Optimizing infectious disease control: how to detect hidden infections? Illustrated by studies on pertussis and hepatitis

Jeanne Heil

CURRICULUM VITAE

Jeanne Heil was born on the 27th of November in 1991. In 2009, she graduated from high school at Carboon-college St. Jan in Hoensbroek. Following this she studied Health Sciences at Maastricht University - Health Promotion and Education and Movement Sciences. In 2013, she obtained her master degree in Health Sciences - Prevention and Public Health at Vrije Universiteit Amsterdam. In the summer of 2013, Jeanne worked as a volunteer in Masaka, Uganda. Thereafter, she started working at the Department of Sexual Health, Infectious Diseases and Environmental Health at the Public Health Service of South Limburg. Under the supervision of Prof. dr. Christian Hoebe, Prof. dr. Jochen Cals and dr. Nicole Dukers-Muijers, she did her PhD at the Department of Medical Microbiology of the Maastricht University Medical Centre (MUMC+) and the Care and Public Health Research Institute (CAPHRI). Currently, she is involved in several research projects and will continue to work at the Public Health Service South Limburg.

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Illustrated by studies on pertussis and hepatitis

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Na afloop bent u van harte welkom op de receptie ter plaatse.

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OPTIMIZING INFECTIOUS DISEASE CONTROL: HOW TO DETECT HIDDEN INFECTIONS?

Illustrated by studies on pertussis and hepatitis

Jeanne Heil

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HOW TO DETECT HIDDEN INFECTIONS?

Illustrated by studies on pertussis and hepatitis

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# List of abbreviations

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<tbody>
<tr>
<td>ALAT</td>
<td>Alanine transferase</td>
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<tr>
<td>CDC</td>
<td>Centers for Disease Control and Prevention</td>
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<td>CHC</td>
<td>Chronic hepatitis C</td>
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<td>CI</td>
<td>Confidence interval</td>
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<td>CT</td>
<td><em>Chlamydia Trachomatis</em></td>
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<td>DAA</td>
<td>Direct-acting antiviral agents</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>ECDC</td>
<td>European Centre for Disease Prevention and Control</td>
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<td>ED</td>
<td>Emergency department</td>
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<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
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<td>GIS</td>
<td>Geographic Information Systems</td>
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<tr>
<td>GP</td>
<td>General practitioner</td>
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<td>GWPR</td>
<td>Geographically Weighted Poisson Regression</td>
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<td>HAV</td>
<td>Hepatitis A virus</td>
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<td>HBV</td>
<td>Hepatitis B virus</td>
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<td>HCP</td>
<td>Health care provider</td>
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<td>HCV</td>
<td>Hepatitis C virus</td>
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<td>HEV</td>
<td>Hepatitis E virus</td>
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<td>HIV</td>
<td>Human immunodeficiency virus</td>
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<td>IDU</td>
<td>Injecting drug use</td>
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<td>MSM</td>
<td>Men who have sex with men</td>
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<td>NG</td>
<td><em>Neisseria Gonorrhoeae</em></td>
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<td>NIP</td>
<td>National Immunisation Programme</td>
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<td>OR</td>
<td>Odds ratio</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>PHS</td>
<td>Public health service</td>
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<td>PT</td>
<td>Pertussis toxin</td>
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<td>PWID</td>
<td>People who inject drugs</td>
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<td>RDS</td>
<td>Respondent Driven Sampling</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
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<tr>
<td>RIVM</td>
<td>Rijks Instituut voor Volksgezondheid en Milieu</td>
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<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SES</td>
<td>Social economic status</td>
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<td>STI</td>
<td>Sexually transmitted infection</td>
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<td>SVR</td>
<td>Sustained virological response</td>
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<td>WHO</td>
<td>World Health Organization</td>
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“Learn continually.  
There's always ‘one more thing' to learn.”

Steve Jobs
Chapter 1

General introduction
1.1 Infectious disease surveillance and control

Infectious diseases are a public health problem worldwide and demand systematic preventive control measures. Infectious disease surveillance aims to monitor the impact of vaccination programmes, identify high-risk areas and detect outbreaks, monitor case management, take timely preventive measures and guide policy [1]. Infectious disease surveillance is the routine ongoing collection, analysis and dissemination of health data. Surveillance includes the monitoring of known infectious diseases and the detection of any emerging infectious diseases that may appear [2]. Efficient and reliable surveillance and notification systems are vital for monitoring public health trends and signalling disease outbreaks [3]. They form the backbone for evidence-based decision-making processes, as well as for infectious disease public health policies [3].

Some infectious diseases are vaccine-preventable. In the Netherlands, childhood vaccinations are covered by the National Immunisation Programme which includes vaccines for diphtheria, pertussis, tetanus, polio, haemophilus influenza type b, hepatitis B, pneumococcal, mumps, measles, rubella, meningococcal and human papillomavirus. Vaccination coverage is high, with 92 to 99%, except for human papillomavirus vaccination where coverage is 58% [4]. Vaccinations for older people are routinely performed in general practice, while travellers may report to travel health clinics for vaccination. Apart from vaccination, certain public health interventions and preventive measures are key components of infectious disease control. Examples are the provision of safe blood and blood products for transfusion, infection prevention in hospitals and the management of needle stick injuries. Health education on hygiene and safe sex can limit the possible transmission of infectious diseases.

Prompt notifications of infectious diseases is important for public health services (PHS) in order to take appropriate and timely control measures and prevent further transmission of the infection. Source and contact tracing are at the centre of these interventions. The PHS identifies the original source of the infection and prevents possible transmission of the infection by tracing contacts of the patient. This may include vaccination or prophylactic use of antibiotics, advice to health care workers and other parties at risk, partner notification, hygiene measures, exclusion from work/school/day-care, monitoring exposed contacts, or in more severe cases isolation of contagious patients and quarantine for contacts.

Outbreak management is required in the case of a (re-)emerging infectious disease (threat) in the population. Surveillance followed by verification, case definition and active case-finding are the fundamentals of outbreak management. An outbreak management team is formed to coordinate control measures. Risk communication of the PHS to the general population, authorities and professionals can play an important role in reducing anxiety among the community and in giving advice on what people should do. Epidemic models and epidemiological investigation, like a case-control, cohort or cross-sectional research, might be necessary to determine the source of infection and transmission route. The choice of adequate public health response measures may depend on the outcome of such investigations.
All notifiable diseases are reported anonymously by PHS to the national Centre of Infectious Disease Control (part of the Dutch National Institute for Public Health and the Environment; RIVM) in accordance with the Dutch Public Health Act. Notification of infectious diseases to regulating authorities is the basis of regional and national infectious disease surveillance, informing guidelines and policy in public health.

1.2 Surveillance pyramid

However, the number of notified infections are likely to represent just the tip of the surveillance pyramid. The total amount of infections in the community is under-estimated at several levels, see Figure 1.

![Surveillance pyramid diagram](image)

Figure 1. Overall surveillance data pyramid including three types of under-estimation of the total amount of infections in the general population.


Starting from the bottom of the surveillance pyramid, some people in the general population are exposed to an infectious agent and some of those exposed become infected. Surveillance data are hampered by under-ascertainment as patients may not present themselves to healthcare for diagnosis, due to common mild and asymptomatic infections, low risk perception or because of care avoidance. Under-ascertainment can be estimated as the number of infections occurring in individuals not attending healthcare services for every case that attends [3]. The fact that many infections are asym-
tomatic in their early stage and thus go unnoticed, hampers the care seeking behaviour of patients and makes it difficult to assess and identify the total pool of infected individuals. For example, persons may be chronically infected with HBV or HCV infections for 20-30 years before they develop any clinical symptoms. Unless persons are tested and diagnosed, they are not aware of their disease and hidden inflammation progresses in the liver [6]. Health care avoidance is associated with serious psychological distress [7], undocumented migrants [8], uninsured homeless persons [9], preference for self-care or alternative care, dislike or distrust of doctors, fear or dislike of medical treatments, and a lack of time or money [10, 11].

General practitioners and physicians working in healthcare institutions and hospitals are the first to see, diagnose and treat a patient and play an important role as the eyes and ears of infectious disease control. In case of pertussis, infections may resemble other respiratory diseases, in particular in the early catarrhal phase when the patient is already highly contagious [12]. In adolescents and adults, the classical pertussis symptoms are often absent and therefore the case is not recognised by the healthcare provider [12-14].

In the case of a suspected infection, the healthcare provider can collect body fluids like blood, stool or saliva from the patient for a diagnostic procedure in a medical microbiological laboratory. If the laboratory confirms an infection and the disease is notifiable, the doctor or medical microbiologist reports the infection to the department of infectious disease control of the regional PHS. Primary schools and healthcare facilities, such as nursing homes, are liable to report cluster of certain syndromes, even in the absence of laboratory confirmation. Notifiable diseases are ranked within four categories (A, B1, B2 and C) according to the severity of the infection and the corresponding preventive measures. Accurate diagnosis of infectious diseases depends on both the awareness and knowledge of physicians, and the sensitivity and specificity of laboratory tests. As the diagnostic process can be quite challenging, not all patients with an infection attending healthcare will be diagnosed correctly (underdiagnosis) or notified (under-notification). Underdiagnosis refers to those cases who attend healthcare but whose infection is not diagnosed or misdiagnosed [3]. Under-notification accounts for the failure to report all positive diagnoses through the notification system [3].

1.3 Hidden infections

Estimates suggest that 40-60% of major infections are currently hidden to care as they are undiagnosed and untreated [15-17]. In this doctoral thesis, as well as in infectious disease control in general, we distinguish the following definitions in the context of hidden infections: hard-to-reach populations, risk groups, hidden key populations, vulnerable and susceptible groups at risk.

Hard-to-reach populations is a term used to describe those sub-groups of the population who are difficult to involve in research or public health programmes due to their physical and geographical lo-
cation or their social and economic situation [18]. Hidden infections might be more prevalent in hard-to-reach populations such as individuals with low socio-economic status (SES) and low health literacy. In the Netherlands, for people with low levels of education life expectancy is six years shorter on average than for people with high levels [19]. In terms of life expectancy in good self-perceived health, the difference is 19 years [19]. Differences in health status also exist between ethnic Dutch versus ethnic minority populations and are strongly associated with work and income [19]. Health literacy is a wider concept than literacy, it is the combination of cognitive and social skills which determine the motivation and ability of individuals to gain access to, understand and use information in ways which promote and maintain good health [20]. Individuals with low health literacy often experience difficulties in understanding the information provided by health care providers and finding their way in the health care system, with consequent increased morbidity and mortality [21]. Low health literacy is associated with low patient activation, low knowledge of the disease and inadequate self-management [22]. Furthermore, individuals with low health literacy consult a general practitioner or medical specialist more frequently while they are less likely to participate in preventive programs [22].

Risk groups include individuals at higher risk of getting (or being) infected. Based on epidemiological research for each infectious disease several specific risk groups are identified based on characteristics such as age, gender and risk behaviour. For example, middle-aged men and injective drug users are risk groups for hepatitis C [23, 24].

In the context of balancing benefits and costs, those individuals at risk of getting infected who do not make use of needed medical care are considered as most important to identify. These hard-to-reach risk groups are defined as hidden key population. For STI control, male sex workers form a hidden key population since they are at a very high risk of contracting STIs and HIV, hard to reach and largely invisible to regular care [25]. Identifying hidden key populations remains a challenge for improving infectious disease surveillance and control.

