The majority of people infected with hepatitis C virus (HCV) are unaware of their infection. Assessment of the prevalence of HCV infection in the general population and in key populations at increased risk is needed for evidence-based testing policies. Our objectives were to estimate the prevalence of antibodies to HCV (anti-HCV), the prevalence of HCV viraemia (HCV RNA), and to describe HCV genotype distribution among pregnant women in Slovenia. Unlinked anonymous testing was performed on residual sera obtained from 31,849 pregnant women for routine syphilis screening during 1999, 2003, 2009, and 2013. Anti-HCV reactive specimens were tested for HCV RNA and HCV genotypes were determined. Annual prevalence of anti-HCV ranged between 0.09% (95% confidence interval (CI): 0.03–0.18) in 2009 and 0.21% (95% CI: 0.12–0.34) in 2003 and HCV RNA positivity between 0.06% (95% CI: 0.02–0.14) in 2009 and 0.14% (95% CI: 0.07–0.25) in 2003. We observed no statistically significant differences in anti-HCV or HCV RNA prevalence between age groups (<20, 20–29 and ≥30 years) in any year and no trend in time. Of 29 HCV active infections, 19 were with genotype 1 and 10 with genotype 3. HCV infection among pregnant women was rare suggesting a low burden in the Slovenian general population. Antenatal screening for HCV in Slovenia could not be recommended.

Introduction
Hepatitis C virus (HCV) is among the most common blood-borne viruses [1]. In ca 75% to 85% of cases of infection, HCV persists as a chronic infection and one third of chronically infected individuals is predicted to develop liver cirrhosis or hepatocellular carcinoma [2]. Although treatment success has substantially improved in recent years [3,4], most infected people are unaware of their infection and/or do not have access to treatment [5]. According to estimates published in 2013, by 2005, more than 185 million people around the world were infected with HCV, of whom 350,000 die annually [1]. The prevalence of antibodies to HCV (anti-HCV) in central Europe was estimated to be 2.4% (2.9 million people infected), in eastern Europe 2.9% (>6.2 million people infected) and in western Europe 2.4% (>10 million people infected) [1]. Compared with other geographical areas in the world these figures indicate a moderate prevalence (1.5%–3.5%) [1]. A recent review of available data from Europe indicated a wide variation in HCV infection prevalence between countries, ranging from 0.1% to 6.0% [6]. The lowest HCV prevalence (≤0.5%) estimates were from Scandinavian countries and the Netherlands, and the highest (>5%) from Romania [7-10].

As HCV shows great diversity in prevalence in different parts of the world, the 2010 World Health Assembly resolution urged Member States to generate reliable information as a foundation for building prevention and control measures that match the local epidemiological profile and health system capacities [11]. In 1998, the United States (US) Centers for Disease Control and Prevention had already recommended routine HCV testing for several population groups at increased risk for HCV, based on HCV risk factors ascertainment, but not for pregnant women and the general population [12]. In 2012, once per lifetime HCV testing for adults born between 1945 and 1965 without prior ascertainment of HCV risk factors was added as a recommendation since the prevalence of anti-HCV among the US population born during these years was estimated to be 3.25% (95% confidence interval (CI): 2.80–3.76) and persons born during these years accounted for approximately three quarters of all chronic HCV infections among adults [13].

In 2014, the World Health Organisation (WHO) recommended to offer anti-HCV testing to individuals who are part of a population with high HCV prevalence or who have a history of HCV risk exposure or behaviour, and suggested that nucleic acid testing for the detection of
HCV RNA be used following a positive HCV serological test to establish the diagnosis of chronic HCV infection as part of the assessment for starting treatment [14]. WHO identified the following populations at increased risk for HCV: persons who inject drugs, recipients of infected blood products or invasive procedures in healthcare facilities with inadequate infection control practices, children born to mothers infected with HCV, people with sexual partners who are HCV-infected, persons with human immunodeficiency virus (HIV) infection, in particular men who have sex with men, people who have used intranasal drugs, and people who have had tattoos or piercings. National testing policies based on the best assessment of the prevalence of HCV infection in the general population and in key populations at increased risk are needed for evidence-based HCV control policy [14].

