Aus dem Robert Koch Institut, Abteilung für Infektionsepidemiologie
Der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Viral Hepatitis in Germany and Europe: Data to Guide Elimination Efforts/ Virale Hepatitiden in Deutschland und Europa: Datengrundlage für die Eliminierung

Zur Erlangung des akademischen Grades

Doctor rerum medicinalium (Dr. rer. medic)

Vorgelegt der Medizinischen Fakultät
Charité – Universitätsmedizin Berlin

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Datum der Promotion: 29. November 2024

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Abbreviations and acronyms

Anti-HBc	antibodies to the hepatitis B core antigen
Anti-HCV	antibodies to the hepatitis C Virus
DAAs	direct-acting antivirals
DEGS 1	the German Health and Examination Survey for Adults
ECDC	European Centre for Disease Prevention and Control
HBsAg	hepatitis B surface antigen
HAV	hepatitis A virus
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HCV RNA	hepatitis C virus ribonucleic acid
HCW	health care workers
HDV	hepatitis D virus
Hep-Epi	assessment of the epidemiological data on viral hepatitis B and C in Germany
HES	health examination survey
HEV	hepatitis E virus
MSM	men who have sex with men
OST	opioid-substitution treatment
PLWH	people living with HIV
PWID	people who inject drugs

RKI	Robert Koch Institute
SPHERE-C	Sero-prevalence of hepatitis C in Europe
TESSy	the European surveillance system
WHO	World Health Organization

Abstract

Viral hepatitis is a major contributor to the global disease burden. Better prevalence data are needed to be able to monitor progress towards the World Health Organization (WHO) goal of eliminating viral hepatitis as a public health problem by 2030 and target the public health response. The aims of the projects in this thesis were to contribute to the understanding of the viral hepatitis epidemic by developing a protocol to enable countries to conduct prevalence surveys, and to create a review of available viral hepatitis prevalence data in Germany.

From 2016 to 2019, as part of the SPHERE-C (sero-prevalence of surveys of hepatitis C in Europe) project, an evidence-based technical protocol was developed outlining three survey approaches for estimating HCV prevalence in the general population. The technical protocol presents best practice and alternative options for all steps needed for conducting an HCV prevalence survey ranging from drawing a probability-based sample to reporting of results and logistical aspects. The protocol was piloted in three European Union (EU) countries. Results from the pilot phase showed the importance of securing a sufficiently large and representative sample through carefully planned recruitment steps.

In the Hep-Epi project (2014-2019) (Assessment of the Epidemiological Data on Viral Hepatitis B and C in Germany), we collected prevalence data in Germany through a systematic literature search. Evidence demonstrated a low prevalence in the general population, but a much higher prevalence was found among certain at-risk populations. Evidence was incomplete or entirely missing for some population groups, indicating a need for a better epidemic understanding, and viral hepatitis prevalence in these population groups to get a complete picture.

Data of better quality are needed for a targeted public health response to eliminate viral hepatitis as a public health problem by 2030.

Zusammenfassung

Virale Hepatitiden tragen wesentlich zur weltweiten Krankheitslast bei. Bessere Prävalenzdaten sind erforderlich, um angemessene Public Health Maßnahmen zu gewährleisten und die Fortschritte auf dem Weg zum WHO-Ziel der Eliminierung der Virushepatitis zu messen.

Von 2016 bis 2019 wurde im Rahmen des SPHERE-C-Projekts ein evidenzbasiertes technisches Protokoll entwickelt, das drei Erhebungsansätze zur HCV-Prävalenzschätzung in der Allgemeinbevölkerung beschreibt. Das Protokoll enthält Mindest- und Goldstandards für alle durchzuführenden Schritte, vom Sampling über den Datenschutz und ethische Fragen, Rekrutierung, Probenentnahme und Labortestoptionen, Schulung des Personals, Datenmanagement und -analyse sowie Budgetüberlegungen. Das Protokoll wurde in drei Ländern der Europäischen Union erprobt. Die Ergebnisse der Pilotphase zeigten, wie wichtig es ist, durch sorgfältig geplante Rekrutierungsschritte eine ausreichend große und repräsentative Stichprobe zu erhalten.

Im Rahmen des Hep-Epi-Projekts wurden Daten zur Prävalenz von Hepatitis B und C in Deutschland in verschiedenen Bevölkerungsgruppen durch Literaturrecherche zusammengetragen. Es zeigte sich eine niedrige Prävalenz in der Allgemeinbevölkerung. In bestimmten Risikopopulationen war die Prävalenz sehr hoch. Für einige Gruppen war die Datenlage unvollständig oder fehlte völlig.

Bessere und vollständigere Daten werden für eine gezielte Reaktion des öffentlichen Gesundheitswesens benötigt, um die Eliminierung der Virushepatitis bis 2030 zu erreichen.

1. Introduction

In the WHO European Region, an estimated 14 and 12 million people are infected with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, respectively [4]. There is a high mortality rate from viral hepatitis, and with its often asymptomatic course of disease, a large proportion of those infected are chronically infected and at risk of liver cirrhosis and hepatocellular carcinoma (HCC). Viral hepatitis accounts for 1.4 million deaths from acute infection, cirrhosis and HCC yearly and is a large contributor to the global disease burden [5, 6].

Viral hepatitis B and C epidemiology varies both within and between countries and over time. Changes in incidence and prevalence have been observed, especially over the last 15 years [7]. The reasons behind the development of the epidemics are multifaceted, and include HBV vaccination, the availability of highly efficient direct-acting antivirals (DAAs) for HCV treatment (since 2014), as well as behavioural changes among key populations including men who have sex with men (MSM) and people who inject drugs (PWID). Moreover, important improvements and advancements in testing of blood products and health care standards including infection control have been implemented [8-11].

The first ever WHO global health sector strategy on viral hepatitis (2016-2021) was published in 2016 [5]. The strategy sets out an ambitious goal of viral hepatitis elimination by 2030. Viral hepatitis elimination is defined by certain targets to be reached by 2030, such as a reduction in incidence of 90%, and 65% reduction in mortality [5]. A viral hepatitis action plan for the European level was approved in 2016 with impact targets to achieve elimination by 2030 in the WHO European Region [12]. In 2016, the German Ministry of Health published an integrated national strategy for HIV, HBV and HCV and other sexually transmitted diseases to improve the response to these diseases [13].

The recognition of viral hepatitis as an important public health challenge and the strategic implementation of measures to eliminate viral hepatitis have further highlighted the need for high-quality and reliable data. Better data are crucial in monitoring the progress towards elimination and identifying where intensified preventive measures are needed to halt the epidemic [4, 14]. This is what is addressed in the strategic direction one in the

WHO strategy, emphasizing the need for strategic information and data for action to be able to target responses and focus strategic and programme planning on national level.

Most European countries, including Germany, have a surveillance system for viral hepatitis based on routine notifications [15, 16]. European Union (EU)/European Economic Area (EEA) countries are requested to upload surveillance data to The European Surveillance System (TESSy) each year using standardised EU case definitions [16]. However, some countries in Europe are unable to provide data on newly diagnosed cases, and for the majority of countries completeness and reporting according to the EU definitions are major issues. Further, surveillance data are often insufficient in providing a clear picture of the incidence (since viral hepatitis can develop into a chronic disease), prevalence, burden, and trends of the disease [17,18].

Ten core indicators for monitoring and evaluation are suggested in the WHO framework, one of which is viral hepatitis prevalence [19]. Knowledge on prevalence is needed to understand the context and needs by identifying the epidemic pattern and the most affected population groups.

2. Objectives and outline of the dissertation

This thesis aims to present key findings from two projects that cover different aspects of prevalence data collection. The thesis is developed into two separate main chapters in which the methodology and results from the two projects are presented. Three papers, published in 2020 and 2021, are included in this doctorate by publication [1-3].

Chapter A is dedicated to the “Sero-Prevalence Survey for Hepatitis C in Europe” (SPHERE-C) project, during which a detailed technical protocol for carrying out HCV prevalence surveys in the general adult population was developed.

Chapter B is dedicated to the “Hep-Epi” project. The aim of this project was to outline available literature and evidence on the HBV, HCV and HDV epidemiology in Germany (2005-2017) to serve as baseline for the work towards, and monitoring of, viral hepatitis in Germany.

3. Chapter A: Development of a technical protocol for conducting HCV prevalence surveys among the general population

3.1 Background

To track progress towards elimination and improve the viral hepatitis response, knowledge of the epidemic through high-quality estimates of the number of people infected with chronic HCV is essential. In the EU/EEA the HCV prevalence studies currently available are heterogeneous, and there is in general a lack of quality data. This makes comparisons and monitoring over time challenging as was also demonstrated in the Hep-Epi review [2, 20] further described in Chapter B. During the SPHERE-C project launched by the European Center for Disease Prevention and Control (ECDC) a standardised protocol on how to conduct an HCV prevalence survey to generate a nationally representative HCV prevalence estimate for the general population was developed, piloted and finalised.

3.2 Methodology

The technical protocol [16] and publication [3] describe the methodology in detail, and is explained in brief below. The protocol [16] is built on available evidence on HCV prevalence surveys and general population prevalence surveys collected through a desktop review. Information on what is needed and what should be prioritised in the technical protocol was collected through an enquiry of national ECDC hepatitis focal points in the EU/EEA Member States.

An expert group was established to provide feedback, and to follow the project as well as the different stages of developing the technical protocol. Members of the expert group had different backgrounds and included public health researchers, epidemiologists and statisticians, as well as medical doctors and laboratory experts from both EU/EEA and the USA. During the project a total of three face-to-face expert group meetings were organised.

In 2018, three pilots were conducted in three EU/EEA countries which were; Bulgaria, Finland and Italy. The aim for all pilots was to evaluate the SPHERE-C protocol methodology and collect experiences with implementing the protocol. To evaluate its usefulness, an evaluation questionnaire was developed with indicators covering the main methodological parts of the protocol. The indicators reflected areas including survey

objectives, sampling frame and sampling, coordination and timeline, as well as ethics and data protection. Moreover, the indicators covered aspects around survey recruitment and awareness-raising, budget and staff, and finally data collection (including development of questionnaire and drawing of blood samples) and data management, and analysis.

The local survey teams completed the questionnaire and provided their experience with the protocol. Thereafter, we interviewed the Finnish and Italian survey teams over the phone to explore further issues more comprehensively. We held a face-to-face meeting with the Bulgarian survey team. Thereafter, we incorporated the results from the three pilots and the evaluation in the revised and updated protocol.

Nested survey approach with retrospective testing of samples

Finland[1]

The primary objective of the survey in Finland was to generate an anti-HCV prevalence estimate and prevalence of chronic infection (anti-HCV and RNA positive) in the Finnish general population (≥ 18 years). Samples from a large general population survey, the national Health Examination Survey (HES) (FinHealth2017) were used. A secondary objective was to estimate the undiagnosed fraction by matching the data from the survey with data from the national infectious disease register.

Stand-alone survey approach

Catanzaro, Italy[2]

The primary survey objective was to generate an estimate of the age- and sex specific chronic HCV prevalence in the general adult population living in Catanzaro in Southern Italy. The secondary survey objective was to estimate the prevalence of undiagnosed HCV, and exposure to HCV in the adult general population in Catanzaro. Initially, the plan was to conduct a nested survey and make use of an already planned HES with focus on

[1] This pilot was conducted by the local survey team in Finland, and prevalence data are not further described in this thesis.

[2] This pilot was conducted by the local survey team in Italy, and prevalence data are not further described in this thesis.

salt consumption (CUORE[3]). It turned out, however, that the sample size in the original HES survey was not sufficiently large to estimate the chronic HCV infection. After re-calculating the sample size, the Italian survey team decided to carry out a stand-alone survey.

Stara Zagora, Bulgaria

The Robert Koch Institute (RKI) and the local survey team in Stara Zagora, Bulgaria conducted this survey in close collaboration. The RKI team developed the locally adapted protocol as well as all needed study materials for the pilot. The pilot methodology is described in detail and published elsewhere [1], and is described in short below. We developed a pilot-specific study protocol and study materials. A cross-sectional population-based survey was set up to estimate the chronic HCV prevalence (anti-HCV and RNA positive) in the adult general population above 18 years in the city of Stara Zagora, Bulgaria.

We calculated a sample size and set the expected chronic HCV prevalence to 1% and a lower precision to 0.25%. The calculated sample size was 999 people, and the total sample size was 1998 people (expecting a 50% non-response).

A probability-based age- and sex stratified sample was retrieved from the local population registry in Stara Zagora. A registered invitation letter was sent to the 1998 people, and a reminder letter followed in case of non-response. The letter was accompanied by a participant information leaflet which included relevant information regarding participation. This included the objectives of the survey, opening hours and contact details, incentive and anonymity [1]. To raise awareness of the survey and promote participation, a local media campaign was organised by the Bulgarian survey team.

Venous blood samples and data via self-administered questionnaires were collected from participants at the local study site, the Regional Health Inspectorate (RHI) in Stara Zagora, from 5 September to 16 November 2018. Anti-HCV testing of all blood samples was performed, and those reactive were tested for HCV RNA. The test result was provided in

[3] http://www.cuore.iss.it/eng/factors/HES2018-2019.asp

person by a doctor at the RHI and if the result was positive, the person was linked to appropriate care.

3.3 Results

3.3.1 Better data for monitoring of HCV

Three probability-based survey approaches are described in the technical protocol. These three are; a stand-alone survey, a 'nested' survey (included in an already planned health survey), and a survey with retrospective testing of already collected samples. To consider different settings and available resources, human and financial, the protocol suggests both gold and minimum standards for all methodological steps and key aspects for conducting a survey. The methodological steps include sampling methods, data protection and ethics, recruitment methodologies, data collection (specimens and questionnaire), laboratory testing as well as budget, staff training, and data management, analysis and reporting [16]. Figure 1 below present the requirements that are mandatory when conducting an HCV prevalence survey, as well as methodological options for all three survey approaches.

It can be challenging to determine which survey approach is most suitable in a given setting and situation. To guide the decision-making, we developed an algorithm which was included in the protocol to guide the reader through the different steps of conducting a survey to support a thorough decision-making process before embarking on a survey (Figure 2).

1) Nested survey

This approach is done by nesting the prevalence survey in an already planned future general population survey, e.g. a HES. By being able to use the already existing infrastructure of the larger survey, the prevalence survey becomes less resource-intensive and costly. Collection of HCV-related behavioural data and testing for HCV can be carried out with little extra effort, and thereby requiring less financial and human resources than when setting up an entire survey only for HCV testing. If a large population-based survey is in planning or already planned, it is suggested to take advantage and nest onto this and test the blood samples for HCV. To include testing for HCV in already developed surveys

and study protocols will involve comparable steps to those required when developing a new survey. However, the steps will mostly be more straightforward as they have already been planned and/or carried out for the original health survey (such as ethical board approval, sampling and recruitment strategy). Moreover, larger population-based surveys often use robust sampling strategies and implement many efforts in securing a high participation rate. Given this and that the sample calculation for the larger survey is sufficient for the expected prevalence of HCV in the general population, there are good chances of a representative sample.

2) Retrospective testing survey

When carrying out a retrospective testing survey, stored samples from a recently conducted population-based survey are retrospectively tested for HCV. In order to use the samples for HCV testing, the criteria of probability-based sampling need to be fulfilled. Also, a sufficient number of samples need to be available, and it is important that these are unbiased and that this sub-set are representative of the samples collected for the original survey.

It is also critical that the participants in the original survey provided informed consent for storing the blood sample, and allowed usage for further and retrospective testing. The costs of this approach will only include the work carried out by the laboratory and analysis of the data. This is the second-best option given that the criteria are fulfilled to ensure high-quality data. For this approach, it is important that the samples were correctly stored, as HCV RNA degradation may cause a biased estimate.

3) Stand-alone survey

The stand-alone HCV prevalence survey entails all steps from sampling to staff training and budget considerations (Figure 1). Therefore, this approach requires the most human- and financial resources, and should only be chosen if there is no planned or recently conducted HES available. As conducting a stand-alone survey requires a lot of resources, a first step could be first to test any available already collected sera (from routine testing or residual) e.g. from antenatal care screening. If there is a low prevalence in these

samples (<1%), it is recommended to focus on key populations and prioritise setting up prevalence surveys in these group rather than in the general population.

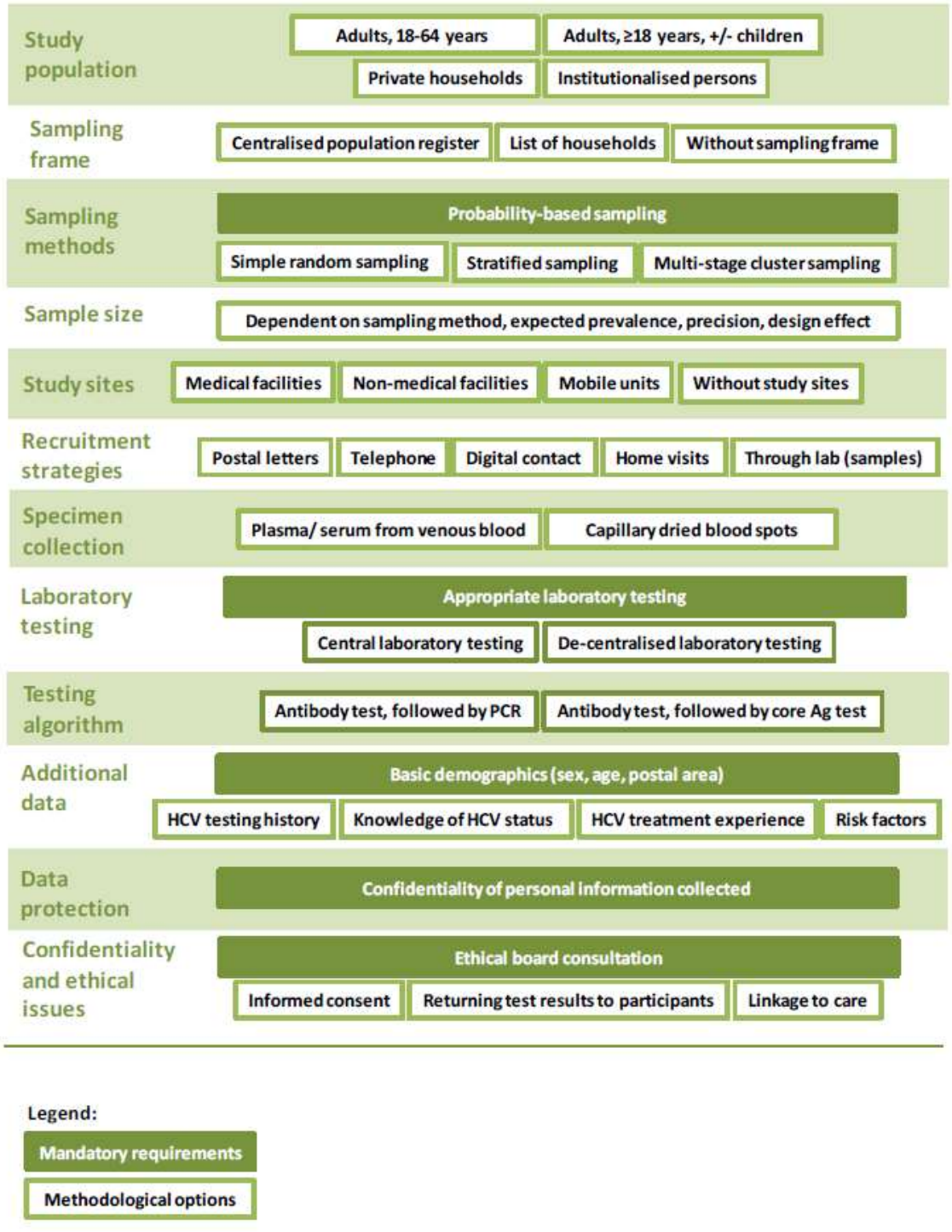
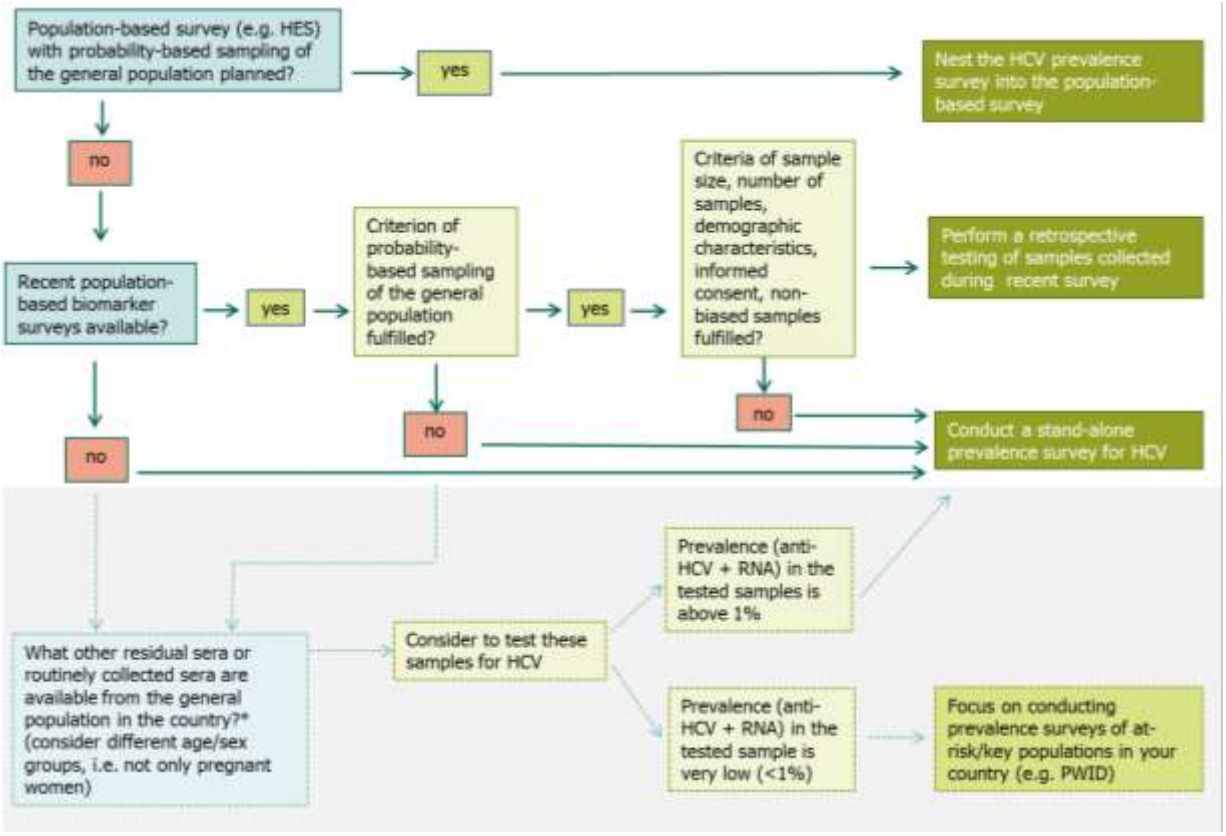


Figure 1: Overview of mandatory requirements and methodological options for an HCV prevalence survey (from 3. Sperle, I., Nielsen, S., Bremer, V., Gassowski, M., Brummer-Korvenkontio, H., Bruni, R., Ciccaglione, AR., Kaneva, E., Liitsola, K., Naneva, Z., Perchemlieva, T., Spada, E., Toikkanen, SE., Amato-Gauci, AJ., Duffell, E., Zimmermann, R 2021)



**Alternative options exist that might be explored by countries to get an idea of the HCV prevalence level in the general population. These can be explored if data from a recent population-based prevalence survey are not available or if there are no plans for a future survey and few resources available for a stand-alone survey [16]*

Figure 2: Decision algorithm (from Sperle, I., Nielsen, S., Bremer, V., Gassowski, M., Brummer-Korvenkontio, H., Bruni, R., Ciccaglione, AR., Kaneva, E., Liitsola, K., Naneva, Z., Perchemlieva, T., Spada, E., Toikkanen, SE., Amato-Gauci, AJ., Duffell, E., Zimmermann, R, 2021)

Results from piloting the protocol

The objectives of the three pilots in Bulgaria, Finland and Italy were achieved. Several challenges associated with both planning and conducting the survey were reported from the local survey teams in the evaluation. Key experiences and lessons from the three pilots are summarised in Table 1.