Overall, infectious diseases can be more severe in certain vulnerable and susceptible groups at risk, e.g. pertussis for unvaccinated young infants [26–29] and HEV for immunocompromised individuals [30–33].

Complications of infectious diseases are potentially severe. Hepatitis C virus for example can develop into progressive scarring of the liver (cirrhosis) and liver cancer (hepatocellular carcinoma). Moreover, the economic (costs) and public health (disease burden) impact of hidden infections is huge. Therefore, identifying and addressing these hidden infections could optimize the current infectious disease prevention, surveillance and control. Better insight into hidden infections allows for example more targeted and effective testing in order to eventually protect the most vulnerable and susceptible groups at risk.
1.4 Methods for identifying hidden infections

Based upon knowledge of characteristics of hard-to-reach populations and specific risk groups, several methods can be used to identify hidden key populations in public health [18]. These methods assess the total number of underestimated (hidden) infections, including under-ascertainment, underdiagnosis and under-notification. Here, we describe the following methods aimed at identifying hidden infections: seroprevalence studies, surveillance studies, network based methods, the Workbook method, capture-recapture approach and the application of geographical analyses.

**Seroprevalence studies**

Seroprevalence studies estimate the prevalence of antibodies against an infectious disease in a defined population and period of time. The prevalence of notified cases may differ from the sampled seroprevalence. The difference may serve as an indicator of the prevalence of hidden infections. In pertussis, for example, the estimated seroprevalence according to national sero-surveys is 100-fold higher than the prevalence of notified cases [34]. For Q-fever in particular, a study among blood donors concluded that the number of infected persons seems to be 10-fold higher than the number of notified cases in the area most affected by Q-fever in 2009 [35].

**Surveillance studies**

During infectious disease surveillance, data is routinely collected by health care providers, laboratories, regional and national public health services. Identifying differences between surveillance systems can reveal under-notification [36]. The surveillance pyramid, as depicted in Figure 1, unravels the underestimation of the true total amount of infections in the general population and studying this pyramid can provide insight into the number of hidden infections.

**Network based methods**

Many infectious diseases spread largely between socially connected individuals, such as household members and schoolchildren, and they often occur in clusters [37, 38]. There is growing evidence on the high potential of network based interventions. By using chain-referral methods such as Respondent Driven Sampling (RDS) and by partner notifications it is possible to reach hidden social and/or sexual networks. The basic idea behind RDS is that individuals with an infection (the indexes/seed) function as an ambassador, they recruit and motivate others in their own network to get tested (first wave), and so on [39]. The RDS methodology has been applied to several risk groups such as injecting drug users, HIV patients and Chlamydia patients [40-43] and on syndromic surveillance [44]. RDS and partner notifications are feasible, promising and highly valuable tools. These network based methods reach hidden key populations, who are currently not in care. On the other hand, stigmatization, confidentiality and privacy issues can be important barriers to successful implementations of these methods [40, 45, 46]. Additionally, the non-random recruitment of participants could bias the estimates of the hidden...
population in unknown ways [39]. Despite possible selection bias, chain-referral methods hold great promise for studying hidden populations.

**Workbook method**

The workbook method has been developed by the 'Joint United Nations program on HIV/AIDS' (UNAIDS) and the WHO, in collaboration with the UNAIDS Reference Group on Estimates, Modelling and Projections [47]. This method was developed for estimating the total HIV/AIDS prevalence in low endemic countries with concentrated epidemics [47], including both diagnosed and undiagnosed infections hidden to care. The workbook method identifies groups at high risk of becoming infected, estimating the size and the prevalence of those risk groups, and combining the groups to obtain a national estimate. In the Netherlands, the Workbook approach has been applied to HCV [15, 48]. This method is easy to use and standardises the approach to measuring surveillance in many countries which allows for estimation of global disease burden. This informs organisations in determining priorities, intervention strategies and treatment delivery. However, a lack of reliable data is one of the main obstacles for the Workbook method [47].

**Capture-recapture method**

The capture-recapture method originally comes from ecological studies estimating an animal population's size. This method is also suitable to estimate population size, coverage or sample success rate in epidemiological studies [18], e.g. for identifying hidden hepatitis C populations [24]. The method is based on at least two observations or sources of this population. The following variables have to be known in order to estimate the size of the total population: the number of individuals in the population observed in the first capture (or in the first source), the number observed in the second capture (or in the second source) and the number of individuals captured twice (observed in both sources) [49]. The primary assumption is that the population is homogeneous without any subgroups meaning each case in the population has the same catchability or probability of ascertainment. Second, for two sources at least, ascertainment of any case by the sources is independent. Third, the population should be closed/stable over the study period. Furthermore, to minimize misclassification, each case should be diagnosed accurately [50]. Considering that all assumptions must be met for the model to be valid, estimates derived from this method may not be very precise. Nonetheless, in the case of populations not well known, even a rather imprecise estimate may yield valuable information [49].

**Geographic Information Systems**

Public health interventions targeted on behavioural risk groups can be effective in identifying and addressing hidden infections. However, the use of Geographic Information Systems (GIS) and spatial epidemiological methods may provide a more feasible basis for interventions through the identification of spatial clusters of infection, so-called hotspots, as well as demographic and socio-economic determinants [51-56]. Overall, by using GIS we can identify who is at risk, and where those at risk are located.
Chapter 1

Using GIS in real-time syndromic surveillance may be a valuable tool to minimise underdiagnosis, as has been shown to be the case retrospectively for the detection of hidden Q-fever outbreaks [57]. The increasing availability of a wide range of population-based variables allows a detailed analysis of demographic and socio-economic determinants of disease risk using spatial regression models at the ecological level. This information is important to facilitate cost-effective and targeted public health interventions. Such interventions are necessary to study to what extent the clusters and the demographic and socio-economic determinants really reflect the hidden population.

1.5 Aim and outline of this thesis

This thesis includes studies on how to detect hidden infections by using GIS (Chapter 3 and 5), a surveillance study (Chapter 2) and a seroprevalence study (Chapter 7). More in-depth studies relate to the underlying factors of hidden infections (Chapter 2 and 4) and on methods to reach (Chapter 6) hidden key populations. The thesis focuses on the evaluation of the current infectious disease surveillance and control in the Netherlands and will provide recommendations for future policy and research.

The first part of this thesis addresses pertussis surveillance and control. Chapter 2 evaluates the accuracy of the Dutch pertussis surveillance and control in the areas of medical care and public healthcare. Chapter 3 assesses whether current pertussis incidence is only the result of testing. The decision making of general practitioners in pertussis diagnostics is studied in Chapter 4.

The second part of the thesis focuses on the detection of hidden hepatitis infections. Spatial distribution of hepatitis C infections is the subject of Chapter 5. Chapter 6 includes a study on the diagnostic yield of a combined public health and primary care birth cohort testing strategy to detect hidden hepatitis B and C cases. The possible sexual transmission route of hepatitis E is studied in Chapter 7.

The overall aim of this thesis is to answer the research question “How to detect hidden infections and optimize infectious disease control?”.
References


48. Koopsen, J., et al., Chronic hepatitis B and C infections in the Netherlands: estimated prev-


Part 1

Pertussis
Chapter 2

Pertussis surveillance and control: exploring variations and delays in testing, laboratory diagnostics and public health service notifications, the Netherlands, 2010 to 2013


Euro Surveill. 2017;22(28)
Abstract

Pertussis is most severe among unvaccinated infants (< 1 year of age), and still leads to several reported deaths in the Netherlands every year. In order to avoid pertussis-related infant morbidity and mortality, pertussis surveillance data are used to guide pertussis control measures. However, more insight into the accuracy of pertussis surveillance and control, and into the range of healthcare and public health-related factors that impede this are needed. We analysed a unique combination of data sources from one Dutch region of 1.1 million residents, including data from laboratory databases and local public health notifications between 2010 and 2013. This large study (n = 12,090 pertussis tests) reveals possible misdiagnoses, substantial under-notification (18%, 412/2,301 laboratory positive episodes) and a delay between patient symptoms and notification to the local public health services (median 34 days, interquartile range (IQR): 27–54). It is likely that the misdiagnoses, under-notification and overall delay in surveillance data are not unique to this area of the Netherlands, and are generalisable to other countries in Europe. In addition to preventive measures such as maternal immunisation, based on current findings, we further recommend greater adherence to testing guidelines, standardisation of test interpretation guidelines, use of automatic notification systems and earlier preventive measures.
Introduction

*Bordetella pertussis* and *Bordetella parapertussis* infections are most severe among unvaccinated infants [1-4]. Complications of the resulting disease, pertussis, include pneumonia, failure to thrive from post-tussive vomiting, seizures, secondary bacterial infection and pulmonary hypertension [2,5]. The full implementation of general pertussis vaccination in the 1950s greatly reduced its incidence in the Netherlands [6] and led to a shift from cases in children to adults [5,7-9]. Even though worldwide vaccination coverage of 86% has been achieved [10], there were around 63,000 pertussis-linked deaths in children under 5 years of age in 2012 [11]. In Europe, the highest number of cases were notified by the Netherlands in 2012 (n = 12,868), accounting for 30% of all notifications in Europe [12]. The Dutch incidence rate of symptomatic pertussis infections in 2011 was estimated to be 107 per 10,000 population [13].

The Netherlands has an extensive free of charge National Immunisation Programme (NIP) to protect all children against 12 infectious diseases, including pertussis [14]. The efficacy of pertussis vaccines has been debated because of waning immunity, incomplete protection of infants younger than 5 months of age, genetic changes in *B. pertussis* and limited duration of protection [3,15,16]. Maternal immunisation is recommended by the WHO, ECDC and by the Health Council of the Netherlands [12,17,18]. In England, maternal immunisation was found to have a vaccine-effectiveness of 91% in infants < 2–3 months of age with no side effects [19,20]. Despite a pertussis vaccination coverage in the Netherlands of 96%, increasing numbers of pertussis notifications have been observed since 1996 [15,21,22], with epidemic peaks every 2–3 years [15] and 1 to 3 pertussis-related deaths per year [14].

