Vertical Transmission of Hepatitis B Virus (HBV)
from Mothers Negative for HBV Surface Antigen
and Positive for Antibody to HBV Core Antigen

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Background. Hepatitis B virus (HBV) can be transmitted by blood donors and organ donors who are positive for antibody to HBV core antigen (anti-HBc) but negative for all other HBV markers. Therefore, we evaluated the risk of infection for babies of mothers with this serostatus.

Methods. A total of 2365 HBV surface antigen (HBsAg)–negative pregnant women were included in the study and screened for anti-HBc. Blood samples for screening were collected 1 day before or after delivery. Infants of mothers with positive anti-HBc test results were tested 3–4 months after birth.

Results. Of 2365 mothers, 147 (6.2%) were anti-HBc positive. Follow-up tests were performed using samples from 105 children. Samples were tested for all HBV markers, including HBV DNA, which was evaluated using a highly sensitive polymerase chain reaction assay (Taq PCR). Seven children (6.6%) had markers of HBV infection; the Taq PCR detected HBV DNA in 5, and HBsAg was detected in the other 2.

Conclusions. This study shows that HBV can be transmitted perinatally even in the absence of HBsAg. None of the children developed chronic HBV infection. Further studies must determine whether routine immunization of infants at the age of 3 months gives enough protection against HBV infection or whether screening of pregnant women for HBV should be extended, with immunization of their newborns beginning immediately after birth.

In areas where hepatitis B virus (HBV) is relatively uncommon, such as Western Europe, 0.5%–2% of pregnant women are positive for HBV surface antigen (HBsAg). At least 10 times more are positive for antibody to HBV core antigen (anti-HBc) but negative for HBsAg, which means that these individuals were once infected with HBV but cleared the virus. According to the World Health Organization, half of the world’s population has been infected with HBV and, thus, is anti-HBc positive. Studies of blood and organ recipients have recently shown HBV transmission from anti-HBc–positive, HBsAg-negative donors, even when HBV DNA was undetectable in the donor [1–5]. This raises the question of whether newborns are at risk of acquiring HBV from mothers with a similar serostatus. Current vaccine programs recommend active hepatitis B immunization for every infant at 2, 3, and 4 months of age. In contrast, newborns delivered by HBsAg-positive mothers additionally receive passive and active immunization 12 h after birth. Because there are therapeutic options for children at risk for HBV infection, it is crucial to study whether mothers with markers of past infection can transmit the virus vertically.

Currently, pregnant women are only screened for HBsAg, and as a result, infants delivered by anti-HBc–positive mothers are not identified. However, chronic HBV infection is now common, predominately because of immunotolerance. Because HBV infection is mostly asymptomatic during childhood, infection is usually only diagnosed when complications such as cirrhosis or hepatocellular carcinoma become evident.

PATIENTS, MATERIALS, AND METHODS

Blood samples were collected from 2365 pregnant women from 3 different maternity clinics over a period of
32 months. All participants provided informed consent. The study was approved by the appropriate ethics committee at Witten-Herdecke University (Witten, Germany) and was performed in accordance with the Declaration of Helsinki.

Blood was collected either the day before or the day after delivery, depending on the circumstances of the delivery. Serum was stored at −80°C. All 2365 samples were tested for HBsAg and anti-HBc. Samples that were anti-HBc positive were further tested for HBV-DNA and antibody to HBsAg (anti-HBs). We asked mothers who were anti-HBc positive whether follow-up tests could be performed on their children; 105 mothers agreed. Follow-up tests were performed on children for detection of anti-HBs if enough serum was available: HBsAg, anti-HBs, HBV e antigen (HBeAg), antibody to HBeAg (anti-HBe), anti-HBc, and HBV DNA. The assays used were Axsym Core (Abbott), for detection of anti-HBc; Axsym HBsAg (V2; Abbott), for detection of HBsAg; Axsym HBsAg Confirmatory (Abbott), for confirmation of HBsAg-positive test results; Axsym AUSAB (Abbott), for detection of anti-HBs; Axsym HBe 2.0 (Abbott), for detection of HBeAg; Axsym Anti-HBe 2.0 (Abbott), for detection of anti-HBe; Axsym Core-M (Abbott), for detection of anti-HBc; and Versant HBV DNA 3.0 (Bayer Diagnostics), for quantitative hybridization of HBV DNA. The manufacturers confirmed that negative and positive controls are not affected by elevated concentrations of hemoglobin (up to 250 mg/dL), lipids (up to 1000 mg/dL), and bilirubin (up to 15 mg/dL). They also excluded cross-reactivity with other viruses, including hepatitis C virus and human immunodeficiency virus (HIV), and antibodies and assured us that repeated freezing and thawing would not affect results. The detection limit for HBsAg is 0.1–0.6 ng/mL (0.031 Paul Erlich Institute units/mL), and the detection limit for HBV DNA in quantitative hybridization is 4342 copies/mL. Moreover, we used the highly sensitive method described by Dreier et al [6] for HBV DNA Taq polymerase chain reaction (PCR). This PCR method reaches a sensitivity of 10 IU of HBV genomes/mL; however, quantification of DNA is not possible. We excluded children with blood transfusions from our analysis. Information about HBV infections in other family members was not gathered.

