccinated children became infected compared with 50% in the unvaccinated group (83% protective effect in vaccines) and true cumulative infection prevalence was 13%. (Viviani S et al 1999)

HBsAg prevalence in girls older than 10 years old was more than twice as high as prevalence in boys, while before vaccination boys in the same age group had higher HBsAg positivity than girls. There are at least two potential explanation for this difference. One is differential access to hepatitis B vaccination by gender among children who were alive when vaccination started. We processed data on HBV vaccination specifically in this age group and no differences by gender were observed among children aged 10 or 11, but at age 12 boys had higher coverage than girls though numbers were very small, only 20 boys and 19 girls were included in the survey. Twelve boys (60%) and 15 girls (79%) did not have accurate information on HBV vaccine status, but among those with vaccine records seven boys were fully vaccinated (7/8) compared with only 2 girls (2/4).

Another explanation for the gender difference might be differences in exposure to HBV. Health workers in the area said that girls are frequent victims of sexual abuse, especially in rural communities. Even in the absence of violence, Colombian girls tend to initiate sexual life earlier than Europeans (16 vs. 20 years) and a study from Puerto Asís, a town in the

Colombian Amazon region, showed that women in these areas start sexual life even earlier than the Colombian average (around 11 years).

One important finding in our study was the difference in hepatitis B infection and HBsAg positivity between rural and urban areas. Though in both, infection prevalence increases with age, it is much sharper in rural areas. On the other hand, HBsAg prevalence seems to increase with age in rural but not in urban areas. Children are more exposed to HBV in rural than in urban areas because more adults and older children are HBV infected or HBsAg positive and HBsAg positive mothers were more prone to be HBeAg positive in rural than in urban areas. This finding is important for public health decisions because our data provides the opportunity to put more emphasis on control measures in rural areas rather than urban. Strengthening vaccine delivery and increasing vaccination opportunities for rural areas should be one of the first steps to be taken.

The only published study comparable to urban Leticia is one from Manaos, in the Brazilian Amazon, where 21% prevalence was found. However, this result was not stratified by age group or sex, so comparison could only be done at an aggregate level (Silveira T et al 1999). Other results from urban areas in endemic Latin American countries came from the Dominican Republic. Overall, prevalence there was 21% but by age it was 9% in children 1 to 10 years while in those aged 16 to 40 years it was 30%. Women had a higher prevalence than men (24% vs. 13%). Socio-economic factors were associated with HBV infection in this study and people classified in the lowest socio-economic classes had higher infection prevalence. An important role for sexual transmission was found in the Dominican Republic and Brazil where prevalence showed a sharp increase in people older than 15 years. It is important to note that this increase is observed in those countries with the highest prevalence but not in those with the lowest ones such as Argentina, Chile and Mexico (Silveira T et al 1999)

Our study has an important strength in the fact that the analysis took into account risk factors for hepatitis B infection other than mother's serological status; this is not a frequent approach in other studies. It is clear from our results that some of these variables continue to be an important predictor of hepatitis B infection even after vaccine introduction. Number of siblings and child's birth condition were the most important individual variables identified. Regarding birth condition, those children whose birth was not attended by a nurse/MD were twice more likely to be found HBsAg positive than those who were attended by a doctor or nurse. This relation was even stronger in rural areas where the odds ratio increased to more than 10-fold. Some factors could explain this difference; one is that being born in a hospital

or health centre would mean receiving the first dose of hepatitis B vaccine closer to the birth date. Another potential explanation would be that practices around birth carried out by traditional midwives or by mothers themselves increase the child's risk for HBV infection. It might be possible that when the child is born in the community he (she) would have more exposure to mother's blood because the procedure to cut the umbilical cord may be delayed. Health workers in rural areas said that in births attended by the mother herself it is not rare that she has to cut the cord with her teeth thus increasing the likelihood of putting children in contact with HBsAg positive body fluids.

Wilson et al also explored the impact of other risk factors in hepatitis B infection. They found that female gender was associated with a higher risk of being infected (any marker positive) as well as having received only two doses of vaccine. Gender differences were similar to those observed by us, but in their study they could not claim that the explanation might be sexual transmission since only children less than 24 months were included.

We found that number of siblings was an important factor associated with HBsAg prevalence. The reason for this lay in the role that horizontal transmission has in maintaining HBV endemicity in the Amazon. More children in a crowded environment mean more opportunities to be in close contact with HBsAg positive children and to be exposed to an infection source. No relation between HBV infection or HBsAg positivity and number of siblings was found in Pacific children (Wilson et al 2001).

One important point raised by Wilson 2000 is the cost effectiveness of HBV vaccination. They estimate that vaccination prevents 10 of every 100 children from becoming HBsAg carriers at a cost of US$ 37 per prevented carrier, assuming that vaccine price is US $0.5 per dose which is the same price that Colombia pays for the Cuban vaccine. Assuming that 25% of HBsAg positive children would die prematurely, these prices, plus the reduction in number of HBsAg carriers, would mean a cost of US $190 per premature death prevented.

