Injections given in healthcare settings as a major source of acute hepatitis B in Moldova

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Background Reported rates of acute hepatitis B are high in many former Soviet Union republics and modes of transmission are not well defined.

Methods Two case-control studies were undertaken in Moldova to identify risk factors for acute hepatitis B in people aged 2–15 years (children) and ≥15 years (adults). Serologically confirmed acute hepatitis B cases occurring between 1 January 1994 and 30 August 30 1995, were matched on age, sex, and district of residence to three potential controls who were tested for hepatitis B markers to exclude the immune. Stratified odds ratios (SOR) were calculated using bivariate and multivariate methods.

Results In multivariate analysis, compared with the 175 controls, the 70 adult cases (mean age 25 years, 66% male) were more likely to report receiving injections in the 6 months before illness during a dental visit (SOR = 21; 95% CI : 3.7–120), a hospital visit (SOR = 35; 95% CI : 7.2–170), or a visit to the polyclinic (SOR = 13; 95% CI : 2.4–74). Among children, receiving injections during a hospital visit (SOR = 5.2; 95% CI : 1.2–23) was the only exposure reported significantly more often by the 19 cases (mean age 8 years, 68% male) compared with the 81 controls.

Conclusion These results, along with reported unsafe injection practices in Moldova, suggest that injections are a major source of hepatitis B virus transmission and highlight the importance of proper infection-control procedures in preventing transmission of blood-borne infections.

Keywords Case-control studies, cross-infection, hepatitis B, infection control, injection adverse effects, Moldova, risk factors, sterilization

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Acute hepatitis B is one of the most common reportable diseases in Moldova (Ministry of Health unpublished data), a former republic of the Soviet Union located between Romania and Ukraine where the prevalence of chronic hepatitis B virus (HBV) infection is high.1 Hepatitis B is also a common cause of chronic liver disease, which is a major cause of death among adults in Moldova.

Although the routes of HBV transmission among children and adults in Moldova have not been studied, injections in healthcare settings were suspected to be a source of HBV infection. We conducted two case-control studies to determine the relative importance of injections and other exposures as a source of acute hepatitis B in Moldova.

Methods

Case ascertainment

Case-control studies were conducted among adults (≥15 years) and children (2–15 years). Potential cases were defined as physician-diagnosed acute hepatitis B in a patient whose serum sample tested positive for HBsAg in the laboratory of the local health department. Potential cases reported between 1 January 1994 and 30 August 1995, in 8 of the 44 Moldovan districts were eligible. Serum samples were also tested at the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA, to confirm the initial diagnosis. Potential cases for which HBsAg was not confirmed on testing at CDC or for which the serum sample was negative for IgM antibody to hepatitis B core antigen (IgM anti-HBc) were excluded from the analysis.
Control recruitment

For each potential case, three potential controls matched for age (5-year age group) and sex were selected at random in the neighbourhood. A serum sample was taken from each subject. Controls for which the sample was positive for total antibodies to hepatitis B core antigen (total anti-HBc) on testing at CDC were considered immune to hepatitis B and excluded from the analysis.

Data collected

The local public health officer interviewed potential case-patients and controls with a standardized questionnaire. Information was collected on age, sex, primary language spoken, and potential exposures during the referent period (i.e. 2–6 months before disease onset for cases or recruitment for controls). Exposures for which information was collected for adults and children included injections received (number and healthcare site), contact with a jaundiced or HBsAg-positive person, hospitalization, surgery, blood transfusion, and blood collection with a lancet. For adults, questions were asked regarding occupational exposure to blood, number of sex partners, blood donation, same sex sexual intercourse, and injecting drug use. For children, additional information was collected on contacts with a person with jaundice or hepatitis in the household, exposure to a jaundiced child while playing, skin lesions, exposure to blood in the household, and sharing of household items.