Due to under-ascertainment and under-reporting, Slovenian HCV surveillance data, which are based on mandatory reporting of new hepatitis C diagnoses, do not provide a full picture of the epidemiology of HCV infection [15]. In Slovenia, we have some anti-HCV prevalence estimates for groups at higher risk (haemodialysis patients, people who inject drugs, HIV infected individuals) and data about the distribution of HCV genotypes among patients with HCV infection [16-20]. During the period from 2009 to 2013, the prevalence of anti-HCV among confidentially tested people who inject drugs entering or re-entering treatment within the network of Centres for the Prevention and Treatment of Illicit Drug Addiction ranged from the lowest 21.5% in 2010 to the highest 31.3% in 2013. These values were relatively low in comparison to a number of other countries in Europe where the prevalence among people who injected drugs during the period from 2011 to 2012 varied from 19% to 84%, with seven of the 11 countries with national data reporting a prevalence exceeding 50% (Austria, Cyprus, Greece, Latvia, Norway, Portugal, Turkey) [21]. We also have data about anti-HCV prevalence among blood donors for the period from 2001 to 2010 with an average of 0.0067% anti-HCV positive donations [22]. By 2013, we had neither reliable data about past and/or active HCV infection prevalence among pregnant women and in the general population nor about possible trends over time.

To complement available information on the prevalence of HCV infection in different population groups in Slovenia, our objectives were to estimate the prevalence of anti-HCV, the prevalence of HCV viraemia (HCV RNA), and to describe HCV genotype and subtype distribution among pregnant women in Slovenia for years 1999, 2003, 2009, and 2013. In addition, we wanted to explore whether there were any differences in anti-HCV and HCV RNA prevalence between different age groups of pregnant women in any of these years and possible changes in anti-HCV and HCV RNA prevalence through time.

**Methods**

**Samples**

In Slovenia, syphilis screening for pregnant women is universal. For this study, 31,849 sera stored at the National Institute of Public Health that were obtained from pregnant women for syphilis screening purposes and were systematically sampled for unlinked anonymous testing for HIV surveillance purposes during 1999, 2003, 2009, and 2013 were included. The sampling strategy for unlinked anonymous testing of pregnant women for HIV surveillance purposes was described previously [23,24]. Briefly, residual sera from specimens obtained from pregnant women for syphilis screening were continuously and consecutively sampled in eight participating laboratories. The eight laboratories were located at seven different sites across Slovenia, whereby one site comprised two laboratories (Figure). The second inclusion of specimens obtained from the same women during the same calendar year was prevented by keeping a separate list of identifying information on women whose sera had already been included into the sample during a particular year, which was checked before storing any new specimen. All specimens were labelled only with the information about the laboratory where samples were collected, sampling period (calendar year), and age group of the pregnant woman (20, 20–24, 25–29, and ≥30 years) from whom the serum specimen had been obtained.

**Sentinel sites involved in collection of residual sera specimens from pregnant women that were used to test for antibodies to hepatitis C virus*, Slovenia, 1999–2013 (n=7)**

**Figure**

Sentinel sites involved in collection of residual sera specimens from pregnant women that were used to test for antibodies to hepatitis C virus*, Slovenia, 1999–2013 (n=7)
for syphilis screening. They were frozen and stored at -20°C until testing [23].