We were able to demonstrate that delay in dose delivery is associated with a higher likelihood of being HBsAg positive. This aspect has not been frequently addressed by other studies either because vaccination timing was standardised (clinical trials) or because the few studies focusing on time between doses have chosen anti-HBs titres as the evaluation outcome. This is important because former studies, focusing on Anti-HBs titres as the main outcome, have concluded that hepatitis B vaccine could be delivered following almost any schedule (0-1-3, 0-2-4, 0-1-6, etc..). Instead, our results showed that while longer intervals could produce higher Anti-HBs titres they might favour infection leading to the HBsAg

carrier status. Ruff T et al showed in Indonesia that a delay to receiving the first dose after the birth of more than week, was associated with a higher risk of being HBsAg+. (Inskip H et al 1991; Hadler S et al 1989; Ruff T et al 1995). Wilson et al did not find a relationship between delays in applying the first or second vaccine dose and hepatitis B infection or HBsAg positivity despite finding that a significant number of children received vaccine doses in a different schedule to that recommended. The proportion of children receiving the first dose on time was 22% to 90% depending on the country while timeliness for the second dose ranged between 46% and 76%, and by 6 months of age fully immunised children ranged between 22 and 84%.

It is interesting to note that our study did not find differences in infection rates between completely vaccinated children and incompletely vaccinated children. Lin D et al found in Taiwan that incompletely vaccinated children had twice the chance of being HBsAg+/anti-HBc+. However, it is important to recall that perinatal transmission is the most important source of HBsAg carriers in Taiwan while it is negligible in the Amazon. (Lin D et al 1998)

Our study is more representative of the true serological HBV infection and HBsAg prevalence after vaccine introduction than others published because it was evaluated routnien vaccination and almost every child who fulfilled the age criteria, and whose guardian agreed to participate, was included. Our results are prompting some changes in the way this program is conducted in the Amazon region. Maternal screening has started in these areas in order to identify high risk children and to ensure them an adequate vaccination schedule while health authorities are studying the feasibility of extending this screening to other endemic areas in the country.

II.3. Anti-HBs titres: A high proportion of participants in our study were found without detectable levels of anti-HBs despite having written vaccination evidence. One year after the third dose, almost 20% of children in our study had anti-HBs levels below 10 IU/ml which is high compared with findings from others studies, where children have been vaccinated under more controlled conditions. One year after being vaccinated with three or four doses of plasma HB vaccine, only 4-5% out of 631 of children was found with anti-HBS levels below 10 IU/ml in Holland. In the same study, only 1% of children vaccinated with four doses of a recombinant vaccine had undetectable anti-HBs levels. (Del Canho et al 1992). Other studies from Taiwan, The Gambia, China, and Canada showed results very similar to Holland. (Wong V et al 1984; Jack A et al 1998; Xu Z et al 1995; Marion S et al 1994)

A large difference was found with the amount of anti-HBs levels as well. In our study, the anti-HBs geometric means titre (GMT) one year after the third dose was 250 IU/ml while it ranged from 1142 IU/ml to more than 15000 in Del Canho's study. For the same length of follow-up, anti-HBs GMT ranged from 270 to 2068 IU/ml in other studies. (Jack A et al 1998; Xu Z et al 1995; Marion S et al 1994).

When longer periods of follow-up were considered, the differences in anti-HBs levels between our study and others became less important. In our results, children bled 5 years after the third dose had an anti-HBs GMT of 135 IU/ml and 19% did not have detectable anti-HBs levels. In more controlled studies and for the same length of follow-up GMT ranged from 41 to 158 IU/ml while the proportion of children without detectable levels was between 12% and 37%. Eight or more years after the third dose our children had an anti-HBs GMT of 78 IU/ml and 33% of them did not have detectable anti-HBs levels. This is very similar to the information reported by Jack et al in The Gambia, where 32 % of children lacked anti-HBs 9 years after being vaccinated with four doses of hepatitis B. At the same time, they found that the anti-HBs GMT was 19 IU/ml. In Canada and after 8 years of follow-up, Marion et al reported that the proportion of children without anti-HBs detectable levels reached 30% while the anti-HBs GMT was 106 IU/ml. (Wainright R et al 1989; Whittle H et al 1991; Xu Z et al 1995; Marion S 1994; Del Canho R et al 1992; Jack A et al 1998).

Other studies, carried out on populations under regular vaccination programs, have found high proportions of vaccinated children without detectable anti-HBs levels. Poovorawan et al found an overall rate of 44% children without anti-HBs, even higher than the rate we found (26%). Around 70% of children aged 1 to 2 years had anti-HBs detectable but by the age of 9 to 10 years only 45% were anti-HBs positive. Wilson et al found that between 21 and 51% of fully vaccinated children did not have detectable anti-HBs which is higher than the proportion we found. Some possible explanations given by Wilson for the lower prevalence of children with anti-HBs protective levels, as compared with the prevalence found in controlled studies, included variations in vaccine storage and handling, particularly vaccine freezing, which could be also a potential explanation for the high proportion of children found without anti-HBs in our study. (Poovorawan et al 2001; Wilson et al 2000). Differences in anti-HBs levels between studies may be due to the type of vaccine used in different studies or in the dose that children receive. The Cuban manufactured vaccine used in the Amazon contains 20 µg of HBsAg per vial and every vaccinated child is intended to receive half dose of it (10 µg). It has been demonstrated that plasma derived vaccine, which has been used in most of the studies presented here, is more immunogenic than the

recombinant especially when anti-HBs levels are compared shortly after completing the scheme. Del Canho et al (1992) and Stevens et al (1992) vaccinated groups of high-risk newborns with plasma or recombinant HBV vaccines and obtained serum samples from them at similar periods of follow up. Table 2.3 shows that in both studies children who received plasma-derived vaccine had consistently higher levels of anti-HBs than children who received the recombinant.