Pertussis is a notifiable disease in the Netherlands according to Wet Publieke Gezondheid, the law that requires notification of NIP-targeted diseases [23]. Local public health services (PHS) must be notified when: (i) patients have typical symptom(s) or (ii) patients have at least 14 days of coughing, combined with either a positive laboratory test or recent contact with a confirmed pertussis case [24]. Legally, both healthcare providers (HCPs) and laboratories are responsible for notification, but in practice, most notifications originate from the laboratories. Local PHS collect and verify all notifications, advise patients’ HCPs on vaccination and/or medical treatment of contacts, and report cases to the National Institute for Public Health and the Environment (RIVM). An overview of the guidelines and criteria for pertussis testing, diagnostics and notifications in the Netherlands is provided in Table 1.
Table 1. Pertussis testing, diagnostics and notification guidelines and criteria, the Netherlands, 2010–2013

<table>
<thead>
<tr>
<th>Actor</th>
<th>Responsibility</th>
<th>Guidelines/criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthcare provider</td>
<td>Clinical diagnostic</td>
<td>Patients with typical symptoms* or, during epidemics, patients with severe coughing who have had contact with a proven pertussis case [45].</td>
</tr>
<tr>
<td>Requests for laboratory testing</td>
<td></td>
<td>When pertussis is suspected in a patient whose family includes unvaccinated or incompletely vaccinated infants &lt; 1 year of age or a woman &gt; 34 weeks pregnant [45]. Test method for:   - Infants &lt; 1 year of age, PCR or culture   - Individuals &gt; 1 year of age and with &gt; 3 weeks of coughing, serologyb   - Individuals &gt; 1 year of age and with &lt; 3 weeks of coughing, PCR [24,40,45].</td>
</tr>
<tr>
<td>Medical treatment of index case and/or at-risk contacts</td>
<td>First confirm the clinical diagnoses of the index case by laboratory test. In a possible index case whose family includes unvaccinated or incompletely vaccinated infants &lt; 1 year of age, a woman &gt; 34 weeks pregnant or a child with severe heart or lung failure, treatment is indicated for all family members and can start before laboratory confirmation of the index case. Medical treatment outside the family only occurs after PHS advice and laboratory confirmation of the index case [24,45]. Preferably, start treatment of the index case within 3 weeks of illness onset [24,45].</td>
<td></td>
</tr>
<tr>
<td>Vaccination of at-risk contacts</td>
<td>Administer first vaccination prior to vaccination of NIP or administer vaccination to unvaccinated or incompletely vaccinated children &lt; 5 years old in the family [24,45].</td>
<td></td>
</tr>
<tr>
<td>Notification of local PHS [23]</td>
<td>Patients with typical symptom(s)* or with at least 14 days of coughing combined with either a positive laboratory test or contact within past three weeks with a confirmed pertussis case [24]. Notify within one workday [44].</td>
<td></td>
</tr>
<tr>
<td>Laboratory</td>
<td>Laboratory diagnostics</td>
<td>Interpret as positive for pertussis when detection of <em>B. pertussis</em> or <em>B. parapertussis</em> or high antibody titre in single serology* or significant increase of titre in multiple serology.</td>
</tr>
<tr>
<td>Notification of local PHS [23]</td>
<td>Patients with typical symptom(s)* or with at least 14 days of coughing combined with either a positive laboratory test or contact within past three weeks with a confirmed pertussis case [24]. Notify within one workday [44].</td>
<td></td>
</tr>
<tr>
<td>Local public health services</td>
<td>Surveillance</td>
<td>Collect notifications and clinical data from HCPs and laboratories and report it to RIVM [23]. Notify within one week [44].</td>
</tr>
<tr>
<td>Medical treatment and/or vaccination advice to the patient’s HCP</td>
<td>Provide advice on medical treatment and vaccination according to national guidelines.</td>
<td></td>
</tr>
</tbody>
</table>


* Typical symptoms include paroxysmal coughing, a whooping sound after coughing or vomiting after coughing [24].

b Single serological testing is not suitable to detect recent infection in individuals vaccinated with an acellular pertussis vaccine within the past year. Multiple serology is also recommended when the first titre is below the cut-off value specific for a pertussis infection [24].
Pertussis surveillance aims to monitor the impact of the vaccination programme, identify high-risk areas and detect outbreaks, monitor case management and take timely preventive measures [25]. However, pertussis surveillance and control are greatly hampered by, for example, under-ascertainment as individuals with mild symptoms or who are asymptomatic may not present to healthcare for diagnosis [26,27]. Clinically, the disease resembles other respiratory diseases, particularly in the early catarrhal phase when an individual is already highly contagious [26]. Also, the classical pertussis symptoms are often absent in adolescents and adults such that these cases may not be recognised by HCPs [26,28,29]. In 2006–2007, the estimated seroprevalence of pertussis infections was 100-fold higher than the reported notifications at that time [15]. Laboratory diagnostic procedures, especially interpretation of serology, are complicated because of changing cut-off value recommendations, in cases of recent immunisation and the limitations of available diagnostic tests [5,27,30]. As the pertussis diagnostic process is quite challenging, not all cases presenting to healthcare are diagnosed (underdiagnosis) or notified (undernotification) [31]. Moreover, there is debate about how to reduce the overall delay until notification to improve pertussis control [32,33].

A quantification of healthcare and public health factors that may compromise the accuracy of pertussis surveillance and quality of pertussis control in infants < 1 year of age in day-to-day practice is missing. This study aims to assess possible pertussis misdiagnosis, under-notification and delay in laboratory testing and delay in notifications to PHS.

Methods

We evaluated the possible pertussis misdiagnosis, under-notification and delay using laboratory testing data and notification data from local PHS.

Study location

This study was conducted in Limburg, the southernmost province of the Netherlands. There are 1.1 million inhabitants and this population is comparable to the rest of the Netherlands in terms of sex composition and urbanisation, although it is slightly older [34]. All six medical microbiology laboratories in this province provided test data for this study. Notification data were retrieved from the two local PHS.

Laboratory records and standardisation of results

Laboratory data from pertussis testing requested by HCPs (general practitioners (GPs) and hospital specialists) and conducted by all laboratories in the study area between 2010 and 2013 were retrieved. The data included patient data on age, type of test performed (serology IgA/IgG, PCR and culture), type of HCP (GP or specialist), date of test request, date of test result, test result and interpretation of test result. No data on sex was reported. Based on regional GP testing-behaviour questionnaires, we estimated that the laboratory data covered at least 90% of all pertussis tests in our region. A total of 12,090 tests on 10,131 individuals were performed.
Laboratory tests

The laboratories used different test assays, cut-off values and algorithms for interpreting test results, including interpretation of disease duration and patient age as the sensitivity of the diagnostic tests for pertussis depends on the age of the patient and the duration of illness [35]. Whether laboratories received clinical data to include into their algorithms depends on the HCP who requested the test. B. pertussis IgA and/or pertussis toxin IgG ELISA from Virion/Serion (Würzburg, Germany) or Virotech (Rüsselsheim, Germany) was used for serology. The sensitivity and specificity of these serological test kits varied between 68% and 89% and between 67% and 87%, respectively [36]. At one laboratory, the serological cut-off values changed on 1 September 2011. Two laboratories switched from Virotech Units (VU) to International Units (IU), one on 1 May 2011 and the other on 16 January 2012. Charcoal blood agar (Oxoid) with and without cephalexin were used for culture. Four of the laboratories used an inhouse multiplex PCR test with target gene IS481 (B. pertussis) and target gene IS1001 (B. parapertussis). The other two laboratories were unable to distinguish between B. pertussis and B. parapertussis because target gene IS1002 or target gene IS481 were used.

Serology interpretation

There is a lack of uniform laboratory guidelines on serology interpretation so different cut-off values are used by laboratories across Europe [27,37]. To correct for the different cut-off values used in our study area and to detect other inter-laboratory differences, we compared laboratory test interpretation to standardised serological test results. The conversion from VU/ml to IU/ml was calculated with the following formula:

$$\left( \frac{\text{number of VU/ml}}{33.3 - \text{number of VU/ml}} \right)^{1.15} \times 112 = \text{IU/ml}.$$  

A single high titre of IgG ≥ 62.5 IU/ml or IgG ≥ 13 VU/ml was defined as positive as these cut-off values have been shown to be sensitive and specific indicators of infection in the past year [15,38,39]. International guidelines recommend measuring IgA antibodies with intermediate IgG levels or when no second sample can be obtained [40,41]. IgA antibodies were not taken into account in our standardisation as most laboratories did not use IgA antibody results in their serology interpretation. Furthermore, measuring IgA antibodies has been proven to be less specific and sensitive [5,36]. The standardised test result was considered positive when multiple serology was applied and all serology tests were positive, and it was considered negative when all serology tests applied were negative. When multiple serology test results were inconsistent, the standardised test result was considered positive when seroconversion occurred from a negative test to a positive test result.

Notification data

Notification data were collected from the two local PHS that serve the study area. These data included date of first day of illness, notifier (laboratory/GP/hospital), date of notification to PHS, date of notification to
Statistical analyses
The laboratory data were analysed at the test-level and at the episode-level. Notably, a possible episode of pertussis disease was considered unique if the same individual was tested once or more within an 8-week interval. However, if an individual was tested twice within an interval longer than 8-weeks it was considered two possible pertussis episodes. Descriptive statistics and chi-squared tests were used to compare categorical variables such as type of HCP, laboratory, type of test and year of test.

Independent sample t-tests were performed to study differences in delay. For analyses of the notification data, we used descriptive statistics. Analyses were performed using the SPSS package version 21.0 (IBM Inc., Somers, New York, United States).

Results

Possible misdiagnosis (underdiagnosis and overdiagnosis) of pertussis
Of all HCP-requested pertussis tests done in the study region in 2010–2013 (n = 12,090), the majority (81%, n = 9,818) were requested by GPs, varying from 72% to 88% per laboratory (p < 0.001). The remaining pertussis tests were requested by hospital specialists (19%, n = 2,272). Most tests (93%, n = 11,190) were serological tests, 6% (n = 729) were PCR tests and culture was performed in 1% (n = 171). This distribution of test type differed between laboratories, ranging from, for example, 77% to 99% for serological tests (p < 0.001). Serological tests were more likely to be requested by GPs (95%, n = 9,275) compared with hospital specialists (84%, n = 1,915), p < 0.001.

In 44% (134/303) of tested infants < 1 year of age, serology was performed instead of the recommended PCR or culture (Table 2). This proportion differed by laboratory, varying from 24% to 70% (p < 0.001) and decreased over time, from 64% in 2010 to 33% in 2013 (p < 0.01). In these infants, GPs requested serology more often (65%, 52/80) than hospital specialists (37%, 82/223), p < 0.001.
Table 2. Recommended tests and performed serology pertussis testing stratified into age groups, the Netherlands, 2010–2013

<table>
<thead>
<tr>
<th>Age group</th>
<th>Recommended test [24,40,45]</th>
<th>Total number of performed tests (n = 12,090)</th>
<th>Number of performed serology tests (n = 11,190)</th>
<th>Percent of performed serology tests (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1 year</td>
<td>PCR or culture</td>
<td>303</td>
<td>134</td>
<td>44</td>
</tr>
<tr>
<td>&gt; 1 year with &gt; 3 weeks of coughing</td>
<td>Serology</td>
<td>11,787</td>
<td>11,056</td>
<td>94</td>
</tr>
<tr>
<td>&gt; 1 year with &lt; 3 weeks of coughing</td>
<td>PCR</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

In total, 10,590 possible pertussis episodes were identified between 2010 and 2013 (Figure 1). Overall, 22% (n = 2,370) of these possible episodes were interpreted by the laboratory as positive, with this varying between laboratories (15% to 28%) and over time (from 11% in 2010 to 27% in 2012) without a clear trend, p < 0.001. Serological tests had higher positivity rates (23%, 2,276/9,736) compared to PCR tests and culture with positivity rates of 12% (83/715) and 8% (11/139) respectively, p < 0.001.

Figure 1. Possible pertussis episodesa considered positive, inconclusive and negative by laboratoriesb, the Netherlands, 2010–2013 (n = 12,090)

a A possible episode of pertussis disease was considered unique if the same person was tested once or more within an 8-week interval. If an individual was tested twice within an interval longer than 8-weeks, it was considered two possible pertussis episodes.
b The laboratories used different test assays, cut-off values and algorithms for interpreting serology, PCR and culture test results as positive, inconclusive or negative.
Furthermore, in 26% (n = 2,788) of the possible episodes, no conclusive laboratory test interpretation was available. These tests were inconclusive due to missing additional serological testing (n = 1,520), missing clinical data such as the first day of illness (n = 321), the possibility of past infections or antibodies after vaccination (n = 214) and no/dubious result available (n = 733). The proportion of possible episodes with an inconclusive laboratory result varied between laboratories (0% to 60%) and increased over time (from 18% in 2010 to 35% in 2013), p < 0.001.

