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Prevalence of hepatitis B, D and C virus infections among children and pregnant women in Moldova: additional evidence supporting the need for routine hepatitis B vaccination of infants

J. DROBENIUC[1], Y. J. F. HUTIN[2]*, R. HARPAZ[3], M. FAVOROV[2], A. MELNIK[1], P. IAROVOI[1], C. N. SHAPIRO[2] AND B. A. WOODRUFF[4]

[1] *National Centre for Scientific and Applied Preventive Medicine, Chisinau, Republic of Moldova*
[2] *Hepatitis Branch, Division of Viral and Rickettsial Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, GA*
[3] *Child Vaccine-Preventable Diseases Branch, Epidemiology and Surveillance Division, National Immunization Program, Centers for Disease Control and Prevention, Atlanta, GA*
[4] *International Emergency and Refugee Health Program, Emergency Response Coordination Group, National Center for Environmental Health, Centers for Disease Control and Prevention, Atlanta, GA*

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SUMMARY

Rates of acute hepatitis B are high in Moldova, but the prevalence of chronic infection is unknown. In 1994, we surveyed children and pregnant women, collected demographic information, and drew blood for laboratory testing. Among the 439 children (mean age, 5 years), the prevalence of antibody to hepatitis B core antigen (anti-HBc) and hepatitis B surface antigen (HBsAg) were 17·1 and 6·8%, respectively. Among the 1098 pregnant women (mean age, 26 years), 52·4% were anti-HBc-positive and 9·7% were HBsAg-positive. Of the HBsAg-positive pregnant women, 35·6% were hepatitis B e antigen (HBeAg) positive and 18·3% had antibodies to hepatitis D virus. The prevalence of antibody to hepatitis C virus was 1·4% in children and 2·3% in pregnant women. The high HBeAg prevalence among HBsAg-positive pregnant women and the high anti-HBc prevalence among children indicate that both perinatal and early childhood transmission contribute to the high hepatitis B virus endemicity in Moldova.

INTRODUCTION

Although hepatitis B virus (HBV) infection is preventable by vaccination, it remains endemic throughout many regions of the world. In highly endemic areas, infection occurs early in life, often resulting in chronic infection [1] and premature death in adulthood because of cirrhosis and hepatocellular carcinoma. In Moldova, a former Soviet Union republic located between Romania and Ukraine, reported rates of acute hepatitis B are high among children and adults (Moldova Ministry of Health, unpublished data) and the prevalence of chronic infection is high [2, 3]. Starting in 1989, the Ministry of Health increased the availability of disposable syringes for children and instituted a programme to prevent perinatal HBV infection. This programme consisted of universal hepatitis B surface antigen (HBsAg) screening of pregnant women and administration of hepatitis B vaccine starting at birth to all infants of HBsAg-positive mothers.

Studies of the prevalence of HBsAg in the adult population and among pre-school children provide useful information to guide prevention strategies. In areas where the HBsAg prevalence is high among

* Author for correspondence: Dr Yvan Hutin, MS G37, Hepatitis Branch, Centers for Disease Control and Prevention, 1600 Clifton Rd, Atlanta, GA 30333.

pre-school children, the prevalence of HBsAg among pregnant women and of hepatitis Be antigen (HBeAg) among those who are HBsAg-positive can help to predict the incidence of perinatal transmission and determine the optimal hepatitis B vaccination strategy. For this purpose, we surveyed children and pregnant women for serological markers of HBV infection during 1994–5 in Moldova. Participants were also tested for antibodies to hepatitis D virus (HDV) and hepatits C virus (HCV) to estimate the prevalence of these infections in Moldova.

MATERIALS AND METHODS

Epidemiological methods

Children and pregnant women were sampled for participation using a two-stage cluster sample. Eleven of the 44 rural and urban districts in Moldova were selected with a probability proportional to size. Within each selected district, a prenatal clinic, a first-grade classroom, and a pre-school group were selected at random. In each pre-school group or classroom, the first 20 children on the registration list were included in the study. The next child on the list replaced children whose parents refused participation. A standardized questionnaire was administered collecting information about age, sex and district of residence. In each prenatal clinic, the first 100 pregnant women presenting during September and October 1994 for routine HBsAg testing were included in the study. Information about age and district of residence was collected on a standardized form.

Laboratory methods

Serum samples were shipped to the Centers for Disease Control and Prevention (CDC) for serological testing using commercial tests (Abbott Laboratories, Abbott Park, IL, USA). All sera were tested for total antibody to hepatitis B core antigen (anti-HBc) using a radio-immunoassay (CORAB). Anti-HBc-positive sera were tested for HBsAg using a radio-immunoassay (AUSRIA II-125). Sera positive for HBsAg were tested for HBeAg, for IgM anti-HBc and for antibody to HDV (anti-HDV) using an enzyme-immunoassay (EIA). All sera were tested for antibody to HCV (anti-HCV) using EIA 2·0 (second generation), and repeatedly reactive specimens were tested with a semi-automated immunoblot supplemental assay (Matrix HCV).