We believe that HW's poor knowledge regarding hepatitis B vaccine may be related to the proportion of children without anti-HBs. Most health workers interviewed by us said that the buttock was the right place to apply the hepatitis B vaccine. It had been demonstrated, in adults that delivering hepatitis B vaccine in the buttock was related to a lower serological response and recently it has been demonstrated in children as well. Alves et al randomly assigned 258 infants to receive a recombinant hepatitis B vaccine either Gluteal or at the anterolateral thigh muscle. The proportion of children who developed anti-HBs levels greater than 10 mIU/ml was similar in both groups but anti-HBs GMT differed (1229 mIU/ml for the buttock group and 1862 mIU/ml for the anterolateral thigh muscle group) (Fessard et al 1988; Alves A et al 2001)

In our study, we found that the amount of anti-HBs was related to the number of days from the child's birth to the first dose. Children who received the first dose in the first two weeks after birth had lower antibodies levels, a higher likelihood of being anti-HBs negative, and a lower chance of having Anti-HBs levels above 1000 IU/ml. Time between doses was not statistically related to these outcomes though delays to receive the second dose (>5 months from the first dose), or the third dose (>7 months from the second dose) were related to higher anti-HBs levels. There have been few attempts before to try to relate dose timing and response to HB vaccine, and they have been done using plasma derived vaccine. Marion S examined the influence of time from birth to first dose in Canadian children born from HBsAg+/HBeAg+ mothers. She found that those who received the first dose more than 2 months from birth had higher anti-HBs levels than those who received it closer to the birth date (GMT 590 IU/ml vs. 110 IU/ml). Hadler S et al in a study among Yucpa Indians found that the inmunologic response to HB vaccine was better among those people who received the third dose later than recommended. This study also found that those receiving the first dose after 20 years of age had a lower immunologic response. Hadler's study results are not completely comparable to those in the present study because it was done on a population with a wider range of age, and no specific analysis was done regarding time from birth to first dose. On the other hand, our inability to find a relation between time doses and anti-HBs titres did not seem to be an issue of low statistical power, since the sample size in the

Hadler's study is similar to that in our study. So, it is probable that in older age groups, young adults and adults, time between doses may be a more important predictor of the serological response than in young children. Results from an study in Gambia seems to be in concordance with this statement. Inskip H et al carried out a study in more than one thousand young children vaccinated with a plasma-derived vaccine and no relation was found between time doses and serological response. (Hadler S et al 1989; Inskip H et al 1991; Marion S 1994)

In summary our study has shown that the process of implementing a new vaccine against hepatitis B in the Colombian Amazon has been successful. We strongly believe that our findings are not the results of potential sources of bias but that they come from true factors in the population where the study was done. HBV vaccine has reached a high coverage especially among children born after the implementation of the program though adherence to vaccine schemes should be improved. It has also been shown that following the vaccine introduction, there has been an important reduction in the prevalence of HBV infection and HBsAg carriers especially among children aged 0 to five years. However, new vaccination strategies should be introduced in order to ensure an adequate and timely access of the population to vaccination activities, especially in rural villages. Based on our recommendations the Amazon Health Service has started a serological surveillance system on pregnant women aimed to identify those mothers HBsAg+ and to provide their children with more adequate HBV vaccination schemes.

III. Conclusions and Public health implications of this work:

The findings from this work have implications for the improvement of the process of the vaccination program as well as for the impact of the hepatitis B control program in the Amazon and, probably, other endemic areas in Colombia. First we found that while the overall coverage of vaccination is good, the age at which children receive the vaccine is far from being that recommended by the MOH. It is highlighted from the results that logistic constraints and inadequate health worker knowledge are important factors associated with delayed vaccination.

Some simple measures may lead to an improvement in the process indicators: 1) Continuing training and supervision of the health workers may reduce lost opportunities by giving them adequate knowledge on vaccine contraindications. 2) The poorest people, especially in rural areas, should be regularly informed about their rights under the social security scheme in

Colombia. Vaccination has been paid for, for all children, by the MOH, so they have the right to be vaccinated whether they hold a health insurance card or not.

Secondly and despite all the problems with the vaccine delivery, the proportion of infected children and carriers seems to be decreasing, which demonstrates that the Cuban manufactured hepatitis B vaccine is as effective as other recombinant hepatitis B vaccine produced in other settings. This is important because the price offered by the Cuban government is lower than that offered by other manufacturers. However the effectiveness of the program could be improved if children get vaccinated with one dose soon after birth (first 15 days) and with the second dose one month after the first, especially in rural areas where the risk is the highest.

