

## Original Report

# Prevalence of Hepatitis Virus Infections in Kosovar Refugees

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### ABSTRACT

**Objectives:** To assess the prevalence of viral hepatitis infections in a sample of Kosovar refugees having arrived in southern Italy as a result of the 1999 war in the Balkans.

**Methods:** The 526 subjects who enrolled on voluntary basis from all age groups were tested for the prevalence of serologic markers for hepatitis virus types A, B, C, D, and E (HAV, HBV, HCV, HDV, HEV).

**Results:** Among the 526 refugees, the prevalence of total anti-HAV antibodies was 81%. A relevant finding was the presence of total anti-HAV antibodies in 61% of the children up to 10 years of age. The prevalence of anti-HEV antibodies was 2.5% among the subjects. Fifteen subjects (2.9%) were positive for hepatitis B surface antigen (HBsAg), whereas 17.5% tested positive for anti-hepatitis B core antigen (anti-HBc). In children up to 10 years of age, the prevalence of HBsAg and anti-HBc was found to be 0.4% and 6%, respectively. In subjects aged 11 to 20 years, 4.2% tested positive for HBsAg and 20.2% for anti-HBc. In the age group 21 to 30 years, 7.1% of the subjects were found to be HBsAg carriers, whereas 25.9% were found to be positive for anti-HBc. Among the refugees over 30 years of age, the prevalence of HBsAg was 4.2%, whereas anti-HBc was 43.7%. None of the refugees tested positive for anti-HDV. The prevalence of anti-HCV antibodies was 0.7%.

**Conclusions:** The results of this seroepidemiologic study indicate a high circulation of HAV in the Kosovar population, whereas the prevalence of HEV antibodies was low and comparable to that of other European countries. The HBV infection seems to be at an intermediate level of endemicity and an immunization policy against HBV infection, through vaccination of all newborns and children before adolescence, may be advisable. Results of this study indicate that the level of endemicity of HCV infection in the Kosovar population is low.

**Key Words:** Kosovar refugees, seroepidemiology, viral hepatitis

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Since the early 1990s, Puglia (southern Italy) has played the important role of frontier region in the Mediterranean area because of its vicinity to the Balkans.

In March and April 1999, as a result of the armed conflict in the Kosovo province of the Federal Republic of Yugoslavia, about 500,000 to 850,000 ethnic Albanians from Kosovo were forced to leave their homes and their land to seek refuge in neighboring countries, mainly Albania, Macedonia, and Montenegro.<sup>1,2</sup> As a result of these massive movements, many Kosovar people reached the coast of Puglia principally via Montenegro and Albania. Upon arrival, the refugees, mainly children, were housed in refugee camps. This had also been the practice in 1991 and 1997, when Puglia experienced an influx of Albanian refugees. The experience achieved in the field of public health during the two previous emergencies had permitted the planning of adequate preventive measures for this more recent exodus of Kosovar refugees. In 1991 and 1997, the purposes of survey studies carried out among the Albanian refugees were the surveillance of infectious diseases and the evaluation of the immunization coverage against vaccine-preventable diseases.<sup>3–9</sup>

Viral hepatitis still represents a major health problem in some areas of Europe, principally Eastern Europe.<sup>10</sup> Previous seroepidemiologic studies on immigrants from Albania revealed a high prevalence of hepatitis A virus (HAV) and hepatitis B virus (HBV) infections.<sup>11–13</sup> In fact, more than 80% of the subjects tested positive for HAV antibodies, and about 13% of the refugees were infected chronically with HBV. The findings of the latter studies suggested the urgent need for a vaccination policy against hepatitis B in the Albanian population.

Little is known about the epidemiology of viral hepatitis in some Eastern European countries. Concerning hepatitis B, no immunization policy existed in Kosovo before the Balkan crisis. In fact, the immunization schedule adopted in Kosovo did not include vaccination against HBV. In addition, it is likely that during the past decade the political and socioeconomic crisis in Kosovo, with the repression and marginalization of ethnic Albanians,

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could have led to a worsening of the medical care system and of sanitary conditions.

The goal of this study was to investigate a sample of Kosovar refugees to assess the prevalence of enteric and blood-borne transmission of viral hepatitis.