Table 1: Summary of methodological details, results of the pilots and lessons learnt (from Sperle, I., Nielsen, S., Bremer, V., Gassowski, M., Brummer-Korvenkontio, H., Bruni, R., Ciccaglione, AR., Kaneva, E., Liitsola, K., Naneva, Z., Perchemlieva, T., Spada, E., Toikkanen, SE., Amato-Gauci, AJ., Duffell, E., Zimmermann, R, 2021)

	Stand-alone survey		Nested with retrospective testing of samples	Implications for the SPHERE-C protocol
	Bulgaria	**Italy**	**Finland**	
Data protection issues/ Ethical approval	Names and addresses of invitees were not allowed to be shared with study team, invitation letters needed to be sent out by the municipality holding the register.	It was required to call every participant for scheduling appointment to return test results.	Data protection issues and ethical approval conducted previously by FinHealth study team. Informed consent form already included possibility of testing for some other diseases.	Plan for getting the ethical approval early to be able to still adjust according to requested changes. Data collection and processing according to the General *Data Protection* Regulation (GDPR) in the EU 2016/679 required, therefore early contact with the national data protection agency advised.
Sampling method	Simple random sample stratified by age and sex.	Simple random sample stratified by age and sex.	Two-stage cluster sampling stratified by age and sex.	Sample should be selected using a probability-based random sampling method. For smaller geographical areas (e.g. cities) simple random sampling may be applied.
Sampling frame	Local population register of the city of Stara Zagora.	Local population register of the city of Catanzaro.	National population registers.	Population registers should be up to date.
Sample size calculation	N=999 (expected prevalence of chronic HCV was 1.0% and a lower precision bound of 0.25%).	N=889 (expected prevalence of chronic HCV infection of 1.0% for age group 35-65 (upper precision bound 2.2%) and 5.0% for age	N=10,305 (expected prevalence of current HCV infection (anti-HCV and HCV RNA positive) of 1% and a lower precision bound of 0.25%).	Ensure large enough sample size to get a valid estimate. (Input and statistical formula on how to calculate sample size included in the SPHERE-C technical protocol [16]).

		group 65+ (upper precision bound 10.0%).		
Recruitment strategy	Tracked invitation letter. Reminders: a second tracked invitation letter.	One invitation letter (in 4 rounds). For each round a new subset of the sample was invited.	First contact with a postcard, followed by an invitation letter. Reminders: postcards, phone calls, SMS reminders.	Emphasise that more recruitment efforts are needed to ensure a high enough response rate and to include the "hard to reach" populations who may have a poorer health. Sending only tracked letters are not recommended. Make at least three attempts to reach participant (invitation letter, reminder letter, phone call, SMS reminders, or house visits). Include a pre-test to test the effectiveness of different incentives.
Promotion of the survey	Information leaflet for invitees; contact with and engagement of local authorities; local media campaign to inform about hepatitis C and encourage participation in the survey including information posters in local pharmacies and outpatient care facilities (general practitioners and medical centres); 3 local press conferences, local radio and television broadcasts.	Information leaflet for invitees; contact with and engagement of local authorities; awareness posters for the survey displayed in waiting rooms of general practitioner practices and in the hospital of Catanzaro.	Information leaflet for invitees; contact with and engagement of local authorities; Press conference, newspaper articles, radio and television broadcasts.	Information leaflet (and website) to inform invitees are strongly recommended for all surveys. Information and promotion of the survey among the general population through media and local authorities, and among health care staff are recommended.
Data collection period	10 weeks (5 September 2018 - 16 November 2018).	4 rounds of 1 week each in a period of 7 months (June 2018 - December 2018).	7 months (January 2017 - July 2017).	Plan extendible data collection period/buffer of time in case sample size is not reached in the planned period. Ideally, the data collection period in Bulgaria should have been prolonged to reach the required sample size.
People invited	1,998	9,000	10,247	Consider expected non-response rate, and consider that the non-

	(1,166 collected their letter at the post office).	(8,655 letters delivered).		response rate may be higher than 50%.
Participants	252	1003	5923 available samples tested	
Incentives	A coffee mug and a pencil.	One day off from work for participants.	Results of the health examinations and laboratory analysis of the collected biological samples.	Consider different incentives for different age-groups. Include a pre-test to test the effectiveness of different incentives.
Response rate	12.6% Net response rate: 21.6% (of those who got the invitation).	11.1% Net response rate: 11.6% (of those who got the invitation).	Overall response rate for questionnaire: 59.6% Net response rate for health examination: 57.8%.	Low response rates in all pilots highlight the challenge of reaching the target set by EHES of 70% [21, 22] and consideration of a more realistic target.
Additional data and questionnaire	Self-administered questionnaire including questions specific to HCV. Migrants were not sufficiently included, and therefore unknown if translation was needed.	Self-administered questionnaire including questions specific to HCV. Migrants were not sufficiently included, and therefore unknown if translation was needed.	Self-administered questionnaire completed before HES either electronically or manually. No HCV-specific questions (e.g. HCV infection risks) included.	Self-administered questionnaires work well in general populations. Prior to data collection, assess whether translation /interviews are needed. In nested surveys, early collaboration with survey team important to ensure that HCV-related questions are included.
Laboratory	Local laboratory for serology and one in capital for confirmatory testing and PCR. Shipping by using routine procedures.	Shipping of samples to a centralised reference laboratory for all testing.	De-freezing, aliquoting and shipping of samples to another laboratory for serology and PCR.	Centralised testing of all steps in one laboratory is recommended. Alternatively, two-step test algorithm in two laboratories when routine shipping procedures can be used. In retrospective design, samples for HCV testing should be aliquoted during data collection.
Testing algorithm	Anti-HCV ELISA, followed by PCR. Immunoblot for PCR negative samples.	Anti-HCV ELISA, followed by PCR. Immunoblot for PCR negative samples.	Anti-HCV ELISA, followed by Immunoblot (HCV ELISA positives and borderlines) and PCR	The number of false positives may be high in low prevalence settings, therefore confirmation of anti-HCV

			(Immunoblot positives and borderlines).	reactive, PCR negative samples is important in these settings.
Returning test results to participants	Test results were returned to all survey participants, who received a letter with their participant ID and a date for when they would receive their test result during an in-person consultation with a medical doctor at the Regional Health Inspectorate.	All participants were contacted via phone to schedule an appointment during which they would receive their test result.	Positive cases were contacted by phone and a letter.	Plan enough time, staff and budget to have appointments with all participants or outsource the scheduling of appointments. Alternatively, only inform positive-tested about test results. Returning test results from retrospective testing only if data was collected recently, and participants consented to being informed.
Data analysis including weighting	Frequencies and percentages were calculated for categorical variables (participants and non-participants). For the chronic HCV prevalence weighting adjustment was performed with age and sex. Prevalence estimates were calculated as crude estimates and weighted estimates with 95% Confidence Intervals. All analyses were carried out in Stata 15.1.	Non-response biases were evaluated by comparing respondents and non-responders with regard to their sex, age distribution and housing deprivation level. Crude, age and sex specific, and standardised anti-HCV prevalence rates were calculated. The associations of HCV infection with the different predictor variables were investigated by log binomial regressions with sampling weights or by exact logistic regressions as appropriate. Variables with a p-value <0.20 at the univariate analysis were considered as potential predictors and included in multivariable analysis.	Post-stratification weights were used to correct the possible for non-response biases by incorporating population distributions of sex, age and other appropriate characteristics into survey estimates. Design based weighted overall and age- and sex-stratified estimates of the HCV prevalence and their 95% confidence intervals are calculated. The associations of HCV infection with multiple explanatory variables are modelled using logistic regression model with sampling weights. Predictive margins of interests are calculated.	Perform non-response analysis to assess bias of results. Consider post-stratification weights to correct for non-response. Calculate crude and weighted overall and stratified estimates of the HCV prevalence, including 95% confidence intervals (considering the design of the survey). Weighting should consider at least age and sex, if possible, further characteristics (e.g., regional or urban–rural distribution, migration status).

		All analyses were carried out in Stata 15.1.		
Budget implications	Most time and resources spent on administrative challenges.	Most resources spent on sending letters and scheduling appointments.	Most time spent on preparing samples for testing.	Allow adequate time for administration. Consider outsourcing the sending of letters/scheduling appointments.

3.3.2 HCV prevalence in Stara Zagora, Bulgaria

Of the 1998 people who were invited to take part in the survey, 1166 received the invitation letter and 252 of those took part in the survey (21.6%) (Figure 3).

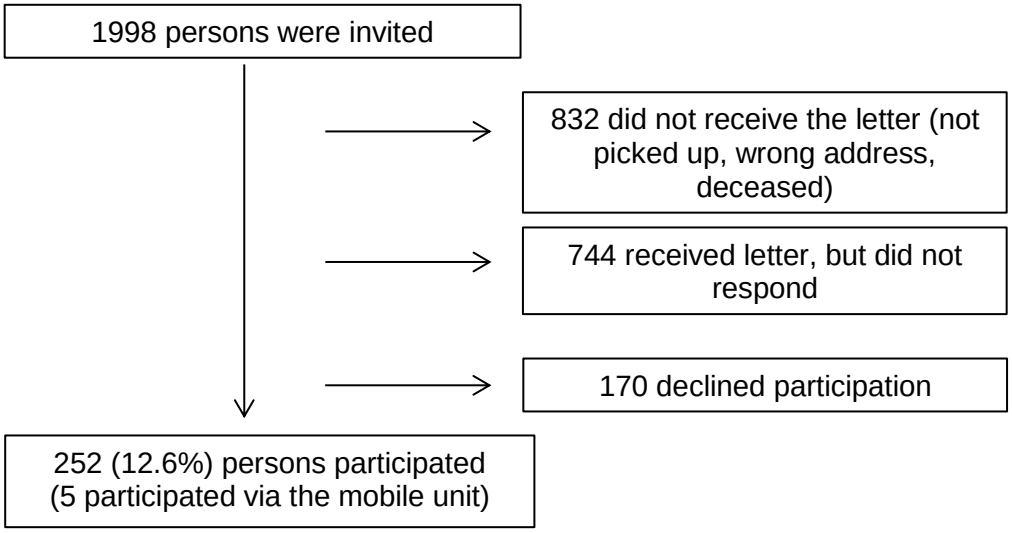


Figure 3: Flowchart of participation (from Sperle, I., Nielsen, S., Gassowski, M., Naneva, Z., Perchemlieva, T., Amato-Gauci, A., An der Heiden, M., Bremer, V., Golkocheva-Markova, E., Hristov, K., Kaneva, E., Simeonova, Y., Tenev, T., Varleva, T., Duffell, E., and Zimmermann, R., 2020)

Two deceased were among the 832 people who did not receive their invitation letter for the survey. The remaining 830 people were either registered with the wrong address or did not go to the post office to collect their letter. The majority of the participants took part during the first 2.5 weeks of data collection (45%), and after 4.5 weeks, 75% of all participants were enrolled in the survey (Figure 4).

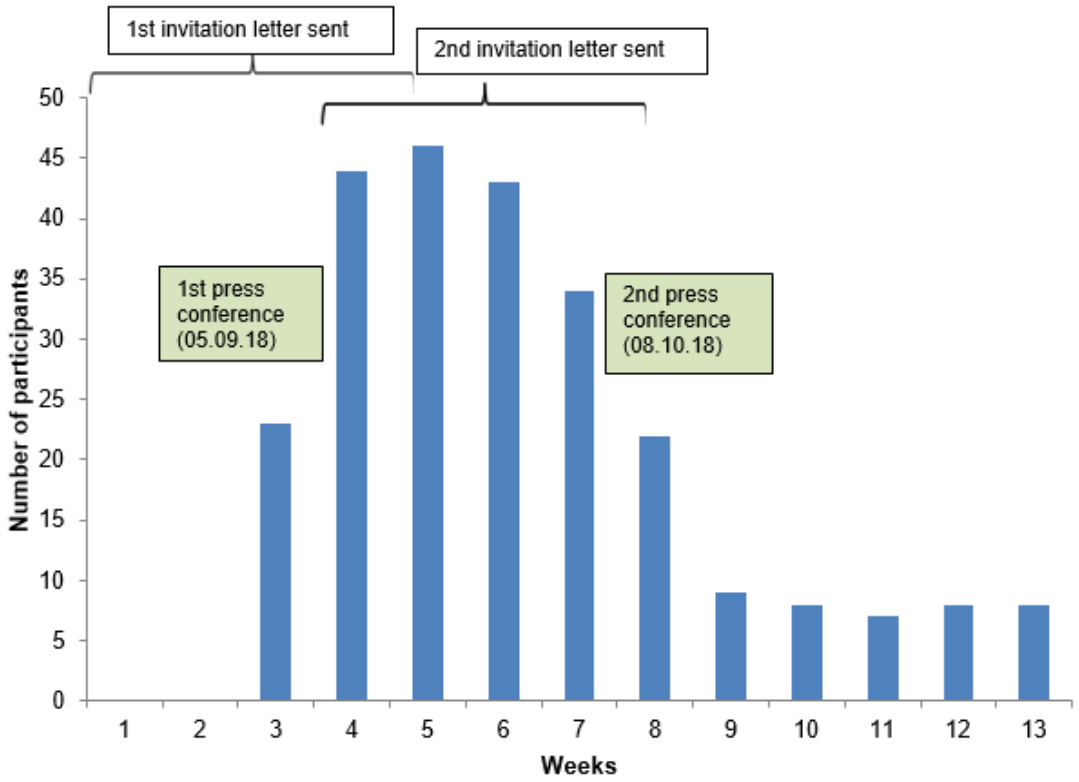


Figure 4: Number of participants per week during the data collection period (5 September-16 November 2018)

The age among the participants ranged from 18-95 years (mean: 55.9 years). Of the 252 participants, 60.3% were female (Table 2).

Table 2: Sociodemographic characteristics of participants (n=252) (from Sperle, I., Nielsen, S., Gassowski, M., Naneva, Z., Perchemlieva, T., Amato-Gauci, A., An der Heiden, M., Bremer, V., Golkocheva-Markova, E., Hristov, K., Kaneva, E., Simeonova, Y., Tenev, T., Varleva, T., Duffell, E., and Zimmermann, R. 2020)

Sociodemographic characteristics		**n (%)**
Sex	Female	152 (60.3%)
	Male	100 (39.7%)
Ethnicity	Bulgarian	248 (98.8%)
	Roma	2 (0.8%)
	Other	1 (0.4%)
	Missing	1 (0.4%)
Highest level of education	Elementary Education	1 (0.4%)
	Primary Education	11 (4.4%)

	Secondary Education	122 (48.4%)
	Higher Education	118 (46.8%)

Prevalence of HCV

Two of the 252 participants were infected with chronic HCV (anti-HCV and HCV RNA positive) (Table 3).

Table 3: HCV prevalence (N=252)

		n	Crude prevalence (%)	Weighted prevalence (%)
Anti-HCV (Elisa) (N=252)	Reactive	2	0.8% [95% CI 0.2–3.1%]	0.9% [95% CI 0.2–4.2%]
	Negative	250		
HCV RNA (N=2)	Positive	2		
	Negative	0		

Testing history and factors associated with HCV

Sixteen participants informed having ever been tested for HCV (6.4%), while 202 reported never being tested (80.5%). One participant informed having tested HCV positive in the past but was tested anti-HCV negative in this pilot survey.

Surgery under general anesthesia (64.1%), followed by blood transfusion before 1992 (11.7%) and having or having had a body piercing (8.8%) were the most commonly reported factors associated with HCV (Table 4). The one participant who reported having injected drugs was also one of the two HCV-positive cases. The other participant with chronic HCV did not inform of any known factors associated with HCV or previous testing for HCV.

Table 4: Factors associated with HCV (N=251)

Factors associated with HCV	n (%)
Ever undergone surgery under general anesthesia	161 (64.1%)
Ever undergone a blood transfusion (1992[a])	29 (11.7%)
Have (or have had) a body piercing	22 (8.8%)
Have (or have had) a tattoo	20 (8.0%)
Ever tried acupuncture	12 (4.8%)

Close family diagnosed with hepatitis C	10 (4.0%)
Ever used drugs (Injected/snorted)	4 (1/2) (1.6%)
Ever been imprisoned	2 (0.8%)
Ever been through haemodialysis	0 (0%)
Ever gone through an organ transplant	0 (0%)

[a] *Routine testing of blood supply for hepatitis C began in 1992 in Bulgaria*

Non-participation analysis

In total, 155 (91.2%) of the 170 non-participants provided reasons for non-participation. A general dislike of surveys (26.5%), living abroad (23.9%), and not being interested in taking part in surveys (17.4%) were the most commonly reported reasons for not taking part in the survey (Table 5).

Table 5: Reasons for non-participation

Reasons for non-participation (N=155)	**n (%)**
Generally dislike surveys	41 (26.5%)
Living abroad	37 (23.9%)
Not interested	27 (17.4%)
No time	26 (16.8%)
Too ill	22 (14.2%)
Known HCV negative	5 (3.2%)
Living outside Stara Zagora (but in Bulgaria)	4 (2.5%)
No suitable appointment	2 (1.3%)
Live too far away	2 (1.3%)
Blood donor	2 (1.3%)
Do not wish to provide a reason	2 (1.3%)
Fear of needles	2 (1.3%)
Got tested in 2018	1 (0.6%)
Known HCV positive	0 (0%)

There was a significant difference in the age and sex distribution among the survey participants (n=252) and the total sample (n=1998). Participant mean age was 55.9 years and slightly lower for the total sample with 48.9 years (t (dr) = 6.3 (2248), p=0.0). Among

participants, 60.9% were female, whereas there were 53.4% females in the total sample (X^2=4.309, p=0.0) (Figure 5).

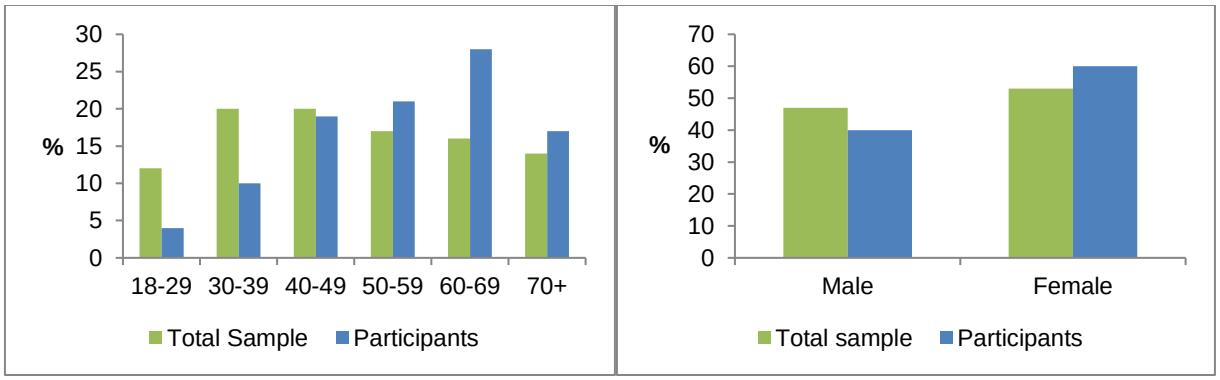


Figure 5: Age and sex among participants (n=252) versus total sample (n=1998)

4. Chapter B: Creating an overview of the viral hepatitis disease burden in Germany – The Hep-Epi Project

4.1 Background

A low prevalence of HBV and HCV (0.3%) was found in the most recent national population-based survey conducted from 2008-2011 in Germany, the German Health and Examination Survey for Adults (DEGS 1) [23]. Some sub-groups of the population are at higher risk of infection. These groups include PWID, MSM, people living with HIV (PLWH), and migrants from high prevalence countries. However, population-based surveys are often good enough at including these groups and they are therefore not well represented in population-based surveys.

Thirteen research questions were developed to identify published literature on HBV, HCV, and HDV epidemiology in Germany and the various sub-populations. The results of the overall review, including all research questions, have been published and are described elsewhere [20]. One of the research questions in this paper covered prevalence: “What is the prevalence of HBV, HCV, and HDV in Germany” [2]. The evidence identified for this question is published in one of the papers included in this dissertation [2].

4.2 Methodology

The methodology applied in the Hep-Epi project is described in detail elsewhere [20,2]. In the section below, the applied methodology is described in short.

4.2.1 Scoping review

We developed the search string and the reporting methods in line with the recommended reporting items for systematic reviews and meta-analysis extension for scoping reviews (PRISMA-ScR) and the Cochrane Collaboration. Our search string covered all 13 research questions formulated for the Hep-Epi project [20]. We conducted the search in six different electronic databases (EMBASE, PubMed, Europe PMC, Scopus, Base Bielefeld and CC Med) on 9 March 2017, and searched for publications published between 1 January 2005 and 9 March 2017 in English or German language, with ended data collection after 1 January 2005. We included and reviewed publications which met our pre-defined inclusion criteria [2]. We screened the included publications on abstract and full-text level. Two independent reviewers performed screening, and we discussed any inconsistencies and consulted a third person, if needed. Relevant information for all 13

research questions was extracted from the included publications and inserted in standardised data extraction sheets.

4.2.2 Data synthesis and analysis

We extracted the information available on HBV, HCV, and HDV prevalence from the overall spreadsheet containing all data from the search. We then summarised and analysed the extracted data based on pathogen (HBV, HCV or HDV) and population group. The pathogens and their markers are listed in Table 6.

Table 6: Viral hepatitis markers

Virus	Serological marker	Meaning
HBV	HBsAG	Indicates acute or chronic infection
	Anti-HBc	Indicates previous or ongoing infection
HCV	Anti-HCV	Indicates previous or ongoing infection
	HCR RNA	Indicates ongoing infection

We defined the population groups according to the WHO Guidelines on hepatitis B and C testing [24], and adapted these to fit the German context (Table 7). If there was no explicit definition of the study population in the publication, we assigned the population group to the most fitting of the pre-defined population groups.

Table 7: Population groups in the review

Population group	Description
1) The general population	The general population in Germany, including children
2) Proxies for the general population	Sub-groups representative of the general population: pregnant women, blood donors and “baby boomers” (individuals born between 1946-1964)
3) Clinical populations	Populations with underlying disease (not viral hepatitis related) and people with viral hepatitis in hepatological care
4) At-risk populations	People with at-risk behaviour or exposure: Household contacts of people with viral hepatitis, health care workers (HCW), PLWH, MSM, PWID, people in prisons and closed settings, sex workers or people part of populations with higher prevalence, such as mobile or migrant populations from intermediate- or high endemic countries

We used a pre-existing checklist [25] to perform a quality appraisal and evaluate the overall risk of bias of each of the publications included in our review. We performed the assessment by scoring the publications according to 10 pre-defined items. The items covered both internal and external validity, and finally the publications were assessed to have either “low” or “high” risk of bias based on the total score. However, the publications were not weighted according to risk of bias in the analyses.

4.3 Results

The general search, covering all 13 research questions, retrieved 18.410 publications, of which 7.454 were duplicates and thereby excluded. Title and abstract screening of the remaining 10.956 publications resulted in the exclusion of 10.329 publications. The remaining 627 publications underwent full-text screening, and 104 fulfilled the inclusion criteria and were included in the overall final review. Of these, 51 covered prevalence and were included in this analysis (Figure 6). Of the 51 included publications, 39 reported on HBV, 33 on HCV and four on HDV. Some of the publications included results on more than one pathogen.

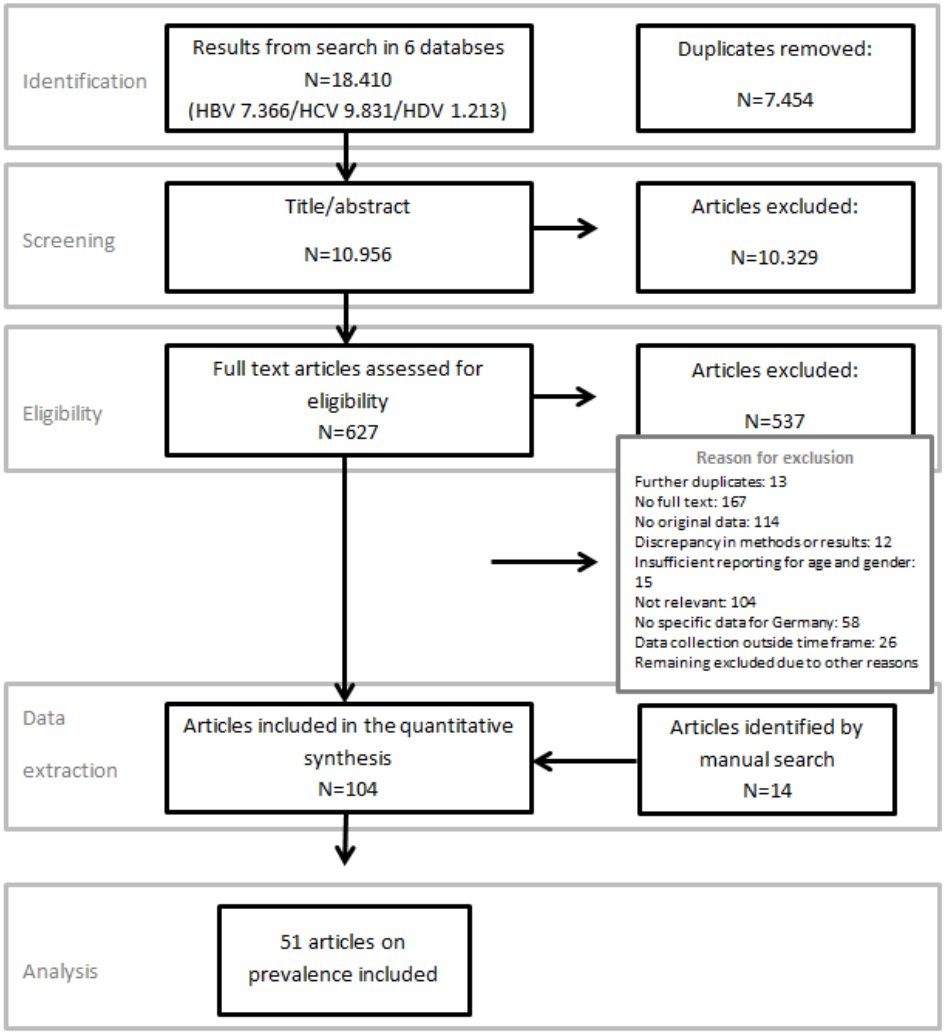


Figure 6: Prisma flowchart for search and selection of articles (from Steffen, G., Sperle, I., Leendertz, SA., Sarma, N., Beermann, S., Thamm, R., Bremer, V., Zimmermann, R., Dudareva, S., 2020)

Overall, 14 of the 51 publications had a high risk of bias according to our quality appraisal. The bias was caused by different reasons, but the most common gaps that contributed to high risk of bias were a lack of a sufficient description of the methodology e.g. how the sample was drawn, of their recruitment strategy, and/or that either the specific viral hepatitis marker was unspecified or that viral hepatitis status was self-reported. The most common study design in the included publications was the cross-sectional design (N=37). Eight studies used a cohort design, and five were surveillance studies and one was a case-control study. Sixteen studies reported national-level data results, whereas the remaining studies reported either local or regional-level data. In one study the level on which data was collected was not reported [26].