Following recommendations from this work the Amazon department have designed a re-training of the rural health workers which has been carried out during this year. They have implemented serological surveillance of pregnant mothers, in order to increase the chance that those children with the highest risk can get the vaccine soon after birth. Mothers who are positive for Anti-HBc or HBsAg are advised that their children need hepatitis B vaccine as soon as they can, so the interest of the parents may ensure a higher compliance with the vaccination schedule. This surveillance did not have an important impact on the cost of the program since less than 1000 births occur yearly in the Amazon.

Annex 1. Univariable analysis of vaccination coverage and related factors

Table I.1. Vaccination coverage with complete/incomplete/ missing data.

Age group	Number with Vaccination card (%)	Number fully vaccinated (%) [95%CI]	Number without vaccination card (%)	Estimates of fully vaccinated among children with missing data (worst-best %) *
1 y	158 (57)	65 (41) [31-49]	120 (43)	22-45
2/3 y	351 (52)	235 (67) [60-72]	324 (48)	49-69
4/5 y	354 (55)	269 (76) [70-80]	289 (45)	60-78
6/7 y	315 (49)	236 (75) [70-80]	327 (51)	62-77
8/11 y	351 (43)	249 (71) [65-76]	465 (57)	50-73
Total	1529 (51)	1054 (69) [64-72]	1525 (49)	50-71

The best coverage was calculated as the mid-point between the point estimate and its upper confidence limit in the third column of the table above. The worst is taken from the lowest limit of the coverage by age group and town. The value of this interval is multiplied by the number of children without a vaccination card in each age group and town in order to calculate the expected number vaccinated by age group. This number is summed across strata and the total is divided by the total number of children without a vaccination card in each category (fourth column in table above) in order to estimate the overall coverage under the worst scenario.

Example: Consider the value for children 1 year old without a vaccination card in the table above with a coverage between 22 and 45%. The highest coverage comes from the mid-point between 41%, the point estimate, and 49%, the upper confidence limit (third column and first row).

To calculate the lowest scenario for these 1 year olds the lowest interval in the first row of the table below (40%, 8%, 1%, 6%, 4%) was used. These percentages were multiplied by the number of children without a vaccination card in that age group and town (53 in urban Leticia, 34 in rural Leticia, 11 in urban P. Nariño, 13

in rural P. Nariño, and 9 in Araracuara). This yielded an expected number of 27 children vaccinated among those without a vaccination card, given that the total number of children in this age group was 120 it results in a worst coverage of 22% (27/120).

Fully vaccination coverage by age group and town

Age group	Urban Leticia % (95%CI)	Rural Leticia % (95% CI)	Urban P. Nariño % (95% CI)	Rural P. Nariño % (95% CI)	Araracuara and P. S/der % (95%CI)
1 y	52 (40-64)	23 (8-50)	11 (1-53)	24 (6-60)	40 (4-91)
2/3 y	70 (61-79)	66 (53-77)	38 (22-57)	64 (41-81)	60 (14-94)
4/5 y	76 (68-83)	78 (67-86)	54 (35-72)	76 (52-90)	72 (63-79)
6/7 y	76 (66-83)	79 (70-86)	44 (31-57)	82 (65-92)	77 (55-90)
8/11 y	74 (67-80)	74 (57-86)	39 (18-65)	68 (46-84)	48 (23-73)

Table I.2. Distribution of people included in the census and vaccination coverage by variables related with children.

Variables	Number surveyed (%)	Number fully vaccinated (%)	OR (CI95%) Fully vaccinated	Number with complete hepatitis B vaccine (%)	OR (CI95%) Hepatitis B vaccination
Age		**P<0.0001**		**P=0.0001**	
1 year	279	65 (41)	1.0	167 (79)	1.0
2/3 years	678	235 (67)	0.34 (0.2-0.5)	468 (91)	0.37 (0.24-0.57)
4/5 years	638	269 (76)	0.22 (0.1-0.3)	374 (93)	0.26 (0.14-0.48)
6/7 years	640	236 (75)	0.22 (0.1-0.3)	316 (88)	0.48 (0.26-0.87)
8/11 years	811	249 (71)	0.28 (0.2-0.4)	379 (88)	0.47 (0.27-0.80)
Sex		P=0.62		P=0.66	
Male	1608	554 (69)	0.95 (0.8-1.2)	896 (89)	1.0
Female	1437	500 (68)	1.0	808 (88)	1.01 (0.77-1.3)
Area		**P=0.02**		**P=0.05**	
Urban Leticia	1485	514 (71)	1.0	745 (89)	1.0
Rural Leticia	677	282 (69)	1.1 (0.7-1.8)	480 (90)	0.98 (0.47-2.04)
Puerto Nariño	310	47 (39)	3.8 (2.4-6.1)	99 (73)	3.13 (2.06-4.75)
Rural Puerto Nariño	316	131 (68)	1.15 (0.5-2.7)	202 (84)	1.41 (0.67-2.98)
Araracuara	260	80 (64)	1.36 (0.8-2.4)	178 (96)	0.37 (0.15-0.92)
Time living in town*		P=0.84		P=0.40	
All of life	2455	895 (69)	1.0	1414 (88)	1.0
Not all of life	558	171 (68)	1.03 (0.72-1.5)	292 (90)	1.18 (0.62-2.26)
Ethnic group		**P=0.009**		**P=0.025**	
Not Indians	1439	408(66)	1.0	616 (87)	1.0
Mestizos	341	150 (74)	0.7 (0.4-1.1)	246 (92)	0.59 (0.33-1.05)
Ticunas	781	290 (64)	1.1 (0.65-1.8)	484 (86)	0.54 (0.21-1.39)
Huitotos	150	43 (60)	1.3 (0.7-2.2)	89 (92)	1.11 (0.59-2.1)
Other groups	337	163 (83)	0.4 (0.2-0.6)	269 (95)	0.32 (0.17-0.59)
Birth order		**P=0.04**		P=0.16	
1	538	205 (74)	1.0	335 (91)	1.0
2/3	933	350 (70)	1.2 (0.8-1.8)	553 (88)	1.61 (1.0-2.61)
4/5	522	209 (65)	1.5 (1.0-2.2)	342 (88)	1.87 (1.12-3.10)
6/20	312	133 (76)	0.9 (0.6-1.4)	220 (92)	1.10 (0.56-2.17)
Number of siblings		P=0.16		P=0.22	
1	85	27 (61)	1.0	72 (94)	1.0
2/3	930	357 (72)	0.6 (0.3-1.2)	562 (89)	1.85 (0.57-5.98)
4/5	746	283 (67)	0.8 (0.4-1.6)	442 (87)	2.36 (0.78-7.13)
6/20	525	221 (74)	0.55 (0.2-1.2)	360 (91)	1.53 (0.52-4.51)
Born in Hospital		P=0.41		P=0.82	
Yes	1146	460 (74)	1.0	710 (91)	1.0
No	885	384 (71)	1.2 (0.8-1.8)	638 (90)	1.07 (0..54- 2.12)
Health security		**P=0.008**		P=0.30	
Affiliated	1638	602 (72)	1.0	1020 (90)	1.0
Not affiliated	393	109 (58)	1.8 (1.2-2.2)	216 (86)	1.67 (0.71-3.97)