**Laboratory testing strategy**

All 31,849 specimens were initially tested for the presence of anti-HCV in pools of 12 specimens by using Ortho HCV Version 3.0 ELISA Test system. Individual specimens from screen reactive pools were retested with the same test. To identify pregnant women with active hepatitis C infection all screen repeatedly anti-HCV reactive specimens were further tested for the presence of HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR)-based COBAS Amplicor HCV 2.0 (Roche Molecular Systems, Branchburg, NJ, US) test. Anti-HCV screen positive pregnant women with measurable HCV RNA were considered as actively infected with hepatitis C. Anti-HCV screen positive pregnant women without measurable HCV RNA were further tested with Hepatitis C Virus Encoded Antigen CHIRON RIBA HCV 3.0 Strip Immunoblot Assay (Chiron Corporation, Emeryville, US) to distinguish pregnant women with false positive anti-HCV screen test (negative with Immunoblot Assay) from those who spontaneously cleared hepatitis C in the past (positive with Immunoblot Assay). In all HCV RNA positive specimens, HCV genotype was determined with InnoLiPa HCV 2.0 test (Innogenetics, Zwijndrecht, Belgium).

**Analyses**

Statistical analyses were performed using STATA package version 10.0 (Stata Statistical Software: release 10.0 College Station. TX: Stata Corporation). We estimated the overall and annual prevalence of anti-HCV and HCV RNA together with 95% CIs, overall and according to age groups of pregnant women. Chi-squared test was used to assess the differences between the prevalence of anti-HCV and HCV RNA in pregnant women of different ages for respective calendar years and for the differences between different calendar years.

**Ethical consent**

Ethical consent to unlinked anonymous testing of pregnant women screened for syphilis for HIV surveillance purposes (consent number 54/09/00) and ethical consent for HCV unlinked anonymous testing of specimens collected in 1999, 2003, 2009, and 2013 (consent number 86/04/13) were obtained from the Medical Ethics Committee at the Ministry of Health in Slovenia.

**Results**

Among a total of 31,849 sera specimens tested, 41 were anti-HCV positive, corresponding to the pooled prevalence estimate of anti-HCV of 0.13% (95% Cl: 0.09–0.17). The 41 positive samples originated from all seven sentinel sites. Among 41 sera specimens positive for anti-HCV, 29 were positive for HCV RNA, corresponding to the pooled prevalence estimate of HCV RNA of 0.09% (95% Cl: 0.06–0.13).

Annual prevalence estimates for anti-HCV ranged between 0.09% (95% Cl: 0.03–0.18) in 2009 and 0.21% (95% Cl: 0.12–0.34) in 2003 and for HCV RNA positivity between 0.06% (95% Cl: 0.02–0.14) in 2009 and 0.14% (95% Cl: 0.07–0.25) in 2003 (Table). We observed no statistically significant differences in anti-HCV or HCV RNA prevalence between age groups (<20, 20–29 and ≥30 years) in any calendar year and no trend in time.

Among a total of 29 pregnant women positive for HCV RNA, 19 were infected with genotype 1 (12 with subtype 1b, 3 with subtype 1a, while in 4 cases subtype could not be determined) and 10 with genotype 3 (all subtype 3a). Infection with HCV genotypes 2, 4, 5 or 6 was not detected.

**Discussion**

The prevalence of antibodies to HCV and HCV viraemia among pregnant women in Slovenia was relatively low and we have not identified any changes during this 15 years period.

In comparison to available data from other European countries, our estimates of prevalence of anti-HCV among pregnant women were more similar to published prevalence estimates among pregnant women in some western European countries (in the United Kingdom (UK): 0.2%, April 1997–June 1998; in Amsterdam, the Netherlands: 0.3%, 2003). Our estimates were however lower than in some southern European countries (in northern Greece: 1.9%, March 1996–February 1997; in Bergamo, Italy: 2.4%, January 1995–December 1998) and eastern European countries (in Moldova: 2.3%, 1994) [25-29]. We should be cautious in comparing our results with the published results of similar studies, as different approaches were used for laboratory testing and for sampling/enrolling pregnant women into the studies (for example invitation to be voluntarily and confidentially tested accompanied with HCV related counselling in contrast to our unlinked anonymous testing of sera specimens obtained from a sera bank, which had been initially collected for syphilis screening purposes).