4.3.1 Prevalence of hepatitis B in Germany

Thirty-nine publications reported on the prevalence of hepatitis B in Germany and they were conducted from 1996 to 2016.

General population, including proxy populations

Thirteen publications covered the general population (including proxy populations). The prevalence of HBsAg in the general population was low (range: 0.3-0.7%), and 0-1.6% in proxy populations. The anti-HBc prevalence was also low in the general population (0.5-0.6%), and ranged from 0.9-1.4% among the proxy populations.

Blood donors

Of the 13 publications, blood donors were covered by six publications. Using surveillance data, HBsAg, anti-HBc, HBV-DNA prevalence (not reported separately) among first time blood donors was presented by four publications showing a range from 0.12-0.15%. Two studies described prevalence of anti-HBc among first time blood donors and found a low prevalence of 1.9% and 0.9%.

Clinical populations

Clinical populations were covered in 13 studies. In four publications the prevalence of HBV (marker not specified) among patients with HCV ranged from 0.1-39.1%. Eight studies described the prevalence of HBsAg which ranged from 0.2-3.4%. Of these eight studies, four reported a prevalence of anti-HBc and HBsAg among emergency and trauma department patients ranging from 0.5-1.3%. Higher prevalence of anti-HBc IgG was found among alcohol dependent patients (8.3%), and among patients with rheumatic disease (5.6%).

At-risk populations

Health care workers

Among health care workers, the anti-HBc prevalence was reported in four studies. The prevalence ranged from 0.5-1.7%. One study among medical doctors found a self-reported prevalence of anti-HBc of 1.6% [27].

Household contacts

Household contacts (partners of people living with HBV) were also studied in one publication, in which a prevalence of 10.7% was self-reported.

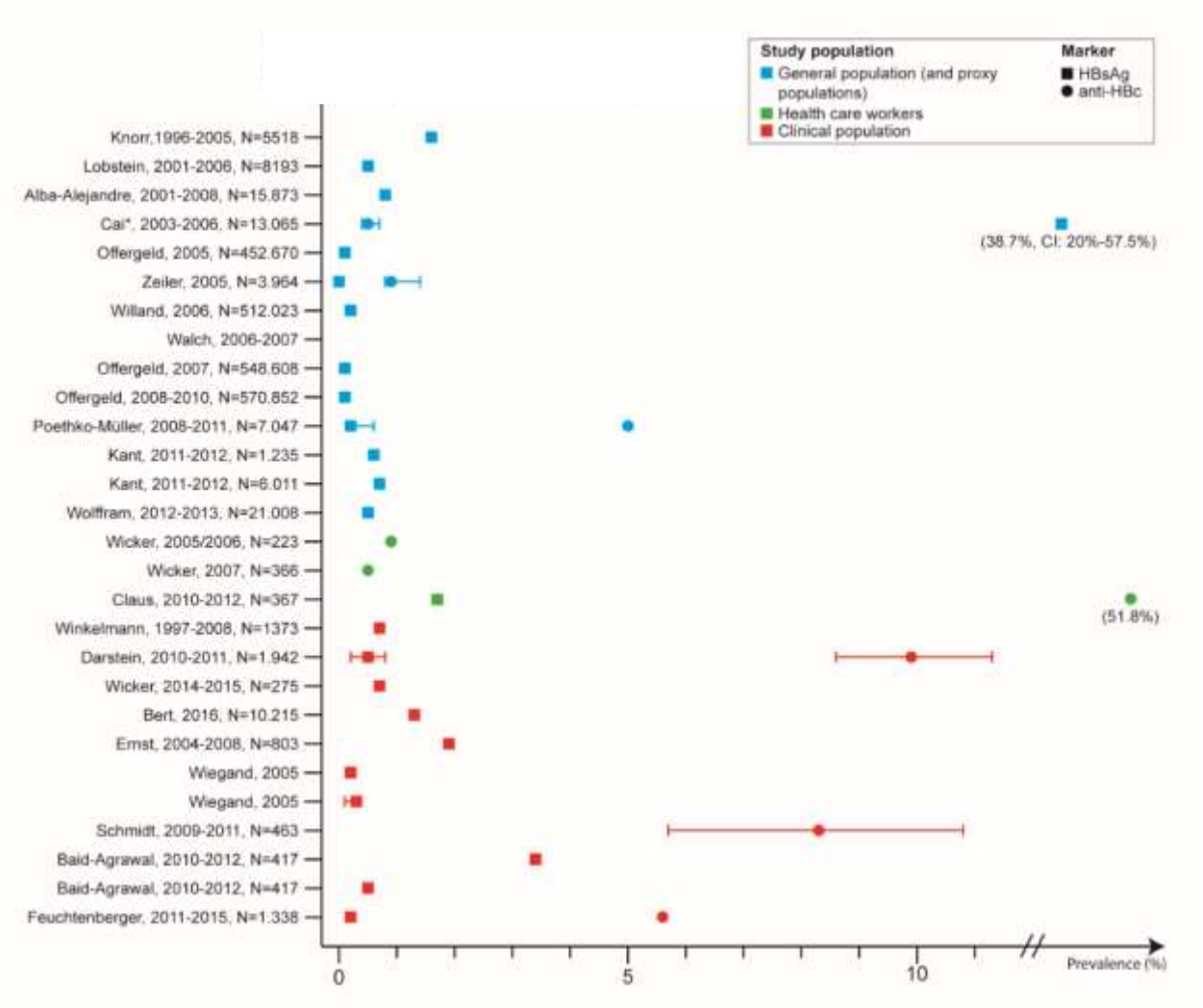


Figure 7: Hepatitis B prevalence in Germany by study population and marker, 2005-2017 (from Sperle, I., Steffen, G., Leendertz, S.A., Sarma, N., Beermann, S., Thamm, R., Simeonova, Y., Cornberg, M., Wedemeyer, H., Bremer, V., Zimmermann, R., and Dudareva, S., 2020)

People with migration background

Three publications reported on HBV prevalence among migrants. One reported results from screening of refugees in an emergency department, where an HBsAg prevalence of 2.3% and an anti-HBc prevalence of 14.0% was found. Among patients (patients or

parents of patient) with migration background, an HBsAg prevalence of 3.6% and anti-HBc of 32.5% was found. No HBV positives (marker not defined) were identified among 488 Syrian refugees screened upon arrival in Germany.

People living with HIV

Two publications were identified which reported on HBV prevalence among PLWH. An HBsAg prevalence of 4.5% was found among HIV patients, and the other study among HIV positive MSM reported a prevalence of 1.7%.

People who inject drugs

One identified study described self-reported HBV infection among PWID recruited from either low-threshold services (drug consumption rooms and substitution clinics) or the streets, and the prevalence found was 14.0% and 14.1%, respectively. Among PWID in specialised methadone substitution centres the HBsAg prevalence was 1.3%. Another study recruited PWID from different low-threshold services in eight cities across Germany. In this study, the anti-HBc prevalence was 25.0% (ranging from 4.6-33.0% in the eight German cities), and 1.1% of these were HBsAg positive (ranging from 0.3-2.5% in the eight German cities).

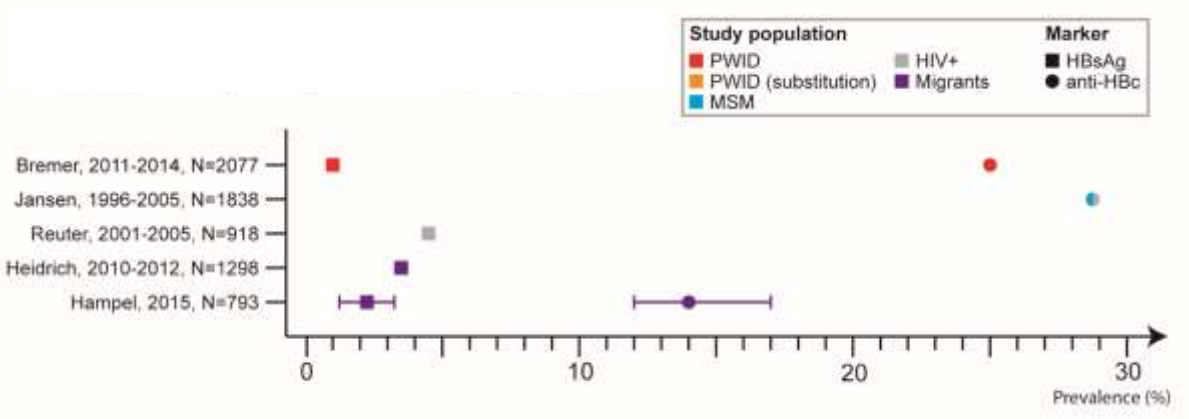


Figure 8: Hepatitis B prevalence in Germany by study population (at-risk) and marker, 2005-2017 (from Sperle, I., Steffen, G., Leendertz, S.A., Sarma, N., Beermann, S., Thamm, R., Simeonova, Y., Cornberg, M., Wedemeyer, H., Bremer, V., Zimmermann, R., and Dudareva, S., 2020)

4.3.2 Prevalence of hepatitis C in Germany

In total, 33 publications reported on prevalence of hepatitis C in Germany, and were conducted from 1996 to 2014.

General population, including proxy populations

Eleven studies reported on HCV prevalence in the general population, including proxy populations. The anti-HCV prevalence in the general population ranged from 0.2-1.9%. One study among baby boomers (proxy population) found an anti-HCV prevalence of 1.5%. Two studies reported on HCV RNA, and found a rate of 0.2% and 0.4%, respectively.

Blood donors

Of the 11 publications, blood donors were covered by four publications. Surveillance of blood donors presented a low anti-HCV prevalence ranging from 0.06-0.08%.

Clinical populations

The prevalence of HCV among clinical populations was reported by 10 studies. Anti-HCV ranged from 0.2-5.2%, and from 0.9-3.5% among emergency and trauma department patients.

One study including two groups of clinical patients also measured HCV RNA. Among chronic haemodialysis patients the HCV RNA prevalence was 2.4%, and among kidney transplant recipients the prevalence was 4.6%. None of the HBV patients studied in one publication were found to be co-infected with HCV.

At-risk populations

Healthcare workers

Three studies on healthcare workers all reported a low anti-HCV prevalence. Two measured the anti-HCV prevalence among healthcare workers to be 0.0% and 0.03%, and one reported a self-reported anti-HCV prevalence of 0.04%.

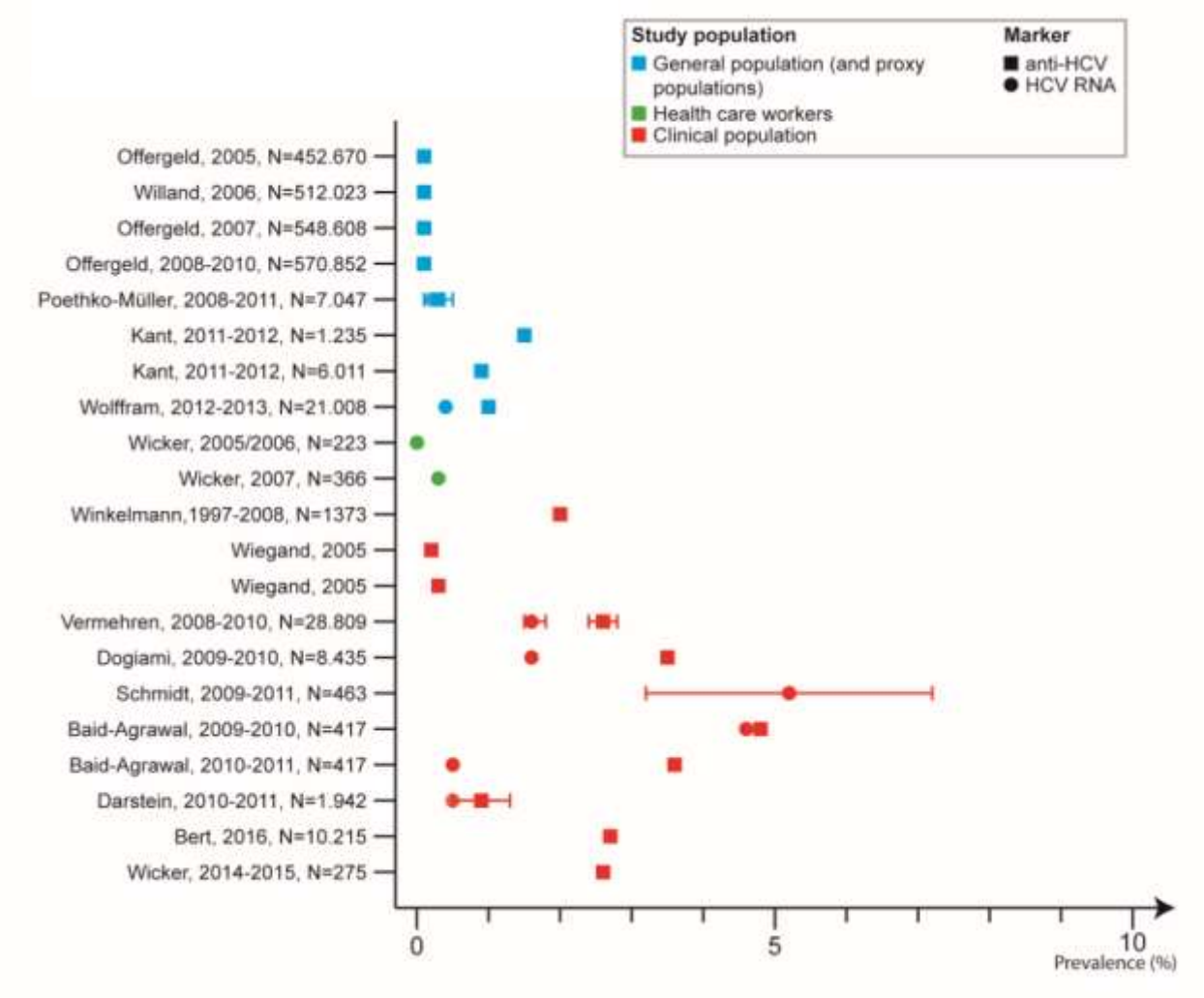


Figure 9: Hepatitis C prevalence in Germany by study population and marker, 2005-2017 (from Sperle, I., Steffen, G., Leendertz, S.A., Sarma, N., Beermann, S., Thamm, R., Simeonova, Y., Cornberg, M., Wedemeyer, H., Bremer, V., Zimmermann, R., and Dudareva, S., 2020)

People with migration background

Two studies examined prevalence of HCV among migrants. One study included patients with migration background in eight different primary care centres in Northwest Germany. Most patients originated from the Eastern Mediterranean area (87.3%), and the second largest group was from Eastern Europe (12.0%). The overall prevalence of anti-HCV was 1.9%, and prevalence of HCV RNA was 0.7%.

Among refugees and asylum seekers (country of origin was not described in the study) routinely screened when arriving in Germany, the anti-HCV prevalence was 0.4%.

People living with HIV

One study described HCV prevalence among patients living with HIV, and the two others (three studies in total described HCV among PLHIV), reported on HCV among MSM living with HIV. For HIV positive patients in general, the anti-HCV prevalence was 10.6%. Among MSM, one study found a prevalence of anti-HCV of 8.2%. Self-reported HCV among MSM who were HIV positive was much higher (8.8%) than among those HIV negative (or not tested for HIV) (0.2%) in the third included study.

People who inject drugs

Anti-HCV among PWID in three included studies was very high and ranged from 63.0% to 68.0%. One of these studies was a cross-sectional study covering eight German cities in which the anti-HCV prevalence ranged from 36.9% in Leipzig to 73.0% in Hanover. The prevalence of HCV RNA in the same eight cities ranged from 23.1% to 54.0%.

PWID recruited from the streets self-reported an HCV prevalence of 58.3% and those recruited from opioid-substitution treatment (OST) programmes 58.7%. In a nationwide study, physicians from 21 different prisons reported an HCV prevalence of 14.3% among people in prisons, and of these 21.9% were also PWID.

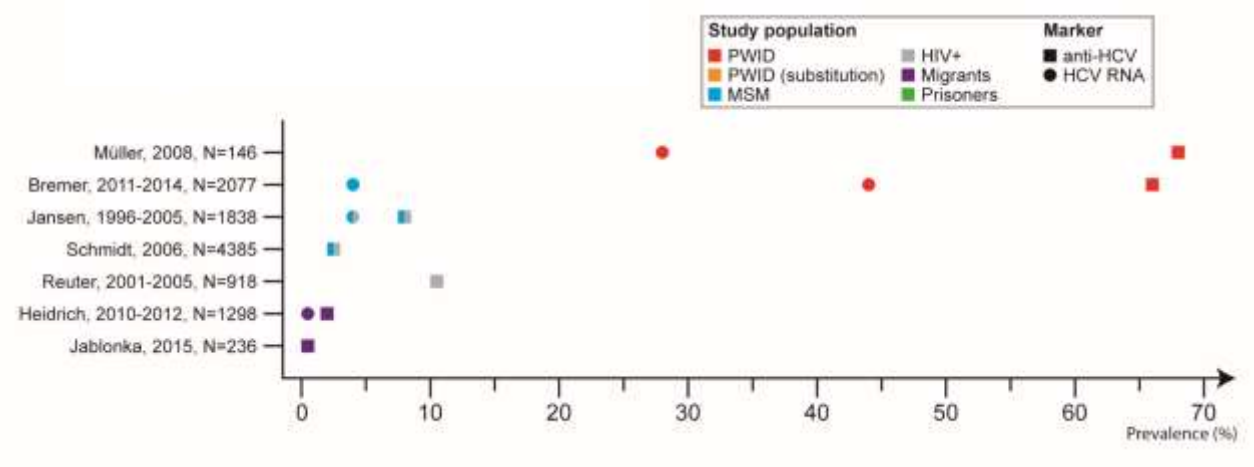


Figure 10: Hepatitis C prevalence in Germany by study population (at-risk) and marker, 2005-2017 (from Sperle, I., Steffen, G., Leendertz, S.A., Sarma, N., Beermann, S., Thamm, R., Simeonova, Y., Cornberg, M., Wedemeyer, H., Bremer, V., Zimmermann, R., and Dudareva, S., 2020)

4.3.3 Prevalence of Hepatitis D in Germany

HDV prevalence was covered by four publications which included patients chronically infected with HBV conducted between 1989 and 2011. In three publications the patients were recruited from hospitals and in one the patient data were provided by physicians. The HDV prevalence ranged from 0-7.4%. One study specified the HDV marker and reported an anti-HDV prevalence of 7.4%, and HDV RNA of 64.5%. One study collected data from 74 hepatology centres across Germany and found a prevalence of 1.4% in the population of HBV positives.

5. Discussion

The papers included in this dissertation contribute to aspects of viral hepatitis prevalence data collection that can help close the gap in strategic information and help inform next steps to reach elimination of viral hepatitis by 2030.

5.1 Improving data collection for viral hepatitis (the SPHERE-C Project)

A population-based survey provides a snapshot of the epidemic, in contrast to surveillance data which often reflect implemented testing strategies. The three probability-based survey approaches recommended in the technical SPHERE-C protocol [16] are all useful in estimating the prevalence of chronic HCV in the adult general population.

It may be necessary to make alterations depending on setting and situation, and it may not always be possible to carry out any survey at any given point in time. However, regardless of which survey approach is chosen, it is critical that the minimum requirements outlined in the protocol are fulfilled to obtain high-quality samples (sample size, representativeness of target population, time of data collection) to be able to generate a robust prevalence estimate.

One of the key aspects of the sample quality is to have a large enough sample. It is crucial that the calculated sample size is reached. This entails a rigorous sampling strategy carefully adapted to local setting and context. When nesting onto a larger population-based survey, an advantage is often that one such strategy is in place. When this is not the case, it is possible to sample further people for HCV testing to reach the calculated

sample size. However, as demonstrated in the pilots, non-response is an issue in most surveys. Implementing several recruitment steps is important to achieve a high response rate. If it is not possible to implement these, it may not be worth the additional efforts associated with a stand-alone as the end sample will not be representative of the target population. Considerations for how to deal with non-response in the analysis are also central, as well as planning for flexibility in terms of timeframe to allow time to increase the number of participants.

The pilot in Stara Zagora, Bulgaria underlined the importance of a solid recruitment strategy. In this pilot, recruitment was only possible through letters, and not through phone calls and house visits as was originally planned. This has probably contributed to the relatively low response rate, and thereby not recruiting enough participants to reach the calculated sample size (N=999). As a consequence, there was a low precision for the HCV prevalence estimate. Although, as done for the pilot in Stara Zagora, weights can be included in the analysis to compensate for non-response, there will inevitably be factors which differentiate responders and non-responders that cannot be accounted for that may increase risk of bias in the result. When too little efforts are made to recruit people less likely to participate, the prevalence will be under- or overestimated. Low participation and selective non-response will bias the results. Some sub-populations are more likely to take part in surveys, and they often represent groups of higher socio-economic status and better health compared to non-participants [28, 29].

A large probability-based sample, which is considered to be representative of the target population, is of high value for many research groups. Therefore, there may be competing proposals from different groups who want to test the samples and include additional content in the questionnaire. Therefore, it is important to be involved in the early planning, and communication and prioritisation with and between research groups.

An HCV prevalence estimate is one of the core indicators in the monitoring and evaluation framework defined by WHO [19]. An HCV prevalence estimate in the general population is just part of estimating the national HCV prevalence. The at-risk groups are disproportionately affected by viral hepatitis. There is a high prevalence in these sub-groups, but they are not sufficiently included in population surveys targeting the general

population. Better prevalence data on at-risk group are also needed, and to reach these groups alternative sampling and recruitment strategies need to be considered. Additionally, modelling techniques can be applied, for which information on the size of and prevalence in at-risk groups is needed.

5.2 Overview of viral hepatitis epidemiology in Germany (the Hep-Epi Project)

The aim of the Hep-Epi Project and the review was to outline the current available evidence on viral hepatitis prevalence in Germany and to describe the baseline situation against which progress towards elimination can be measured. As far as we are aware, the Hep-Epi review is the first to systematically search for all available evidence on viral hepatitis epidemiology in Germany.

We found large variations in the available evidence depending on virus but also population group. The review identified a large amount of evidence on viral hepatitis prevalence (51 publications), but primarily evidence on the general population (HBV and HCV) as well as certain clinical population sub-groups. Little evidence was found among certain at-risk populations and no evidence was identified for people who have received blood transfusions, persons with tattoos/piercings or sex workers.

We found a low prevalence of HBV and HCV in the general population, which corresponds with other European studies [8]. Among proxy populations, a slightly higher prevalence was found. Precaution is necessary when extrapolating data from proxy populations for the general population as these for various reasons, and depending on which group, will differ from the general population.

It is known that some at-risk populations are disproportionately affected by viral hepatitis [30] which was also confirmed in this review. The most frequent routes of transmission affect which groups have the highest prevalence. Sexual transmission is most common for HBV, whereas blood-to-blood transmission is most common for HCV. We found a higher prevalence of both HBV and HCV among PLWH in the studies included in this review, and also other at-risk groups such as MSM and PWID. That the prevalence is high among PWID corresponds to injecting drug use being the main driver of the epidemic in

the EU/EEA. Injecting drug use causes 40% of the cases in Europe for which the transmission route is known [31].

Varying results were reported for migrants. Two of the three studies found a higher HBV prevalence among migrants compared to the general population, and for HCV a relatively lower prevalence and closer to that of the general population was reported. The reasons behind the variations in prevalence are the result of a complex relationship between factors in the new country and country of origin. Furthermore, terminology and sub-groups of migrants also differed, ranging from newly arrived people seeking asylum screened at reception centres to German residents with migration background. The complexity is large, and the importance of distinguishing country of origin, groups and terminology, e.g. refugee versus migrant, is important for clarity.

Although the heterogeneity of the identified evidence makes conclusions as well as comparisons across groups and time challenging, the review provides a good foundation for deciding on viral hepatitis response actions, in particular which survey method to apply to estimate the national prevalence as part of monitoring the viral hepatitis elimination. The data from the Hep-Epi project also provides a baseline of the previous years, and needs to be regularly updated in order to monitor the development of the viral hepatitis epidemic in Germany.

Since early 2020 the entire world has been affected by the COVID-19 pandemic. The pandemic has posed significant challenges to public health and health systems and has led to re-allocation of attention and resources. Now that the COVID-19 pandemic has been contained, the viral hepatitis community will need to reconvene to ensure focus on reaching the elimination targets. Despite challenges, there are also important lessons to be drawn. New attention to the importance of infectious disease epidemics, as well as synergies and collaborations may be used to better implement efforts to respond to viral hepatitis and gather the data necessary to monitor these efforts.

5.3 Limitations

The central limitations that the two projects in this dissertation are subject to, need to be considered when interpreting the results.

For the SPHERE-C project, we did not pilot the retrospective and nested survey approaches in their pure form. Further, we only piloted the stand-alone survey on city level, and not nationally. Experiences collected to further develop the technical protocol may therefore lack nuances that were not captured in the pilot phase [3]. A low response rate was a large limitation in the survey in Stara Zagora, Bulgaria, and the HCV prevalence is therefore not representative for the general population [1].

For the Hep-Epi project [2], although a robust search string was developed to capture all published evidence on viral hepatitis prevalence, risk of publication bias remains as well as delayed publishing of data relevant for the review. The review provided an idea of how the prevalence differs according to population group and virus. It also demonstrates what evidence is available, and importantly highlights gaps in knowledge. However, a comparison and evaluation of prevalence over time and across geographical areas in Germany was not possible due to the heterogeneity of the publications included in the review.