+ Time that the person has been living in the same place where he (she) was interviewed.

Table I.3. Distribution of people included in the census and vaccination coverage by variables related to parental characteristics.

Variable	Number surveyed (%)	Number fully vaccinated (%)	OR (CI95%) Fully vaccinated	Number with complete hepatitis B vaccine (%)	OR (CI95%) Hepatitis B vaccine
Living with father		P=0.69		P=0.38	
Yes	1913 (58)	773 (70)	1.0	1240 (89)	1.0
No	329 (10)	120 (73)	0.9 (0.5-1.5)	201 (92)	0.73 (0.36-1.47)
Mothers age at child's birth		P=0.42		P=0.51	
13-19 y	469	165 (71)	1.0	306 (90)	1.0
20-29 y	1233	499 (71)	1.0 (0.7-1.4)	821 (89)	1.09 (0.65-1.81)
30/45 y	546	215 (68)	1.21 (0.8-1.8)	363 (88)	1.33 (0.78-2.29)
Mother's age at survey		**P=0.04**		P=0.43	
16/20	105	32 (55)	1.0	86 (91)	1.0
21/25	468	200 (75)	0.4 (0.2-0.8)	313 (89)	1.19 (0.54-2.61)
26/30	621	246 (73)	0.45 (0.2-0.9)	388 (91)	0.96 (0.37-2.44)
31/35	493	191 (66)	0.6 (0.3-1.27)	323 (86)	1.52 (0.59-3.92)
36/57	542	219 (73)	0.4 (0.2-0.8)	339 (91)	1.0 (0.43-2.37)
Father's age at survey		P = 0.18		P =0.65	
16-25 y	171	57 (60)	1.0	115 (87)	1.0
26-30 y	348	135 (71)	0.59 (0.33-1.03)	228 (89)	0.83 (0.45-1.51)
31-35 y	389	164 (72)	0.56 (0.3-1.05)	243 (89)	0.80 (0.37-1.73)
36-57 y	884	361 (72)	0.56 (0.3-0.92)	568 (90)	0.71 (0.42-1.17)
58-76 y	50	17 (61)	0.92 (0.3-2.7)	30 (81)	1.54 (0.50-4.76)
Mothers education level (number of years in school)		P=0.22		P=0.69	
0/4	768	298 (70)	1.0	534 (90)	1.0
5	518	222 (73)	0.8 (0.6-1.3)	356 (90)	1.0 (0.62-1.62)
6/8	281	102 (75)	0.8 (0.4-1.3)	184 (94)	0.60 (0.28-1.25)
9/10	114	51 (81)	0.5 (0.2-1.4)	68 (92)	0.82 (0.26-2.63)
11/17	320	135 (74)	0.8 (0.5-1.4)	204 (90)	1.0 (0.51-2.10)
Fathers education level (number of years in school)		P=0.34		P=0.31	
0/4	1049	416 (68)	1.0	685 (88)	1.0
5	65	18 (62)	1.3 (0.6-2.8)	35 (82)	1.58 (0.61-4.09)
6/8	345	140 (75)	0.7 (0.4-1.1)	220 (91)	0.67 (0.37-1.22)
9/10	364	157 (73)	0.8 (0.5-1.2)	234 (91)	0.73 (0.40-1.33)
11/17	49	21 (76	0.7 (0.3-1.5)	31 (94)	0.49 (0.11-2.12)

Table I.4 Distribution of people included in the census and vaccination coverage by variables related to socio-economic characteristics.