RESULTS

Mothers. Of 2365 HBsAg-negative women who provided blood specimens for analysis, 147 (6.2%) were anti-HBc positive. A total of 105 anti-HBc–positive women provided informed consent to have their children tested at the age of 3–4 months. At the time of delivery, 3 women were <20 years old, 15 were aged 20–25 years, 39 were aged 26–30 years, 33 were aged 31–35 years, 9 were aged 36–40 years, and 6 were aged >40 years.

Mothers of the children who were tested had the following anti-HBs status: 18 were negative for anti-HBs, 6 had an anti-HBs level of 1–10 IU/L, 13 had an anti-HBs level of 11–99 IU/L, and 72 had an anti-HBs level of >100 IU/L. Among the 91 mothers in whom anti-HBs was detected, the mean anti-HBs level was 505.9 IU/L. Three mothers were positive for HBV DNA, with anti-HBs titers of 7 IU/L, 151 IU/L, and 215 IU/L. One of the 3 had a child who became HBsAg positive 4 months after birth (the child in mother-child pair 1 in table 1), and 2 had children who showed no markers of HBV infection.

Children Seven children had markers of HBV infection; 2 were HBsAg positive, and 5 were HBV DNA positive. Table 1 shows test results for 7 HBV-infected children and their mothers. In some cases, all HBV markers could not be analyzed because the volume of the blood sample was limited. All but one of the children were positive for anti-HBs. Anti-HBe was detected in 1 child, and anti-HBc was present in 4 children.

DISCUSSION

Recently it became evident that individuals with an anti-HBc–positive but HBsAg-negative serostatus can be viremic at a very low level. However, this low level of virus is sufficient to infect others, even in persons for whom the HBV DNA level is below the detection limit [1–5]. Similarly, this study shows that women who are anti-HBc positive but HBsAg negative can transmit HBV to their infants; ~7% of these infants were found to be positive for HBV DNA or HBsAg.

To date, serologic screening of pregnant women includes tests for HBsAg to search for newborns at risk for vertical transmission of HBV infection. However, use of highly sensitive nucleic acid amplification tests has shown that up to 30% of individuals with a past history of HBV infection retain viral DNA in their serum or blood cells. Such individuals have so-called occult HBV infection [7, 8]. Twenty years ago, Descos et al [9] evaluated peripartal HBV transmission from HBsAg-negative, anti-HBc–positive mothers. Vertical transmission occurred in 3 of 52 children. Surprisingly, these results did not prompt further studies. The infants in the study by Descos and colleagues did not receive vaccination against HBV nor were further follow-up findings reported, so it is unknown whether they developed chronic HBV infection [9]. In our study, the incidence of HBV transmission was similar, occurring in 7 of 105 children. The risk of vertical transmission is similar to the risk of transmission associated with blood transfusion and organ transplantation. Of the mothers of these 7 children, only 1 was HBV DNA positive at the time of delivery, which shows that, as with blood donors, even individuals without any mark-
Table 1. Serological Data on Hepatitis B Virus (HBV) Markers in Mother-Child Pairs Involving a Child with HBV Infection