6. Conclusions

Viral hepatitis poses a major burden on health systems worldwide, and requires a strong response including prevention, testing and treatment to prevent transmission and reduce mortality. Data on mortality and morbidity are needed to keep the attention to and momentum for eliminating viral hepatitis. Data sources for these indicators need to be defined, and could include data from laboratories, health services and hospitals or registry data depending on quality and completeness. Data on prevalence, combined with modelling data, is important to monitor the progress towards elimination of viral hepatitis as a public health problem. And importantly, to use this information for determining public health actions to overcome gaps on the path to elimination.

The papers included in this dissertation [1-3] each highlight methodological aspects of improving prevalence data collection on viral hepatitis. The technical SPHERE-C protocol

was proven to be useful in estimating the prevalence of HCV in the general population. The prevalence found in the general population was low, but there are challenges in reaching a high participation rate and carefully planned recruitment steps are needed to be able to generate a robust prevalence estimate.
A large body of evidence on viral hepatitis in Germany exists, and this can serve as a good baseline against which progress towards elimination can be measured. Nonetheless, information was scarce for some groups and missing for important key groups including sex workers and people who have received blood transfusions. There is a need for more research to close the evidence gaps as well as a continuous update to monitor the road to elimination. Creating an overview of the current state of the epidemic through review of literature, and conducting prevalence surveys are good methodological options to create knowledge on the state of the viral hepatitis epidemic.

With less than ten years to go to the deadline of the elimination targets, standardised and reliable data and evidence-based decisions are crucial to improve the epidemic response and finally eliminate viral hepatitis as a public health problem by 2030.

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Statutory declaration

I, Ida Sperle-Heupel, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic Viral Hepatitis in Germany and Europe: Data to Guide Elimination Efforts/ Virale Hepatitiden in Deutschland und Europa: Datengrundlage für die Eliminierung, independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.

Date Signature

Declaration of own contribution to the publications

Ida Sperle worked as a coordinator for the SPHERE-C project and worked on the hep-epi project while employed as an epidemiologist at the Robert Koch-Institute (2017-2019). For SPHERE-C she took part in the development of the technical protocol and the evaluation of the two pilot surveys in Finland and Italy. She took part in planning, conducting and evaluation of the pilot survey in Stara Zagora, Bulgaria. This also included the organising of double data entry of the 252 questionnaire (using EpiData) and analysing the data for the scientific paper including in this dissertation.

Ida Sperle contributed the following to the below listed publications:

Publication 1:

Sperle, I., Nielsen, S., Gassowski, M., Naneva, Z., Perchemlieva, T., Amato-Gauci, A., An der Heiden, M., Bremer, V., Golkocheva-Markova, E., Hristov, K., Kaneva, E., Simeonova, Y., Tenev, T., Varleva, T., Duffell, E., and Zimmermann, R., Prevalence of hepatitis C in the adult population of Bulgaria: a pilot study. *BMC Res Notes*. 2020;13(1):326. Published 2020 Jul 7. doi:10.1186/s13104-020-05158-3

Overall estimated contribution: 80%

Details of contribution:

- Data analysis and interpretation of results together with statistician (for prevalence estimate)
- All tables, figures and supplementary materials in the publication
- Identifying relevant literature for background information and references
- Development of the first draft of the manuscript and revising according to feedback from co-authors
- Submission to journal and incorporation of feedback from review process

Publication 2:

Sperle, I., Steffen, G., Leendertz, S.A., Sarma, N., Beermann, S., Thamm, R., Simeonova, Y., Cornberg, M., Wedemeyer, H., Bremer, V., Zimmermann, R., and Dudareva, S.,

Prevalence of Hepatitis B, C, and D in Germany: Results From a Scoping Review. *Frontiers in Public Health*. 2020; 8: 424. Published 2020 Aug 28. Doi: 10.3389/fpubh.2020.00424

Overall estimated contribution: 60%

Details of contribution:

- Data analysis and interpretation of data collected from the included publications in the review
- Development of all tables and figures except Figure 1
- Identifying relevant literature for background information and references
- Development of the first draft of the manuscript and revising according to feedback from co-authors
- Submission to journal and incorporation of feedback from review process

Publication 3:

Sperle, I., Nielsen, S., Bremer, V., Gassowski, M., Brummer-Korvenkontio, H., Bruni, R., Ciccaglione, A.R., Kaneva, E., Liitsola, K., Naneva, Z, Perchemlieva, T., Spada, E., Toikkanen, S.E., Amato-Gauci, AJ., Duffell, E., Zimmermann, R., Developing and Piloting a Standardized European Protocol for Hepatitis C Prevalence Surveys in the General Population (2016–2019). Frontiers in Public Health. Front. Public Health 9:568524. doi: 10.3389/fpubh.2021.568524 (accepted)

Overall estimated contribution: 80%

Details of contribution:

- Data analysis and interpretation of results from evaluation
- Development of all tables and figures except Figure 1 where the first draft was developed by a co-author
- Development of the first draft of the manuscript and revising according to feedback from co-authors
- Submission to journal and incorporation of feedback from review process

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Signature, date and stamp of first supervising university professor / lecturer

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Signature of doctoral candidate

Printed copies of the publications

Sperle et al. BMC Res Notes (2020) 13:326
https://doi.org/10.1186/s13104-020-05158-3

BMC Research Notes

RESEARCH NOTE Open Access

Prevalence of hepatitis C in the adult population of Bulgaria: a pilot study

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Abstract

Objective: This study piloted a European technical protocol for conducting chronic hepatitis C prevalence surveys in the general population. The pilot study took place in the Bulgarian city of Stara Zagora in 2018, and results of setting up, conducting and evaluating the survey are presented.

Results: A probability-based sample of the general adult population was drawn from the local population registry, stratified by age and sex. A sample size of 999 was calculated, and accounting for 50% non-response, 1998 registered invitation letters were sent. Venous blood samples and questionnaire data were collected by the Regional Health Inspectorate in Stara Zagora. Blood samples were tested for anti-HCV, and if reactive for RNA. 252 (21.6%) of the participants were included in the study. Mean age and sex distribution differed between the participants (55.9 years, 60.3% females) and the total sample (48.9 years, 53.4%). The weighted chronic HCV prevalence among participants was 0.9% [95% CI 0.2–4.2%]. The approach of only sending registered letters contributed to a low response rate, and more efforts are needed to reduce non-response, especially among men and younger age groups. Results of the evaluation were integrated in the final technical protocol.

Keyword: Hepatitis C, HCV, Prevalence, General population, Prevalence survey, Bulgaria

Introduction

The World Health Organization global strategy on viral hepatitis calls for elimination as a public health threat by 2030 [1] and national prevalence of chronic hepatitis C virus (HCV) infection is one of ten core indicators to be monitored [2].

HCV is primarily transmitted through infected blood and in European Union (EU) countries it mainly affects people who inject drugs (PWID) [3]. However, a higher prevalence may be found in birth cohorts of the general population (GP) exposed through nosocomial or transfusion-related transmission [4–7].

A recent systematic review found an anti-HCV prevalence in the GP in EU/European Economic Area (EEA) ranging from 0.1% (Belgium, Ireland and the Netherlands) to 5.9% (Italy) [3, 8]. Differences in prevalence between 16 countries with available estimates were difficult to interpret due to heterogeneous methodological approaches [3]. To address this, the European Center for Disease Prevention and Control contracted the Robert Koch Institute (RKI) from 2016 to 2019 to develop and pilot an evidence-based technical protocol with the aim to contribute to the standardisation of chronic HCV prevalence surveys in the GP. The protocol was developed in conjunction with an international and interdisciplinary expert panel and was published in March 2020

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[9]. The stand-alone survey approach is one of three recommended approaches in the technical protocol and was piloted in the city of Stara Zagora, Bulgaria.

Stara Zagora is the sixth largest city in Bulgaria with an adult population of 120,849.[1] The city has one of the country's best economies [10]. Stara Zagora was selected as study site because of a strong collaboration between the Regional Health Inspectorate (RHI) and the Ministry of Health and a good laboratory testing infrastructure.

Robust data on HCV prevalence in Bulgaria are limited. One multi-centre study (1999–2000) among healthy volunteers in the five largest cities found an overall anti-HCV prevalence of 1.3% with a range from 1.1% in Stara Zagora and Plovdiv to 1.6% in Sofia [11]. Another study (2010–2011), found an 0.7% anti-HCV prevalence among outpatients in the Plovdiv Region [12].

This paper presents the results of the HCV prevalence pilot survey and reports on the feasibility of the protocol and key lessons learnt.

Methods

A cross-sectional survey was undertaken to measure the chronic HCV prevalence (anti-HCV and RNA positive) in the adult GP (≥ 18 years) in Stara Zagora.

Sampling

Based on an expected chronic HCV prevalence of 1% and a lower precision bound of 0.25%, a sample size of 999 was calculated. Accounting for an expected non-response rate of 50%, the total sample size was 1998.

A probability-based sample of the GP with current address in Stara Zagora, stratified by sex and six age groups (18–29, 30–39, 40–49, 50–59, 60–69, 70 + years), was drawn from the local population registry "Esgraon-TDS" [13, 14].

Recruitment

Registered invitation letters (Additional file 1: S1a) were sent from the local population registry to the invitees. The first batch (400 letters) was sent two weeks prior to onset of data collection (05.09.2018). A reminder letter followed if no response within three weeks of sending the first letter (Additional file 2: S1b). If people were not home when the letter arrived, a note was delivered informing of the letter available to be collected at the local postal office.

The letters described the aims of the survey, selection of participants, opening hours and contact details of the study site and the availability of a mobile unit which could facilitate participation close to home and the incentive (coffee mug and pen) provided after participation. Voluntary participation, anonymity and confidentiality of data were underlined. A participant information leaflet (Additional file 3: S2) accompanied the letter providing more details about HCV, the survey, the importance of taking part, and that test results would be provided followed by linkage to care if HCV positive.

A local awareness campaign including posters in pharmacies and medical centers, announcements on RHI Facebook page and local press conferences to encourage participation was launched.

Ethical approval, data protection

Persons in the sample were assigned an identification number and all identifiable information was kept at the local population registry. Participants provided written informed consent. Original data were kept at the RHI and copies were sent to RKI via an online server allowing an encrypted secure transfer. The survey protocol was approved by the local ethics committee established at the RHI.

Data collection

Data were collected from 5 September to 16 November 2018, Monday–Friday: 8:30 am–7:00 pm and Saturday: 8:30 am–1:30 pm at the RHI, and on Saturdays also in a mobile unit.

On site, participants self-completed a questionnaire on socio-demographics, HCV testing history, knowledge of HCV status and risk factors. Basic sociodemographic information on non-responders who called to decline participation was collected over the phone.

Venous blood samples were tested for HCV antibodies [Bioelisa, antibody HCV 4th generation (by Biokit)] at the RHI laboratory. Anti-HCV reactive samples were tested for RNA (Additional file 4: S3) (HCV Real Time PCR, Abbott, USA) at the National Reference Laboratory "Hepatitis viruses", Sofia. RNA negative samples were tested by immunoblot (Inno-Lia HCV score, Fujirebio, Belgium) to confirm the positive anti-HCV result.

During a face-to-face consultation at the RHI a medical doctor informed participants about their test results. Those with chronic HCV were referred to a gastroenterologist in the hospital of Stara Zagora where liver function was assessed and treatment initiated in line with national guidelines.

Data analysis

Double data entry was performed using EpiData (version 4.4.2.1), and analyses in STATA 15. Descriptive analysis was performed for all variables. T-test was used to compare the mean age among the participants and the total

[1] Registered as currently living in Stara Zagora as of 15 March 2018 (Information from the Local Population Registry, "Esgraon-TDS", in Stara Zagora)

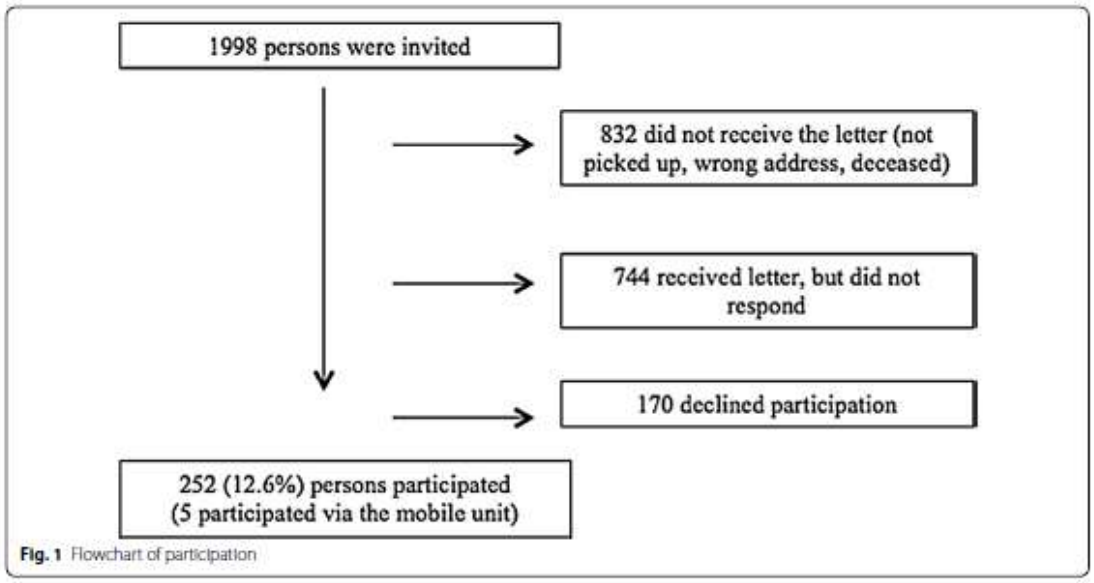

Fig. 1 Flowchart of participation

sample, and chi-squared test was used for sex with the statistical significance defined as p value < 0.05. Chronic HCV prevalence was calculated as crude and weighted estimates with 95% confidence intervals (CI). For the latter, we applied post-stratification weights according to age and sex to adjust for non-response.

Evaluation of the draft technical protocol

Indicators were developed and transformed into a questionnaire with 10 main questions covering objectives of the survey, methodology, time, structure, coordination and collaboration, ethical approval, data protection, staff and budget to be completed by the RHI study team. During a 2 day evaluation workshop recommendations for improvement of the protocol were discussed with 14 survey staff members.

Results

Of 1998 invited people, 1166 received the invitation letter of which 252 participated (21.6%) (Fig. 1).

Of the 832 who did not receive the letter, two were deceased. The rest did either not pick up the letter or were registered with a wrong address.

Non-participation analysis

Among 170 declining participation, 155 (91.2%) provided reasons for non-participation (Table 1).

The age and sex distribution among participants differed significantly from the total sample (n = 1998). The mean age for participants was 55.9 years versus 48.9 years for the total sample (p < 0.0001). There were 60.3% females among participants versus 53.4% in the total sample (p < 0.0001) (Additional file 4: S3).

Survey participants characteristics

Participants' mean age was 55.9 years (18–95 years) (Table 2) (Additional file 4: S3).

Table 1 Reasons for non-participation

Reason for non-participation (n = 155)	n (%)
Generally dislike surveys	41 (26.5%)
Living abroad	37 (23.9%)
Not interested	27 (17.4%)
No time	26 (16.8%)
Too ill	22 (14.2%)
Live too far away	6 (5.8%)
Known HCV negative	5 (3.2%)
No suitable appointment	2 (1.3%)
Blood donor	2 (1.3%)
Fear of needles	2 (1.3%)
Got tested in 2018	1 (0.6%)
Known HCV positive	0 (0%)
Do not wish to provide a reason	2 (1.3%)

Table 2 Sociodemographic characteristics of participants (n = 252)

Sociodemographic characteristics	n (%)
Sex	
Female	152 (60.3%)
Male	100 (39.7%)
Ethnicity	
Bulgarian	248 (98.8%)
Roma	2 (0.8%)
Other	1 (0.4%)
Missing	1 (0.4%)
Highest level of education	
Elementary education	1 (0.4%)
Primaryeducation	11 (4.4%)
Secondary education	122 (48.4%)
Higher education	118 (46.8%)

Prevalence of HCV

Two participants were both anti-HCV and HCV-RNA positive, crude chronic HCV prevalence: 0.8% [95% CI 0.2–3.1%], weighted prevalence: 0.9% [95% CI 0.2–4.2%].

Factors associated with HCV

Among the 252 participants, the most frequently reported factors associated with HCV were surgery under general anesthesia (64.1%), followed by blood transfusion before 1992 (11.7%) (Additional file 4: S3). One of the two HCV positive participants reported having injected drugs, the other did not report any known factors associated with HCV.

Results from the evaluation

Sufficient staff training was provided and the protocol was evaluated as useful and understandable. The extended opening hours helped accommodate participation of people who work, whereas the mobile unit was less utilised. Planning took more time than expected (one full-time equivalent staff for nine months) particularly on administrative tasks and data protection issues. In total, 19 RHI staff were involved in the data collection.

Discussion

We performed a cross-sectional survey with the aim to pilot the draft technical protocol, assess its feasibility and to generate an HCV prevalence estimate in the GP of Stara Zagora. As the Data Protection Commission denied RHI access to contact information for the invited participants, the initially planned recruitment strategy (involving house-visits to non-responders) was changed, allowing only recruitment via letters which resulted in not reaching the calculated sample size (n = 999). As consequence a low precision for the HCV prevalence estimate, for which reason weighting was performed to adjust for non-response. The prevalence may under- or overestimate the true prevalence due to the failure of including persons less likely to participate. Low participation and selective non-participation cause bias to survey results [15, 16]. Lower socio-economic status, a poorer health profile and higher mortality have previously been found among non-participants compared to participants [17, 18]. In this survey, non-participation was more frequent among men and younger age groups. Higher participation among women and older age groups corresponds with findings from other similar surveys [19]. Reasons for non-response are likely multifaceted, and may differ depending on sex and age group.

Of the 170 people who actively declined participation, 41 (24%) lived outside Stara Zagora. It is plausible that a similar proportion among the 832 who did not receive the letter also migrated to other cities or countries e.g. for work. This indicates that the sampling frame was not up-to-date which is a key requirement for surveys [20].

More efforts are needed to reduce non-participation, but their effectiveness may differ between settings [21, 22]. In a German Health Survey phone calls and house visits increased participation from 37 to 49%, with greater effect among younger persons, males and non-Germans [23]. In Finland, SMS reminders have shown a positive effect [24]. We used registered letters allowing monitoring of whether letters were received or not, but in Bulgaria registered letters are often associated with "bad news" (e.g. fines or unpaid taxes). In addition to the inconvenience of collecting the letter at the postal office, this may explain why many letters were not picked up. Also, recruitment via mobile unit might have worked better if addresses had been available to RHI staff enabling them to then proactively visit people.

The incentives provided were well accepted, but different incentives for different age groups might have impacted positively on the response rate. In Germany gift vouchers work well.[2] Some studies have shown that monetary incentives are preferred [25] whereas in others, participants considered them to impose an unwanted commercial feature and undermine confidence in the survey [26]. Pre-survey qualitative assessments, e.g. focus groups, are recommended to identify the most effective measures to increase participation [27].

The HCV prevalence weighted for age and sex was 0.9% [95% CI 0.2–4.2%], and similar to that found in the 1999–2000 study among healthy volunteers in Stara Zagora [1.05% (anti-HCV)] [11]. Although non-response bias

[2] Dr. Antje Gößwald, RKI, personal communication, April 2, 2019

cannot be ruled out, the use of weights likely reduced non-response bias.

Two thirds of participants reported having been exposed to risk factors for HCV infection, with surgery under general anesthesia being reported by two thirds of participants. Nosocomial transmission was the second most common transmission-route among acute HCV cases in 2017 in the EU/EEA (17%) [28]. In Bulgaria, recipients of a transfusion of unscreened blood (prior to 1992) are a key risk group. Recent reports of breaches of infection control procedures also indicate that iatrogenic transmission may be a current risk factor for HCV in Bulgaria [29, 30], however in our sample, even in those reporting potential exposure, none tested positive for HCV. Two participants reported injecting drug use, and one of them tested HCV positive. The highest rates of chronic HCV prevalence in Europe are found among PWID ranging from 13.8 to 84.3% (anti-HCV) [31]. Studies in Bulgaria have found high levels of HCV transmission among PWID and other groups [32], with one study in Sofia reporting 73.9% of 773 PWID being anti-HCV positive [33]. GP surveys are not ideal to collect representative data on PWID. Other recruitment strategies are needed for this vulnerable population [34].

Self-reported data may be prone to social-desirability bias. Questions about drug use, imprisonment and previous test results are sensitive and people may tend to provide answers perceived as more socially acceptable. Social-desirability bias however is often reduced when the questionnaire is self-administered [35]. Recall bias might also have played a role in this survey.

Our survey methodology was found to be feasible, understandable and helpful in providing a step-by-step approach on how to implement a HCV prevalence survey in the GP. Despite the low response rate, the survey approach was found to be useful in estimating the prevalence but also resource intensive in terms of time, staff and costs. All lessons learnt were included in the final version of the technical protocol [9].

The technical protocol targets the GP [9], and estimating the prevalence among the GP is one step needed to estimate the overall national HCV burden. The technical protocol provides an opportunity to improve the availability of reliable and robust data to describe the HCV epidemiology and contribute to monitoring progress towards the elimination of viral hepatitis.

Limitations

The main limitation in this study is the low response rate which reduced the reliability and validity of the results. Therefore, we cannot draw conclusions regarding HCV prevalence in the GP in Stara Zagora.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13104-020-05158-3.

Additional file 1. S1a: First invitation letter

Additional file 2. S1b: Second invitation letter

Additional file 3. S2: Participant information leaflet

Additional file 4. S3: Additional tables and figures

Abbreviations

Anti-HCV: Antibody to hepatitis C virus; CI: Confidence Intervals; EU/EEA: European Union/European Economic Area; GP: General population; HCV: Hepatitis C virus; HCV-RNA: Hepatitis C virus ribonucleic acid; PWID: People who inject drugs; RHI: Regional Health Inspectorate; RKI: Robert Koch Institute.

Acknowledgements

We would like to thank all the survey participants and all staff members at the Regional Health Inspectorate and the staff from the local population registry ("Esgraon-TD5") in Stara Zagora, Bulgaria. The contributions of the SPHERE-C Expert Group who participated in one or more of the in total three meetings held to discuss how best to measure HCV prevalence in the general population between 2016–2019 are also highly appreciated.

Authors' contributions

RZ, SN, MG, ED and AA conceptualised the SPHERE-C project. RZ supervised the survey. VB provided overall supervision and feedback throughout the SPHERE-C project. IS, SN, YS, RZ, EGM, EK, ZN, TT, TP carried out the study, and TV and KH provided support for the planning of the study.IS, SN, RZ, MG drafted the study protocol. YS entered the data, and IS, SN, RZ and MH performed the analyses. IS drafted the manuscript and all authors critically revised the manuscript and approved the final version. IS is corresponding author. All authors read and approved the final manuscript.

Funding

This work was funded by a tender from the European Centre for Disease Prevention and Control (ECDC).

Availability of data and materials

The study protocol and the datasets analysed are available from the corresponding author upon request.

Ethics approval and consent to participate

Ethics approval was received from the local ethics committee in Stara Zagora, Bulgaria. Written informed consent was collected from all participants prior to participation in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 21 January 2020 Accepted: 26 June 2020
Published online: 07 July 2020

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Publisher's Note

frontiers in Public Health

SYSTEMATIC REVIEW
published: 28 August 2020
doi: 10.3389/fpubh.2020.00424

Prevalence of Hepatitis B, C, and D in Germany: Results From a Scoping Review

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OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

Received: 17 March 2020
Accepted: 14 July 2020
Published: 28 August 2020

Citation:
Sperle I, Steffen G, Leendertz SA, Sarma N, Beermann S, Thamm R, Simeonova Y, Cornberg M, Wedemeyer H, Bremer V, Zimmermann R and Dudareva S (2020) Prevalence of Hepatitis B, C, and D in Germany: Results From a Scoping Review. Front. Public Health 8:424. doi: 10.3389/fpubh.2020.00424

Background: One of the five strategic directions in the World Health Organization global health sector strategy on viral hepatitis 2016–2021 is to generate strong strategic information for focused action to understand the viral hepatitis epidemic and focus the response. Knowledge of national prevalence is a cornerstone of strategic information. Germany is considered to be a low prevalence country for viral hepatitis B, C, and D, however the prevalence is likely to be higher among at-risk groups.

Methods: The aim of this work was to give a detailed overview of the prevalence of viral hepatitis B (HBsAg, anti-HBc), C (anti-HCV, HCV RNA), and D (anti-HDV, HDV RNA) in different population groups in Germany. Therefore, we analyzed the results of a comprehensive literature search on various aspects of the epidemiological situation of hepatitis B, C, and D in Germany. Eligible publications including information on hepatitis B, C, and D prevalence were extracted from the overall spreadsheet table and summarized and analyzed based on virus and different population groups. A quality appraisal was performed using a checklist developed by Hoy et al. to assess risk of bias in prevalence studies.

Results: Overall, 51 publications were identified through the literature search. The overall prevalence of HBsAg in the general (and proxy) population ranged from 0.3 to 1.6%. Among at-risk groups, including clinical populations and health care workers, the HBsAg prevalence ranged from 0.2% (among rheumatic patients) to 4.5% among HIV positive patients. The overall prevalence of anti-HCV in the general (and proxy) population ranged from 0.2 to 1.9%. Among at-risk groups, including clinical populations and health care workers, the anti-HCV prevalence ranged from 0.04% (among health care workers) to 68.0% among people who inject drugs.