Of all possible pertussis episodes with an available IgG-titre (n = 8,929), 22% (n = 1,998) were positive according to the laboratory and 19% (n = 1,700) appeared positive after standardisation. This standardised positivity rate varied between laboratories (15% to 23%) and over time (9% in 2013 to 24% in 2012), p < 0.001. Of all episodes considered positive by the laboratory, 32% (n = 644) were negative after standardisation. This was due to high IgA-titres in combination with IgG-titres below 62.5 IU/ml. Of all inconclusive episodes, 9% (n = 248) were positive after standardisation. This was due to the use of a grey area in both the interpretation of IgA- and IgG-titres of some laboratories in combination with IgG-titres higher or equal to 62.5 IU/ml. Of all negative episodes, 2% (n = 98) were positive after standardisation (Table 3).

Table 3. Standardised test results for possible pertussis episodes using serology with available IgG-titres, Limburg province, the Netherlands, 2010–2013

<table>
<thead>
<tr>
<th></th>
<th>Standardised* result negative (n = 7,229)</th>
<th>Standardised* result positive (n = 1,700)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number (n)</td>
<td>Percent (%)</td>
</tr>
<tr>
<td>Laboratory interpretation of possible episodes with IgG titres (n = 8,929)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive (n = 1,998)</td>
<td>644</td>
<td>32</td>
</tr>
<tr>
<td>Inconclusive (n = 2,672)</td>
<td>2,424</td>
<td>91</td>
</tr>
<tr>
<td>Negative (n = 4,259)</td>
<td>4,161</td>
<td>98</td>
</tr>
</tbody>
</table>

* Cut-off values of IgG ≥ 62.5 IU/ml and IgG ≥ 13 VU/ml were used for standardisation. A single high titre at/above these values was defined as positive. The standardised test result was considered positive when multiple serology was applied and all serology tests were positive, and it was considered negative when all serology tests applied were negative. When multiple serology test results were inconsistent, the standardised test result was considered positive when seroconversion occurred from a negative test to a positive test result.

<sup>b</sup> p < 0.01.

<sup>c</sup> p < 0.001.
Under-notification of pertussis

Of all notifications to local PHS between 2010 and 2013 (n = 2,241), 93% (n = 2,090) were notified by a laboratory. The remaining notifications were by a GP (3%), a hospital (2%) or others (2%). Of the total number of laboratory-positive episodes of persons living in the study region (n = 2,301), 412 (18%) were not notified to the PHS. This under-notification varied between laboratories from 10% to 39%, and varied from 13% in 2011 to 59% in 2010 with no trend, p < 0.001. All notifications were evaluated and verified by the local PHS, which were only able to take timely preventive measures, such as giving advice or providing vaccination or prophylaxes to at-risk contacts, in 1% (n = 26) of all notifications.

Delay in pertussis control cascade

The median time between patients’ first day of illness and HCP request for a laboratory test, patient and HCP delay, was 28 days (interquartile range (IQR): 21–47) (Figure 2). It was longer for serological test requests (median = 29 days, IQR: 21–49) compared with PCR or cultures (median = 18 days, IQR: 13–24), p < 0.001. For infants, this delay was shorter (median = 12 days, IQR: 6–19) compared with all other ages (median = 28 days, IQR: 21–48), p < 0.05. Of all laboratory notifications, 28% (n = 571) were tested within 3 weeks. Median time from a requested laboratory test to a test result, median test delay, was 4 days (IQR: 3–7). The median test delay was longest for culture (7 days) and shortest for PCR and serology (4 days), p < 0.001. In terms of notification delay, the median time between patients’ first day of illness to local PHS notification was 34 days (IQR: 27–54). Of all laboratory notifications, 12% (n = 245) were notified to the local PHS within 3 weeks. It then took local PHS a median of 2 days (IQR: 0–5) to collect all patient and patient contacts information for a proper risk assessment and to notify RIVM. This is usually done by contacting HCPs for further essential risk information in order to decide on any necessary preventive measures. In 96% (1,538/1,601) of the notifications, it took less than 7 days for the local PHS report to the national system of RIVM.

Figure 2. Median time of Bordetella pertussis and Bordetella parapertussis infection from first day of illness to notification of the RIVM, Limburg province, the Netherlands, 2010–2013

Discussion

This study reveals possible pertussis misdiagnosis by both HCPs and laboratories, substantial under-notification of positive pertussis episodes by laboratories, and a large delay in the pertussis control cascade. All these factors negatively impact control strategies and jeopardise the effectiveness of the national pertussis surveillance system. The accuracy of pertussis surveillance is of urgent interest to all countries using notification data to guide pertussis surveillance and control. In our study, large variations in test behaviour, laboratory assays used, test interpretation and notification behaviour were observed between HCPs and between laboratories in the pertussis diagnostic process. This variation is likely to cause the accuracy of surveillance data to be different at the local level, yet which areas are more accurate than other areas is unknown. These results are also likely applicable to the other countries in Europe with similar surveillance systems considering the generalisability of misdiagnosis, under-notification and overall delay in surveillance data.

Just slightly over half of the infants were tested for pertussis using the recommended PCR or culture. Furthermore, a quarter of possible episodes in all ages lacked a conclusive laboratory test result. These results show the possibility of misdiagnosis and the complexity of the pertussis diagnostic process both for HCPs and for the laboratories. It is unknown whether the possible misdiagnoses caused underdiagnosis or overdiagnosis. The standardisation of test results using IgG-titres revealed that positivity rate differences between laboratories remained after correcting for the different cut-off values used. Laboratory differences in test interpretation, the variation in tests used and positivity rates have also been found in prior studies [5,27,30].

Our study shows that almost one fifth of all laboratory pertussis diagnoses were not notified to the local PHS. A comparable under-notification rate has been reported in Italy [42]. Administrative and logistical problems are possible contributors to this under-notification. In the laboratory that used an automatic digital notification system, there was less under-notification (10%) compared to the other laboratories, p < 0.001.

There was a considerable delay in the national surveillance data. Overall, it took a median of 34 days from first day of illness before the local PHS was notified, and only 12% of all laboratory notifications were reported within three weeks, which is comparable to other Dutch findings [32,43]. Time from the laboratory test result to local PHS notification and from local PHS to RIVM was in accordance with the guidelines [44]. However, time from the first day of illness to a laboratory test request was 28 days, while pertussis remains contagious up to 3–4 weeks after the first symptoms [5]. Clinical diagnosis and diagnosis after laboratory testing is therefore made too late to start treatment or take any necessary preventive measures. Adequate and early diagnosis followed by antibiotic treatment is particularly important as it can prevent further transmission to infants, HCPs and pregnant women [21]. For optimal effectiveness, treatment has to be started early after onset of illness as pertussis is no longer present in respiratory secretions after about 3 weeks [45]. The long patient and/or HCP delay seen in this study therefore limits early treatment and optimal pertussis control.
This study benefited from a large sample size and having complete regional data via a large database that included around 90% of laboratory tests for pertussis in one geographical area. Another strength was the availability of information on advice given or preventive measures taken by the local PHS for all notifications. Moreover, we studied data from across the pertussis control chain for both healthcare as well as public health. However, data on patient symptoms, disease awareness or healthcare-seeking behaviour were lacking. We were therefore unable to assess these patient-related factors as an explanation for patient delay. More information on the HCP’s reasons to test for pertussis would have been desirable. It would be of interest to know whether they initiated any preventive measures for close contacts since notification often comes too late for local PHS to take action. Given that date of consultation was not known, we were unable to identify how much delay was attributable to patient and HCP delay, respectively. We were also unable to estimate under-notification specifically for notifications by GPs or hospital specialists since we have no data on the number of clinical diagnoses. At last, the standardisation of laboratory results was only based on IgG antibodies and did not take any clinical data like vaccination status, duration of coughing or age into account. This meant that we were not able to estimate possible false positives or false negatives, but this limited standardisation does illustrate differences between laboratories that could lead to local differences in diagnosis or misdiagnosis.

Conclusion and recommendations

In conclusion, this study revealed several factors that prevent good pertussis control by PHS by contributing to misdiagnosis, under-notification and delay in notifications. These factors include suboptimal testing behaviour, laboratory diagnostic procedures, and notification behaviour. While the number of notifications are the current basis for pertussis surveillance, the accuracy of this indicator for disease occurrence and as a management tool is likely poor.

The accuracy of surveillance would be improved by focusing on the factors identified here. First, to reduce misdiagnosis and the variation in pertussis diagnostics, we recommend that laboratories and HCPs improve their adherence to national guidelines about when to perform which type of test and on whom. A national uniform guideline on serology cut-off values and the use of IgA and/or IgG is desirable. Testing all patients presenting with cough is not feasible as previous research has shown that only 3% of adult patients in 12 European countries presenting with acute cough in primary care had evidence of an acute pertussis infection [46]. In the Netherlands, the current pertussis incidence is largely the result of testing and more testing would not necessarily improve pertussis control [47]. Therefore, HCPs should focus more on diagnosing patients with pertussis-like symptoms who have pregnant women or infants in their proximity. Second, to reduce under-notification, laboratories and HCPs could benefit from using an automated notification system. Third, as public health is almost always too late to intervene, preventive measures should be carried out earlier in the pertussis control
chain of actions. GPs, midwives and child care workers could play a major role here. Creating awareness among these professionals and patients about taking timely preventive measures could lead to lower individual disease burden and increased pertussis care cost-effectiveness. Additional preventive measures such as the recommended maternal vaccination [18] and shortening the chain of actions would contribute to improving the surveillance system and more importantly, preventing pertussis infection, morbidity and mortality prevention in infants.

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References


Chapter 3

Is the current pertussis incidence only the result of testing? A spatial and space-time analysis of pertussis surveillance data using cluster detection methods and geographically weighted regression modelling

Boris Kauhl, Jeanne Heil, Christian J.P.A. Hoebe, Jürgen Schweikart, Thomas Krafft, Nicole H.T.M. Dukers-Muijrrers

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Abstract

Background
Despite high vaccination coverage, pertussis incidence in the Netherlands is amongst the highest in Europe with a shifting tendency towards adults and elderly. Early detection of outbreaks and preventive actions are necessary to prevent severe complications in infants. Efficient pertussis control requires additional background knowledge about the determinants of testing and possible determinants of the current pertussis incidence. Therefore, the aim of our study is to examine the possibility of locating possible pertussis outbreaks using space time cluster detection and to examine the determinants of pertussis testing and incidence using geographically weighted regression models.

Methods
We analysed laboratory registry data including all geocoded pertussis tests in the southern area of the Netherlands between 2007 and 2013. Socio-demographic and infrastructure related population data were matched to the geocoded laboratory data. The spatial scan statistic was applied to detect spatial and space-time clusters of testing, incidence and test positivity. Geographically weighted Poisson regression (GWPR) models were then constructed to model the associations between the age-specific rates of testing and incidence and possible population-based determinants.

Results
Space-time clusters for pertussis incidence overlapped with space-time clusters for testing, reflecting a strong relationship between testing and incidence, irrespective of the examined age group. Testing for pertussis itself was overall associated with lower socio-economic status, multi-person-households, proximity to primary school and availability of healthcare. The current incidence in contradiction is mainly determined by testing and is not associated with a lower socioeconomic status.

Discussion
Testing for pertussis follows to an extent the general healthcare seeking behaviour for common respiratory infections, whereas the current pertussis incidence is largely the result of testing. More testing would thus not necessarily improve pertussis control. Detecting outbreaks using space-time cluster detection is feasible but needs to adjust for the strong impact of testing on the detection of pertussis cases.
Is the current pertussis incidence only the result of testing?