## MATERIALS AND METHODS

Between March and April 1999 about 21,000 Kosovar refugees arrived in Puglia in an uninterrupted flow. After identification controls, the refugees were housed in eight refugee camps in Puglia. However, the number of refugees in the camps varied daily, because they quickly moved to other camps in different areas of Italy. For the purpose of this study, a team of physicians and nurses went to the Puglian refugee camps. Every subject present in the camps on that day from 8:00 AM to 12:00 AM was asked to participate in a seroepidemiologic survey. After obtaining informed consent, a blood sample was taken on a voluntary basis. The consent to take a blood sample from the children was obtained from their parents or legal guardians. More than 90% of refugees gave their consent, the remaining having fear of blood withdrawing.

Personal data (age, gender, place of origin) were obtained from each of the 526 Kosovar refugees who were recruited for blood sampling. The blood samples were taken to the laboratory, centrifuged and stored at  $-20^{\circ}\text{C}$  until testing.

The sera collected were tested within 1 month for the following hepatitis markers: total anti-HAV (IgG and IgM), anti-HEV, hepatitis B surface antigen (HBsAg), anti-HBs, total anti-HBc (IgG and IgM), HBeAg, anti-HDV and anti-HCV. Hepatitis A and hepatitis B markers were detected in serum samples by IMX-Microparticle Enzyme Immunoassay MEIA (Abbott Diagnostics, Chicago, IL). Antibodies to hepatitis E virus were detected by an enzyme immunoassay (HEV IgG, Nuclear Laser Medicine, Milan, Italy). The repeatedly reactive samples were then confirmed by an immunoblot assay (RecomBlot HEV IgG/IgM, Mikrogen GmbH, Martinsried, Germany). Antibodies to hepatitis delta virus (anti-HDV IgG) were detected in HBsAg-positive subjects by a commercial enzyme immunoassay (HDV-Ab, Nuclear Laser Medicine). The presence of antibodies to hepatitis C virus (anti-HCV) was ascertained by a third generation enzyme-linked immunosorbent assay (ELISA) test (HCV 3.0 ELISA, Ortho-Clinical Diagnostics, Milan, Italy). The repeatedly reactive serum samples were confirmed for the presence of anti-HCV antibodies using a recombinant immunoblot assay (Chiron RIBA HCV 3.0 SIA, Ortho Diagnostic System). The interpretation of the results was carried out according to the manufacturer's directions.

Data were processed using the software package Epi Info (version 6.04c). Confidence intervals were calculated by the quadratic method and chi-squared test was

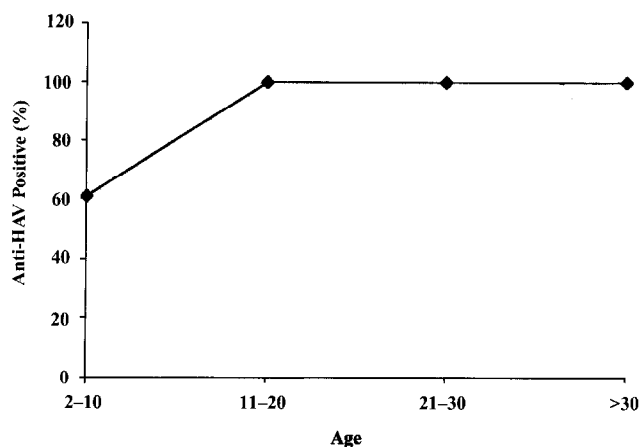


Figure 1. Prevalence of anti-HAV antibodies in the Kosovar refugees by age group.

used to compare proportions. If a P-value was below 0.05, the difference between proportions was considered to be statistically significant.

## RESULTS

Among the 526 Kosovar refugees, 255 (48.4%) were females and 271 (51.6%) were males. The refugees were from the areas of Kosovo most involved in the armed conflict: Pec (34%), Decane (15%), Djakovica (14%), Prizren (6%), Mitrovica (6%), Pristina (5%), Istok (4%), and Klina (3%). The remaining 13% of subjects were from other small villages in Kosovo. Children up to 10 years of age comprised 47.7% (251/526) of the study group; 119 (22.6%) were 11 to 20 years of age; 85 (16.1%) were 21 to 30 years of age, and 71 (13.5%) were older than 30 years. The mean age of the recruits was 13 years (SD = 11.35 y; range, 2-72 y).

Figure 1 shows the prevalence of anti-HAV antibodies in the Kosovar refugees by age group. The overall prevalence of total anti-HAV (IgG and IgM) was 81% (95% CI = 78.0-84.7). A relevant finding was the presence of anti-HAV antibodies in 61% (95% CI = 54.9-66.9) of the children 2 to 10 years of age, whereas in the other age groups the prevalence was 100%. No significant difference in the prevalence rate for anti-HAV antibodies was found between males and females (71% vs. 75%;  $P = 0.296$ ).