Laboratory methods

Blood samples were drawn and centrifuged within 6 h of collection. Reverse passive haemagglutination assays and non-standardized enzyme-immunoassay were used in Moldova to screen potential case-patients for HBsAg. Additional serum supernatants from controls and from case-patients who tested positive for HBsAg in Moldova were frozen at −20 °C, shipped to CDC and tested using commercial assays (Abbott Laboratories, North Chicago, Illinois). All serum samples were tested for total anti-HBc using a radio-immunoassay; total anti-HBc positive serum samples were tested for HBsAg using a radio-immunoassay. The HBsAg-positive serum samples were then tested for IgM anti-HBc using an enzyme-immunoassay.

Sample size and data analysis

For each age group, the initial objective was to recruit 200 cases with three matched controls per case to detect, with a statistical significance, a difference between a 20% and a 10% prevalence of exposure among cases and controls (with an alpha error of 0.05 and 80% power). Mantel-Haenszel stratified odds ratios (SOR), Cornfield CI, and the sample size were calculated using Epi-Info version 6 (CDC, Atlanta, Georgia). Variables for which the P-value in stratified analysis was ≤0.2 and variables that had been associated with HBV infection in previous studies were tested in a stepwise forward conditional logistic regression model using EGRET software (SERC, Seattle, Washington). Population attributable risks for exposures independently associated with acute hepatitis B in logistic regression were calculated using a method compatible with a stratified estimate of the odds ratio.2

Human subjects

Informed written consent was obtained from study participants or from parents of children. The study protocol was approved by the Ethical Committee of the Moldovan Ministry of Health and by the Institutional Review Board at CDC.

Results

Case-control study in adults

Of the 140 potential cases identified for enrolment, 67 were excluded. The reasons for exclusion were absence of a serum specimen (N = 16), being IgM anti-HBc negative (N = 49), and being HBsAg negative on testing at the CDC (N = 2). Of the 413 potential controls, 219 were excluded. Reasons for exclusion were absence of serum sample (N = 3), being total anti-HBc positive (N = 214), and being HBsAg positive (N = 2).

Exclusion of cases and controls that were not biologically confirmed at CDC disrupted matched sets, leading to further exclusion of cases and controls. To avoid excessive power loss, the initial case-control match was broken. New strata were reorganized on the basis of identical area of residence (using four more broadly defined areas), sex, and age (using five age groups). The analysis was based upon 70 cases and 175 controls re-arranged in 20 strata, which limited the exclusion to an additional 3 cases and 19 controls.

The mean age of case-patients was 25 years (range 15–68, standard deviation [SD] = 11.2 years) and 46 (66%) were male. The primary language spoken by case-patients was Romanian (N = 46, 66%), Russian (N = 14, 20%), Ukrainian (N = 4, 6%), and other (N = 6, 9%).

Compared with controls, case-patients were more likely to report having received injections at a hospital, a dental clinic a polyclinic, or an outpatient department (Table 1). Receiving any injection was also associated with being a case when the study participants who had received injections with sterilizable syringes were excluded (SOR = 14, 95% CI : 4.5–44, data not shown). Other healthcare-related exposures associated with being a case included reporting having spent a night at the hospital, undergoing major surgery, receiving stitches, and receiving a blood transfusion (Table 1). Casual contact with a jaundiced person or with an HBsAg-positive person was reported significantly more often by case-patients than by controls. Other exposures examined were not significantly associated with acute hepatitis B.

When the analysis was restricted to study participants who did not report receiving injections, none of the other healthcare-associated exposures were associated with acute hepatitis B (data not shown). However, there was an association between reported casual contact with an HBsAg-positive person and acute hepatitis B (SOR indeterminate, lower limit of the 95% CI of the OR in univariate analysis: 1.6, P = 0.06, data not shown).

In multivariate analysis, report of injections received at the hospital, at the dentist or at the polyclinic were the only exposures associated with being a case (Table 2). The population attributable risk associated with receiving an injection in any of these healthcare settings was 52% (95% CI : 47–54).