Sample size and statistical methods

The sample size of 200 children was chosen to obtain 95% confidence limits of ± 3 percentage points around the point estimate of the prevalence. This calculation was based upon an estimated population size of 250000 in each of the two age groups and a 40% expected prevalence of anti-HBc. The sample size of 1000 pregnant women was chosen to obtain 95% confidence limits of ± 2 percentage points around the point estimate of the prevalence. This calculation was based upon an estimated population size of 54000 and a 10% expected prevalence of HBsAg.

For the purpose of the analysis, the 11 selected districts of Moldova were grouped into a northern region (2 districts), a central region (5 districts) and a southern region (4 districts) [3]. Confidence intervals for proportions were calculated in the CSAMPLE module of Epi-Info to take into account the design effect of the cluster sample [4]. Prevalences were compared by the calculation of the prevalence ratio [PR] using Mantel–Haenszel adjustment when appropriate. Sample size and other statistical calculations were done using Epo-Info version 6 [5].

Human subjects

Parents of children gave informed written consent and the network of public medical care providers returned all serological results to them. Serum samples for pregnant women were collected as part of the Moldovan routine HBsAg screening programme; results of the tests conducted at CDC were not returned to study participants, and test results for anti-HCV and anti-HDV were not linked to individual pregnant women to preserve their anonymity. The study protocol was approved by the Scientific Advisory Board of the Prophylactic and Clinical Medicine Research Institute in Chisinau, by the ethical committee of the Ministry of Health of the Republic of Moldova, and the Institutional Review Board of the CDC.

RESULTS

Children

Of the 440 children recruited, 439 children had a serum sample available and were included in the study. The mean age of the pre-school children was 3·5 years (standard deviation [SD] 1·3 years; range 0–6

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Table 1. *Prevalence of serological markers of hepatitis B and C virus infection in children, by age and sex, Republic of Moldova, 1994*

	HBsAg			Total anti-HBc			Anti-HCV			
	Positive	(%)	95% CI	Positive	(%)	95% CI	Positive	(%)	95% CI	Total
Age										
Pre-school	13	4·6	2·3–6·9	31	11·0	6·8–15·2	2	0·7	0–1·6	281
First-grade	17	10·8	5·1–16·6	44	27·8	12·7–42·9	4	2·5	0–6·4	158
Sex										
Boys	27	10·3	5·2–15·4	53	20·2	11·3–28·9	3	1·1	0–2·8	263
Girls	3	1·7	0–3·4	22	12·5	6·2–18·8	3	1·7	0–4·0	176
Total	30	6·8	3·6–10·1	75	17·1	9·6–24·7	6	1·4	0–3·2	439

years). Four (1·4%) of the pre-school children were under 2 years of age. The mean age of the first-graders was 7 years (S.D. 0·5 years, range 7–13 years). Seventy-five children (17·1%) were anti-HBc positive, consistent with past or present HBV infection (Table 1). Thirty children (6·8%) were HBsAg positive. The prevalence of both anti-HBc and HBsAg was higher among first-graders than among pre-school children (anti-HBc: PR = 2·5, 95% CI 1·7–3·8; HBsAg: PR 2·3, 95% CI 1·5–3·8). The prevalence of anti-HBc was higher among boys than girls, although this difference was not statistically significant (age-adjusted PR 1·4; 95% CI 0·9–2·1). However, the prevalence of HBsAg was significantly higher among boys than girls (age-adjusted PR 5·4, 95% CI 1·6–18). The prevalence of serological markers of HBV infection did not vary significantly among the three regions of the country (data not shown). Of the 29 HBsAg-positive children whose serum could be tested for total anti-HDV, 4 (13·8%) were positive. Among the 439 samples of the study children, 6 (1·4%) were positive for anti-HCV.

Pregnant women

Of the 1100 pregnant women recruited, 1098 had a serum sample available and were included in the study. Among the women studied (mean age 26 years, SD 6), 575 (52·4%; 95% CI 45·4–59·3) were anti-HBc-positive and 106 (9·7%; 95% CI 7·1–12·3) were HBsAg positive. Of the 104 HBsAg-positive pregnant women whose serum could be tested, 37 (35·6%; 95% CI 28·7–42·5) were HBeAg positive, none was IgM anti-HBc-positive, and 19 (18·3%; 95% CI 11·4–27·1) were anti-HDV positive. The prevalence of anti-HBc increased from the northern to the southern region (29·5%, 37·1 and 47·3% in the northern, centre and southern regions, respectively; $P < 0·001$). The prevalence of HBsAg also presented the same north to south gradient (2·5, 10·2 and 12·5% in the northern, centre and southern regions, respectively; $P < 0·001$).