Variables	Number of surveyed (%)	Number fully vaccinated (%)	OR (CI95%) Fully vaccinated	Number with complete hepatitis B vaccine (%)	OR (CI95%) Hepatitis B vaccine
Roof made with:		**P=0.003**		**P=0.01**	
Tile	2574	899 (70)	1.0	1488 (89)	1.0
Palm tree leaf	383	67 (52)	2.0 (1.4-2.96)	183 (82)	1.91 (1.14-3.18)
Unknown					
Floor made with:		**P=0.07**		P=0.58	
Cement	1050	389 (72)	1.0	551 (90)	1.0
Wood	1829	622 (64)	1.4 (1.0-2.0)	1085 (88)	1.20 (0.80-1.81)
Soil	99	32 (74)	0.9 (0.4-1.9)	51 (89)	1.02 (0.40-2.58)
Unknown	278 (8)				
Walls made with:		P=0.19		P=0.54	
Bricks	657	228 (72)	1.0	341 (90)	1.0
Wood	2271	791 (65)	1.3 (0.9-1.8)	1316 (88)	1.16 (0.71-1.91)
Crowding: # of people by room		P=0.11		P=0.78	
1/3	1416	495(71)	1.0	736 (89)	1.0
4/6	896	305(63)	1.4 (1.05-1.9)	540 (88)	1.03 (0.68-1.56)
7/9	339	134 (68)	1.1 (0.7-1.8)	223 (89)	0.95 (0.62-1.45)
10/15	242	74 (67)	1.2 (0.8-1.8)	141 (91)	0.76 (0.35-1.60)
Freezer in house		**P=0.0013**		**P=0.09**	
Yes	684	266 (77)	1.0	424 (0.92)	1.0
No	1515	513 (65)	1.8 (1.2-2.6)	913 (88%)	1.55 (0.92-2.62)
Unknown					
TV in house		**P=0.06**		P=0.36	
Yes	1018	360 (73)	1.0	586 (91)	1.0
No	1195	421 (65)	1.45(0.97-2.15)	756 (88)	1.27 (0.75-2.17)
Unknown					
Radio at home		**P=0.05**		P=0.22	
Yes	1044	374 (73)	1.0	628 (81)	1.0
No	1169	407 (66)	1.39 (1.0-1.96)	714 (88)	1.34 (0.83-2.15)
Unknown					
Outboard Motor		P=0.11		**P=0.06**	
Yes	214	73 (76)	1.0	132 (94)	1.0
No	1999	704 (69)	1.48 (0.9-2.4)	1210 (89)	1.78 (0.97-3.27)
Unknown					
Piped water		P=0.98		P=0.65	
Yes	1468	487 (68)	1.0	742 (88)	1.0
No	1481	536 (68)	1.0	929 (89)	0.90 (0.57-1.42)
Excretal disposal		P=0.89		P=0.73	
Toilet	1104	419 (67)	1.0	705 (89)	1.0
Pit	1074	331 (68)	0.96 (0.65-1.4)	500 (88)	1.14 (0.70-1.90)
None	837 (26)	246 (66)	1.1 (0.7-1.5)	429 (87)	1.20 (0.77-1.88)

Table I.5 Relationship between ecological variables and individual vaccination status against all vaccines. Health worker perceptions.

Variable	Total number N	Complete schedule N (%)	OR (CI95%) Incomplete schedule	Hepatitis B vaccination N (%)	OR (CI95%) Incomplete HB vaccination
Are there children in your community that do not come to the health center for vaccination?		**P=0.002**		**P=0.000**	
Yes	1421	892 (64)	1.9 (1.3-2.9)	1451 (88)	3.2 (2.4-4.2)
No	136	104 (77)	1.0	179 (96)	1.0
Are there children in your community who have right to be vaccinated by another health provider?		P=0.18		P=0.17	
Yes	778	515 (66)	0.8 (0.5-1.1)	747 (89)	0.7 (0.4-1.2)
No	692	411 (60)	1.0	763 (85)	1.0
Do you believe that in your community there are parents who do not agree with vaccination?		P=0.19		P=0.88	
Yes	1230	768 (64)	1.4 (0.8-2.3)	1210 (87)	1.1(0.4-3.4)
No	112	78 (71)	1.0	123 (88)	1.0
Why do you believe that hepatitis B is an important disease in your area?		**P=0.001**		**P=0.0003**	
Infectiousness	1226	756 (63)	1.9 (1.3-2.8)	1190 (87)	3.2 (1.7-6.0)
Severity	275	207 (77)	1.0	366 (95)	1.0
What do you believe is an important reason for children not being vaccinated in your area?		**P=0.007**		**P=0.000**	
Parent education/ Parent fear of vaccine side effects	1112	747 (63)	1.8 (1.2-2.8)	1162 (86)	4.1 (3.1-5.3)
Logistic reasons/ Poverty	158	120 (76)	1.0	210 (96)	1.0
Do you believe that there is not enough time in the health center for vaccination activities?		**P=0.02**		**P=0.03**	
Yes	1145	701 (63)	1.5 (1.1-2.2)	1128 (87)	1.9(1.04-3.4)
No	412	295 (72)	1.0	502 (93)	1.0
Do you believe that in your community parents do not spare enough time to take children to health center?		**P=0.008**		**P=0.000**	
Yes	1458	952 (66)	0.46 (0.3--0.8)	1549 (89)	0.3 (0.16-0.48)
No	99	44 (47)	1.0	81 (71)	1.0

Annex 2. Questionnaire for vaccination coverage for field work.