Analyses showed that 20 subjects (3.8%) (95% CI = 2.1-5.4) yielded repeatedly reactive results by enzyme immunoassay for antibodies to hepatitis E virus (Table 1), and 2.5% were confirmed to be seropositive by immunoblot assay. Subjects seropositive for anti-HEV were from various areas of Kosovo and did not cluster.

The prevalence of hepatitis B markers by age is reported in Table 2. Of the 2.9% (95% CI = 1.4-4.2) of samples that yielded HBsAg-positive results (0.8%) (95%

**Table 1.** Prevalence of Antibodies to HEV in Kosovar Refugees by Age

Age Group (y)	Number of Patients (n = 526)	Reactive by EIA			Confirmed by Immunoblot Assay		
		Number (n = 20)	Positive (3.8%)	95% CI (2.2-5.4)	Number (n = 13)	Positive (2.5%)	95% CI (1.1-3.8)
2-10	251	15	(6.0)	3.0-8.1	9	(3.6)	1.3-5.9
11-20	119	1	(0.9)	0.0-2.5	1	(0.8)	0.0-2.5
21-30	85	2	(2.3)	0.0-5.6	2	(2.3)	0.0-5.6
>30	71	2	(2.8)	0.0-6.7	1	(1.4)	0.0-4.1

EIA = enzyme immunoassay; CI = confidence interval.

CI = 0.0-1.5) were also HBeAg-positive. Positivity for total anti-HBc (with or without anti-HBs) was detected in 17.5% (95% CI = 14.2-20.7) of the refugees. When the results were stratified by age, the 21 to 30 age group was found to have the highest rate of HBsAg (7.1%; 95% CI = 1.61-12.5), followed by the 11 to 20 (4.2%; 95% CI = 0.6-7.8), the over 30 (4.2%; 95% CI = 0.0-8.9), and the 2 to 10 (0.4%; 95% CI = 0.0-1.1) age groups. The proportion of refugees positive for HBV markers increased with age. None of the subjects was positive for anti-HBs alone. No difference between males and females emerged in the distribution of hepatitis B serologic markers (Table 3). In addition, none of the subjects yielded a positive result for antibodies to hepatitis D virus. A low prevalence of anti-HCV antibodies was found in the Kosovar refugees. In fact, among the 526 subjects tested, only 4 proved reactive by screening assay for anti-HCV; 3 adults (0.7%; 95% CI = 0.0-1.2) were confirmed as seropositive by RIBA test (1 female; 2 males). No information was available about the possible risk factors.

## DISCUSSION

The present study reports the results of a survey to evaluate the seroprevalence of viral hepatitis markers in a sample of refugees who arrived in Puglia region. Undoubtedly the decision of enrolling subjects on a voluntary basis may have introduced a selection bias. The sample population studied may be considered biased with respect to

the population in Kosovo and to the primarily young age of the subjects studied. In particular, the presence of a majority of younger people in the sample tested could have led to a possible underestimation in the population of the prevalence rate of some viral hepatitis markers. Nevertheless, some indications emerge from the present study. A high prevalence of total anti-HAV was found among refugees. Since none of the subjects present in the refugee camps showed clear symptoms of acute viral hepatitis at enrollment and there was no indication for anti-HAV IgM detection, the high prevalence found strongly indicates previous infections. This finding suggests that Kosovo shows an epidemiologic pattern typical of hyper-endemic areas. These areas are characterized by poor hygienic, sanitary, and socioeconomic conditions and by the fact that HAV infection is largely acquired during childhood.<sup>14-16</sup> In fact, the prevalence of total anti-HAV among the 2- to 10-year-old children reached 61%, confirming the high level of circulation of hepatitis A virus in the population since early life. The high prevalence rate for anti-HAV found in the Kosovar refugees was similar to that previously reported for Albanian immigrants.<sup>11,17</sup> It is conceivable that this high prevalence rate reflects the worsening of hygienic and sanitary conditions in Kosovo as a result of ethnic conflict during the past decade. In contrast, the overall prevalence of anti-HEV antibodies in this sample of refugees was low and comparable to that of other European countries.<sup>18-22</sup> An intriguing finding is the presence of seropositive subjects among the 2- to 10-year-olds. In non-endemic areas, HEV infection is rare among children.<sup>23,24</sup> Although no information on the risk factors of the refugees coming into Puglia is available, it is unlikely that the presence of antibodies in children 2 to 10 years of age could be ascribable to their having travelled to