Of the 1098 pregnant women tested, 25 (2·3%, 95% CI 1·0–3·5) were anti-HCV positive.

DISCUSSION

The results of this study demonstrate that chronic HBV infection is common in Moldova. The prevalence of HBsAg among pregnant women, a surrogate group for the general population, was 9·7%. This level of HBsAg prevalence indicates that HBV infection in Moldova can be characterized as highly endemic (prevalence of HBsAg exceeding 8% in the general adult population [6]). However, despite a high prevalence of anti-HBc, almost half of the adult population was susceptible to HBV infection.

Based on the HBsAg prevalence among pregnant women (9·7%), the HBeAg prevalence among HBsAg-positive pregnant women (36%), and on reported risk of HBV transmission to infants born to HBeAg-positive and HBeAg-negative HBsAg-positive mothers (85 and 30% respectively [7]), an estimated 5% of Moldovan infants acquire HBV infection perinatally. Thus, the 27·8% prevalence of anti-HBc among children in first-grade in our study is higher than expected from perinatal transmission alone, suggesting a high incidence of HBV infection in early childhood. The exact incidence of HBV infection in children could not be inferred from these cross-sectional data. The two age groups included in this study may have been exposed to different risks of HBV infection in children, as prevention activities, including use of hepatitis B vaccine, were implemented in Moldova starting in 1989.

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Modes of HBV transmission in children are difficult to identify precisely [8]. Other children probably represent an important source of infection for children in Moldova, because the prevalence of HBsAg is high in this age group. Clustering of infection in households [9] suggests that HBV is acquired from other infected household members, probably through exposure to small quantities of blood [10–12]. In addition, one study showed that approximately 30% of acute hepatitis B cases among children under 15 years of age in Moldova are associated with injections given in various health-care settings [13].

Boys in Moldova tended to have a higher prevalence of anti-HBc than girls, although the difference was not statistically significant. However, compared with girls, boys had a higher prevalence of HBsAg. A higher HBsAg prevalence among boys has also been observed in West Africa [12, 14] and in Taiwan [15]. A more prolonged active, HBsAg-positive phase of HBV infection in boys is the proposed explanation for the differences in HBsAg prevalence observed between boys and girls [14].

The overall HBsAg prevalence among pregnant women in the Republic of Moldova is similar to that reported in Romania [16, 17]. However, the HBeAg prevalence among HBsAg-positive pregnant women was lower (2%) in north-eastern Romania than in our study [17]. The reason is unclear for the differences in HBeAg prevalence between populations that would be expected to be close in background and socio-economic status.

Co-infection with HDV in HBsAg-positive persons may increase the risk of rapid development of chronic liver disease. The prevalence of anti-HDV among HBsAg-positive children and pregnant women suggests moderate HDV endemicity [18]. This endemicity level probably contributes to the burden of chronic liver diseases in Moldova. The prevalence of anti-HCV among pregnant women in Moldova was intermediate (ie between 0·1 and 5% [19]), as seen in the United States and in European countries [20]. Modes of HCV transmission have not been studied in Moldova.

The high HBeAg prevalence in HBsAg-positive pregnant women and the high HBsAg prevalence in children indicate that both perinatal and early childhood transmissions are important sources of infection among children in Moldova. Starting in 1989, universal HBsAg screening of pregnant women and vaccination of infants born to HBsAg-positive mothers starting at birth was initiated in Moldova to prevent perinatal HBV transmission. In 1994, hepatitis B vaccination of all infants at birth was added. Hepatitis B vaccine, when started at birth, has high efficacy in preventing perinatal HBV infection even when hepatitis B immune globulin is not given [21]. Therefore, some countries or regions (eg Alaska) have either not included maternal HBsAg screening as part of their hepatitis B prevention strategies or stopped maternal HBsAg screening [22] [CDC, unpublished data]. Stopping maternal HBsAg screening might be considered in Moldova where a high proportion of infants receive their first dose of hepatitis B vaccine at birth.

Routine infant hepatitis B vaccination should reduce the risk of HBV infection and its sequelae among children in Moldova [23]. However, infant vaccination alone will not eliminate the risk of HBV infection in older cohorts for many years; furthermore, it will not protect persons from infection with HCV and other bloodborne pathogens. Thus, prevention programmes, including initiatives to improve injection practices [13], should be initiated particularly among older cohorts.