Conclusions: The hepatitis B and C prevalence in the general population in Germany is low. Prevalence is high to very high among at-risk populations, however for some

groups evidence was incomplete or missing completely. To reach the elimination goals in Germany and implement a targeted response, more research among at-risk groups is needed.

Keywords: hepatitis B, hepatitis C, hepatitis D, scoping review, epidemiology, prevalence

INTRODUCTION

Viral hepatitis (VH) is a major global public health concern. Worldwide, an estimated number of 257 and 71 million people are chronically infected with hepatitis B virus (HBV) and hepatitis C virus (HCV), respectively (1). In total, 15–20 million people are infected with hepatitis D, which corresponds to 5% among those with hepatitis B (1). In the World Health Organization (WHO) European Region, an estimated 15 and 14 million people suffer from chronic HBV and HCV infection, respectively (2).

The WHO global health sector strategy for VH (2016–2021) (3), the WHO European level action plan (2016) (2) and the German integrated national strategy for HIV, HBV, and HCV and other sexually transmitted diseases (2016) (4) represent steps forward in terms of elimination of VH. Nevertheless, they shed light on the lack of comprehensive data to monitor progress and to identify where intensified efforts are needed.

The VH viruses, HBV, HCV, and HDV, show diversity in their prevalence, but also in their modes of transmission depending on country, context, and population group. Data on the country specific epidemic in Germany as well as on population groups most at risk and the effectiveness of prevention and treatment measures are urgently needed to intensify efforts and to reach the elimination goals by 2030.

The most recent national population-based survey among adults in Germany (2008–2011) (DEGS1) found a low HBV and HCV prevalence (0.3%) (5). However, it is known that the prevalence of VH is higher in some groups more vulnerable to VH infection. More research among population groups that are often poorly represented in population-based surveys and more vulnerable to VH (hereafter populations at-risk) is needed.

The aim was to create an overview of existing evidence on the epidemiology of HBV, HCV, and HDV in different population groups in Germany in the time period from 2005 to 2017 to serve as baseline information and guide to improve monitoring of VH in Germany. In this paper, the prevalence in Germany is described.

Abbreviations: DAAs, Direct-acting antiviral treatment; BASE, Bielefeld Academic Search Engine; CC Med, Current Contents Medizin; DEGS 1, National population-based survey among adults in Germany; GP, General population; EMBASE, Excerpta Medica Database; Europe PMC, Europe PubMed Central; HCWs, Health care workers; IDU, Injecting drug use; IMIRA, Improving Health Monitoring in Migrant Populations; MEDLINE, Medical Literature Analysis and Retrieval System Online; MSM, Men who have sex with men; PICO, Participants, Interventions, Comparator, Outcome; PWID, People who inject drugs; PLWH, People living with HIV; PLWVH, populations with non-VH related underlying disease and people with VH in hepatological care; PRISMA-ScR, Preferred reporting items for systematic reviews and meta-analysis extension for scoping reviews; PROSPERO, International prospective register of systematic reviews; RKI, Robert Koch Institute; VH, Viral hepatitis.

MATERIALS AND METHODS

Review Process

The aim of the overall scoping review was operationalised into 13 specific research questions to identify available evidence in the form of published literature on VH epidemiology in Germany (6). One of the 13 questions was "What is the prevalence of HBV, HCV, and HDV in Germany?"

The detailed methods of the review are described elsewhere (6). In brief, the search and reporting methods were based on the *reporting items for systematic reviews and meta-analysis extension for scoping reviews* (PRISMA-ScR) and the Cochrane Collaboration (https://training.cochrane.org/handbook). Included in the review were available full-text (peer- and non-peer-reviewed) publications of original works in German or English language, published between 01/01/2005 and 09/03/2017 with end of data collection after 01/01/2005 and content relevant to one or more of the research questions. The literature search was conducted in six electronic databases (MEDLINE, EMBASE, Europe PMC, Scopus, Bielefeld Academic Search Engine (BASE), and CC Med) with a detailed search string developed from the research questions **Supplementary S1**. The final search was conducted on 09/03/2017. The reference list of all publications retrieved from the electronic search and eligible for full-text screening as well as national surveillance reports not cited in the six electronic databases were also screened for references of further publications meeting the inclusion criteria **Supplementary S2**.

The screening was performed on abstract and full-text level. After full-text screening, relevant information according to the research questions was extracted from the eligible publications using standardized extraction sheets. The screening and data extraction process was performed by two independent reviewers. All discrepancies between the reviewers were discussed. A validation of the screening and extraction process was conducted.

Data Analysis

The extracted data was assigned to different pre-defined categories based on the research questions and sorted by population groups using the definition of the target population in the corresponding publication. Population groups were defined based on the WHO guidelines on Hepatitis B and C testing (7) and adapted to the German context. Population groups were (a) the general population (GP), (b) sub-populations being representative of the national population, which are not considered at higher risk for VH and therefore act as a proxy for the GP (blood donors and pregnant women), (c) clinical populations [populations with non-VH related underlying disease and people with VH in hepatological care (PLWVH)], (d) populations at risk for VH due to risk behavior/exposure

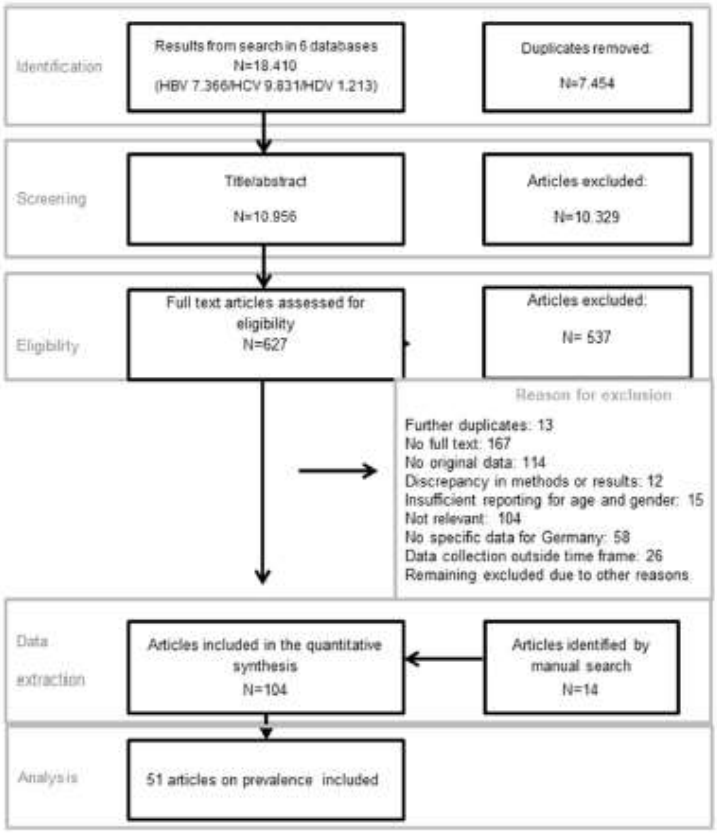

FIGURE 1 | Study flow of study selection.

(household contacts of PLWVH, health-care workers (HCW), people living with HIV (PLWH), men who have sex with men (MSM), people in prison and closed settings, people who inject drugs (PWID), sex workers) or because they are part of a population with high VH seroprevalence (e.g., mobile or migrant populations from intermediate- and high-endemic countries). When no definition of the target population was available in the corresponding publication, the review team allocated the publication to a population group. In this paper, the evidence identified on HBV, HCV, and HDV prevalence is presented which includes all publications from the scoping review allocated to the category "prevalence."

A quality of the evidence on prevalence was assessed using a checklist developed by Hoy et al. (8). This tool allows a judgement of the overall risk of bias based on the assessment of 10 individual items covering internal and external validity and reliability (8). The assessment was performed by one of the reviewers, and then checked by the other reviewer and categorized as either at "low risk" or "high risk" of bias. Discrepancies were discussed to reach agreement, and a third reviewer was consulted if needed. The publications were not weighted according to their quality of evidence in the analyses.

RESULTS

Overall, the electronic literature search retrieved 18,410 publications, and an additional 14 publications were identified by manual search. After removal of duplicates, abstract and full-text screening 104 publications were included in the scoping review which covered all 13 research questions. Fifty-six publications of the 104 were allocated to the category "prevalence." Five of 56 publications were excluded due to the lack of relevance for the analysis, and the remaining 51 were included (**Figure 1**). Some of the included publications reported on the prevalence of more than one pathogen or marker (**Table 1**).

The results of the quality appraisal performed for the publications included in this paper are summarized in **Table 2**.

TABLE 1 | Number of publications.

Total	HBV	HCV	HDV
51	39 (HBsAg: 23, anti-HBc: 19, marker not specified: 6)	33 (anti-HCV: 26, HCV RNA: 13, marker not specified: 6)	4 (anti-HDV: 1, HDV RNA: 1, marker not specified: 3)

Fourteen of the 51 publications were assessed to have a high risk of bias due to either lack of properly describing their sampling and recruitment strategy, and/or that the VH markers were either not specified or that VH status was self-reported.

Prevalence of Hepatitis B, C, and D in Germany

Of the 51 publications reporting on VH prevalence 37 had a cross-sectional design, eight a cohort design, five were surveillance studies, and one was a case-control study (37). For seven publications the origin of the data was national surveillance. National level data were reported by 16 publications, while regional or local level data were reported by the remaining publications, except one which did not report on which level the data were from (16).

Hepatitis B

The 39 publications covering HBV prevalence were on studies conducted between 1996 and 2016. The prevalence of HBV in the GP, including proxy populations, was reported in 13 publications of which 11 were at national level (5, 10, 13, 25, 27, 30–32, 35, 58, 59). One publication did not describe on which level the data were from (16).

The prevalence of HBsAg in the GP ranged from 0.3 to 0.7%, and 0 to 1.6% among proxy populations. The prevalence of anti-HBc ranged from 0.5 to 0.6% in GP, and 0.9 to 1.4% in proxy populations. Six publications (16, 18, 30–32, 35) included surveillance data among blood donors. Four of these reported on the prevalence of HBsAg, HBcAg, HBV-DNA (not separately) among first time blood donors and reported a range from 0.12 to 0.15%. Two studies described anti-HBc prevalence among first time blood donors and found a prevalence of 1.9% (18) and 0.9% (16).

Four studies described anti-HBc prevalence among HCWs which ranged from 0.5 to 1.7%, one identifying a self-reported anti-HBc prevalence among medical doctors (1.6%) (36).

One study included HBV infection among household contacts (partner and children) and reported a self-reported prevalence of 10.7% (17).

Thirteen studies looked at HBV prevalence among clinical populations, of which four were VH patients in hepatologic care. These four described the proportion of patients with HCV who were co-infected with HBV (markers not specified) which ranged from 0.1 to 39.1%. The prevalence of HBsAg was reported by eight studies and ranged from 0.2 to 3.4%. Four of these were among emergency and trauma department in which the prevalence ranged from 0.5 (anti-HBc and HBsAg) to 1.3%. One study reported an anti-HBc IgG prevalence of 8.3% among alcohol dependent patients (38), and one study an anti-HBc prevalence of 5.6% among patients with rheumatic disease (21) (**Figure 2**).

Eight publications described HBV prevalence in at-risk populations, and three were among people with migration background. One study was among refugees screened in an emergency department and found a prevalence of HBsAg and anti-HBc of 2.3 and 14.0%, respectively. The country of birth was not specified (24). Another study screened patients with migration background (patient or parents of patient) and found an HBsAg prevalence of 3.6% and anti-HBc of 32.5%. In total, 87.3% of the patients were from the Eastern Mediterranean Area, 12.0% were from Eastern Europe, and 0.7% originated from other countries (40). The third study tested 488 Syrian refugees upon arrival in Germany, but none were HBV positive (markers not specified) (23).

HBV prevalence among PLWH was reported by two studies, one of which was among HIV positive MSM. The prevalence of HBsAg was 4.5% among HIV patients (28) and 1.7% among HIV positive MSM (25).

Three studies included results on HBV prevalence among PWID. One study included results on self-reported HBV infection among PWID recruited from the streets or drug consumption rooms and from substitution clinics, and found a rate of 14.1 and 14.0%, respectively (41). One study reported an HBsAg prevalence of 1.3% among PWID in specialized methadone substitution centers (34) and another reported an anti-HBc prevalence of 25% (range in the cities: 4.6–33%), among which 1.1% were HBsAg positive (range in the cities: 0.3–2.5%). among PWID recruited from low threshold services (43) (**Figure 3**).

Hepatitis C

The 33 publications covering HCV prevalence were on studies conducted between 1996 and 2014. The prevalence of HCV in the GP, including proxy populations, was reported in 11 publications of which 10 were on the national level.

The anti-HCV prevalence in the GP ranged from 0.2 to 1.9%, and was 1.5% among baby boomers (proxy population) (42). Two studies reported an HCV RNA prevalence of 0.2% (5) and 0.4%, respectively (44).

Four publications (30–32, 35) reported on surveillance data among blood donors, describing an anti-HCV range from 0.06 to 0.08%.

Three studies on prevalence of anti-HCV among HCWs reported a prevalence of 0 and 0.03% (29, 33) and of 0.04% self-reported anti-HCV (36).

Ten studies analyzed HCV prevalence among clinical populations (26, 27, 37–39, 45, 46, 51, 52, 55), including one in HBV patients in care (55). Anti-HCV ranged from 0.2 to 5.2% with the lowest prevalence in autologous blood donors (giving blood for themselves). One study reported on HCV RNA among two groups of clinical patients and reported a prevalence of 2.4% among chronic haemodialysis patients and 4.6% among kidney transplant recipients (37). One study reported on the proportion of HBV patients with HCV without specifying the marker where 0% were co-infected with HCV (55). Six studies

TABLE 2 | Continued

Publication (references nr.)	Data collection period	Place of data collection	Study type	Recruitment setting	Study population (*n*)	Virus	Mean/median age (range)	Prevalence								Risk of bias
								HBc-Ag	HBs-Ag	HBV DNA	Anti-HCV	HCV RNA	Anti-HDV	HDV RNA	Not specified	
Wiese et al. 2014 (19)	2011–2012	East Germany (Leipzig, Dresden, Rostock, Chemnitz, Potsdam, Berlin, Magdeburg, Cottbus, Jena, Erfurt, Halle)	Cohort	Referral centers, multi-centric	PLWH in care (HCV) (*N* = 718)	HBV	At HCV-infection: 24 yrs. (median), after 35 yrs.: 57 yrs. (median)	x	x	x	x	x	x	x	0.1%	Low risk
Claus et al. 2016 (20)	Aug 2010–2012	Rhineland-Palatinate	Cross-sectional	Schools for handicapped (*n* = 13) (questionnaires)	Health care staff (staff at the schools) (*N* = 367)	HBV	45 yrs. (mean) (not reported)	1.7%	51.8%	x	x	x	x	x	x	Low risk
Feuchtenberger et al. 2016 (21)	2011–2015	Würzburg	Cross-sectional	Hospital, single center	Clinical population (rheumatic disease) (*N* = 1,338)	HBV	60.98 yrs. (mean)	5.6%	0.2%	x	x	x	x	x	x	High risk
Kartashev et al. 2016 (22)	2011–2015	Cologne	Cross-sectional	University hospital	PLWH in hepatologic care (chronic HCV) (*N* = 1208)	HBV	Not reported	x	x	x	x	x	x	x	39.1%	High risk
Mockenhaupt et al. 2016 (23)	Oct 2013–Nov 2015	Berlin	Cross-sectional	Clinic (*n* = 1)	Migrants (unaccompanied minors) (*N* = 488) (at-risk population)	HBV	(6–17 yrs.)	x	x	x	x	x	x	x	0%	High risk
Hampel et al. 2016 (24)	Aug 2015	Northern Germany	Cross-sectional	Central refugee stations (*n* = 6)	Migrants (refugees) (*N* = 793) (at-risk population)	HBV	28.8 yrs. (median) (3–76 yrs.)	14.0% (95% CI:11,9–16,9)	2.3% (95% CI: 1,3–3,4)	x	x	x	x	x	x	Low risk
Jansen et al. 2015 (25)	Jun 1996–May 2012	Nationwide	Cohort	Clinics	MSM (HIV positive) (*N* = 1,838) (at-risk population)	HBV, HCV	33 yrs. (mean age at HIV seroconversion) (17–76 yrs.)	28.8%	x	x	8.2%	4.0%	x	x	x	Low risk
Winkelmann et al. 2016 (26)	Jan 1997–Dec 2008	Hannover	Cross-sectional	Hospital, Hannover Medical School, trauma department (*n* = 1)	Clinical population (*N* = 1,373)	HBV, HCV	64.2 yrs. (mean)	x	0.7%	x	2.0%	x	x	x	x	Low risk
Wiegand et al. 2009 (27)	2000–2005	Nationwide	Cross-sectional	21 transfusion centers throughout Germany	Autologous blood donors (clinical population) (*N* = >35,000)	HBV, HCV	Not reported	x	0.2% [95% CI 0.1–0.2] East 0.3% [95% CI 0.2–0.4] West	x	0.2% [95% CI 0.1–0.3] East 0.3% [95% CI 0.3–0.4] West	x	x	x	x	Low risk
Reuter et al. 2011 (28)	Jan 2001–Dec 2005	Cologne and Düsseldorf	Cross-sectional	University Hospitals	HIV positives (*N* = 918) (at-risk population)	HBV, HCV	37 yrs. (median) (17–77)	42.8%	4.5%	x	10.6%	x	x	x	x	High risk

(Continued)

TABLE 2 | Hepatitis B, C, and D prevalence in Germany.

Publication (references nr.)	Data collection period	Place of data collection	Study type	Recruitment setting	Study population (*n*)	Virus	Mean/median age (range)	Prevalence								Risk of bias
								HBc-Ag	HBs-Ag	HBV DNA	Anti-HCV	HCV RNA	Anti-HDV	HDV RNA	Not specified	
Knorr et al. 2015 (9)	Jan 1996–Dec 2005	Heidelberg	Cross-sectional	Hospital	Pregnant/reproducing (*N* = 5,518) (GP proxy)	HBV	(16–45 yrs)	x	1.6%	x	x	x	x	x	x	Low risk
Marcellin et al. 2015 (10)	Jan 2000–Dec 2006	Nationwide	Cross-sectional	Hospital	PLWH in hepatologic care (chronic HCV) (*N* = 996)	HBV	48.9 yrs. (mean)	x	x	x	x	x	x	x	4.5%	High risk
Lobstein et al. 2011 (11)	2001–2006	Leipzig	Cross-sectional	Hospital	Pregnant/reproducing (*N* = 8,193) (GP proxy)	HBV	Not reported	x	0.5%	x	x	x	x	x	x	Low risk
Alba-Alejandre et al. 2009 (12)	2001–2008	Munich	Cross-sectional	Clinic (all women who gave birth in clinic; HBsAg collected retrospectively) (medical records, serology)	Pregnant/reproducing women (*N* = 15,873) (GP proxy)	HBV	Not reported	x	0.8%	x	x	x	x	x	x	Low risk
Cai et al. 2011 (13)	May 2003–2006	Nationwide	Cross-sectional	At physicians and via self-completed questionnaires	GP (children) (*N* = 13,065)	HBV	Not reported (3–17 yrs.)	0.5% [CI: 0.4–0.7]	38.7% [95% CI 20.0–57.5] (of the 0.5%)	x	x	x	x	x	x	Low risk
Hüppe et al. 2008 (14)	Mar 2003–May 2006	Nationwide	Cohort	Hepatitis centers and outpatients units	PLWH in hepatologic care (chronic HCV) (*N* = 10,326)	HBV	43.4 yrs. (mean)	x	x	x	x	x	x	X	1.5%	High risk
Ernst et al. 2012 (15)	Aug 2004–2008	Potsdam	Cross-sectional	Hospital	Hospital patients but not only hepatitis related patients(Clinical population) (*N* = 803)	HBV	61 yrs. (mean)	x	1.9%	x	x	x	x	X	x	Low risk
Zeiler et al. 2006 (16)	2005	Not reported	Surveillance	German blood donation services	Blood donors (GP proxy) (*N* = 3964)	HBV	Not reported	0.9% [95% CI 0.8–1.4]	0%		x	x	x	X	x	Low risk
Deterding et al. 2012 (17)	Not specified (a collaboration project of Northern Expert Network for Hepatitis established 2005–2007)	Hannover	Cross-sectional	Hospital/treatment centers	Child/partner of chronic HBV patients (*N* = 312) (at-risk population)	HBV	42 yrs. (mean)	x	x	x	x	x	x	X	10.7%	High risk
Walch 2010 (18)	2/2006–11/2007	Baden-Württemberg/ Hesse	Cross-sectional	5 Transfusion centers of the blood donation service in Baden-Württemberg/Hesse provided blood samples of blood donors	Blood donors (GP proxy)	HBV	Not reported	1.4%	x	0.1%	x	x	x	x	x	Low risk

(Continued)

TABLE 2 | Continued

Publication (references nr.)	Data collection period	Place of data collection	Study type	Recruitment setting	Study population (n)	Virus	Mean/median age (range)	Prevalence								Risk of bias
								HBc-Ag	HBs-Ag	HBV DNA	Anti-HCV	HCV RNA	Anti-HDV	HDV RNA	Not specified	
Wicker et al. 2007 (29)	Winter semester 2005/2006	Frankfurt	Cross-sectional	University hospital	Health care workers (*N* = 223)	HBV, HCV	23.4 yrs. (mean) (20–45 yrs.)	0.9%	x	x	0%	x	x	x	x	High risk
Offergeld et al. 2007 (30)	2005	Nationwide	Surveillance data	All blood donor centers provide data on demographics/test results of routine testing.	Blood donors (GP proxy) (*N* = 452,670, new donors)	HBV, HCV	Not reported		0.1%			0.1%	x	x	x	Low risk
Willand et al. 2008 (31)	2006	Nationwide	Surveillance	German Blood Donor Centers	Blood donors (GP proxy) (*N* = 512,023 first donors)	HBV, HCV	Not reported		0.2%			0.1%	x	x	x	Low risk
Offergeld et al. 2010 (32)	2007	Nationwide	Surveillance data	All blood donor centers provide data on demographics/test results of routine testing.	Blood donors (GP proxy) (*N* = 548,608 new donors)	HBV, HCV	Not reported	0.1% (2008), 0.1% (2009), 0.1% (2010)			0.1% (2008), 0.1% (2009), 0.1% (2010)					Low risk
Wicker et al. 2009 (33)	Apr–May 2007	Frankfurt	Cross-sectional	University hospital	Health care workers (*N* = 366)	HBV, HCV	24.4 yrs. (mean) (19.8–48.2 years.)	0.5%	x	x	0.3%	x	x	x	x	High risk
Müller et al. 2009 (34)	Feb 2008–Dec 2008	Munich	Cross-sectional	Specialized methadone substitution center in Germany	PWID (*N* = 146) (at-risk population)	HBV, HCV	35 yrs. (mean)	x	x	x	68.0%	28.0%	x	x	1.3% (chronic HBV)	High risk
Offergeld et al. 2012 (35)	2008–2010	Nationwide	Surveillance data	All blood donor centers provide data on demographics/test results of routine testing.	Blood donors (GP proxy) (N=570,852)	HBV, HCV	Not reported		0.1%			0.1%	x	x	x	Low risk
Poethko-Müller et al. 2013 (5)	2008-2011	Nationwide	Cohort	Population-based. Participants were the invited to fill out questionnaire and visit examination clinics (DEGS1)	GP (*N* = 7,047)	HBV, HCV	Not specified (18–79 yrs.)	0.3% [0,2–0,6], 0.6% (only Anti-HBc)	x	0.3% [95% CI 0.1-0.5]	0.2%	x	x	x	Low risk	
Baars 2011 (36)	2009–2010	Lower Saxony	Cross-sectional	Company doctors (all medical staff in company doctor practices invited to participate in survey, self-reported)	Health care workers (HBV: *N* = 831, HCV: *N* = 2295)	HBV, HCV	Not reported	1.6% (self-reported)	x	x	0.0% (self-reported)	x	x	x	x	Low risk
Baid-Agrawal et al. 2014 (37)	2009–2011	Berlin	Case-control	Outpatient transplant clinic, Charité University Hospital (medical records, serum sampling)	Kidney transplant recipients (clinical population) (*N* = 417)	HBV, HCV	53.0 yrs. (mean) (53.0 yrs. +/−12.8)	*x*	*3.4%*	*x*	*4.8%*	*4.6%*	*x*	*x*	*x*	High risk
					Chronic haemodialysis patients) (*N* = 417) (clinical population)	HBV, HCV	66.1 yrs. (mean) (66.1 yrs. +/−14.9)	x	0.5%	x	3.6%	2.4%	x	x	x	

(Continued)