Introduction

Pertussis is a highly infectious respiratory disease caused by *Bordetella Pertussis* and is especially severe in unvaccinated and incomplete vaccinated children [1]. Despite the implementation of extensive vaccination schemes, the incidence of pertussis is increasing in many countries with a shifting tendency towards adults and elderly [2-7]. In fully vaccinated children and adults with waning immunity, the symptoms are often mild and indistinguishable from other respiratory diseases [5]. The clinical diagnosis of pertussis is challenging, not only because symptoms are often unspecific, but also because co-infection with respiratory diseases complicates diagnosis [5,8,9]. Additionally, sensitivity and specificity of the applied laboratory tests are influenced by vaccination coverage, frequency of mild cases within the population, exposure to pertussis and age of the patient so that no single laboratory test can be considered as "gold standard" for confirming pertussis cases [10]. The lack of universal standards to confirm pertussis infections thus further facilitates the spread of undiagnosed infections.

This is problematic, as transmission through infected, but undiagnosed members of the same household are held responsible for most transmissions to not or incomplete vaccinated infants [11]. To further reduce transmission, several countries such as France, USA and Australia have incorporated adult booster doses in their respective vaccination schemes [12-15] and the Dutch health council recently recommended the introduction of maternal vaccination to the national vaccination program [16].

In the Netherlands, the pertussis incidence is amongst the highest in Europe and rates have increased since 1996 [17]. The underlying reasons of this increase are not fully conclusive. Several studies attribute the increase of pertussis to a waning immunity in adults [2,17] and new emerging strains of *Bordetella Pertussis* [18,19]. Other studies suggest that an increase of detected pertussis infections occurs mainly because of an increased awareness of the population and general practitioners (GPs) [20-22] and enhanced notification systems [21-23].

According to current general practitioner guidelines in the Netherlands, a clinical pertussis diagnosis is considered in patients having typical symptoms such as severe coughing who had contact with a proven pertussis case. Additional testing for pertussis is only recommended for patients in a household with an unvaccinated or incomplete vaccinated child younger than one year old and in households with a woman, which is more than 34 weeks pregnant [24]. For all other groups, testing is rather induced by the patient than the GP [25].

As pertussis is a notifiable disease in the Netherlands [26] and many other countries, the resulting surveillance data on testing and infections is used to monitor changes over space and time [27-29]. Despite previous findings that pertussis is highly heterogeneously distributed in space as well as in space-time [30,31], a substantial amount of current surveillance activities on pertussis is still restricted to a temporal analysis only [7,28,29,32], masking important regional variations and thus complicating an effective public health response.

Geographic Information Systems (GIS) and cluster detection methods both, purely spatial as
well as in time and space have proven useful to locate possible outbreaks of infectious diseases [33-35], including pertussis [30], resulting in a timely and effective response in affected areas. Such an approach might ultimately help to minimize the spread of pertussis at an early stage when the risk of transmission is highest [36].

Efficient pertussis control however, requires additional background knowledge about the determinants of pertussis testing and the determinants of the current pertussis incidence. However, personal patient information such as occurrence of infections within the same household, household composition, vaccination status and socio-demographic characteristics are mostly unavailable on an individual level due to privacy restrictions of surveillance data [37]. Detailed population information on household composition, socio-economic variables and information on available healthcare and infrastructure is in the Netherlands only available on an aggregated level such as neighbourhoods or municipalities [38,39].

In this context, spatial regression models at the ecological level have been increasingly applied in epidemiological studies of infectious diseases in recent years as regression modelling based on aggregated population data allows an analysis of possible risk factors that are unavailable on an individual level [39-41].

Geographically weighted regression modelling (GWR) is an extension of traditional global spatial regression models and measures how the association between disease risk and sociodemographic population characteristics varies over space. This approach often led to the conclusion that the key populations for certain diseases depend largely on the place of residence, resulting in more cost-effective, targeted public health interventions aimed at those groups who are most at risk in specific locations [39,42,43].

This approach has shown to be effective in revealing associated determinants of several infectious diseases such as Hepatitis C [39], HIV [44] and Japanese encephalitis [42] but has also been useful to examine determinants of treatment seeking behaviour i.e. for Malaria [45] and could thus provide a feasible basis to examine the determinants of pertussis testing and the associated determinants of the current pertussis incidence.

The aim of our article is therefore (i) to examine spatial and space-time clustering of pertussis testing, incidence and test-positivity and (ii) to model the associations between sociodemographic, healthcare and infrastructure related determinants and pertussis testing and incidence using geographically weighted regression models.

Data and methods

Ethics statement
The medical ethics committee of the Maastricht University Medical Centre (Maastricht, the Netherlands) approved the study (11-4-136) and waived the need for consent to be collected from participants.
Since retrospective data originated from standard care (in which one can opt-out for the use of their data for scientific research) and were analysed anonymously, no further informed consent for data analysis was obtained.

**Laboratory data**

Pertussis laboratory data were collected between Jan. 1st 2007 and Dec. 31st 2013 in the province Limburg, the Netherlands, comprising a population of 1,121,820 inhabitants [46]. Testing for pertussis was performed by GPs and hospitals in the area and the test-samples were then sent to the six laboratories in the region, which are capable of analysing the collected samples. The data for this study were therefore retrieved from the registries of these six laboratories in the province and included all pertussis tests requested by health care providers. In total, the data consisted of 15,429 tested persons of which 3,312 (21.5%) persons were tested positive. Positivity was either based on the test result of the PCR (5.5% of tests), culture (2.4% of tests) or serology (IgG, 92.2% of tests); for the latter the international standard cut-off value of IgG≥62.5 IU/ml or IgG≥13 VU/ml was used to be a sensitive and specific indicator of a pertussis infection in the past year [47,48]. This standardisation was made, instead of using the laboratory interpretation, because laboratories used test cut-offs that differed between the laboratories and over time. When multiple serology was applied to test a person and test results were inconsistent, the standardised test result was positive when seroconversion occurred from a negative test to a positive test result. In 8.8% (1,361) of the tested persons, standardization was not possible because IgG-titres were unavailable for the serological test. We were able to filter the laboratory result for most of these tests, but for a total of 1.0% (151) the test result remained missing.

Besides the results of the laboratory diagnosis, the available information included the four-digits postal code, sex, age and date of testing. In total 14,810 tested persons (96.0%) and 3,150 positive persons (95.1%) had valid postal codes assigned and were therefore included in our analyses.

**Outcomes**

As outcomes, we examined three different rates: (i) the proportion of tested persons per inhabitants (testing); (ii) the incidence of pertussis expressed as proportion of positive tested persons per inhabitants (incidence) and (iii) the proportion of positive to tested persons (test-positivity). Due to the different vulnerability between age groups [7], four demographic strata were used to calculate the rates outlined above: (i) children aged 0-14, (ii) adults between 15-64, (iii) persons aged 65 and older and (iv), total population. Although infants display the highest vulnerability to pertussis [1], an analysis of pertussis testing and incidence among 0-5 year olds was not advisable due to the low number of tests and infections in this age group.

**Explanatory variables**

We assessed several socio-demographic, infrastructure and healthcare-related variables for their association with the proportion of tested persons and pertussis incidence among the different age strata.
The data and map sources were available from Statistics Netherlands [49]. The data were available per neighbourhood and had to be aggregated to the four-digits postal codes to match the pertussis laboratory data. In the Netherlands, a neighbourhood is an administrative area within a municipality with a homogenous socio-economic structure [38,39,49]. Due to privacy restrictions of statistics Netherlands, data for each variable is only reported for a minimum of inhabitants or households. For example, the number of persons in a specific age group is only reported for neighbourhoods with more than 50 persons, while average income is only reported for neighbourhoods with more than 100 persons [38,39,49]. The population-weighted aggregation was therefore only based on the neighbourhoods, for which data were made available. The age stratified population data, which were used for the calculation of the proportion of tested persons and the Pertussis incidence, was only available for 2013 from the Central Bureau for statistics Netherlands. As transmission occurs mainly between members of the same households [11], testing between different demographic strata is expected to follow a comparable pattern. We therefore included testing among the other age groups as independent variables in a regression analysis for testing in a specific age group. To analyse the determinants of the incidence, we included the rate of tested persons within the same age group and the rates of infected persons in the other age groups as independent variables. This should in a later stage confirm through GWR in which areas the effect of testing and potential intra-household transmission is stronger than in other areas.

**Exploratory disease mapping**

As our study aimed to highlight geographic heterogeneity among different demographic strata and different epidemiological outcomes on a small spatial scale, the numbers in the numerator for each examined demographic strata and epidemiological outcome can be considered as fairly low. This leads to a large variance of the respective rate simply due to varying population densities of relatively arbitrary administrative boundaries. As pertussis testing and infections are highly depending on local characteristics, neighbouring areas can therefore be expected to display similar rates and abrupt changes are more likely to occur due to the effect of relatively arbitrary administrative boundaries - also referred to as the modifiable area unit Problem (MAUP) [50]. We therefore applied a local empirical Bayesian smoothing approach where the respective rates are smoothed towards a local mean. The neighbours were defined as an area sharing a common edge or boundary [51]. The analysis was carried out in GeoDa 12.0 [52]. The resulting rates were then imported in ESRI ArcGIS 10.2.

**Local cluster detection**

*Purely spatial cluster detection.* The spatial scan statistic is a local cluster test, which identifies the geographic location and statistical significance of local clusters [39,53,54]. The rationale behind a cluster analysis in our study was to detect significant local clusters of high rates within one demographic stratum and compare the location of clusters within the other demographic strata. In this study, we used two different models in SaTScan: For the proportion of tested persons and the incidence of pertussis, we used a purely spatial Poisson model [39,55]. The input data for this model consisted of...
Is the current pertussis incidence only the result of testing?

the number of tested persons / positive tested persons and the population per demographic stratum as well as the centroid coordinates of each postal code [56]. For the proportion of positive tested persons, we used a purely spatial Bernoulli model [54, 57]. For this model, the input data consisted of the number of positive tested persons, the number of negative tested persons per demographic stratum and the centroid coordinates for each postal code [56]. The spatial scan statistic then uses a circular scanning window, which is flexible in size up to a user-specified maximum or the standard setting of including up to 50% of the population inside a cluster. The scanning window gradually moves over the coordinates over the study area, evaluating all possible cluster locations and sizes. The statistical significance is evaluated by computing 999 Monte-Carlo replications [58]. In our study, we set the maximum population at risk to be included in a possible cluster not to exceed 5%. This was done since the default settings in SaTScan are more likely to produce very large clusters and therefore contain locations of low relative risk simply because of the circular scanning window [59]. The value of 5% of the maximum population at risk was based on the experience of a previous study in the area, which met our criterion of including only locations of elevated risk in a cluster [39].

Space-time cluster detection. A space-time cluster analysis was employed in this study to evaluate whether testing, incidence and test-positivity occur at the same geographical locations and in the same time periods, providing background information whether space-time clusters of pertussis incidence are correlated in space-time with testing.

The space-time cluster analysis in SaTScan is comparable to a purely spatial model, except that the scanning window may be represented as a cylinder, where the base of the cylinder represents the geographic location and the height of the cylinder represents the time component of the scanning window. The scanning window then moves over all centroid coordinates across the study area and evaluates all possible space-time clusters within the study area and study period [60]. Similar to the purely spatial cluster analysis, we used for both, proportion of tested persons and incidence a space-time Poisson model and for the proportion of positive to tested persons, a space-time Bernoulli model [61]. In this study, we used a scanning window that may contain up to 10% of the background population and up to 12 months of the study period. The calculation of purely spatial and space-time clusters was carried out using SaTScan v9.4.1.