<table>
<thead>
<tr>
<th>Serologic characteristic</th>
<th>Mother-child pair</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mother</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
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<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Anti-HBs level, IU/L</td>
<td>215</td>
<td>246</td>
<td>174</td>
<td>&gt;1000</td>
<td>652</td>
<td>&gt;1000</td>
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<tr>
<td>HBV DNA PCR result</td>
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<td>NA</td>
<td>Negative</td>
<td>Negative</td>
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</tr>
<tr>
<td><strong>Child</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, months</td>
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<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
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<tr>
<td>Vaccinations, no.</td>
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<td>0</td>
<td>0</td>
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<td>2</td>
<td>1</td>
<td>0</td>
<td></td>
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<tr>
<td>HBsAg test result</td>
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<td>Positive</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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<tr>
<td>Anti-HBs level, IU/L</td>
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<td>10</td>
<td>39</td>
<td>634</td>
<td>71</td>
<td>237</td>
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<td></td>
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<tr>
<td>Anti-HBe test result</td>
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<td>Negative</td>
<td>Positive</td>
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<td>Negative</td>
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<td>Negative</td>
<td></td>
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<tr>
<td>Anti-HBc test result</td>
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<td>Positive</td>
<td>Negative</td>
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<td>HBV DNA hybridization finding, copies/mL</td>
<td>Below LLQ</td>
<td>NA</td>
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<tr>
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<td>5</td>
<td>15</td>
<td>6</td>
<td>ND</td>
<td>10</td>
<td></td>
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<tr>
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<td>3</td>
<td>3</td>
<td>3</td>
<td>ND</td>
<td>2</td>
<td></td>
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<tr>
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<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
<td>Negative</td>
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<tr>
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<td>&gt;1000</td>
<td>&gt;1000</td>
<td>&gt;1000</td>
<td>ND</td>
<td>NA</td>
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<tr>
<td>Anti-HBe test result</td>
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<td>Negative</td>
<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
<td>NA</td>
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<tr>
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<td>NA</td>
<td>Negative</td>
<td>Marginal</td>
<td>ND</td>
<td>NA</td>
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<tr>
<td>HBV DNA hybridization finding</td>
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<td>NA</td>
<td>Negative</td>
<td>Negative</td>
<td>ND</td>
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<tr>
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<td>Negative</td>
<td>Negative</td>
<td>ND</td>
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<td></td>
</tr>
</tbody>
</table>

**NOTE.** Vaccinations were done using HBV surface antigen (HBsAg). Anti-HBc, antibody to HBV core antigen; anti-HBe, antibody to HBV e antigen; anti-HBs, antibody to HBsAg; LLQ, lower level of quantification (4342 copies/mL for the HBV DNA hybridization assay and 10 IU/mL [80 gEq/mL] for the HBV DNA Taq polymerase chain reaction [PCR]); NA, not analyzed; ND, not done because of loss of follow-up.

er of active HBV infection (ie, HBsAg and HBV DNA) can transmit the infection.

Probably the most clinically important issue is whether HBV-infected children develop a chronic infection. The development of chronic infection after perinatal transmission depends on the HBV serologic status and the level of HBV viremia. During the first phase of infection (when HBeAg is detectable), up to $10^{12}$ genome copies/mL circulate in the blood, but during the second phase, after seroconversion to anti-HBe, $<10^6$ genome copies/mL are detected. The third phase is the HBsAg-negative state, in which 2 serologic conditions can be distinguished. The first condition involves anti-HBc positivity alone (termed “anti-HBc alone”), and the second involves both anti-HBc and anti-HBs positivity. The anti-HBc–alone serostatus reflects either an unresolved HBV infection that has become chronic but remains in a late or “low-grade” productive state [10] or a state of late immunity after resolution of infection. Soon after infection, anti-HBs levels are known to decrease below the lower limit of detection, whereas anti-HBc persists. The second condition, anti-HBc and anti-HBs positivity, is believed to reflect a full recovery from HBV infection. However, up to 30% of individuals who are HBsAg negative still test positive for HBV DNA by PCR, though at very low titers, with median HBV DNA loads of 380 copies/mL [8, 11, 12]. Without vaccination, chronic carriage acquired via perinatal transmission occurs in 90% of children born to HBeAg-positive mothers and in ∼20% of those born to anti-HBe–positive mothers. Since a relatively high level of HBV DNA is necessary for chronic infection, only a small proportion of children born to HBsAg-negative mothers are likely to develop such infections. In our study, none of the 105 children developed chronic HBV infection, and the 7 children who had markers of HBV detected at birth seemed to clear the infection over the following months. However, the number of children for whom we have subsequent follow-up data is too small to precisely define the risk for chronic infection.

Although viral concentrations in HBsAg-negative, anti-HBc–positive individuals are usually low, rarely exceeding $10^6$ genome copies/mL, concentrations of HBV DNA can be much higher (ie, $10^9$ copies/mL) in individuals concomitantly infected with hepatitis C virus or HIV [13]. The condition of low viremia but high HBV DNA level may well be similar to that of patients...
receiving immunosuppressive therapy. In our study, no women were known to be coinfected or immunosuppressed.