Case-control study in children

Of the 56 potential cases identified for the study, 35 were excluded. The reasons for exclusion were absence of a serum specimen (N = 12), being IgM anti-HBc negative (N = 20), and being HBsAg negative on testing at CDC (N = 3). Of the 168
potential controls, 57 were excluded because they were total anti-HBc positive. The analysis was therefore based upon 19 cases and 81 controls re-arranged in 12 strata defined, like for the adults, on the basis of identical area of residence (using the same four geographical areas), sex, and age (using three age groups). This re-arrangement limited the further exclusion to an additional 2 cases and 30 controls.

The mean age of case-patients was 8 years (range 2–14, SD = 3.8 years) and 13 (68%) were boys. The primary languages spoken by case-patients were Romanian (N = 15, 79%) and Russian (N = 4, 21%).

Compared with controls, case-patients were more likely to report having received injections at the hospital (Table 3), but there was no significant association with reported injections received in other healthcare settings. Other healthcare-related exposures were not significantly associated with being a case (Table 3). Contact with a person with hepatitis or jaundice was not significantly associated with acute hepatitis B.

In multivariate analysis, the association between receiving an injection in the hospital and acute hepatitis B was not confounded by any other exposure, and no other exposure was associated with illness when adjusted for receipt of an injection at the hospital. The population attributable risk associated with receiving an injection at the hospital was 21% (95% CI: 4.4–25).

Discussion
The results of this study suggest that injections received in various healthcare settings were associated with acute hepatitis B in Moldova. This association was stronger and accounted for a higher proportion of cases in adults than children. In addition, injections in a greater variety of healthcare settings were associated with illness in adults compared with children. Nevertheless, since the incidence of HBV infection is high during childhood,1 and people infected in childhood are at higher risk of long-term consequences of HBV infection,3 HBV infection secondary to injections is a major public health problem for both adults and children in Moldova.

In this study, report of contact with a HBsAg-positive person was also associated with illness. However, we could not identify a statistically significant association between acute hepatitis B and other exposures that have been associated with hepatitis B in other published studies (e.g. multiple sex partners, male homosexual behaviour or occupational exposure to blood). Few patients reported these exposures, limiting the power to detect an association with illness.

Overuse of injections is common in Moldova and in other parts of the world.4–7 Reasons for popular demand for injections include beliefs that the pain of the injection is a marker of efficacy, that a drug is more efficient when entering the body
directly, and that injections represent a more advanced technology. Among healthcare workers, motivations for overuse of injections include belief of a better efficacy of injected drugs, ability to directly observe therapy, and, sometimes, financial incentives.

In Moldova, sterilizable and disposable syringes were commonly re-used after sterilization procedures that were not quality-controlled, and use of multi-dose medication vials was common (Ministry of Health, unpublished data). Among children, the hospital was the only healthcare facility for which injections were associated with acute hepatitis B, suggesting that children may be exposed to relatively safer injection practices in Moldova. Since 1987, the Moldovan Ministry of Health has recommended that the limited supply of disposable injection equipment be used primarily for children.

The risk of HBV transmission following percutaneous exposure is high (at least 30%) after a needle-stick from an infected source-patient. An association between HBV infection and injections has been reported in India, Taiwan, Egypt, and the Sudan, but the proportion of HBV infection attributable to unsafe injection practices has not been commonly measured. In Moldova, calculation of the population attributable risk suggests that injections may explain 52% of acute hepatitis B cases occurring in adults and 21% of such cases in children.

Although not directly assessed in our study, injections in Moldova may transmit other blood-borne pathogens. The risk of hepatitis C virus (HCV) infection following a needle-stick from an infected source patient averages 5%. Transmission of HCV infection through unsafe injection practices has been reported in Egypt and in Pakistan. Transmission of human immunodeficiency virus (HIV) through unsafe injection practices has also been reported. However, because the risk of HIV infection is approximately 0.3% following a needle-stick from an infected source patient, injections received in healthcare settings probably account for a small proportion of HIV infections. Other adverse effects of injections include provocation paralysis during the course of infection with wild or vaccine-strain polioviruses, aseptic abscesses, and transmission of viral hemorrhagic fever viruses.