Relatively low estimated anti-HCV and HCV RNA prevalence among pregnant women in Slovenia in comparison to many other European countries may correspond to relatively low prevalence of anti-HCV among confidentially tested people who inject drugs [21]. Although some researchers have reported that anti-HCV prevalence among pregnant women increases with age, we did not found a statistically significant association between age group and prevalence in our study [25,30].

Only genotypes 1 and 3 were identified in our study which is consistent with the results of another Slovenian study in which chronic hepatitis C patients were enrolled and 93.8% of patients had genotypes 1 and 3 [20]. The fact that we did not find any patients with genotypes 4, 5 and 6, could be partly explained by the observation that the introduction of genotype 4,
Annual prevalence of antibodies to hepatitis C virus (HCV) and HCV viraemia among pregnant women, overall and by age group, 1999, 2003, 2009, and 2013, Slovenia (n=31,849)

<table>
<thead>
<tr>
<th>Age group in years</th>
<th>1999</th>
<th>2003</th>
<th>2009</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anti-HCV % (95% CI)</td>
<td>HCV RNA % (95% CI)</td>
<td>N</td>
<td>Anti-HCV % (95% CI)</td>
</tr>
<tr>
<td>120</td>
<td>0.27 (0.01–1.51)</td>
<td>0.27 (0.01–1.51)</td>
<td>367</td>
<td>0.00 (0.00–1.41)</td>
</tr>
<tr>
<td>20–29</td>
<td>0.11 (0.04–0.25)</td>
<td>0.09 (0.02–0.22)</td>
<td>4,573</td>
<td>0.27 (0.14–0.47)</td>
</tr>
<tr>
<td>330</td>
<td>0.10 (0.01–0.36)</td>
<td>0.05 (0.00–0.28)</td>
<td>1,990</td>
<td>0.12 (0.02–0.34)</td>
</tr>
<tr>
<td>Total</td>
<td>0.12 (0.05–0.23)</td>
<td>0.09 (0.03–0.19)</td>
<td>6,930</td>
<td>0.21 (0.12–0.34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Year</th>
<th>N</th>
<th>Anti-HCV % (95% CI)</th>
<th>HCV RNA % (95% CI)</th>
<th>N</th>
<th>Anti-HCV % (95% CI)</th>
<th>HCV RNA % (95% CI)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>367</td>
<td>0.27 (0.01–1.51)</td>
<td>0.27 (0.01–1.51)</td>
<td>259</td>
<td>0.00 (0.00–1.41)</td>
<td>0.00 (0.00–1.41)</td>
<td>134</td>
</tr>
<tr>
<td>2003</td>
<td>4,573</td>
<td>0.11 (0.04–0.25)</td>
<td>0.09 (0.02–0.22)</td>
<td>4,475</td>
<td>0.07 (0.01–0.21)</td>
<td>0.01 (0.01–0.21)</td>
<td>4,185</td>
</tr>
<tr>
<td>2009</td>
<td>1,990</td>
<td>0.10 (0.01–0.36)</td>
<td>0.05 (0.00–0.28)</td>
<td>2,547</td>
<td>0.11 (0.03–0.27)</td>
<td>0.05 (0.01–0.19)</td>
<td>3,745</td>
</tr>
<tr>
<td>2013</td>
<td>6,930</td>
<td>0.12 (0.05–0.23)</td>
<td>0.09 (0.03–0.19)</td>
<td>7,281</td>
<td>0.09 (0.03–0.18)</td>
<td>0.06 (0.02–0.14)</td>
<td>8,064</td>
</tr>
<tr>
<td></td>
<td>169</td>
<td>0.00 (0.00–2.16)</td>
<td>0.00 (0.00–2.16)</td>
<td>134</td>
<td>0.00 (0.00–2.16)</td>
<td>0.00 (0.00–2.16)</td>
<td>4,465</td>
</tr>
</tbody>
</table>

Anti-HCV: antibodies to HCV; N: number of sera collected from pregnant women for syphilis serology screening subjected to unlinked anonymous testing for anti-HCV or HCV RNA; CI: confidence interval.