In most countries with highly developed health care systems, blood and organ donors are screened for both HBsAg and anti-HBc [14]. Individuals positive for either marker are usually disqualified from donating, because of ongoing or past infections. Our data currently do not allow a recommendation regarding use of extended screening during pregnancy, because of the limited number of cases. Children born to HBsAg-negative but anti-HBc-positive women could be treated by active immunization or simultaneous active and passive vaccination immediately after birth, rather than only after 2–3 months. In our study, 6.2% of women were positive for anti-HBc. This prevalence is in accordance with results from studies by Jilg et al [15] and Thierfelder et al [16], who found 7%–8.7% of adults to be anti-HBc positive. In a country like Germany, with 680,000 live births per year, ~42,000 children are born to anti-HBc positive women. If only 1% of these children develop chronic infection, every year >40 children would become chronic carriers. Although this hypothesis is speculative, it may help explain the 20% of chronic HBV infections that are of unknown origin.

In our study, none of the infected children were positive for both HBsAg and HBV DNA. It is possible that they had very low levels of viral DNA and viral antigens, and, if so, insufficiently high lower limits of detection may have affected test results. Another possible explanation of these findings is that the assays yielded false-negative results. This would be the case especially, as for child 3, if the viral load was too low to create an HBsAg level that can be detected. Another explanation for negative HBsAg results could be mutations in the major antigenic determinant of HBsAg [17]. These mutated determinants are known to escape detection by commercially available enzyme-linked immunosorbent assays. Other mutations, such as deletions in the core promoter (eg, 8 nucleotide deletions in positions 1768–1775) and direct-repeat sequences, suppress HBV gene expression and lead to down-regulation of HBsAg expression [10, 18, 19]. False-positive results of anti-HBc tests have also been published. Although the manufacturer of the kit reports a specificity of 99.9%–100%, it is not possible to rule out that a number of our mothers had false-positive test results.

The clearance of HBV in our study children might be due to initial protection conferred by maternal antibodies and to subsequent protection by the child’s own antibody response, induced by regular immunization starting at 2–3 months.

Nevertheless, our results are alarming because their long-term implications are unknown. Several cases of HBV reactivation have been observed in immunosuppressed persons with seroconversion from anti-HBs to HBsAg [20–22].

In countries where passive HBV immunization is available, children born to HBsAg-positive mothers receive active and passive vaccination. With this strategy, 92%–94% of cases of chronic carriage can be avoided [23, 24]. However, even in countries where anti-HBV immunoglobulins are not available, active immunization alone, given immediately after birth, is able to prevent chronic infection in ~90% of children [25–27]. Thus, if there are subgroups with a high risk for HBV infection, active vaccination after birth represents an effective prevention strategy without significant adverse effects or costs.

There were some limitations in the present study. The follow-up schedule did not include testing of mothers. Such tests would have allowed us to detect whether mothers were first tested during a period of acute HBV infection. However, since none of the mothers reported any symptoms of hepatitis either at birth or afterwards, this seems unlikely. Another limitation is that not all 7 children with signs of HBV infection were tested a second time. Two patients were lost to follow-up because their parents did not consent to further examinations. This reflects an inherent difficulty of the study: because anti-HBc positivity without HBsAg is generally not believed to be a pathologic condition, it was challenging to convince parents that further investigations were warranted.

In conclusion, our findings showed occult HBV infection after perinatal transmission in a substantial number of infants born to anti-HBc-positive mothers. Although blood transfusions and organ transplants from persons positive for anti-HBc can result in chronic HBV infection in recipients, more studies are needed before further diagnostic or prophylactic measures can be implemented. The following 2 steps are necessary: genome sequence analyses of HBV DNA from mothers and their babies must be performed, and it must be demonstrated that despite current vaccination strategies some children develop chronic, not only acute, infection.

The estimated costs for an anti-HBc screening program for all pregnant women can be calculated using data collected from Germany. In 2003 there were 706,700 live births. A total of 6.2% of mothers are estimated to be positive for anti-HBc. The current recommendation is to start active vaccination of all children at the age of 3 months. To achieve complete active and passive vaccination immediately after birth, 1 additional dose of the active vaccine plus the HBV-specific immunoglobulin must be administered. This means an additional cost of $254 for the vaccine plus an estimated $241 for monitoring babies after immunoglobulin treatment, for a total cost of $495 per child. Therefore, an HBV prevalence of 6.2% among mothers during a year in which 706,700 live infants were delivered yields a cost of $2.2 million (costs were calculated in euros and converted to US dollars at a rate of 1.34). Furthermore, additional testing of all pregnant women with an anti-HBc test would be necessary. This could be done together with the HBsAg test and would mean additional costs of $13.1 million,
or $19 per pregnancy. This strategy could be cost-effective, because it would avoid lifetime costs for managing chronic hepatitis B and all of its known complications.

References