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Household Census. (This form will be used for a census in all the households selected in the random cluster sampling. It will provide a reference for the main demographic characteristics of the children under study)

1) Interview Date: mm__/ dd__ / yy__ 2)Town or village:_____

3) Interviewer name:_____ 4) Cluster #_____ 5) House #_____

People who answer the questionnaire: Children's mother __Children's father ___ Other___

Household characteristics:

5) How many people live in this household?: ____ 6) Number of sleeping rooms:_____

7) Roofs made with:_____ 8) Walls made with:_____

10) Floor made with:_____ 11) Is there a water tap in the house?: Y__ N__

If not, how do you manage to obtain drink water?: Rain___ River___ Well___

12) Is there a toilet at home?: Y__ N__ 13) If not is there any other form of excretal disposal? Y__N___

If yes, Please describe: _____

13) Are you or your children affiliated to the social security system? Y___N____

14) You or your husband owned a: Motorcycle___ Refrigerator____ TV____ Radio____

The following form will only be filled for children less than 10 years. If you do not find a vaccination card at home for a child, please ask the mother if it may be at the school. If yes, please ask the school's name and child's grade. These data can be written beside the name of the child on the external area of the table. After finishing the interview at this household, please go to the school and ask there if the vaccination card can be searched by the school's head-teacher.

	1	2	3	4	5	6	7	8	9
Child number									
Birth date									
Time living in town (years)									
Relation with the family head									
Vaccination card Y/N									
Does the child have the vaccination card in the school? Y/N									
Hep B Number of doses									
1st dose date									
2nd. dose date									
3rd dose date									
DPT Number of doses									
1st dose date									
2nd dose date									
3rd dose date									
Booster date									
Measles Number of doses									
1st dose date									
Booster date									
Yellow fever. First dose date									
BCG first dose date									

Annex 3. Questionnaire for risk factors

1) Child ID: _____ 2) Cluster #_____

3) Town or village:_____4) Area: Urban__ Rural__

5)Interviewer Name:_____6)Code:_____

7) Date of interview: mm__/ dd__ /yy__

8) Name of the child:_____ 9) Sex: M__ F__

10) Date of birth:_____ mm/dd/yy

11)Age: Years_____ Months____ (record age just if date of birth is unknown)

12) Birth order:____ (please ask the number of siblings older than the child under interview. Ask even about those deceased)

13) What was the age of the mother when the child was born _____ Years

14) Place of birth of the child:_____15)Area: Rural/Urban

16) Born in: Hospital __ Local Health Centre __ At home ____

17) Birth attended by: Doctor__ Nurse___ Relative____ Other_____ Specify_____ None___

18) How long has the children been living here?: Years _____Months_____ (please specify the number of years and months)

General information:

19) Was the child breastfed?: Y__N__ 20) For how long?:_____ (put the number of months)

21) Has the child had hepatitis?: Y__N__ 22) If yes, what age ?: _____

23) Was he/she taken to the doctor?: Y__N__ 24) If yes, where?:
Local health centre ___
Hospital __
Private __

Mother's information:

25) Mother's Name:_____ 26) Date of birth:_____

27) Age:____ (put age just if date of birth is unknown)

28) Place of birth:_____

29) Town of birth:_____

30) Time living in town where they are living now: Years_____ Months____

31) Have you ever been told about having hepatitis? Y __N__

32) If yes, when? _____

33) What was the last level that you reached in school?:
Primary __ Number of years __
Secondary __ Number of years__
University __ Number of years__

34) How old is the father? ___

35) What was the last level that the father reached in school?
Primary __ Number of years
Secondary __ Number of years__
University __ Number of years__

36) Are you or your husband from an aboriginal family? Y __N__

If yes, could you please tell me the name of your aboriginal family? _____

37) Has anybody in the household had hepatitis before? Y__ N__

If yes, how many people in the household have had hepatitis before?:_____

Name of the first person having hepatitis_____ Date of starting symptoms:_____ Relationship with the child _____
Died Y__N__

Name of the second person with hepatitis_____ Date of starting of symptoms _____ Relationship with the children_____ Died Y__N__

Have anybody in the household had before any of the following diseases?:

38) Cirrhosis : Y__N__ If yes, how many people have had this?_____ How many died from this disease? ____
Name _____ Relationship with the children _____ Died Y__ N__
Name _____ Relationship with the children _____ Died Y__N__

39) Fulminant Hepatitis: Y__N__ If yes, how many people have had this?_____ How many died from this disease? ____
Name_____ Relationship with the children _____ Died Y/N
Name_____ Relationship with the children _____ Died Y/N

40) Hepatocarcinoma: Y__N__ If yes, how many people have had this? _____ How many died from this disease? ____
Name _____Relationship with the children _____ Died Y__N__
Name _____Relationship with the children _____ Died Y__N__

Annex 4. Questionnaire for health workers (To be answered by the people in charge of the EPI programme if it is run in the health centre)

1) Name of the health centre?:_____

2) Town or village:_____ 3) Area: Rural__ Urban__ (Rural areas are those where population is under 5000)

4) How long have you been in this health centre?_____

5) Name of the interviewer _____

6) Profession: MD ____ Nurse____ Health promoter _____ Auxiliary nurse_____

7) Date of the interview: mm__/ dd__ / yy __

8) Does the centre provide EPI vaccination? Y __ N __ (If not please interrupt the interview and thanks to the director for his time)

If not, 9) Has it ever provided EPI vaccines? Y __ N__ If yes, 10) Why was it stopped?