**Table 2.** Serologic Markers of HBV Infection in Kosovar Refugees

Age Group (y)	Number of Patients	Number Positive (%) 95% Confidence Interval			
		HBsAg	anti-HBc	anti-HBs	HBeAg
2-10	251	1 (0.4) 0.0-1.2	15 (6.0) 3.0-8.9	12 (4.8) 2.1-7.4	0 (0.0) 0.0-0.0
11-20	119	5 (4.2) 0.6-7.8	24 (20.2) 12.9-27.3	16 (13.4) 7.3-19.5	2 (1.7) 0.0-3.9
21-30	85	6 (7.1) 1.6-12.5	22 (25.9) 16.5-35.1	11 (12.9) 5.8-20.0	1 (1.2) 0.0-3.4
>30	71	3 (4.2) 0.0-8.9	31 (43.7) 32.1-55.2	24 (33.8) 22.8-44.8	1 (1.4) 0.0-4.1
Total	526	15 (2.9) 1.4-4.2	92 (17.5) 14.2-20.7	63 (12.0) 9.2-14.7	4 (0.8) 0.0-1.5

**Table 3.** HBV Markers by Gender in Kosovar Refugees

	HBsAg +	anti-HBc +	anti-HBs +	HBeAg +
	Number (%)	Number (%)	Number (%)	Number (%)
Male (n = 271)	11 (4.0)	44 (16.2)	30 (11.0)	3 (1.1)
Female (n = 255)	5 (1.9)	45 (17.7)	36 (14.1)	1 (0.3)
P-value	0.161	0.666	0.291	0.659*

\*Yates' corrected chi-square.

disease-endemic zones. Therefore, a low-level circulation of hepatitis E virus in the Kosovo region could be supposed. More extensive studies, such as molecular studies on HEV, would be needed to clarify this aspect.

Concerning hepatitis B, results of this study are consistent with an intermediate level of endemicity of HBV infection in the Kosovar population. Since low prevalence of HBsAg carriers was found among the children sampled (0.4%), vertical transmission does not seem to be an important route of transmission. This hypothesis is also supported by the low prevalence of HBeAg in the elderly age categories and in particular among the females (0.3%). Therefore, the increasing prevalence of HBV markers with age seems to indicate that hepatitis B infection in the Kosovar population is mainly acquired by sexual or parenteral exposure and by horizontal transmission.

The prevalence of hepatitis B infection in the Kosovar population is significantly lower than that previously found in Albanian refugees coming into Puglia in 1997 and clustered in the same age groups,<sup>11-12,25</sup> thus showing a profound difference in the risk factors between Kosovar and Albanian populations. In particular, Albanian children showed a higher prevalence rate of HBsAg carriers (16%) than Kosovar children. Moreover, HBV infection in the Kosovars seems to be at an endemic level comparable to that of other populations of the former Yugoslavia.<sup>26</sup>

None of the Kosovar refugees was vaccinated against hepatitis B as suggested by the lack of antibodies to hepatitis B surface antigen alone. On the basis of these findings, the authors suggest that a universal immunization policy for hepatitis B may be advisable in this population to control the spread of HBV infection. It has been reported that universal HBV vaccination is cost-effective even in countries with moderate to low HBV endemicity.<sup>27,28</sup> In Italy, vaccination against hepatitis B has been compulsory since 1991 in newborns and children. Therefore, all Kosovar children staying in the refugee camps were immunized against hepatitis B, as recommended by the Italian Ministry of Health.

On the basis of the results of the present survey, the authors suggest that an immunization strategy like that applied in Italy (vaccination to all newborns and children at the age of 12 years) may be adopted in Kosovo to control HBV infection consistently with the available economic resources. In addition, also risk groups could be vaccinated against HBV. Alternatively, only preadolescent children may be considered for immunization. The absence of antibodies to hepatitis delta virus confirms that HDV infection is not endemic in Kosovo, although it has been reported to be endemic for the other areas of the former Yugoslavia.<sup>29</sup> Finally, antibodies to hepatitis C virus were detected in a low percentage of Kosovar refugees. This data is in agreement with the prevalence rates reported in the general population of other European countries, ranging from 0.04% to 2%.<sup>30</sup> However, the adoption of general preventive measures in the

refugee population could help avoid the potential spread of HCV infection.

Further studies on the Kosovar population may be necessary to confirm the present observations. Moreover, a monitoring of the Kosovar population could be helpful during the reconstruction phase in that region, to evaluate and plan adequate preventive measures.

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