* One-sided, 97.5% confidence interval.

5 and 6 in European countries has been related mostly to immigration from Africa and in Slovenia immigration from Africa has been relatively low [20].

Since the available estimates of HCV infection among pregnant women in Europe are generally relatively low, only two countries (Norway and Spain) introduced antenatal screening programs for hepatitis C [31], while for example, in the UK, routine antenatal screening for hepatitis C virus infection was decided against [25].

Our approach to obtain estimates of anti-HCV and HCV RNA prevalence by unlinked anonymous testing of rather large convenience samples of stored residual sera specimens obtained from pregnant women for syphilis screening in four calendar years spanning the period from 1999 to 2013, has proven to be logistically feasible. The strengths of such unlinked anonymous monitoring are minimised participation bias, non-invasive specimen collection and a very cost-efficient approach to collecting substantial number of specimens in laboratories. By repeating cross-sectional studies using the same methodology over time, we can monitor possible trends. As syphilis screening in Slovenia is universal and the numbers of residual sera tested corresponded to substantial proportions of pregnancies in respective calendar years (equivalent to 38% to 46% of deliveries), we believe that our prevalence estimates reflect quite accurately the true HCV prevalence among Slovenian pregnant women in those years. Pregnant women cannot be assumed to be representative of the general population. However, we believe that the estimated level of HCV infection prevalence among pregnant women may fairly well reflect the level of HCV infection prevalence in the Slovenian general population of reproductive ages, as suggested by others [32]. Other countries with constrained resources may consider using similar, logistically relatively simple and rather cost-effective, approaches to obtain better population HCV prevalence data.

Our study had several limitations. We tested residual sera specimens from convenience and not probability samples of pregnant women in Slovenia during the respective years. We did not have information on additional risk behaviour for pregnant women (for example, information on possible history of sharing injecting equipment) and women whose sera specimens had not been included into our samples may have been at a different risk for HCV infection. Finally, we may have slightly underestimated the prevalence of HCV RNA, as only screen repeatedly anti-HCV reactive specimens were tested for the presence of HCV RNA and not all 31,849 sera specimens. However, we believe that because of very low anti-HCV prevalence and consequently extremely low probability for a specimen to be collected before seroconversion (recently infected person who is still anti-HCV negative while already HCV RNA positive), little, if any, loss of sensitivity for ascertainment of HCV RNA positivity would result from our testing algorithm. Thus we assumed that the estimation of HCV viraemia prevalence by our laboratory testing algorithm fairly well reflected the true prevalence of HCV viraemia.

To conclude, our data represent the first reliable estimates of the relatively low burden of hepatitis C among pregnant women in Slovenia and suggest a relatively low burden of hepatitis C in the Slovenian general population. This suggests that the anti-HCV prevalence estimate for central Europe (2.4%) published in 2013 [1] may have been an overestimation and should be revised according to new information available. But it should be noted, that considerable heterogeneity in the HCV infection prevalence may exists among different countries of central Europe. Based on our results, opportunistic screening for HCV should not be recommended for pregnant women or the general population in Slovenia, however, voluntary HCV testing should be offered when there is a history of risk exposure or behaviour or a medical condition suggestive of HCV infection.
infection. Opportunistic screening for HCV should only be targeted to groups at increased risk, such as people who inject drugs, persons with HIV infection, in particular men who have sex with men, and other groups at increased risk for HCV as defined by the WHO [14].

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Conflict of interest
None declared.

Authors’ contributions
BK contributed to the design of the study, analysed the data and drafted the manuscript. MP contributed to the design of the study, supervised the testing and commented on the final version of the manuscript. KS contributed to the supervision of testing and commented on the final version of the manuscript. IK designed the study, supervised analyses and contributed to drafting the manuscript. All authors participated in interpretation of the results and approved the final version of a manuscript.

References