_____

If answer to question number 6 was yes, please continue to the following questions.

11) How does the health centre provide vaccination?: Just inside_____
Using outreach teams____

12) Size of population covered by the health centre: _____
13) Number of daily external medical consults in the last month: ____
14) Does the centre have medical service every day? Y/N
15) If yes, does the centre provide medical attention 24 hours a day? Y__N__

16) If not, How frequent is the medical service in the centre by week? (Please record the week days when medical service is provided in the centre) Monday __ Tuesday__ Wednesday__ Thursday__ Friday __ Saturday__

18) How many health workers does the centre have under contract?__
19) How many doctors work in the centre? ___
20) How many nurses? ___
21) How many technicians? __

22) Which vaccines are available in this moment at the centre?
Measles__ # of doses
Polio__ # of doses
DPT__ # of doses
Hep B__ # of doses
BCG___ # of doses

23) Where are the vaccines stored? _____

24) Do you have a record of temperature? Y__N__

25) If yes, may I see the temperature records in the last week?: 1 day _____ 2 day ___ 3 day____ 4 day ____ 5 day ____ 6 day ____ 7 day ____

26) What days does the centre provide EPI vaccination inside? Monday___ Tuesday__ Wednesday__ Thursday__ Friday__ Saturday__

27) What days does the centre provide EPI vaccination outreach? Monday___ Tuesday__ Wednesday__ Thursday__ Friday__ Saturday__ (please tick each day given by the interviewed)

28) What time in a day does the centre provide EPI vaccination? All day___ in the morning____ in the afternoon___

29) In the last month, have you rejected any children coming here for vaccination because there were no supplies of the vaccine? Y__N__

30) If yes, could you remember how many times it happened? _____

31) How many workers are in charge of EPI vaccination? ____

32) How long have you been working in your profession? _____

33) How long have you been working in this health centre? _____

34) Do you know what the diseases preventable by vaccination are? Y__N__

35) If yes, could you please tell me their names?
_____
_____
_____
_____
_____
_____

36) By what age should a child be completely vaccinated? _____

37) Have you ever heard about hepatitis B vaccine? Y__N__

38) If yes, how many doses are needed to immunise completely a person? _____

39) Do you know in what part of the body should be the hepatitis B vaccine administered? Y__ N ____

40) Do you think that there is any contraindication for administration of hepatitis B vaccine? Y__N__

41) If yes, could you please tell me what are these contraindications?:_____
_____
_____
_____

42) Do you know how hepatitis B vaccine should be stored?: Y/N
_____

43) What are the most important diseases in your area?
_____
_____
_____
_____

44) Is hepatitis B an important disease in your area? Y/N

45) If yes, why? _____

46) If not, why? _____

47) A two year old child comes for vaccination today (1999) and his hepatitis B vaccination history is:

1a. Dose: 01/01/97 2a. Dose: 01/ 02/97. What would you do now?

- Start the schedule again ___

- Apply the third dose__

48) Are health education materials about immunisation against hep B clearly displayed in the clinic?
Yes___ NO____

49) What materials about immunization are present in the clinic that you can see?

Pamphlets _____

Posters_____

Audiovisual material_____

Others_____

50) This table shows same conditions that children may have, as well as the commonly administered vaccines. Please indicate whether a child with any one of these conditions may receive each vaccine. Please mark " + "if yes or "-" if not

Conditions	DPT	OPV	HPB
Fever <101'F			
Fever >101'F			
Prematurity			
Febrile convulsions			
Non-febrile convulsions			
Relative with convulsion			
Prior vaccine reaction			
Cough			
Leukaemia			
AIDS			
Diarrhoea			

51) What are the 3 most important childhood diseases in this area?
1._____2._____3._____
____

Was hepatitis B mentioned above? Y__ N__

52) Are there children in this community who do not have access to the vaccination services in this clinic? Y__ N__

53) If not, Why do you think those children do not have access to vaccination?_____
_____
_____
_____

54) Why do you think those children don't have access to vaccination?
_____

55) Do you keep immunisation records from children vaccinated in the area?
Y_____
N ____

56) Dou you charge any fee for vaccination service? Yes ____No____

If yes; how much do you charge for vaccination services?
_____

57) What do you think are some of the difficulties or problems in getting children immunised?

Inadequate supplies ___
Insufficient instruction on vaccine administration__
Not enough time in clinic session___
Not enough staff working at clinic ___
Need for doctor's order delays immunization__
Other_____ Specify_____

58) Do you have written guidelines on hepatitis B vaccine issues? Yes ___No____

59) If yes, may I see them? _____

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