TABLE 2 | Continued

Publication (references nr.)	Data collection period	Place of data collection	Study type	Recruitment setting	Study population (*n*)	Virus	Mean/median age (range)	Prevalence								Risk of bias
								HBc-Ag	HBs-Ag	HBV DNA	Anti-HCV	HCV RNA	Anti-HDV	HDV RNA	Not specified	
Schmidt et al. 2013 (38)	Sep 2009–Mar 2011	Hamburg	Cross-sectional	Hospital	Alcohol dependent (*N* = 463) (clinical population)	HBV, HCV	Not reported	8.3% [95% CI: 5.7–10.8%]	x	x	5.2% [95% CI: 3.2–7.2%]	3.2%	x	x	x	Low risk
Darstein 2015 (39)	Aug 2010–Nov 2011	Berlin	Cross-sectional	Accident and emergency unit, hospital (*n* = 1)	Emergency department patients (Clinical population) (*N* = 1,942)	HBV, HCV	59.5 yrs. (median) (18–97 yrs.)	0.5% [95% CI: 0.2–0.8] (anti-HBc & HBsAg), 9.9% [95%CI 8.6–11.3%] (anti-HBc), 6.1% [95% CI:5.0–7.2], (anti-HBc and anti-HBs), 1.9% [95% CI: 1.3–2.5] (anti-HBc and anti-HBs negative)		x	0.9% [95% CI 0.5–1.3]	0.5% (HCV RNA)	x	x	x	Low risk
Heidrich et al. 2014 (40)	Nov 2010–Jan 2012	North-Western Germany	Cross-sectional	Primary care centers (*n* = 8)	Migrants (*N* = 1,298) (at-risk population)	HBV, HCV	49.1 yrs. (mean) (49.1 +/– 15.8 yrs.)	32.5%	3.6%	2.2%	1.9%	0.7%	x	x	x	Low risk
Mone 2015 (41)	Jan 2011–Mar 2011	Aachen, Berlin, Bochum, Cologne, Essen/Hamm, Hamburg, Frankfurt am Main, Münster, Saarbrücken, Wuppertal	Cross-sectional	On the street and in drug consumption places	PWID ("street clients") (*N* = 420) (at-risk population)	HBV, HCV	38.4 yrs. (mean) (38.4 +/– 8.4 yrs.)	x	x	x	x	x	x	x	14.1% (HBV+), 58.3% (HCV +) (self-reported)	High risk
				Substitution clinics (*n* = 12)	PWID ("substitution patients") (*N* = 404) (at-risk population)	HBV, HCV	40.8 yrs. (mean) (40.8 +/– 8.6 yrs.)	x	x	x	x	x	x	x	14.0% (HBV +), 58.7% (HCV +) (self-reported)	
Kant et al. 2018 (42)	Feb 2011–Jan 2012	Leipzig	Cross-sectional	Hospital, department of internal medicine and neurology	Baby boomers (born 1955–1965) (*N* = 1,235) (GP proxy)	HBV, HCV	(only available for GP)	x	0.6%	x	1.5%	x	x	x	x	Low risk
					GP (*N* = 6011)	HBV, HCV	62.4 yrs. (mean)	x	0.7%	x	0.9%	x	x	x	x	
Bremer et al. 2018 (43)	2011–2014	Berlin, Cologne, Essen, Frankfurt am Main, Hamburg, Hannover, Leipzig, Münich	Cross-sectional	Low threshold drug services[a] (questionnaires, serology)	PWID (*N* = 2,077) (at-risk population)	HBV, HCV	38.0 yrs. (median) (17–65 yrs.)	25.0%	0.1%	x	66.0%	44.0%	x	x	x	Low risk
Wolffram et al. 2015 (44)	Jan 2012–Jun 2013	North Rhine Westphalia	Cross-sectional	General practitioner practices (*n* = 51)	GP (*N* = 21,008)	HBV, HCV	57.5 yrs. (mean) (7–107 yrs.)	x	0.5%	x	1.0%	0.4%	x	x	x	Low risk

(Continued)

TABLE 2 | Continued

Publication (references nr.)	Data collection period	Place of data collection	Study type	Recruitment setting	Study population (*n*)	Virus	Mean/median age (range)	Prevalence								Risk of bias
								HBc-Ag	HBs-Ag	HBV DNA	Anti-HCV	HCV RNA	Anti-HDV	HDV RNA	Not specified	
Wicker et al. 2016 (45)	Feb 2014–Jan 2015	Frankfurt/Main	Cross-sectional	Accident and emergency unit, University Clinic Frankfurt	Clinical population (*N* = 275)	HBV, HCV	46.7 yrs. (mean) (8–91 yrs.)	x	0.7%	x	2.6%	x	x	x	x	Low risk
Bert et al. 2016 (46)	2016	Frankfurt am Main	Cohort	Emergency department of hospital (medical records)	Emergency department patients (Clinical population) (*N* = 10,215)	HBV, HCV	59.0 yrs. (mean) (24–94 yrs.)	x	1.3%	x	2.7%	x	x	x	x	High risk
Schmidt et al. 2011 (47)	2006	Nationwide	Cross-sectional	Online survey	MSM (*N* = 4,385) (at-risk population)	HCV	32 yrs. (median, HIV-neg/not tested) and 40 yrs. (median, HIV-pos.) (16–79 yrs.)	x	x	x	2.4% (HIV negative/untested: 0.8%), HIV positive: 8.8%)	x	x	x	x	Low risk
Schulte et al. 2009 (48)	Mar 2006 (not further specified)	Nationwide	Cross-sectional	Prison	People in prisons (*N* = 14,187) (of which 21.9% (*n* = 3,111) were PWID) (at-risk population)	HCV	Not reported	x	x	x	x	x	x	x	14.3%	High risk
Torneczkowski et al. 2015 (49)	2007–2011	Nationwide	Cohort	Health insurance	GP proxy (*N* = 5 464,191)	HCV	Not reported	x	x	x	x	x	Projected prev.: Average of 0.2% per year and 0.2% over three years; 19.0% of the patients were first diagnosed with acute hepatitis.	x	Low risk	
Thönnes et al. 2017 (50)	2007–2013	Nationwide	Cohort	German company health insurance funds	GP proxy (*N* = 3,200,000 million)	HCV	Not reported	x	x	x	x	x	X	x	0.2% (projected prevalence)	Low risk
Vermehren et al. 2012 (51)	May 2008–Mar 2010	Berlin Frankfurt/Main	Cross-sectional	Hospital emergency units	Clinical population (*N* = 28809)	HCV	51.9 yrs. (mean) (31.9–71.9)	x	x	x	2.6% [95% CI 2.4–2.8]	1.6% [95% CI 1.5–1.8]	x	x	x	Low risk
Dogjami 2014 (52)	Jun 2009–Jun 2010	Bochum	Cross-sectional	Hospital, accident and emergency unit of the St. Josef Hospital	Clinical population (*N* = 8,435)	HCV	51.15 yrs. (median) (10–100)	x	x	x	3.5%	1.6%	x	x	x	Low risk

(*Continued*)

TABLE 2 | Continued

Publication (references nr)	Data collection period	Place of data collection	Study type	Recruitment setting	Study population (n)	Virus	Mean/median age (range)	Prevalence							Risk of bias
								HBc-Ag	HBs-Ag	HBV DNA	Anti-HCV	HCV RNA	Anti-HDV	HDV RNA	Not specified	
DEtonaventura 2010 et al. 2012 (53)		Nationwide	Cross-sectional	Online survey (Self-reported)	GP (N= 15,070)	HCV	Not reported (18-65 yrs.)	x	x	x	x	x	x	x	0.4% (self-reported (projected)	High risk
Jablonka et al. 2017 (54)	Aug 2015	Northern Germany	Cross-sectional	Reception center for refugees (n = 6)	Migrants (N = 236) (at-risk population)	HCV	28.7 yrs. (mean) (6–76)	x	x	x	0.4%	x	x	x	x	Low risk
Magiorkinis 2014 (55)	Jan 2000–Dec 2008	Ulm	Cross-sectional	Hepatology Unit, University Hospital	PLWH in hepatologic care (chronic HBV) (N = 327)	HCV, HDV	41.9 yrs. (mean) (17-61 yrs.)	x	x	67.7% 19.6% (HBsAg)	x	x	x	x	0% (HCV, HCV)	Low risk
Erhardt 2010 (56)	Jan 1999–Dec 2008	Düsseldorf	Cohort	Hospital	PLWH in hepatologic care (chronic HBV) (N = 1,307)	HDV	Not reported	x	x	x	x	x	5.1% (chronic HDV)	x	x	Low risk
Rothkreimer et al. 2012 (57)	Jan 2000–Oct 2011	Frankfurt am Main	Cross-sectional	University Hospital Frankfurt	PLWH in hepatologic care (chronic HBV) (N = 2,844)	HDV	Not described	x	x	x	x	x	7.4%	64.5%	x	Low risk
Fischer et al. 2012 (58)	Dec 2004–Mar 2007	Nationwide	Cross-sectional	German centers with a predominantly hepatologic focus (n = 74)	PLWH in hepatologic care (chronic HBV) (N = 1,535)	HDV	36.8 yrs. (mean) (58.9 +/- 136 yrs.)	x	x	x	x	x	x	x	1.4%	Low risk

* *Harm reduction-based health care service with minimal demands for their clients.*

were among emergency and trauma department in which the anti-HCV prevalence ranged from 0.9 to 3.5% (**Figure 4**).

Nine publications reported HCV prevalence in at-risk populations. Two studies (40, 54) reported an anti-HCV prevalence among mobile/migrant populations of 1.9% among patients with migration background in eight primary care centers in Northwest Germany (40) and 0.4% among refugees and asylum seekers who went through routine screening for infectious diseases upon arrival in Germany (54). The first study with patients largely originating from the Eastern Mediterranean area (87.3%) followed by Eastern Europe (12.0%) and other countries (0.7%) also reported an HCV RNA prevalence of 0.7% (40). The country of origin of the refugees and asylum seekers in the second study was not described (54).

Three studies reported on HCV prevalence among PWID (34, 41, 43) in which the anti-HCV prevalence ranged from 63.0 to 68.0%. One cross-sectional study covered eight cities where the anti-HCV prevalence ranged from 36.9% in Leipzig to 73.0% in Hannover. The HCV RNA prevalence ranged from 23.1 to 54.0% (43).

One study included results on self-reported HCV prevalence among PWID recruited from the street and PWID in opioid-substitution treatment (OST) and found a prevalence of 58.3 and 58.7%, respectively (41). One nationwide study including 21 prisons found an HCV prevalence reported by prison physicians of 14.3% among people in prisons, of which 21.9% were PWID (48).

Three studies described prevalence among PLWH, and for two studies these were MSM. Among HIV positive patients the anti-HCV prevalence was 10.6% (28). Among MSM with HIV the anti-HCV prevalence was 8.2% (25). One study described self-reported HCV prevalence among MSM who were HIV positive and HIV negative (or untested) and the prevalence was 8.8 and 0.2%, respectively (47) (**Figure 5**).

Hepatitis D

Four publications covered HDV prevalence based on studies conducted between 1989 and 2011. All four included results on the prevalence in patients with chronic HBV, three recruited patients from hospital settings and in one physicians provided patient data (58). The overall prevalence of HDV ranged from 0 to 7.4%. One study specified the HDV marker and reported an anti-HDV prevalence of 7.4%, and HDV RNA of 64.5% (57). One study collected nationwide data from 74 centers across Germany with focus on hepatology and the prevalence was 1.4% in the population of HBV positives.

DISCUSSION

The aim of this analysis was to assess the state of evidence on HBV, HCV, and HDV prevalence in Germany. To our knowledge this is the first time that all available evidence on HBV, HCV, and HDV prevalence has been systematically searched for. The results demonstrate that there is a large body of evidence on prevalence of HBV and HCV in Germany, but less on HDV.

The available evidence is highly variable. Good coverage was found for the GP and some clinical populations but there are gaps

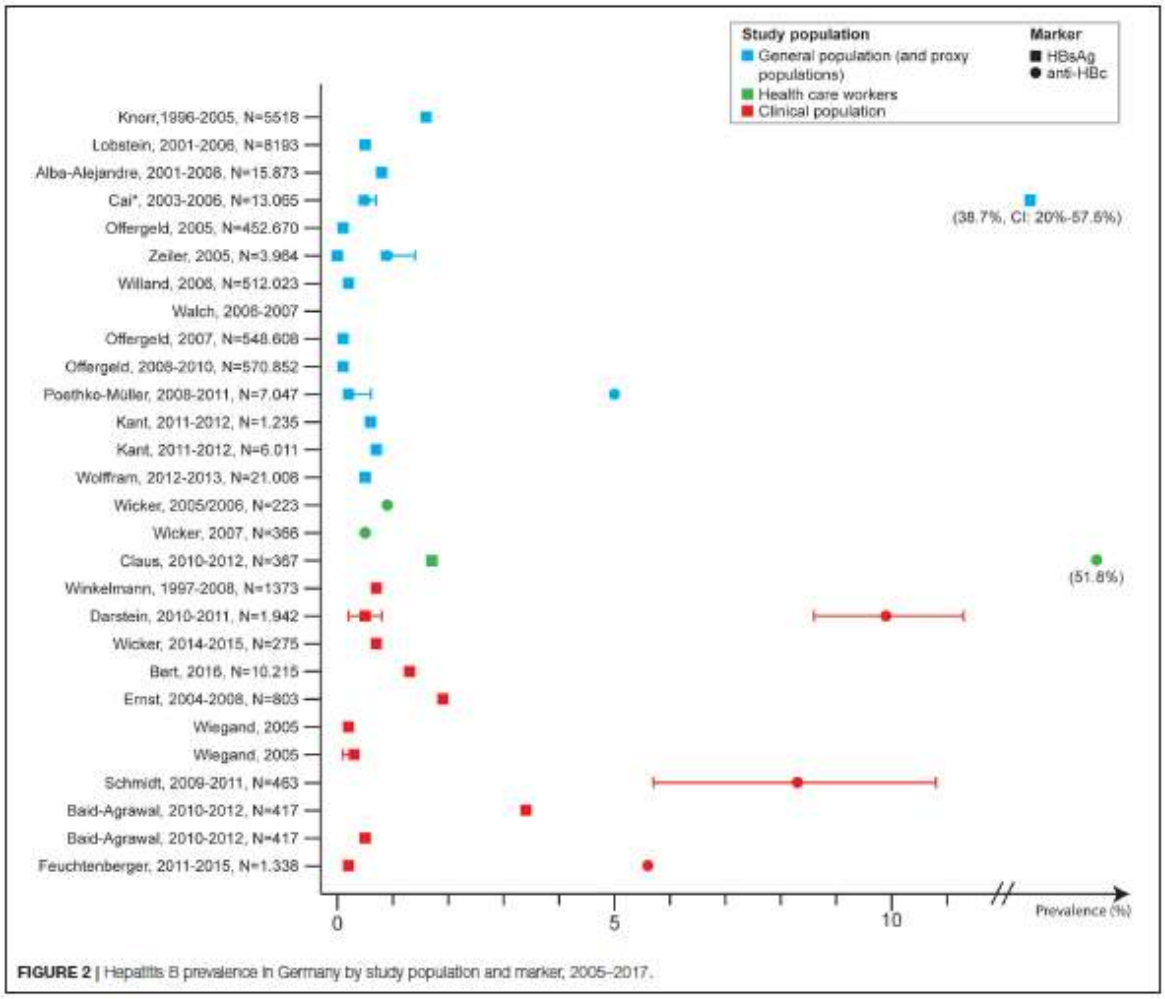

FIGURE 2 | Hepatitis B prevalence in Germany by study population and marker, 2005–2017.

in knowledge for some at-risk populations and missing for sex workers, people who have received blood transfusion and persons with tattoos/piercings.

Prevalence in General Population

The low prevalence of HBV and HCV found in the GP is similar to what has been found in other European countries (60). A higher prevalence was found for proxy populations for the GP [e.g., some patient groups or among pregnant women compared to larger health examination surveys which use a random sample of the GP such as DEGS1 (5)]. The robustness of estimates based on proxy populations for the GP has its limitations. On the one hand, pregnant women may serve as a good proxy as women with migration background are likely to be better represented, however in some cases even over-represented, compared to the larger population-based surveys. On the other hand, they represent a group in more frequent contact with health care services and women of younger age only, and not all pregnant women attend all routine screenings potentially introducing selection bias.

A higher prevalence can be found in birth cohorts of the GP exposed through nosocomial or transfusion-related transmission. These are often referred to as baby boomers. Although the epidemiology is changing with injecting drug use now being a primary risk factor, the prevalence of VH is associated with age and sex. A higher prevalence is often found among males and with increasing age (5, 61, 62), and using baby boomers as proxy population for the GP in Germany should be carefully considered. The study which compared baby boomers and GP in this study found similar prevalence of HBsAg prevalence in the two groups, but higher anti-HCV prevalence among the German baby boomer population (42). Data from first time blood donors were included as this group is more likely to resemble the GP compared to multiple blood donors.

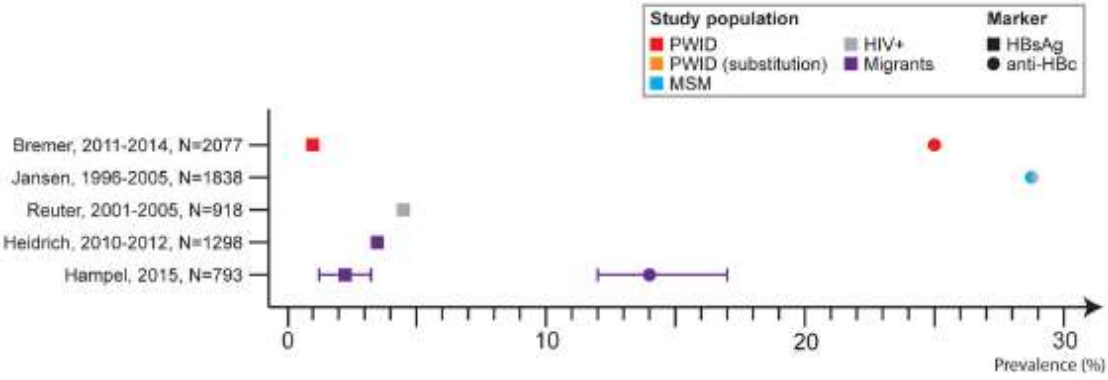

FIGURE 3 | Hepatitis B prevalence in Germany by study population (at-risk) and marker, 2005–2017.

The prevalence of HDV was above 5% in two of the four studies identified. While HDV is relatively rare compared to HBV and HCV, as it requires the envelope of HBV for its entry into hepatocytes, it has important implications for mortality and morbidity (56). The prevalence of HDV found in this review is similar to that in other European countries (e.g., Spain (4.0%) (63), and Switzerland (4.4%) (64), but lower than for example in Italy with 11.9% (65)). A recent systematic review found that globally 10.6% of HBsAg carriers without risk factors (IDU or high risk sexual behavior) are infected with HDV, but higher prevalences were found among those with risk factors with 37.6% in PWID and 17.0% in populations with high risk sexual behavior (66).

Improving screening for people with migration background from areas of high prevalence (e.g., from Turkey, who represent the majority of migrant populations in Germany and which is a high prevalence area (67)), may improve early diagnosis, treatment, and data on HDV in Germany.

Prevalence in At-Risk Populations

The VH burden disproportionately affects some population groups more (61) which was also confirmed in this review.

Sexual transmission of HBV is more common than of HCV, whereas HCV is largely transmitted via blood-to-blood contact with infected fluids. The most common transmission paths ultimately affect which groups are at highest risk and where there is the highest prevalence (68, 69).

The HBV prevalence among populations with migration background was higher than in the GP among refugees who were screened in an emergency department (country of origin not specified) (24), and among patients with migration background primarily from the Eastern Mediterranean Area and Eastern Europe (40). The study among refugees arriving from Syria, where none were tested positive for HBV (23) was among unaccompanied minors who may have a different prevalence than the adult population.

The reasons for a higher prevalence found in the two studies are likely multifacetted. Firstly, people with migration background and refugees are two groups of people that need to be distinguished. Refugees are more likely to have been exposed to risks during flight from war and or persecution in home country and to have lack of access to well-functioning health care services and timely medical care. For people with migration background, prevalence will depend in part on the prevalence in the country of origin. This was however only described in two of three studies (23, 40). The wide ranges of prevalence (from 0 to 3.6%) found in this review coincide with results from other European countries demonstrating large heterogeneity depending on country of origin, ranging from 0 to 22.2% among mobile/migrant populations with the highest prevalence reported among migrants from countries in Southeast Asia (20%) and Sub-Saharan Africa (22.2%) (70). The highest rates of prevalence were found among refugees from east European (1.6–53.1%) and Southeast Asian countries (57.7%) (70).

For HCV, a relatively lower prevalence than HBV and closer to that of the GP was found for people with migration background. One study (not with specific focus on people with migration background) looking at HCV prevalence among patients arriving at an emergency room observed that 67.8% of those HCV positive were of German origin (51).

In one study of 236 refugees and asylum seekers screened for anti-HCV at a reception center upon arrival in Germany, one tested anti-HCV positive (54) (country of origin not specified), and in the other 1.9% were anti-HCV positive among 1,298 people with migration background, primarily from the Eastern Mediterranean Area and Eastern Europe, tested in primary care centers (40). The most HCV affected regions are the WHO Eastern Mediterranean and European Regions (71), corresponding to the higher prevalence found among the people from the Eastern Mediterranean Area and Eastern Europe. HCV estimates from other European countries range from 0 to 19.2% with the highest prevalence rates reported among migrants from countries in Eastern Europe (9.3%) and Sub-Saharan-Africa (19.2%). Among refugees, the highest rates were found among refugees from South Asia (9.1%) and Sub-Saharan Africa (26.7%) (70).

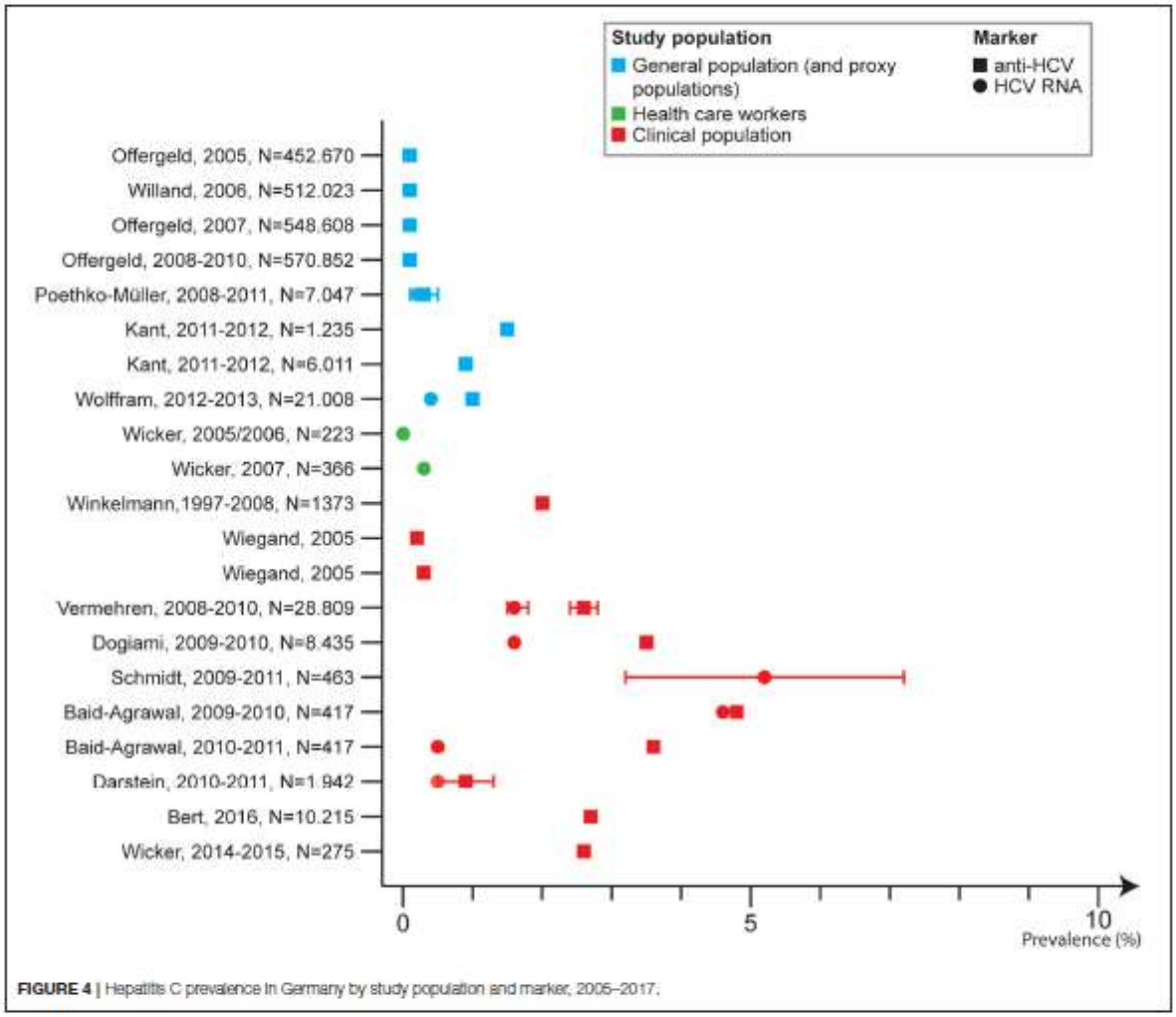

FIGURE 4 | Hepatitis C prevalence in Germany by study population and marker, 2005–2017.

Factors relating to higher vulnerability as a result of a migration background are intertwined and related to social and political factors, either in the country of origin or the new country. Further, drawing any general conclusions for migrants based on this review is challenging. The country of origin differed in the included publications ranging from patients with parents with migration background to newly arrived refugees from Syria. Moreover, the publications that reported on prevalence among mobile/migrant populations categorized the countries/regions of origin differently. A standardization of countries/regions of origin reported in literature would improve the comparison across countries and over time to improve the understanding of the epidemic. Moreover, strengthening the terminology is crucial, as different terminology has very distinct and different meanings, and confusing these terms (e.g., migrant vs. refugee, or nationality vs. country of residence) hinders standardization of data and generation of comparable estimates.

More efforts are needed to reach migrant/mobile populations in the larger health surveys conducted in Germany and to include VH testing in these larger population-based surveys. This is currently being piloted and planned to be implemented at the Robert Koch Institute (RKI) as part of the Improving Health Monitoring in Migrant Populations (IMIRA) Project (72, 73).