Selection of explanatory variables. To select a meaningful set of explanatory variables for the regression analysis, we used a data-mining tool called "exploratory regression" in ESRI ArcGIS 10.2. This tool is comparable to a forward step-wise regression. In each step, one additional variable is added to the regression equation and evaluated based on following criteria in our analysis: (i) The regression coefficients are statistically significant (p < 0.05) and (ii) do not display multicollinearity (Variance Inflation Factor < 7.5) [62]. We then chose a set of statistically significant explanatory variables as suggested by the exploratory regression that delivered a plausible explanation of the respective outcome (the proportion of tested persons or the pertussis incidence in the respective age group).

Geographically weighted poisson regression. We constructed spatial regression models based on the variables suggested by the exploratory regression, which delivered a plausible explanation of the
respective outcome. Global spatial regression models are often applied to determine the strength of
the association between the dependent variable and a set of explanatory variables, but the obtained
coefficients are averaged over the whole study area [51,63]. Our study area however, consisted of 258
postal codes and the socio-demographic composition and available infrastructure varies at local level.
It is therefore unlikely that one single coefficient per explanatory variable would be a good estimator
of the strength of the association for the whole study area. We therefore favoured a geographically
weighted regression (GWR) approach over a global approach. Geographically weighted regression mod-
elling measures how the relationship between a set of explanatory variables and an epidemiological
outcome varies over space, resulting in more detailed understanding of the spatially varying key popula-
tions and local characteristics of the study area for different epidemiological outcomes [39,41,43,45,64].

The Poisson distribution among the available GWR models is most suitable for diseases, especial-
ly if observed counts of cases are low in certain areas [39,65-67]. The dependent variable was in the
analysis for testing specified as the number of tests and in the analysis for incidence as positive cases
per postal code. The offset variable was specified as the number of inhabitants per postal code. The
centroids of each postal code area were used as input coordinates. The geographically weighted Pois-
son regression (GWPR) calculates an additional global Poisson model to allow a comparison between
a global and a local approach. The GWPR uses a kernel function to fit a regression equation for each
postal code area, where the centre of the kernel is the regression point. The kernel function assigns
decreasing weights to the observations, depending on the distance (bandwidth) of the respective ob-
servation to the centre. The bandwidth of the kernel in GWPR can be either fixed or adaptive and the
shape of the kernel can follow a Gaussian or a bi-square distribution. The optimization of the band-
width in a GWPR model can be based on one of the three available criteria: (i) Akaike's Information
Criterion (AIC); (ii) Akaike's corrected Information Criterion (AICc) and (iii) Bayesian Information Criteri-
on (BIC) [63,68]. We thus evaluated all 12 possible combinations of kernel shape, bandwidth type and
bandwidth optimization method for the eight different dependent variables. The models without clus-
tered residuals were further considered and out of those, the models with the lowest AICc value and
highest adjusted R² were then chosen as the final models. The statistical significance of each coeffi-
cient per postal code was calculated using pseudo t-values [63]. The statistic behind GWPR is de-
scribed in detail elsewhere [63].

We assessed clustering of the residuals of the GWPR using the global Moran's I test in ESRI ArcGIS
10.2. The computation of GWPR was carried out in the GWR4 software [68]. The coefficients were stan-
dardized to allow a direct comparison of the strength of association among the examined explanatory
variables. To enhance visualization of the spatially varying coefficients, we used the software's "pre-
diction at non-sample points" function and calculated the predicted values for a grid of Limburg based
on a cell size of 100m x 100m. The obtained values were then interpolated using ordinary kriging in ESRI
ArcGIS 10.2.
Results

Purely spatial analysis

Testing. The spatial distribution of the proportion of tested persons among the different demographic strata displayed strong local variations and local clustering (Fig 1). The proportion of tested persons differed widely between the examined age groups and is highest in children (Table 1).

All demographic groups displayed strong local clustering. Despite the differences in the proportion of tested persons, local clustering displayed similar patterns among the different demographic strata. Local clusters were observed especially in the central and northern parts of the study area. In the southern part in contradiction, no clusters could be observed.

Table 1. Rates of testing, test positivity and population incidence

<table>
<thead>
<tr>
<th>Age</th>
<th>Tested (%)</th>
<th>Positive tested (%)</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
</tr>
<tr>
<td>0-14</td>
<td>2.39</td>
<td>1.52</td>
<td>29.77</td>
</tr>
<tr>
<td>15-64</td>
<td>1.27</td>
<td>0.75</td>
<td>18.45</td>
</tr>
<tr>
<td>&gt;65</td>
<td>0.92</td>
<td>0.93</td>
<td>18.22</td>
</tr>
<tr>
<td>All ages</td>
<td>1.36</td>
<td>0.72</td>
<td>21.27</td>
</tr>
</tbody>
</table>

SD = standard deviation.

Incidence. The incidence of pertussis varied between the different demographic groups and was also highest in children (Table 1). Overall, the clusters of the pertussis incidence followed closely the locations of the observed clusters for testing (Fig 1), reflecting a strong spatial correlation to the patterns of testing. The clusters of pertussis incidence among children partially overlapped with clusters among adults and the clusters among adults overlapped in certain areas with those for seniors.

Test-positivity. Test-positivity was again highest in children, but the positivity rate did not differ much between adults and seniors (Table 1). Test-positivity in children was the only rate that did not display any local clusters (Fig 1). Among adults, seniors and the total tested persons, only a small number of clusters were observed. Except for one cluster in the southwestern part of the study area for the total tested persons, the clusters observed for test-positivity overlapped with the clusters for testing and incidence.

Space-time analysis

Testing. In all demographic groups, space-time clustering started generally in the beginning of 2012 and lasted partially until the beginning of 2013 (Fig 2). Only in children, one cluster in 2007 and one cluster starting in 2011 could be observed. Space-time clustering for testing thus started relatively uniform across the study area in the beginning of 2012.
Figure 1. Spatial distribution of a) pertussis testing, b) incidence and c) test-positivity, 2007-2013
**Incidence.** In adults, seniors and among the total population, the space-time distribution of clusters for the pertussis incidence followed closely the space-time distribution of clusters for testing (Fig 2). The majority of clusters were observed in the beginning of 2012, lasting partially until the beginning of 2013 and were located in the same locations as space-time clusters for testing. In children however, space-time clusters were also observed in 2007 and 2009, for which no space-time clusters for testing were observed.

**Test-positivity.** The distribution of space-time clusters for positivity differed strongly from the observed clusters from testing and pertussis incidence (Fig 2). In children, a cluster observed in 2009 in the centre of the study area overlapped with a cluster for pertussis incidence. In seniors, a cluster observed in 2012 in the northwestern part overlapped with the clusters for testing and pertussis incidence. The other clusters were scattered over the entire study area and study period.

**Geographically weighted poisson regression**

**Determinants of testing.** For pertussis testing, the Gaussian kernel type using a fixed, AICc optimized bandwidth fulfilled the requirements of the residuals not displaying spatial autocorrelation among all evaluated demographic groups (Table 2). The local models generally outperformed the global models, reflecting important local differences in the associations between testing and the examined explanatory variables.

**Testing in children:** For testing in children, testing in adults was the strongest predictor, followed by a moderate association to testing in seniors. The negative association to unmarried persons reflects that parents, which have ever been married, are a determinant for testing in children. The negative association to mean property value indicates that children in deprived neighbourhoods are more likely to get tested for pertussis.

**Testing in adults:** Testing in children and seniors had the strongest impact on testing in adults. The positive association to household size indicates that adults in multi-person households are more likely to get tested. Similar to testing in children, mean property value was negatively associated. Additionally, testing in adults was associated with proximity to primary schools and GPs, but was also associated with increasing distance to pharmacies.

**Testing in seniors:** Testing in adults had the strongest impact on testing in seniors. The strength of the association to testing in children was relatively weak. Additionally, testing in seniors was negatively associated with proportion of households with high income.

**Testing in the total population:** The strongest predictor for testing among the total population was the positive association to household size, followed by the negative associations to mean property value, proximity to GPs and primary schools. The proportion of children and distance to pharmacies were overall positively associated. However, none of the predictors was significant in the entire study area. The results of the GWPR model additionally point out important local variations of the association between testing among the total population and the examined explanatory variables (Fig 3).
Figure 2. Space-time clusters of a) testing, b) incidence and c) test-positivity, 2007-2013
Table 2. Poisson regression models for pertussis testing, stratified by age

<table>
<thead>
<tr>
<th>Standardized coefficients of pertussis testing</th>
<th>0-14</th>
<th>15-64</th>
<th>&gt;65</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested 0-14 (%)</td>
<td>0.2033***</td>
<td>0.0818**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tested 15-64 (%)</td>
<td>0.2655***</td>
<td>0.3874***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tested seniors (%)</td>
<td>0.0659**</td>
<td>0.2360***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (%)</td>
<td>0.0610***</td>
<td>0.0525***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarried (%)</td>
<td>-0.0418*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One person households (%)</td>
<td>-0.0769***</td>
<td>-0.1567***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Property value (Euro)</td>
<td>-0.0576***</td>
<td>-0.0419***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HH with high income</td>
<td>-0.0750**</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. Prim. school (km)</td>
<td>-0.0354*</td>
<td>-0.0973***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. GP (km)</td>
<td>-0.1930***</td>
<td>-0.1367***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. Pharmacy (km)</td>
<td>0.0700***</td>
<td>0.0441***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AICc global</td>
<td>608</td>
<td>1151</td>
<td>523</td>
<td>2722</td>
</tr>
<tr>
<td>Deviance expl. global</td>
<td>0.35</td>
<td>0.51</td>
<td>0.40</td>
<td>0.17</td>
</tr>
<tr>
<td>AICc local</td>
<td>543</td>
<td>697</td>
<td>469</td>
<td>925</td>
</tr>
<tr>
<td>Deviance expl. local</td>
<td>0.44</td>
<td>0.83</td>
<td>0.49</td>
<td>0.81</td>
</tr>
<tr>
<td>Moran's I of residuals</td>
<td>I = 0.06; p&gt;0.05</td>
<td>I = -0.00; p&gt;0.05</td>
<td>I = 0.03; p&gt;0.05</td>
<td>I = -0.01; p&gt;0.05</td>
</tr>
</tbody>
</table>

Significance levels: * = 0.05; ** = 0.01; *** = 0.001. Only significant predictors are reported.