Our study has limitations. Firstly, the combination of a matched study design with inclusion criteria based upon laboratory testing conducted in another country led to the exclusion of several cases and controls. Reorganization of the sets minimized adverse consequences of this design. Secondly, the association between receiving an injection at the dentist and acute hepatitis B may be difficult to differentiate from exposures to other dental instruments potentially contaminated with blood. Adverse effects of injections may not be immediately apparent and they cause a burden of chronic infections often difficult

### Table 2

Factors associated with an acute hepatitis B in multivariate analysis, adults ≥15 years of age, Moldova, 1994–1995

<table>
<thead>
<tr>
<th>Exposures reported by study participants</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injections at the hospital</td>
<td>35</td>
<td>7.2–170</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Injections at the dentist</td>
<td>21</td>
<td>3.7–120</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Injections at the polyclinic</td>
<td>13</td>
<td>2.4–74</td>
<td>0.003</td>
</tr>
</tbody>
</table>

### Table 3

Potential exposures to hepatitis B virus infection in acute hepatitis B cases and controls, children <15 years, Moldova, 1994–1995

<table>
<thead>
<tr>
<th>Exposures reported by study participants</th>
<th>Cases (N = 19)</th>
<th>Controls (N = 81)</th>
<th>SOR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection at the polyclinic</td>
<td>5</td>
<td>5</td>
<td>1.0</td>
<td>0.1–10</td>
</tr>
<tr>
<td>Injection at the hospital</td>
<td>6</td>
<td>7</td>
<td>2.5</td>
<td>1.2–5.2</td>
</tr>
<tr>
<td>Injection at school</td>
<td>2</td>
<td>6</td>
<td>3.1</td>
<td>1.3–8.1</td>
</tr>
<tr>
<td>Anaesthetic injection at the dentist</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
<td>0.0–1.4</td>
</tr>
<tr>
<td>Injection by the paramedic</td>
<td>1</td>
<td>3</td>
<td>0.3</td>
<td>0.0–4.4</td>
</tr>
<tr>
<td>Injection with sterilizable syringe</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
<td>0.0–79</td>
</tr>
<tr>
<td>Blood taken with a lancet</td>
<td>2</td>
<td>5</td>
<td>1.5</td>
<td>0.2–9.5</td>
</tr>
<tr>
<td>Night spent at a hospital</td>
<td>4</td>
<td>6</td>
<td>3.7</td>
<td>1.2–11.6</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
<td>0.0–1.4</td>
</tr>
<tr>
<td>Major surgery</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>0.1–10</td>
</tr>
<tr>
<td>Stitches</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>0.1–10</td>
</tr>
<tr>
<td>Skin lesions</td>
<td>3</td>
<td>10</td>
<td>16.0</td>
<td>4.4–44.4</td>
</tr>
<tr>
<td>Playing with a jaundiced child</td>
<td>3</td>
<td>2</td>
<td>1.5</td>
<td>0.7–3.7</td>
</tr>
<tr>
<td>Exposure to blood at home</td>
<td>0</td>
<td>1</td>
<td>0.0</td>
<td>0.0–1.4</td>
</tr>
<tr>
<td>Sharing tooth brushes</td>
<td>1</td>
<td>1</td>
<td>1.0</td>
<td>0.1–10</td>
</tr>
<tr>
<td>Sharing nail clippers</td>
<td>16</td>
<td>71</td>
<td>0.91</td>
<td>0.1–5.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Stratified odds ratio.
to quantify. However, transmission of blood-borne pathogens through unsafe injection practices is a problem increasingly identified worldwide. Routine infant hepatitis B vaccination through unsafe injection practices is a problem increasingly identified worldwide.  Studies should be conducted to measure the risk of infection with blood-borne pathogens associated with injections in various settings worldwide, including in other former Soviet Union republics where treatment protocols were comparable to those used in Moldova. In the future, priority should be given to studies evaluating the effectiveness of various interventions aiming at preventing injection adverse events through improvement of injection safety and reduction of injection frequency.

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References