People living with HIV (PLWH) are also disproportionately affected by VH, and higher rates of HBsAg prevalence was found among PLWH in this review. Sexual transmission of HBV may occur in particular among MSM and/or heterosexual persons with multiple sex partners, making the interaction between different at-risk groups important to consider.

A higher prevalence of HCV among PLWH was also found, which mirrors the global pattern where a 5.8 times (95% CI 4.5–7.5) increased odds of HCV antibody positivity in HIV-positive people compared with HIV-negative people across all population groups has been documented (74). There is particularly a high

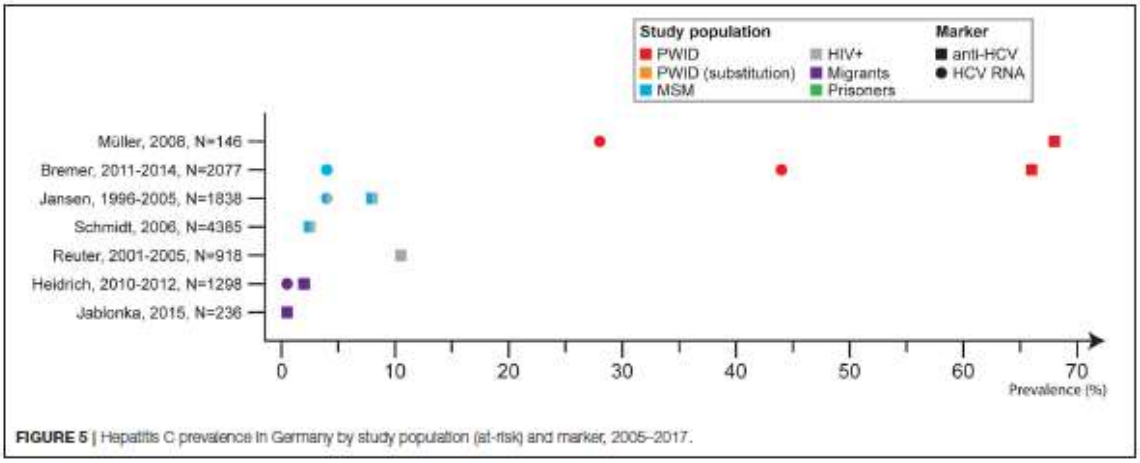

FIGURE 5 | Hepatitis C prevalence in Germany by study population (at-risk) and marker, 2005–2017.

rate among at-risk groups with rates as high as 6.4% in MSM and 82.4% in PWID. Sexual behaviors linked to blood exposure and use of drugs may contribute to the high prevalence among MSM and HIV positive MSM. Chemsex, referring to voluntary intake of psychoactive and non-psychoactive drugs to facilitate and/or enhance sexual intercourse mostly among MSM, has been shown to be associated with higher risk of HIV and HCV transmission and contribute to increased risk among MSM (75).

High prevalence rates of HBV and HCV were shown among PWID in this analysis, corresponding to rates found in the EU/EEA ranging from 0.5 to 6.1% (HBsAg) and 13.8 to 84.3% (anti-HCV), respectively (61).

This coincides with the pattern of IDU being the main driver of HCV transmission in Europe accounting for more than 40% of new reported infections where the transmission route is known (76). A recent modeling study found that if the increased risk of HCV transmission among PWID was removed, an estimated 43% (95% CrI 25–67) of incident HCV infections globally would be prevented from 2018 to 2030 (77), and the population attributable fraction was higher in high-income countries. Focusing on prevention, testing, and treatment of PWID is important in targeted settings as part of harm reduction services.

In total, 14.3% of the prison population throughout Germany were anti-HCV positive (48). In the EU/EEA some of the highest rates of anti-HCV are detected among prison populations (4.3–86.3%). Further, 21.9% of the included prison populations were PWID demonstrating the intertwined relationship between at risk-groups. However, recent data are missing.

This paper aimed to describe the prevalence among GP and at-risk populations in Germany. This is however a simplistic approach given that populations at higher risk of VH may be exposed to several risk factors contributing to their vulnerability such as migration from a high prevalence country and sex work or prisoners who are sentenced due to IDU combined with potentially lack of access to health care services. Large-scale studies that focus on at-risk populations may determine differences in the prevalence of VH and identify frequent intersections between different at-risk groups in order to identify sub-populations in particular need of intensified testing and treatment efforts.

Some at-risk populations were missing in the identified literature including sex workers, persons with frequently changing sex partners, recipients of blood transfusions, and persons with tattoos and piercings. This indicates a need for more research to generate valid estimates of the prevalence in these groups to know the true burden of VH in Germany.

Methodology—Strengths and Limitations

The broad search string used in this overall scoping review ensured that all relevant outcomes were included and reviewed. By running the search string also in CC Med Base Bielefeld, it was ensured that evidence published only in German was included. Almost half of the identified publications in the "prevalence" category were published in German (24 of 51 publications), which highlights the need to search for publications in both German and English to gain insights into ongoing research and results from Germany.

The quality of the evidence was overall good with risk of bias being low in the majority of the included publications. We used the tool developed by Hoy et al. (8) specifically developed with the purpose of assessing risk of bias in prevalence studies with the focus on looking at the attempt made by the studies in minimizing the risk of bias. The majority of the studies were not population-based prevalence surveys aiming to estimate the national prevalence of HBV, HCV, or HDV, but rather studies with non-probability based sampling methods and small sample sizes. Therefore, they failed to address some of the critical items necessary to reduce bias as set forth in the risk assessment tool by Hoy et al. Although the results were not analyzed based on the risk of bias, this was an important step in order to allow critical interpretation of data and be aware of their strengths and limitations.

Our scoping review has limitations. There is a risk of publication bias and delays in the available and published data. Attempts were made to compensate this by including

non-published articles from the RKI Epidemiological Bulletin (EpiBull) and relevant regional journals. Moreover, a manual search was performed of reference lists in the included publications of the overall scoping review on VH epidemiology (6), and 14 references were identified but none were on prevalence. Further efforts, such as conducting a search for gray literature through other sources would potentially increase the number of relevant non-published literature.

This analysis was part of a large comprehensive review covering all aspects of viral hepatitis B, D, and D epidemiology in Germany and presents data on VH prevalence until 2017 (6). With this comprehensive review, information on the baseline situation which is necessary for better monitoring of VH elimination in Germany was collected. The time period before 2017 is of special interest as it serves as baseline to identify where the evidence gaps are and where the prevalence data are missing. An update of the overall review, including prevalence data, is planned to be conducted within the next few years, where the current review will serve as baseline.

Comparisons between the publications in this analysis are challenging because of their heterogeneity. The publications have made use of different study design, population, age-groups, and marker etc. which hinders the drawing of conclusions on patterns and temporal trends of prevalence. Similarly, geographical trends were not possible to analyse due to too few publications with same methodology from the same regional areas in Germany.

Publications with self-reported data and data where the diagnostic marker was not specified were included in this review. However, it is important to emphasize that these cannot be compared to studies based on laboratory data and data with specific diagnostic markers. Therefore, they are mentioned in the text and **Table 2** as our aim with the review was also to outlay where there is evidence and where there is not, but excluded from the figures as direct comparisons are not possible.

The majority of the studies included were large cross-sectional screening studies in which patients attending general practices or emergency rooms were offered screening for VH. There is a gap in evidence from longitudinal studies, which could contribute to an understanding of how the VH epidemic is evolving and would allow calculation of incidence and the effects of prevention and control measures on reaching the VH elimination targets. Differences identified in this review are more likely the results of heterogeneous methodology rather than reflections of changes in the VH epidemic. Nonetheless, blood donors represent a group for which standardized data are collected nationwide and over time. The six studies included in this review covered the period from 2005 to 2010, and throughout this 5 years' time period the HBV and HCV prevalence was low, and slightly lower in the later years [2005: 0.14% (HBV), 0.08% (HCV), 2010: 0.12% (HBV), 0.07% (HCV)].

During the time period in which the evidence identified in this review was published, the assays used to test for VH have changed. This may have contributed to a difference in prevalence found in the different studies. In particular for anti-HBc where patterns need to be carefully evaluated due to the risk of differences in sensitivity with the more recent tests having a higher sensitivity than the older tests.

It is also important to underline that some HBsAg positive may be inactive chronic carriers and thereby not sick, eligible for treatment or at risk for developing sequelae. When screening people with migrant background, in particular, many inactive HBsAg carriers with low viremia are identified. However, although not eligible for clinical treatment inactive HBsAg carriers can still transmit the virus to other persons. In this review, of the 39 publications that reported on HBV prevalence, 11 reported on either HBV DNA or HBeAg among those testing HBsAg positive. Further, screening for anti-HBc is important, as while it detects past infection, HBV can reactivate in people who are immunocompromised (e.g., PLWH).

Of the 33 publications covering HCV prevalence, only 13 tested for HCV RNA in addition to anti-HCV, which is important to demonstrate chronic HCV and replication. And importantly, our results include articles published until 2017, which means that the potential impact of the highly effective direct-acting antiviral (DAAs) treatment options on the HCV epidemic are not sufficiently covered in this review.

CONCLUSION

Globally, the elimination of VH is still gaining momentum. The progress of the interventions needed to reach the WHO elimination goals are being monitored (78) and the continuous need to collect strategic information to target the response is key. This review contributes to the understanding of the existing knowledge about the VH epidemic in Germany.

A comprehensive evidence-based overview of the available evidence on VH prevalence in Germany was provided. While there is overall good evidence, this is largely on HBV and HCV prevalence in the general and clinical populations. Gaps in knowledge exist for HDV and at-risk populations, and longitudinal studies are needed to uncover trends in the epidemic. Although Germany is considered a low prevalence country, high and very high rates are found among at-risk populations, in particular among PWID. Further research is needed on these groups and representative samples at the national level to gain much needed insights into the large-scale patterns of VH and the progress toward reaching the WHO elimination goals by 2030 in Germany.

AUTHOR CONTRIBUTIONS

RZ and VB conceptualized this study. SD supervised the study. GS, NS, and SL carried out the study. GS, NS, and RZ developed the research questions and drafted the a priori protocol. GS, NS, SL, SB, RT, RZ, and SD extracted the eligible data. IS and SD performed the analyses. IS, GS, NS, MC, HW, YS, VB, RZ, and SD interpreted the results and contributed to the discussion. IS drafted the manuscript. All authors critically revised the manuscript and approved the final version.

FUNDING

This work was partly funded by a grant from the German Center for Infection Research (DZIF) and the German Ministry of Health (BMG). The funders did not play a role in the design and data collection, analysis, and interpretation of data or in writing the manuscript.

ACKNOWLEDGMENTS

We thank Henriette Senst, Sarah Friedländer, and Thomas Harder for their help and inspiration in the data extraction.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2020.00424/full#supplementary-material

Supplementary S1 | Filename: S1_Text_search strategy.docx.
Title of data: Search strategy.
Description of data: The search strategy used for the study to identify the included publications.

Supplementary S2 | In- and exclusion criteria.
Filename: S2_Figure_In- and exclusion criteria.tiff.
Title of data: In- and exclusion criteria.
Description of data: Table listing the criteria for selection of the publications retrieved through the search.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Public Health

ORIGINAL RESEARCH
published: 28 May 2021
doi: 10.3389/fpubh.2021.568524

Developing and Piloting a Standardized European Protocol for Hepatitis C Prevalence Surveys in the General Population (2016–2019)

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Specialty section:
This article was submitted to Public Health Policy, a section of the journal Frontiers in Public Health

Received: 09 September 2020
Accepted: 28 April 2021
Published: 28 May 2021

Citation:
Sperle I, Nielsen S, Bremer V, Gassowski M, Brummer-Korvenkontio H, Bruni R, Ciccaglione AR, Kaneva E, Liitsola K, Naneva Z, Perchemlieva T, Spada E, Toikkanen SE, Amato-Gauci AJ, Duffell E and Zimmermann R (2021) Developing and Piloting a Standardized European Protocol for Hepatitis C Prevalence Surveys in the General Population (2016–2019). Front. Public Health 9:568524. doi: 10.3389/fpubh.2021.568524

Background: A robust estimate of the number of people with chronic hepatitis C virus (HCV) infection is essential for an appropriate public health response and for monitoring progress toward the WHO goal of eliminating viral hepatitis. Existing HCV prevalence studies in the European Union (EU)/European Economic Area (EEA) countries are heterogeneous and often of poor quality due to non-probability based sampling methods, small sample sizes and lack of standardization, leading to poor national representativeness. This project aimed to develop and pilot standardized protocols for undertaking nationally representative HCV prevalence surveys in the general adult population.

Methods: From 2016 to 2019a team from the Robert Koch-Institute contracted by the European Centre for Disease Prevention and Control synthesized evidence on existing HCV prevalence surveys and survey methodology and drafted a protocol. The methodological elements of the protocol were piloted and evaluated in Bulgaria, Finland and Italy, and lessons learnt from the pilots were integrated in the final protocol. An international multidisciplinary expert group was consulted regularly.

Results: The protocol includes three alternative study approaches: a stand-alone survey; a "nested" survey within an existing health survey; and a retrospective testing survey approach. A decision algorithm advising which approach to use was developed. The protocol was piloted and finalized covering minimum and gold standards for all steps to be implemented from sampling, data protection and ethical issues, recruitment, specimen collection and laboratory testing options, staff training, data management and analysis and budget considerations. Through piloting, the survey approaches were effectively implemented to produce HCV prevalence estimates and the pilots highlighted the strengths and limitations of each approach and key lessons learnt were used to improve the protocol.

Conclusions: An evidence-based protocol for undertaking HCV prevalence serosurveys in the general population reflecting the different needs, resources and epidemiological situations has been developed, effectively implemented and refined through piloting. This technical guidance supports EU/EEA countries in their efforts to estimate their national hepatitis C burden as part of monitoring progress toward the elimination targets.

Keywords: hepatitis C, HCV, general population, prevalence, technical protocol, surveys, questionnaires

BACKGROUND

The World Health Organization (WHO) has set ambitious targets for the elimination of viral hepatitis as a public health threat by 2030 in the global health sector strategy on viral hepatitis 2016–2021 (1).

One of the five strategic directions outlined in the strategy entails information for focused action, underlining the importance of collecting robust data on the viral hepatitis epidemic in order to improve and guide implementation of efforts in the response. An update on the progress of the implementation of the strategy was recently published by WHO, stressing the need to strengthen and more regularly update viral hepatitis data in order to improve implementation (2). Robust estimates of the number of people with chronic hepatitis C virus (HCV) infection are needed and its prevalence is one of 10 core indicators (C.1.b), identified by the WHO in their framework on monitoring and evaluation for viral hepatitis (3).

Data on newly diagnosed and notified cases of viral hepatitis are collected through the surveillance systems, which are in place for HCV in the majority of countries in the European Union (EU). However, completeness of data is a major issue, and reporting of data according to EU case definitions to enable a clear comparison across countries and time remains challenging (4, 5). Furthermore, the data collected through the surveillance systems are largely influenced by the local testing strategies rather than actual epidemiological trends or burden of disease.

HCV prevalence surveys provide key information on the epidemiology of HCV infection. These surveys, in contrast to surveillance data, provide a snapshot of the current epidemiological situation, as all individuals in the sample infected with HCV are identified, regardless of their diagnostic status. However, a recent systematic review found that up-to-date estimates of prevalence are lacking from many EU/European Economic Area (EEA) countries (5, 6). This review also found that studies that have been undertaken in the EU/EEA are heterogeneous and often of poor quality due to non-probability based sampling methods, small sample sizes and lack of standardization leading to poor national representativeness (5, 6).

The HCV epidemiology varies between countries and depends on multiple factors. In countries with low prevalence, injecting drug use (IDU) is an important risk factor and a main contributor to the HCV epidemic (7). In these countries, people who inject drugs (PWID) are often the group with the highest prevalence and a key population to target with prevention and treatment measures. In other countries, where higher levels of transmission occurred in the past through unsafe injections, via blood transfusions or other nosocomial transmission routes such as unsafe use of glass syringes, as reported in Italy (8), HCV is more widespread in the older general population (9). This type of more generalized epidemics has been observed in some European countries such as Czechia, Italy, Poland and Romania (10–14).

Knowing the HCV prevalence in the general population, and standardizing the way data are collected and estimates generated will contribute to more robust data allowing monitoring and comparisons between countries and over time (15). This will positively contribute to the monitoring and tracking of the progress toward the WHO viral hepatitis elimination goal (3).

To address this issue and support EU/EEA Member States (MS) in their efforts to generate robust estimates of HCV prevalence, the European Center for Disease Prevention and Control (ECDC) launched the "Sero-Prevalence Survey for Hepatitis C in Europe" (SPHERE-C) project. The Robert Koch Institute (RKI) was formally contracted by ECDC between 2016 and 2019 to develop a detailed technical protocol, with the aim to develop and pilot standardized protocols for undertaking nationally representative prevalence surveys of HCV in the general adult population (15).

METHODS

A short inquiry was sent to all ECDC national focal points for hepatitis in the EU/EEA MS in September 2016 to gain insight in the countries' availability of HCV prevalence data from previous surveys and around future plans for undertaking work in this area, as well as gauging interest in participating in a pilot of the SPHERE-C protocol in 2018. Responses from 22 MS were obtained and used to guide the development of the protocol.

The development of the protocol was based on synthesis of scientific information and evidence on HCV prevalence surveys. A desktop review was conducted to define all the objectives for the survey and to suggest methods for each objective. To inform these objectives, a literature review was undertaken to gain understanding of the local epidemiological gaps and political needs. Thereafter, to identify the most appropriate methods for the defined objectives, available information on the methods used in previously conducted HCV prevalence surveys was collected, and efforts were made to also identify surveys outside the EU/EEA. The identified surveys and key information were entered into a table, and study protocols were collected through online searches or through contact with the researchers who performed the surveys. Methodological criteria to achieve minimum or gold standard for each objective was identified

and a conceptual matrix presenting the findings was constructed with areas covering selection of sites/population, sampling and stratified sampling methods, specimen/data collection, laboratory testing methods, storage and transport of samples, confidentiality and ethical issues, data management, quality control and training materials needed.

An expert group was set up to guide the direction of the project and to provide feedback to the development of the protocol. The expert group consisted of researchers, laboratory experts, statisticians, medical doctors and epidemiologists from across Europe and the USA. Three face-to-face consultations were held with the expert group between 2016 and 2019. The group was asked to comment on draft versions of the protocol over the course of the project. The expert group agreed upon the most relevant methodological approaches to be included in the protocol based on the evidence presented by the RKI project team and through consensus.

Three EU countries were selected to pilot the technical protocol. Methodological elements in the protocol were piloted to gather practical experience and evaluate its usability and applicability. Lessons learnt were collected to guide the further development of the protocol.

The following three pilots were carried out during 2018:

- A retrospective survey with testing of blood samples from the FinHealth2017 national health examination survey in Finland
- A stand-alone survey in the city of Stara Zagora, Bulgaria
- A stand-alone survey in the city of Catanzaro, Italy

A pilot-specific study protocol based on the overall protocol and study materials were developed for the pilot of the stand-alone survey conducted in Stara Zagora, Bulgaria by the RKI. The local survey teams in Finland and Italy developed their own pilot-protocols and materials, based on the recommendations from the technical SPHERE-C protocol. The aim for each of the three surveys was formulated and tailored to the local context drawing on the recommended aim in the technical protocol. All three pilots were performed in close collaboration with the team at RKI, and regular teleconferences were held with the local survey teams to ensure that decisions made locally were coherent with the technical protocol.

Indicators were developed to evaluate the feasibility of the protocol and the methodological approaches. The evaluation indicators were transformed into an evaluation questionnaire with 10 main questions covering all sections in the technical protocol including objectives of the survey, sampling and sample frame, time spent, structure, coordination and collaboration, ethical approval, data protection and informed consent, awareness-raising, recruitment, personnel, budget, data management and data collection (blood sampling and questionnaire). The evaluation questionnaire was completed in writing by the local survey teams in the three countries, and then sent electronically to the RKI. Interviews to explore issues in further depth were conducted with the local survey teams on the phone with the survey teams from Finland and Italy, and face to face during a 2 day evaluation workshop in December 2018, at the RKI in Berlin, Germany with the survey team from Bulgaria.

RESULTS

The technical protocol provides background as well as more detailed information demonstrating the importance of undertaking prevalence surveys to generate robust estimates of hepatitis C prevalence. Importantly, it provides options and steps for planning and conducting a population-based hepatitis C survey which can be adapted to the local context. The technical protocol consists of two main parts:

1) Selection of a survey approach
2) Planning and conducting a survey

This is explained in detail in the published protocol (15), and in brief below.

Three Survey Approaches

The technical protocol includes three survey approaches which were identified as the best approaches through the desktop review and through discussions with the expert group. The three survey approaches are: a survey "nested" within an upcoming health survey; a retrospective testing survey; and a stand-alone survey.

The three survey approaches all fulfill the pre-defined criteria outlined in the protocol and are variations of a survey with probability-based sampling. The protocol covers minimum and gold standards for key aspects including: sampling; data protection; ethical issues; recruitment; specimen collection; laboratory testing; staff training; data management; quality assurance and budget considerations (15). As an example, for the type of specimen, the minimum requirement is dried blood spots, and the gold standard is venous blood samples (15).

Mandatory requirements and methodological options for an HCV prevalence survey (for all three survey approaches) are illustrated in **Figure 1** and described in more details in the published protocol (15).

Nested Survey

The nested survey approach requires an upcoming larger population-based health survey of the general population, e.g., a national health examination survey (HES). In this approach, the prevalence survey is nested in this larger survey, which makes it less resource intensive and costly due to the use of the existing infrastructure of the already planned survey. This allows additional testing of the participants for HCV, as well as collection of HCV-related behavioral data, with little extra effort. Therefore, this option requires relatively small amounts of financial and human resources. The chances of a representative sample are increased if the sample size calculations for the HES are sufficient for the expected prevalence of HCV due to the often rigorous sampling strategy and efforts to reduce non-response, that are part of a larger population-based survey.

Retrospective Testing Survey

This approach requires a recently conducted population-based survey. From stored blood samples of a former survey, HCV testing can be performed retrospectively. The criterion of probability-based sampling needs to be fulfilled. Furthermore, it is important to ensure that there is a sufficient number of

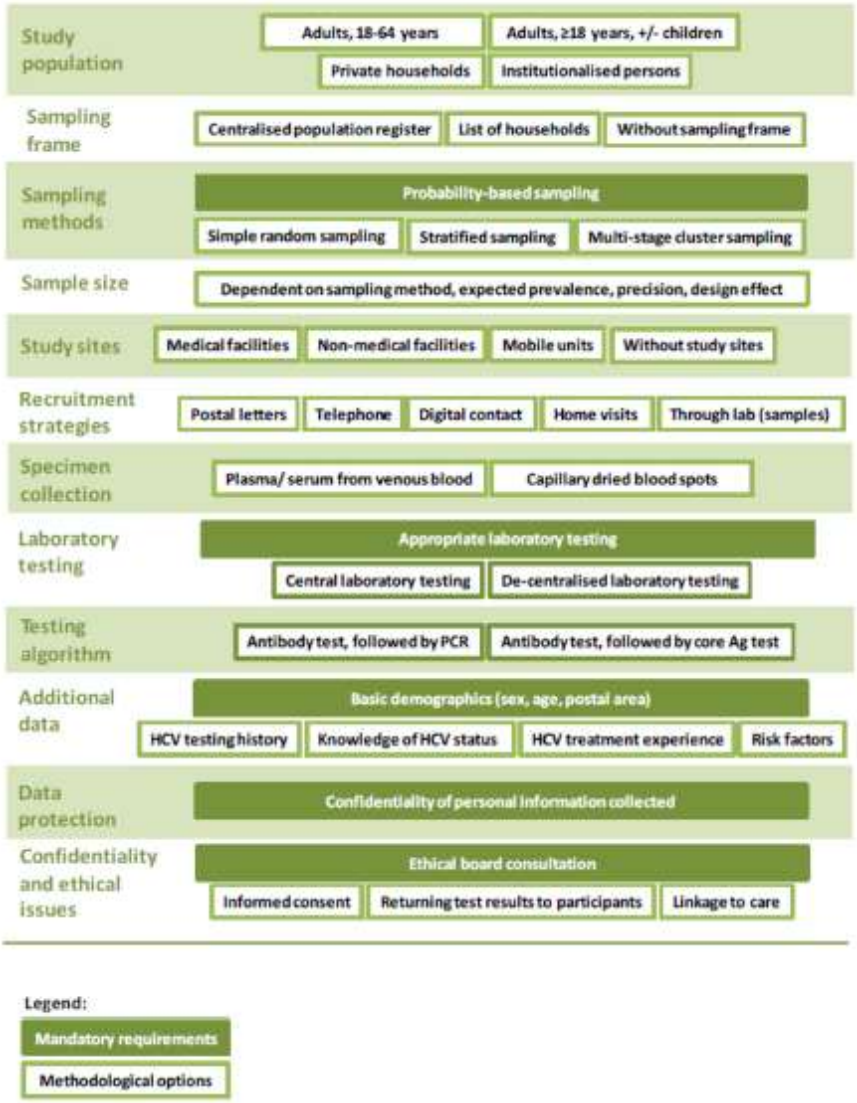

FIGURE 1 | Overview of mandatory requirements and methodological options for an HCV prevalence survey.

samples with enough material left for testing, and that these do not represent a biased sub-set of the original samples collected. Further, informed consent that was given by participants needs to include storage of samples for further research and retrospective testing. If the abovementioned requirements are fulfilled, extra costs for this approach will mainly arise from the laboratory work and analysis of the data.

Stand-Alone Survey

The third option is to embark on a stand-alone HCV prevalence survey where the primary aim is to estimate the HCV prevalence (by age and sex). This is the most staff- and financial resource intensive approach, as all steps needed to do a survey, including sampling, data protection and ethical issues, recruitment, specimen collection and laboratory testing options, staff training, data management and budget considerations, need to be performed.