**Determinants of pertussis incidence.** For modelling the pertussis incidence, the Gaussian kernel type using a fixed, AIC optimized bandwidth fulfilled the requirements of the residuals not displaying spatial autocorrelation among all evaluated demographic groups (Table 3). The local models outperformed the global models, although the difference was not as pronounced as the difference between global and local models in testing.
Table 3. Poisson regression models of pertussis incidence stratified by age

<table>
<thead>
<tr>
<th>Standardized coefficients of pertussis incidence</th>
<th>0-14</th>
<th>15-64</th>
<th>&gt;65</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tested 0-14 (%)</td>
<td>0.5191***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tested 15-64 (%)</td>
<td></td>
<td>0.4425***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tested seniors (%)</td>
<td></td>
<td></td>
<td>0.5591***</td>
<td></td>
</tr>
<tr>
<td>Tested total (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.4692***</td>
</tr>
<tr>
<td>Incidence 0-14 (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.0819**</td>
</tr>
<tr>
<td>Incidence 15-64 (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.3093***</td>
</tr>
<tr>
<td>Incidence senior (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.0654**</td>
</tr>
<tr>
<td>Children (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One pers. households (%)</td>
<td></td>
<td></td>
<td></td>
<td>-0.0975***</td>
</tr>
<tr>
<td>Immigrants (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Property value (Euro)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HH with low income (%)</td>
<td></td>
<td></td>
<td></td>
<td>-0.0546**</td>
</tr>
<tr>
<td>Dist. Kinder garden (km)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. Pharmacy (km)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dist. Hospital (%)</td>
<td></td>
<td></td>
<td></td>
<td>-0.1481***</td>
</tr>
<tr>
<td>AIC global</td>
<td>273</td>
<td>285</td>
<td>275</td>
<td>375</td>
</tr>
<tr>
<td>Deviance expl. global</td>
<td>0.53</td>
<td>0.60</td>
<td>0.36</td>
<td>0.65</td>
</tr>
<tr>
<td>AIC local</td>
<td>268</td>
<td>280</td>
<td>249</td>
<td>309</td>
</tr>
<tr>
<td>Deviance expl. local</td>
<td>0.55</td>
<td>0.65</td>
<td>0.47</td>
<td>0.78</td>
</tr>
<tr>
<td>Moran's I of residuals</td>
<td>l = 0.02; p&gt;0.05</td>
<td>l = -0.00; p&gt;0.05</td>
<td>l = -0.04; p&gt;0.05</td>
<td>l = -0.01; p&gt;0.05</td>
</tr>
</tbody>
</table>

Significance levels: * = 0.05; ** = 0.01; *** = 0.001. Only significant predictors are reported.

**Incidence in children:** In children, testing was the only significant predictor for the pertussis incidence.

**Incidence in adults:** In adults, testing was by far the strongest predictor. The strength of the association to the incidence in children and seniors was comparably small. One-person households were similar to testing in adults—negatively associated. A significant association to proximity to hospitals could also be observed.

**Incidence in seniors:** Testing was also the strongest predictor in seniors, followed by the incidence in adults.

**Incidence in the total population:** Among the incidence for the total population, testing had again the strongest impact on the pertussis incidence. The strength of the negative association to households with low income and proximity to hospitals was comparatively weak. The visualized results of the GWPR model point out that testing was significant in the total study area, despite strong local differences in the strength of this association. The negative association to households with low income and proximity to hospitals was only significant in specific areas (Fig 4).
Is the current pertussis incidence only the result of testing?

Figure 3. Results of the geographically weighted Poisson regression of pertussis testing among the total population

Figure 4. Results of the geographically weighted Poisson regression of pertussis incidence among the total population
Discussion

The main findings of this study are: (i) The determinants of pertussis testing reflect to a certain extent the healthcare seeking behaviour of the general population for common respiratory infections; (ii) the current pertussis incidence is mainly determined by testing and (iii) outbreak detection for pertussis is feasible using surveillance data but needs to adjust for the strong impact of testing.

Determinants of pertussis testing

Pertussis testing follows to an extent the healthcare seeking behaviour of the general population for common respiratory infections. This can be seen by the associations of proximity to GPs and distance to pharmacies as both compliment each other. Especially in areas where distance to GPs was negatively associated, distance to pharmacies was positively associated. Self-medication for common respiratory infections resulting in coughing is typical in countries where over the counter medication is available [69]. Our results thus could indicate that in areas, where a pharmacy is easily accessible, self-medication for coughing is the first option to choose, whereas in areas where a physician is close, patients with symptoms related to coughing are more likely to consult a GP [70]. As a result, testing for pertussis is to an extent related to treatment-seeking behaviour for common respiratory infections.

The association to proximity to primary schools in certain areas indicates potentially an increased awareness of pertussis vulnerability among parents and GPs for children enrolled in primary schools [71]. An overall positive association of pertussis testing among the general population to the proportion of children within the study area was expected as the incidence of pertussis in children is generally higher than in other age groups [72]. The negative association to unmarried persons and mean property value both display a comparable spatial pattern. This could indicate that in families, where the parents have ever been married and are located in a more deprived area are tested more frequently for pertussis, at least in the areas where both associations were significant. Previous studies noted a lower participation rate in childhood immunization schemes among persons with a lower socio-economic status [73]. These associations could therefore be seen as an indicator for an increased awareness of a higher vulnerability to pertussis among this population group, although it has to be noted that the vaccination coverage in the Netherlands is generally very high with approximately 95% [74]. Additionally, higher healthcare use is typical for persons living in deprived areas [75].

When analysing the determinants of pertussis testing in specific age groups, we found that testing in the adjacent age group had the strongest impact on testing in the examined age group. This strong association between the examined age groups corresponds well to previous studies suggesting that intra-household transmission is the most likely route of infection [11,76] and thus logically leads to testing among the same households.
Determinants of pertussis incidence

The spatial and space-time distribution of clusters for pertussis incidence followed closely the spatial and space-time distribution of pertussis testing, reflecting a strong spatial and space-time correlation between testing and incidence.

We found evidence that space-time clustering of testing for pertussis and the pertussis incidence itself increased across the total study area in the first quarter of 2012, irrespective of the examined demographic group. An increase of diagnosed pertussis cases between 2011 and 2012 has also been noted by the US [77], Great Britain [78] and Spain [79]. When we searched for newspaper articles related to pertussis in the Netherlands within the NexisLexis database (www.nexislexis.com), we found that the number of newspaper articles related to pertussis increased sharply in 2012 as compared to the previous years.

Other studies also suggest a “positive feedback loop” where an increase of diagnosed pertussis cases subsequently causes an increase in testing, which in turn results in an increase of diagnosed pertussis cases again [80,81]. In our study area, the strong increase of testing and pertussis incidence in 2012 could be the result of a combination of several factors; an increased awareness of GPs due to the noted increase in other countries [77-79], stronger media attention and the noted positive feedback loop [80,81]. The effect of the positive feedback loop is indicated by the fact that space-time clusters for testing and incidence occurred mostly in the same areas and time periods across all evaluated age groups. These findings highlight the importance of monitoring changes in pertussis testing in addition to monitoring the pertussis incidence.

The GWPR analyses confirmed what became already apparent during the spatial and space-time analyses. Regardless of age group, testing was the most important predictor of the pertussis incidence. The second most important predictor for the pertussis incidence in a specific age group was the incidence of pertussis in the adjacent age groups. As our analysis is based on aggregated data, we see these associations as an indicator for intra-household transmission [11,76].

In children, testing was the only predictor for pertussis. No other variables were found to be significant in a regression analysis. Thus, the current incidence among the most vulnerable group does not exhibit any socio-economic associations and can be explained by testing only. In adults, we found an additional association to proximity to hospitals. Increased exposure to pertussis and outbreaks among persons employed in hospitals has been previously noted in several countries such as the US [82] and France [83]. The results of our analysis for adults thus indicate a possible exposure to pertussis in our study area. The positive association to household-size indicates, that for adults living in multi-person households, intra-household transmission might be an important risk factor as well, despite the findings of previous studies that intra-household transmission occurs mainly from parents to children [11,76].

The results of the GWPR model for the pertussis incidence among the total population confirm that testing is the most important predictor for pertussis, despite local differences in the strength of this association. The negative association to households with low income in a small part of the study area...
was the only significant variable related to socio-economic status. An overall assumption that pertussis is related to specific socio-economic characteristics is therefore not possible. Similar to adults, an association to proximity to hospitals was observed in a small part of the study area. However, further research on an individual level would be necessary to confirm whether an increased pertussis exposure in hospitals exists in this area.

**Outbreak detection using space-time cluster detection**

Our results clearly demonstrate that space-time cluster detection is feasible using surveillance data and the methods described in this study. However, in light of the similar increase in testing and incidence in 2012 and its potential association to an increased awareness of the population and GPs, it is necessary to adjust for the impact of testing. This is reflected by the fact that we mostly detected space-time clusters for pertussis incidence in 2012, where the majority of clusters overlap with those for testing. We found only in children clusters in earlier years in 2007-2009, which did not overlap with clusters for testing. It is clear that the incidence of pertussis has to be strongly correlated to testing, no matter which algorithm is used. Logically, pertussis incidence is not suitable in our study area to detect outbreaks. By using test-positivity as indicator however, we could locate space-time clusters within the whole study area and study period, which were not the results of testing only. Test-positivity as indicator is to a certain extent adjusted for testing in a space-time analysis as a similar increase in positive cases and tested persons would not increase the ratio of positive cases to tested persons in the three dimensional cylindrical scanning window used by the spatial scan statistic. Our results clearly demonstrate that test-positivity could be a better indicator for locating outbreaks, which are not the results of testing only. This conclusion is supported by previous studies, which evaluated the temporal correlation of pertussis incidence to testing behaviour over time [80,81].

When surveillance data-as in our study are used to locate outbreaks, a prospective space time cluster analysis using daily updated surveillance data would allow a detection of recent outbreaks in an automated surveillance system as early as possible. Prospective space-time cluster detection has been widely applied in syndromic surveillance [84,85] and an automated, space-time analysis could be implemented for pertussis as well [86].

**Implications for pertussis surveillance**

The surveillance data for pertussis in our study were collected by the respective local laboratories and had to merged manually for our study area to allow a retrospective analysis for a whole province. Surveillance of pertussis would thus greatly benefit from an automated near real-time data transfer of each respective laboratory to a centralized institution to allow a detailed, prospective analysis of possible disease outbreaks as early as possible. However, such an approach should focus on a day-wise analysis rather than a month-wise analysis as employed in this study to capture possible outbreaks as precisely as possible [35].
Strengths and limitations of this study

Strengths. First, a major strength of this study is that the design of this study can be repeated for the whole country to confirm whether testing is also in the whole of the Netherlands the most important predictor of pertussis.

Second, the majority of studies on pertussis focus on a purely temporal analysis [7,28,29,32]. Our approach of analysing the spatial and space-time distribution of pertussis therefore provides a novel level of detail as our approach of detecting clusters in space as well as in space-time could allow a more focused detection of outbreaks, resulting in a more timely and cost-effective response.

Third, we analysed the spatial distribution of pertussis testing and incidence at the smallest possible spatial scale, for which surveillance data and population data can be combined without violating privacy restrictions of surveillance data. The four-digit postal code areas are very suitable for small-scale spatial-epidemiological analyses in the Netherlands [38] and thus allow a very detailed analysis of the determinants of pertussis testing and incidence.

Fourth, the use of GWPR allowed us to examine the spatially varying associations between the evaluated outcomes (testing and incidence) and the examined predictor variables. We could therefore clearly see in which areas several predictors such as distance to GPs, pharmacies and hospitals were significant. Our results can therefore be used to allow more targeted investigations for example if working in hospitals in the areas outlined in our study comprises an exposure factor on an individual level as well.

Fifth, our study highlighted that the detection of outbreaks using space-time cluster detection is feasible, when using test-positivity as indicator.

Limitations. First, we used the international standard IgG cut-off value of 62.5 to consider a diagnosed pertussis infection. As a consequence, the incidence in our study does not necessarily reflect the notification data, which is based on laboratory interpretation.

Second, our analysis was based on aggregated data. The associations detected in our study may not necessarily reflect determinants of pertussis testing and incidence on an individual level. Given the current privacy protection of surveillance data [37], the ecological analysis employed here allowed us to analyse potential determinants that are unavailable on an individual level. Therefore, further research is necessary to confirm whether the associations captured in the ecological analysis really reflect associations on an individual level as well.