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REFERENCES

1. McMahon BJ, Alward WL, Hall DB, Heyward WL, Bender TR, Francis DP. Acute hepatitis B virus infection: relation of age to the clinical expression of

disease and subsequent development of the carrier state. J Infect Dis 1985; **151**: 599–603.
2. Kostritsa SS, Kusin SN, Khukhlowitch PA, Shakhgildian IV, Dorsheno NV, Blinov VM. The frequency of antibody to hepatitis C virus and HBsAg detection in blood donors, patients with viral hepatitides and oncological diseases in Moldova Republic. Vopr Virusol 1994; **39**: 93–4.
3. Drobeniuk ZA, Mikhailov MI, Bokancha GV, Iarovoi PI, Anan'ev VA, Shakhgil'dian IV. The frequency of detection of HBsAg, anti-HBs and anti-HBc in the healthy population of the Moldovan SSR. Vopr Virusol 1988; **33**: 621–4.
4. Bennett S, Woods T, Liyanage WM, Smith DL. A simplified general method for cluster-sample surveys of health in developing countries. World Health Stat Q 1991; **44**: 98–106.
5. Dean AG, Dean JA, Coulombier D, et al. Epi Info, Version 6: A word-processing, database, and statistics program for public health on IBM-compatible microcomputers. Atlanta, Georgia, USA; Centers for Disease Control and Prevention, 1994.
6. Maynard JE, Kane MA, Hadler SC. Global control of hepatitis B through vaccination: role of hepatitis B vaccine in the Expanded Programme on Immunization. Rev Infect Dis 1989; **11** (Suppl 3): S574–8.
7. Beasley RP, Trepo C, Stevens CE, Szmuness, W. The e antigen and vertical transmission of hepatitis B surface antigen. Am J Epidemiol 1997; **105**; 94–8.
8. Davis LG, Weber DJ, Lemon SM. Horizontal transmission of hepatitis B virus. Lancet 1989; **i**: 889–93.
9. Whittle HC, Bradley AK, McLauchlan K, Ajdukiewicz AB, Howard CR, Zuckerman AJ. Hepatitis B virus infection in two Gambian villages. Lancet 1983; **i**: 1203–6.
10. Petersen NJ, Barrett DH, Bond WW, Berquist KR, Favero MS, Bender TR. Hepatitis B surface antigen in saliva, impetiginous lesions, and the environment in two remote Alaskan villages. Appl Environ Microbiol 1976; **32**: 572–4.
11. Vall Mayans M, Hall AJ, Inskip HM, Chotard J, Lindsay SW, Coromina E. Risk factors for transmission of hepatitis B virus to Gambian children. Lancet 1990; **336**: 1107–9.
12. Martison FEA, Weigle KA, Royce RA, Weber DJ, Suchindran CM, Lemon SM. Risk factors for horizontal transmission of hepatitis B virus in a rural district in Ghana. Am J Epidemiol 1998; **147**: 478–87.
13. Hutin YJF, Harpaz R, Drobeniuc J, et al. Injections given in health care settings as a major source of acute hepatitis B in Moldova. Int J Epidemiol 1999; **28**: 782–6.
14. Coursaget P, Yvonnet B, Chotard J, Vincelot P, Sarr M, Diouf C. Age- and sex-related study of hepatitis B virus chronic carrier state in infants from an endemic area (Senegal). J Med Virol 1987; **22**: 1–5.
15. Beasley RP, Hwang LY, Lin CC, Leu ML, Stevens CE, Szmuness W. Incidence of hepatitis B virus infections in preschool children in Taiwan. J Infect Dis 1982; **146**: 198–204.
16. Paquet C, Babes VT, Drucker J, Senemaud B, Dobrescu A. Viral hepatitis in Bucharest. Bull WHO 1993; **71**: 781–6.
17. Woodruff BA, Popovici F, Beldescu N, Shapiro CN, Hersh BS. Hepatitis B virus infection among pregnant women in northeastern Romania. Int J Epidemiol 1993; **22**: 923–6.
18. Polish LB, Gallagher M, Fields HA, Hadler SC. Delta hepatitis: molecular biology and clinical and epidemiological features. Clin Microbiol Rev 1993; **6**: 211–29.
19. Alter MJ. Epidemiology and disease burden of hepatitis B and C. Antiviral Ther 1996; **1** (suppl. 3): 9–14.
20. Alter MJ. Epidemiology of hepatitis C in the West. Semin Liver Dis 1995; **15**: 5–14.
21. Poovorawan Y, Sanpavat S, Pongpunlert W, Chumderpadetsuk S, Sentrakul P, Safary A. Protective efficacy of a recombinant DNA hepatitis B vaccine in neonates of HBe antigen-positive mothers. JAMA 1989; **261**: 3278–81.
22. Hall AJ, Roberston RL, Crivelli PE, Lowe Y, Inskip H, Snow SK. Cost-effectiveness of hepatitis B vaccine in The Gambia. Trans R Soc Trop Med Hyg 1993; **87**: 333–6.
23. Centers for Disease Control and Prevention. Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR 1991; **40** (RR-13).