Selecting a Survey Approach

A decision algorithm was developed and included in the protocol to guide MS through a careful decision making process when

selecting the most suitable survey approach for their respective setting and situation (**Figure 2**) (15).

If a large population-based survey is planned, where blood samples are collected (e.g., a HES with a probability-based sampling of the general population), it is suggested to nest the HCV prevalence survey into this population survey. The precondition is that the planned survey fulfills the minimum criteria outlined in the protocol, e.g., has a sufficiently large sample size and is representative of the populations of interest. Including HCV testing in existing survey protocols involves steps similar to those for designing a new survey, although some steps may be simpler as they have already been done for the original survey (such as ethical approval, sampling process, and the recruitment strategy).

If no population-based survey is planned, but a former survey such as a HES or another study with a probability-based sample of the general population was conducted recently and included the collection of blood samples, an option is to test the sera left over from this survey retrospectively. Again, the above mentioned criteria need to be met to ensure the quality of the data generated. Furthermore, proper sample storage should be assured to prevent bias due to HCV RNA degradation.

If none of the two above options are available, then a third option is to do a stand-alone survey, where the primary purpose is to estimate the HCV prevalence. When conducting a stand-alone survey, all the steps for undertaking a survey need to be carefully planned and undertaken. Setting up a stand-alone survey in the general population is time- and budget intensive. Therefore, a preliminary first step is to test any residual or routinely collected sera (e.g., from antenatal care screening). If the prevalence in those samples is found to be low (<1%), it is recommended that prevalence surveys in key populations at higher risk of infection, e.g., among PWID should be prioritized over a population-based survey in the general population (**Figure 2**).

If none of the three survey approaches are possible there are several alternative methods to consider, although these methods may be more subject to potential bias. These include testing residual sera from laboratory samples (16, 17), samples from proxy populations of the general population such as pregnant women (18) or first-time blood donors (6) or general practitioner or health insurance registries as well as linking information from multiple national registries and applying various modeling techniques (19, 20). These and more

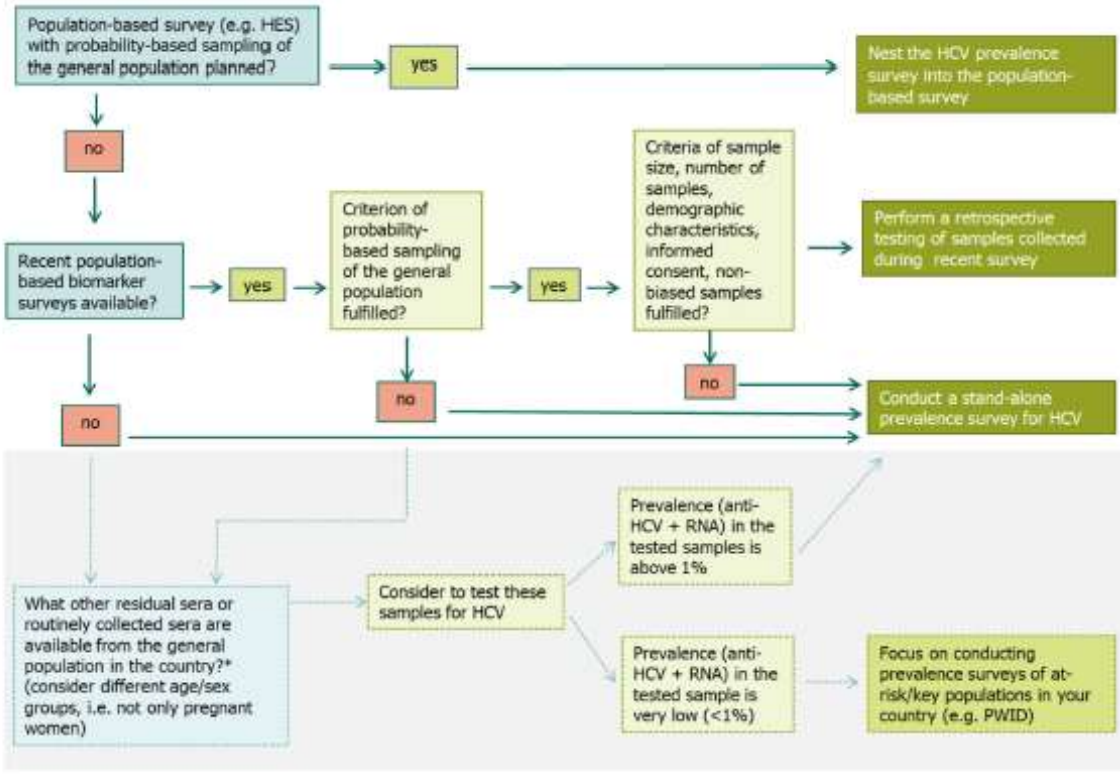

FIGURE 2 | Decision algorithm to select the most suitable survey approach when planning a prevalence survey for hepatitis C in the general population (15). *Alternative options exist that might be explored by countries to get an idea of the HCV prevalence level in the general population, if they do not have data from a recent population-based prevalence survey or plans for a future survey and few resources for a stand-alone survey (15).

alternative methods are explained in further detail the technical protocol (15).

Results From Piloting the Protocol

The three separate survey approaches were planned to be piloted in three different EU countries. However, these plans were subsequently adapted on account of the local situation in each of these three sites, so that finally the stand-alone survey approach was piloted in two countries and the retrospective testing approach was piloted in the third country. Furthermore, due to local circumstances the recommended steps in the protocol for the different survey approaches were adapted to fit with what was feasible and in agreement with the local context in the three countries.

Stand-Alone Survey Approach

Stara Zagora, Bulgaria

The main objectives of this pilot were to estimate the prevalence of chronic HCV infection, by sex and age group, in the adult population in the city of Stara Zagora, Bulgaria and to test the feasibility and proposed methodological approach in the draft technical SPHERE-C protocol.

Italy

In Italy, the initial plan was to nest the HCV survey onto a planned HES focused on salt consumption (CUORE[1]). However, this needed to be adapted as the sample size in the CUORE survey was too small. Therefore, the sample size was re-calculated and the local team took the decision to undertake a stand-alone survey.

The objectives of the survey pilot in Italy were to estimate the age- and sex specific prevalence of chronic HCV infection, age- and sex specific prevalence of exposure to HCV and the prevalence of undiagnosed HCV in the adult population of the city of Catanzaro, Southern Italy. All these objectives were fulfilled.

Nested Survey Approach With Retrospective Testing of Samples

Finland

The main objectives were to estimate the anti-HCV and prevalence of chronic infection in the Finnish general population (above 18 years of age) using the samples from the FinHealth2017 national health examination survey. A secondary objective was to match the data with the national infectious disease register, in order to generate an estimate of the undiagnosed fraction. The objectives of the survey were fulfilled.

General Results

From the evaluation of the pilots and the technical protocol, various challenges were reported by the local survey teams. In **Table 1** below, the sections included in the technical protocol are listed together with key lessons learnt from the three pilot surveys, and implications for the protocol. The detailed results of the pilot in Bulgaria are published elsewhere (22).

[1] Available online at: http://www.cuore.iss.it/eng/factors/HES2018-2019.asp.

DISCUSSION

The survey approach selected to estimate HCV in the adult general population needs to be carefully considered. Conducting a population-based survey is challenging, resource intensive, requires a good survey infrastructure, and a sufficient number of well-trained staff members. Therefore, the preferred option is to make use of an already planned population-based health survey, or to make use of retrospective testing of already collected samples, providing that requirements are fulfilled to ensure representativeness. However, these approaches also have their limitations, as, for example, nesting a survey onto a pre-planned survey may not fit in with the scope or logistical capacity of the pre-planned survey.

The evaluation of the three pilot surveys indicated that the different survey approaches selected are suitable methodological designs for estimating the anti-HCV and the chronic HCV infection prevalence in the adult general population. Nonetheless, the pilots were associated with several important limitations. The stand-alone surveys were only conducted on city level, and conducting these on national level is likely to be more complex. The nested survey design outlined in the protocol was not fully piloted, as the survey in Finland adapted the approach and retrospectively tested the samples for HCV. Nonetheless, methodological elements in the technical protocol for conducting HCV prevalence surveys has been demonstrated to be a useful and effective tool for EU/EEA MS as expressed by the local survey teams in the qualitative evaluation (15). Importantly, the protocol considers different situations in different settings by assisting countries through careful decisions that need to be made to select the most appropriate survey approach for any given context.

The technical protocol refers to chronic HCV. Having an up to date estimate of chronic HCV is particularly important given the availability of the direct acting antiviral treatments (DAAs) for HCV. It has been demonstrated that increased access to DAAs leads to a decrease in HCV incidence and prevalence (23, 24). Although low, monitoring the HCV burden and estimating the number of people in need of treatment is of critical importance in the response to viral hepatitis.

Lessons From the Pilots

Although the nested survey approach is the first approach to consider, it is first and foremost critical that the minimum requirements are fulfilled. This was not the case in the pilot in Italy where the CUORE survey was not powered to estimate the HCV prevalence. However, while it would have been possible to nest onto the survey, and then sample additional people for HCV testing to reach the sample size calculated for the HCV prevalence survey, the Italian survey team decided to change survey approach to a stand-alone approach. This approach however required more efforts in terms of organization and time as well as human and financial resources.

The original plan in Finland was a nested survey. However, delay in getting access to the samples for HCV testing meant that it ended up resembling more a retrospective testing approach. Lessons from the retrospective testing of samples in Finland

TABLE 1 | Summary of methodological details, results of the pilots, lessons learnt, and implications for the technical protocol (15).

	Stand-alone survey		Nested with retrospective testing of samples	Implications for protocol
	Bulgaria	**Italy**	**Finland**	
Data protection issues/Ethical approval	Names and addresses of invitees were not allowed to be shared with study team, invitation letters needed to be sent out by the municipality holding the register	Required to call every participant for scheduling appointment to return test results	Data protection issues and ethical approval conducted previously by FinHealth study team. Informed consent form already included possibility of testing for some other diseases	Plan for getting the ethical approval early to be able to still adjust according to requested changes Data collection and processing according to the General Data Protection Regulation (GDPR) in the EU 2016/679 required, therefore early contact with the national data protection agency advised
Sampling method	Simple random sample stratified by age and sex	Simple random sample stratified by age and sex	Two-stage cluster sampling stratified by age and sex	Sample should be selected using a probability-based random sampling method For smaller geographical areas (e.g., cities) simple random sampling may be applied
Sampling frame	Local population register of the city of Stara Zagora	Local population register of the city of Catanzaro	National population register	Population registers should be up to date
Sample size calculation	$N = 999$ (expected prevalence of chronic HCV was 1.0% and a lower precision bound of 0.25%)	$N = 889$ (expected prevalence of of chronic HCV infection of 1.0% for age group 35–65 (upper precision bound 2.2%) and 5.0% for age group 65+ (upper precision bound 10.0%)	$N = 10,305$ (expected prevalence of current HCV infection (anti-HCV and HCV RNA positive) of 1% and a lower precision bound of 0.25%)	Ensure large enough sample size to get a valid estimate [input and statistical formula on how to calculate sample size included in technical protocol (15)]
Recruitment strategy	Tracked invitation letter. Reminders: a second tracked invitation letter	One invitation letter (in 4 rounds). For each round a new subset of the sample was invited	First contact with a postcard, followed by an invitation letter. Reminders: postcards, phone calls, SMS reminders	Emphasize that more recruitment efforts are needed to ensure a high enough response rate and to include the "hard to reach" populations who may have a poorer health Only tracked letters are not recommended Make at least three attempts to reach participant (invitation letter, reminder letter, phone call, SMS reminders, or house visits) Include a pre-test to test the effectiveness of different incentives
Promotion of the survey	Information leaflet for invitees; contact with and engagement of local authorities; local media campaign to inform about hepatitis C and encourage participation in the survey including information posters in local pharmacies and outpatient care facilities (general practitioners and medical centers); 3 local press conferences, local radio and television broadcasts	Information leaflet for invitees; contact with and engagement of local authorities; awareness posters for the survey displayed in waiting rooms of general practitioner practices and in the hospital of Catanzaro	Information leaflet for invitees; contact with and engagement of local authorities; Press conference, newspaper articles, radio and television broadcasts	Information leaflet (and website) to inform invitees are strongly recommended for all surveys Information and promotion of the survey among the general population through media and local authorities, and among health care staff are recommended
Data collection period	10 weeks (5 September 2018–16 November 2018)	4 rounds of 1 week each in a period of 7 months (June 2018–December 2018)	7 months (January 2017–July 2017)	Plan extendible data collection period/buffer of time in case sample size was not reached in the planned period. The data collection period in Bulgaria should have been prolonged to reach sample size

(Continued)

TABLE 1 | Continued

	Stand-alone survey		Nested with retrospective testing of samples	Implications for protocol
	Bulgaria	**Italy**	**Finland**	
People invited	1,998 (1,166 picked up their letter)	9,000 (8,655 letters delivered)	10,247	Take into account expected non-response rate, and consider that the non-response rate may be higher than 50%
Participants	252	1,003	5,923 available samples tested	
Incentives	A coffee mug and a pencil	One day off from work for participants	Results of the health examinations and laboratory analysis of the collected biological samples	Consider different incentives for different age-groups. Include a pre-test to test the effectiveness of different incentives
Response rate	12.6% Net response rate: 21.6% (of those who got the invitation)	11.1% Net response rate: 11.6% (of those who got the invitation)	Overall response rate for questionnaire: 59.6% Net response rate for health examination: 57.8%.	Low response rates in all pilots highlight the challenge of reaching the target set by EHES of 70% (15, 21) and consideration of a more realistic target
Additional data and questionnaire	Self-administered questionnaire including questions specific to HCV Migrants were not sufficiently included, and therefore unknown if translation was needed	Self-administered questionnaire including questions specific to HCV Migrants were not sufficiently included, and therefore unknown if translation was needed	Self-administered questionnaire completed before HES either electronically or manually No HCV-specific questions (e.g., HCV infection risks) included	Self-administered questionnaires work well in general populations Prior to data collection, assess whether translation /interviews are needed In nested surveys, early collaboration with survey team important to ensure that HCV-related questions are included
Laboratory	Local laboratory for serology and one in capital for confirmatory testing and PCR. Shipping by using routine procedures	Shipping of samples to a centralized reference laboratory for all testing	Defreezing, aliquoting and shipping of samples to another laboratory for serology and PCR	Centralized testing of all steps in one laboratory is recommended Alternatively two-step test algorithm in two laboratories when routine shipping procedures can be used In retrospective design, samples for HCV testing should be aliquoted during data collection
Testing algorithm	Anti HCV ELISA, followed by PCR. Immunoblot for PCR negative samples	Anti HCV ELISA, followed by PCR. Immunoblot for PCR negative samples	Anti HCV ELISA, followed by immunoblot (HCV ELISA positives and borderlines) and PCR (immunoblot positives and borderlines)	The number of false positives may be high in low prevalence settings, therefore confirmation of anti-HCV reactive, PCR negative samples is important in these settings
Returning test results to participants and linkage to care	Test results were returned to all survey participants, who received a letter with their participant ID and a date for when they would receive their test result in person at the Regional Health Inspectorate. Those who were tested positive were linked to specialised medical care	All participants contacted via phone to schedule an appointment during which they would receive their test result Those who were tested positive were linked to specialised medical care	Positive cases were contacted by phone and a letter. Those who were tested positive were linked to specialised medical care	Plan enough time, staff and budget to have appointments with all participants or outsource the scheduling of appointments Alternatively only inform positive-tested about test results Returning test results from retrospective testing only if data was collected recently, and participants consented to being informed
Data analysis including weighting	Frequencies and percentages were calculated for categorical variables (participants and non-participants). For the chronic HCV prevalence weighting adjustment was performed with age and sex.	Non-response biases were evaluated by comparing respondents and non-responders with regard to their sex, age distribution and housing deprivation level. Crude, age and sex specific,	Post-stratification weights were used to correct the possible for non-response biases by incorporating population distributions of sex, age and other appropriate characteristics into survey estimates	95% confidence intervals (taking into account the design of the survey) Weighting should take into account at least age and sex, if possible, further characteristics (e.g., regional or urban–rural

(Continued)

TABLE 1 | Continued

	Stand-alone survey		Nested with retrospective testing of samples	Implications for protocol
	Bulgaria	**Italy**	**Finland**	
	Prevalence estimates were calculated as crude estimates and weighted estimates with 95% Confidence Intervals All analyzes were carried out in Stata 15.1	and standardized anti-HCV prevalence rates were calculated. The associations of HCV infection with the different predictor variables were investigated by log binomial regressions with sampling weights or by exact logistic regressions as appropriate. Variables with a $p < 0.20$ at the univariate analysis were considered as potential predictors and included in multivariable analysis All analyzes were carried out in Stata 15.1	Design based weighted overall and age- and sex-stratified estimates of the HCV prevalence and their 95% confidence intervals were calculated The associations of HCV infection with multiple explanatory variables were modeled using logistic regression model with sampling weights. Predictive margins of interests were calculated	distribution, migration status) 95% confidence intervals (taking into account the design of the survey) Weighting should take into account at least age and sex, if possible, further characteristics (e.g., regional or urban–rural distribution, migration status)
Budget implications	Most time and resources spent on administrative challenges	Most resources spent on sending letters and scheduling appointments	Most time spent on preparing samples for testing	Allow adequate time for administration Consider outsourcing the sending of letters/scheduling appointments

underlined the importance of communication and mutual understanding between the two teams (main survey team and HCV prevalence survey team) in order to keep the timeline for the HES and ensure the testing of samples for HCV. Early and clear communications may also increase the chances of including extra HCV relevant questions in the questionnaire. For the survey in Finland, questions on past or present drug use were not included to keep the questionnaire short. It is important to be able to standardize results across Europe, and therefore important to collect a minimum set of sociodemographic data for each participant, regardless of survey approach. These include information on sex,) at the time of blood sample collection, and a postal or geographical code. The core set of data, as well as recommended questions on HCV testing and status and risk factors, are provided in the technical protocol (15). There are various strengths using the nested approach, but also important limitations. While a significant advantage is the possibility to make use of an established survey including its sampling approach and the associated socio-economic data, the disadvantage is that there may be limited opportunities to influence the sampling strategy and the overall schedule of the survey, which was a barrier for the Finnish pilot.

Another challenge with the nested approach is interest from different research groups with focus on different disease areas. With a probability-based sampling and rigorous recruitment strategy, the samples are considered of high value and can contribute to valuable knowledge for several disease areas. There are often competing proposals and research ideas from different groups, all wanting to include specific questions in the questionnaire, making early planning and prioritization crucial. In the retrospective testing approach in Finland, more time was needed for sample handling. Therefore, the Finnish team recommends to draw specific samples for infectious diseases testing during the HES, as opposed to only one blood sample which then needs to be tested by multiple groups.

It was not possible to pilot all recruitment steps recommended in the technical protocol (letter, phone calls, short message service (SMS) reminders, and house visits). In Italy, only letters were sent in several rounds, and for each round, a new subset of the sample was invited to participate. While the sample size was reached, the recruitment strategy implemented for the Italian survey may have led to a less representative sample as those who take part after one recruitment attempt are easier to reach and thereby likely in better health or more interested or have more time. Additional recruitment steps are needed to reach initial non-responders, who might differ in socioeconomic and other characteristics from those who more easily accept to participate (25–27). Other innovative approaches may help to increase the number of respondents, e.g., by self-sampling or by offering telephone interview (28, 29). Similarly, the low response rate in Bulgaria is likely to have been caused by the change in recruitment strategy which only allowed invitation via letter. Further recruitment steps are needed to ensure a higher response rate such as e.g., phone calls and house visits (27, 30), which could not be piloted. In Finland, SMS reminders have previously proved successful in increasing participation among young invitees (30). Implementing several recruitment steps, as outlined in the SPHERE-C protocol, is important to ensure a high response rate. If unable to implement enough steps to ensure a high response rate, the large efforts needed to conduct a stand-alone survey may be unwarranted as the end sample will not be representative. In which case,

a better choice may be an alternative approach for estimating HCV prevalence.

The impact of incentives depends on the context in which they are offered. While the incentive provided in Stara Zagora was well-received (22), different incentives tailored to different age groups may have resulted in a greater response rate. For all surveys, the most efficient incentives and recruitment efforts need to be locally evaluated, e.g., through a pre-test prior to the survey, and decided upon according to context (26).

It may be that neither of the recommended three approaches are an option for some countries. Therefore, if there are no resources available for a stand-alone survey and testing stored samples or samples from a planned survey is not possible, alternatives may be explored. These may include testing residual sera from clinical laboratories, looking at data from first-time blood donors or looking at data from routine screening of pregnant women (15). These possibilities may also be used to get an idea of what the prevalence is before embarking on a stand-alone HCV prevalence survey. Even if such alternative approaches are likely to be based on non-probability-based sampling which increases the risk of bias, they may provide sufficient evidence for focusing future prevalence surveys in at-risk populations. By testing residual sera from different groups, bias can be reduced (16). It is of crucial importance that regardless of approach and method selected, efforts are made to ensure that the minimum requirements outlined in the technical protocol are met to ensure that results are representative and useful for estimating the HCV prevalence.

If a country sets out to do a stand-alone survey, it is highly advisable to include testing for other infectious diseases, such as hepatitis A, B, D, E, HIV, other sexually transmitted infections, in addition to HCV. It may also be relevant, depending on country and context, to consider including vaccine preventable diseases or relevant non-communicable diseases. A lot of work needs to be put into the planning and conducting of a stand-alone prevalence survey, especially if recommended approaches are taken to ensure a good response rate, and therefore it will make sense to make use of the rigorous sampling strategy to test for other infectious diseases.

Moving From HCV Prevalence Estimate in General Population to National Prevalence Estimate

Estimating the HCV prevalence in the general population is only one part of getting a national estimate of the HCV prevalence, which is one of the WHO core indicators in the monitoring and evaluation framework (3).

More data and additional methodological approaches are needed in order to generate a national prevalence estimate. Some countries have combined data from multiple registers and applied various modeling techniques to generate national HCV prevalence estimates (20, 31). Others have applied the workbook method (32) or the Bayesian multi-parameter evidence synthesis (MPES) (33). For these approaches, additional activities beyond what is covered in the technical protocol are needed. These activities include identifying the at-risk groups for HCV, which include PWID (both current and former), prison population, men who have sex with men (MSM) and migrants (documented and undocumented), then estimating the sizes and the prevalence in these groups. It is important to consider that many populations are not sufficiently captured in general population surveys but may contribute considerably to the total burden of HCV. Modeling studies from the UK and the USA suggest that the majority of people living with chronic HCV are either current or former PWID—with so-called "never injectors" contributing much less to the total burden of HCV (estimates from the UK suggest only around 15%) (31, 33–36). However, the epidemiology varies across Europe, with iatrogenic transmission an important driver of infection in some countries and non-PWID groups, such as migrants and MSM, affected in other countries (5, 37).

In conclusion, an evidence-based technical protocol for undertaking HCV prevalence surveys in the general population reflecting the different needs, resources and epidemiological situations across Europe has been developed and found useful through piloting (15). This technical protocol will help support EU/EEA countries in estimating their national viral hepatitis burden.

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DATA AVAILABILITY STATEMENT

The data analysed in this study is subject to the following licences/restrictions: The raw data supporting the conclusions of this article will be made available by the authors, without undue

reservation. Requests to access these datasets should be directed to sperle-heupeli@rki.de.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the local ethics committees in the pilot countries. The ethics committee at the Regional Health Inspectorate in Stara Zagora, Bulgaria and the Ethics Committee at the Istituto Superiore di Sanità in Rome Italy. In Finland, ethical approval was already provided for the large population based study FinHealth2017 and the informed consent formed covered HCV, and therefore it was not needed for the nested survey. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

RZ, SN, MG, ED, and AA-G conceptualised the SPHERE-C project. RZ supervised the project. IS, SN, RZ, and MG drafted the SPHERE-C protocol. HB-K, RB, AC, EK, KL, ZN, TP, ES, and ST prepared and implemented the pilot surveys in Finland, Italy, and Bulgaria and collected and interpreted the data. IS drafted the manuscript. All authors critically revised the manuscript and approved the final version.

FUNDING

This work was funded by the European Center for Disease Prevention and Control (ECDC) through a contract.

ACKNOWLEDGMENTS

We would like to thank all the survey participants and all staff members in the three pilot countries, Bulgaria, Finland, and Italy. The contributions of the SPHERE-C Expert Group are highly appreciated. Further, we thank Yanita Simeonova for translation and support during the Bulgarian pilot, as well as Sandra Dudareva, Lina Nerlander, and Peter Markov for providing valuable advice and support to the SPHERE-C project.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Curriculum Vitae for Ida Sperle

My CV will not be published in the electronic version of my thesis for privacy reasons.

Publication list

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Acknowledgements

I would like to thank the SPHERE-C survey participants, the local survey teams, and the SPHERE-C Expert Group, including staff at ECDC, for providing time and insights and for the much-valued collaboration.

I am grateful to several people who have supported me during the achievement of this degree. First of all, I would like to thank my supervisor, Ruth Zimmermann, for giving me the opportunity to come and work at the Robert Koch Institute in Berlin and Stine Nielsen for the great collaboration. Thanks to both of you for the laughs, inspiration and friendship.

I would also like to thank Sandra Dudareva for giving me the chance to contribute to the Hep-Epi project and for the support during the writing of the publication. Finally, a thanks also to Klaus Stark for his input and time as a supervisor for this thesis.

This thesis is dedicated to my father – for his endless encouragement and confidence in me and tasks ahead.