Third, we may have missed associations simply because several variables were not available within the data of Statistics Netherlands such as educational level. Lower educational level has been shown to be an important risk factor for poorer health outcomes [87] and could thus also be a possible risk factor for pertussis as well.

Fourth, the GWR4 software currently allows only a purely spatial geographically weighted regression but does not account for space-time differences [63]. Given the strong temporal component of Pertussis testing and infections, a geographically weighted temporal regression might yield more de-
tailed results. However, given the mathematical complexity of such a model [88], this approach could not be implemented in this study.

Fifth, we could not account for the role of vaccinations in our study, as this information is not available in the examined surveillance data. Potentially, data on vaccination among children and booster doses among adults could have a strong impact on the serological test results presented here and could be of additional value for future analyses.

Sixth, we could not verify whether the space-time clusters detected for pertussis incidence or test-positivity capture true outbreaks. However, we think it is reasonable to assume that space-time clusters for the pertussis incidence, which are the results of testing only, are not of interest for the detection of outbreaks. Ultimately, verifying the detected space-time clusters with known outbreaks should be discussed with public health officials residing in the area.

Seventh, we performed a month-wise space-time cluster analysis because a day-wise analysis did consume considerable computation time [35]. Our results are therefore not as temporally precise as it would be technically possible with the surveillance data of our study.

Eight, it would be useful to compare both approaches to detect outbreaks—purely temporal algorithms with the results of the space-time cluster analysis to evaluate (i) which method allows a more focused outbreak detection and (ii), to evaluate whether the purely temporal algorithms or the space-time cluster detection delivers fewer false alarms. However, such a comparison was beyond the scope of this paper.

Conclusion

We found empirical evidence that testing for pertussis and the current pertussis incidence in our study area increased similar to other countries in 2012. Testing was higher in deprived areas and was also associated with proximity to primary schools and availability of healthcare. Testing for pertussis thus reflects to an extent the overall healthcare seeking behaviour for common respiratory infections. The pertussis incidence in our study area is largely the result of testing and does not display a specific socio-economic association. The main population at risk for pertussis remains therefore still unknown. Given the strong association of detected pertussis cases to testing, it is questionable whether more testing would enhance pertussis control.

The detection of outbreaks using space-time cluster detection is feasible and could help to facilitate an early and appropriate public health response. However, this approach has to be adjusted for the strong dependency to testing and is probably most efficient when using test positivity as indicator.
Is the current pertussis incidence only the result of testing?

Acknowledgments

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Is the current pertussis incidence only the result of testing?


62. ESRI Interpreting Exploratory Regression results. ESRI.


78. Service NH (2012) Sharp rise in whooping cough cases. NHS.


Chapter 4

Reasons for testing and non-testing for pertussis are not well known


Fam Pract 2018 (submitted)
Dear editor,

Pertussis infections are underdiagnosed and underreported. In Europe, the highest number of cases were notified by the Netherlands in 2012 (n=12,868), representing 30% of all notifications in Europe [1] and 70% of all national notifications [2]. Pertussis infections can develop severely in unimmunised infants with the highest rates of complications and death [3]. These young infants can be protected by preventive measures in their close contacts. However, pertussis notifications are often reported too late for public health authorities to take any preventive measures [4, 5]. We showed that the median time from the onset of symptoms to the notification of the public health authorities in the Netherlands was 34 days [4]. Of all healthcare providers, general practitioners (GPs) request the majority (81%) of diagnostic test for pertussis [4]. It was shown that a substantial number of GPs do not utilise pertussis testing at all and may not be able to recognise the clinical symptoms in adolescents [6]. The clinical diagnosis of pertussis is difficult because of often unspecific symptoms as well as co-infection with respiratory diseases. Currently, it is largely unknown why GPs decide to test specifically for pertussis or refrain from doing so, and whether they base these decisions on clinical symptoms of patients or their proximity to vulnerable groups at risk. As many cases remain undetected at present, unvaccinated or incompletely vaccinated infants <1 year of age, women >34 weeks pregnant and children with severe heart or lung failure cannot be adequately protected. To improve pertussis surveillance and control, we evaluated GP-reported diagnostic practices as well as their reasons for (not) testing and intentions towards pertussis testing using an online standardised questionnaire. In contrast to national guidelines, our results suggest that GPs test mainly based on clinical symptoms rather than based on protecting vulnerable groups at risk.

Study setting

This pilot study was conducted in Limburg, the southernmost province of the Netherlands. Its population of 1.1 million is comparable to the rest of the Netherlands in terms of sex composition and urbanisation, although it is slightly older [7]. According to the national guidelines, pertussis testing is recommended when pertussis is suspected in patients living with infants aged under one year old who are not or not completely vaccinated, with women who are more than 34 weeks pregnant or with children who have severe heart or lung failure in the household [8]. The Netherlands provides an extensive National Immunisation Programme (NIP) free of charge to protect all children against 12 infectious diseases, including pertussis [3]. The introduction of general pertussis vaccination in the 1950s has tremendously reduced its incidence [9]. Currently, the efficacy of pertussis vaccines has been debated because of waning immunity, incomplete protection of infants younger than 5 months of age, genetic changes in Bordetella pertussis and the limited duration of protection [10-12]. Maternal immunisation is recommended by the WHO, the ECDC and the Health Council of the Netherlands [1, 13, 14], and im-
munisation will be offered to pregnant women from 2019 onwards in the Netherlands. As yet, maternal immunisation is not part of routine care or guidelines in the Netherlands.

General practitioner questionnaire

All GPs in the study area (n=594) were invited by email to complete an online standardised questionnaire, in which they were asked to reflect on their clinical diagnosis and diagnostic testing of pertussis patients in the year 2013. A reminder email was sent to non-responding GPs after three weeks. We asked the GPs about their demographics, number of clinical diagnoses (based on clinical history), number of laboratory diagnoses (based on medical microbiology laboratory testing), reports of additional laboratory diagnostic testing after a clinical diagnosis and total patients tested during one year. We also provided a list of reasons for (not) testing for pertussis. We measured the intention to test by presenting GPs with eight clinical vignettes on a five-point scale. These clinical pertussis vignettes were grouped into three different types on the basis of the national GP guideline [8]: one typical pertussis case vignette, four symptom-based vignettes and three vignettes of vulnerable groups at risk (see Table 1). Of the 594 invited GPs, 122 (21%) completed the questionnaire. Respondents were on average 48 years old (SD=9.4), of which most were male (61%), worked at a practice with 3,336 patients on average (SD=1712.1), had on average 18 years of clinical experience (SD=9.7) and 41% worked fulltime. In comparison, Dutch GPs are on average 49 years old, of which 55% are male and 40% work fulltime [15]. GPs sent their pertussis samples mainly to the six medical microbiology laboratories in the study region, while only a minority of 7% (9/122) sent their tests primarily to laboratories outside the region.

GP reported pertussis diagnostic testing

GPs annually diagnosed 6.1 (SD=7.0, range 0–40) pertussis cases on average and 84% (n=103) of the GPs had at least one diagnosed case of pertussis in that year. In case of a clinical pertussis diagnosis (n=93), 88% (n=82) of GPs indicated that they requested a confirmatory laboratory test. Of all GPs, 14% (n=17) did not perform any pertussis diagnostic testing during the study year, while 39% (n=47) of the GPs requested one to four tests and 48% (n=58) of the GPs requested five or more tests (mean=5.9, SD=6.5, range=0–30). GP characteristics were similar for the groups of testing and nontesting general practitioners.

General practitioners’ reported reasons for testing, reasons for not testing and intention to test for pertussis can be seen in Table 1. When asked about their considerations to perform diagnostic testing for pertussis, the most frequently mentioned reasons to test were to confirm the clinical pertussis diagnosis (70%), on patients’ request (57%), to rule out pertussis (51%), to give more information on the duration of symptoms such as coughing (50%) or to start preventive measures (43%). Only 7% of the
GPs tested in order to enable treatment to start. The most important reported reason for not testing was that GPs considered the diagnostic test result not to influence the treatment (70%; see Table 1).

The clinical vignette in which GPs were most likely to perform diagnostic testing for pertussis was the typical pertussis case vignette presented (a patient with two weeks of persistent coughing who had direct contact with a person diagnosed with pertussis; 64%). GPs reported that they were more likely to perform laboratory testing for pertussis in the symptom-based vignettes than in the vignettes of vulnerable groups at risk. Frequent testers (GPs performing five or more laboratory pertussis tests annually) were more likely to test on the basis of vulnerable groups at risk (mean=0.1, SD=0.9) compared to non-frequent testers (mean=-0.3, SD=0.8, p=0.006).

Limitations

As no medical records were available for research purposes, we collected self-reported data for this pilot study. The use of these self-reported data may introduce recall bias, whereas the numbers depicted in this study are estimates rather than exact figures. In general, the use of vignettes can influence the construct-, internal and external validity of study results [16]. Whether the reported reasons and intention to test for pertussis accurately reflect GPs’ actual clinical practice is not completely known from our study. However, studying this in an observational study or self-reported study without clinical vignettes will have ethical, practical and other scientific limitations [16]. Moreover, our study may be prone to selection bias due to the low response rate of 21%. This could influence the generalizability of our results. Yet, the response rate was only slightly lower than other questionnaire studies in GPs [17, 18] and the characteristics of responding GPs were comparable to the demographic characteristics of Dutch GPs in general [15].

Implications for practice

Our findings suggest that GPs mostly decide whether or not to test for pertussis based on clinical symptoms rather than on vulnerable groups at risk, GPs reasons for (not) testing for pertussis confirm this symptom-based testing. In conclusion, we recommend to focus pertussis testing practices of GPs more on vulnerable groups at risk instead of symptom-based testing on patients’ request, as advised in the Dutch national guidelines. GPs need to be aware that it is recommended to consider testing in families with incompletely vaccinated infants <1 year of age, women >34 weeks pregnant and children with severe comorbidities such as heart or lung failure. Therefore, they are advised to check whether patients with symptoms suggestive of pertussis have vulnerable groups at risk in their household.
### Table 1. General practitioners’ reported reasons for testing, reasons for not testing and intention to test for pertussis, the Netherlands, 2013 (N=122)

<table>
<thead>
<tr>
<th>Reasons for testing</th>
<th>% (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>To confirm the clinical pertussis diagnosis</td>
<td>70 (86)</td>
</tr>
<tr>
<td>On patients’ request</td>
<td>57 (70)</td>
</tr>
<tr>
<td>To rule out pertussis</td>
<td>51 (62)</td>
</tr>
<tr>
<td>To give more information on the duration of symptoms</td>
<td>50 (61)</td>
</tr>
<tr>
<td>To start preventive measures</td>
<td>43 (53)</td>
</tr>
<tr>
<td>To start treatment</td>
<td>7 (8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reasons for not testing</th>
<th>% (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The diagnostic test result does not influence treatment</td>
<td>70 (86)</td>
</tr>
<tr>
<td>No direct contact with risk groups (hence no need for treatment)</td>
<td>52 (64)</td>
</tr>
<tr>
<td>Clear diagnosis due to direct link to a confirmed pertussis case</td>
<td>41 (50)</td>
</tr>
<tr>
<td>Clinical assessment alone is sufficient to diagnose pertussis</td>
<td>33 (40)</td>
</tr>
<tr>
<td>Costs for patients</td>
<td>28 (34)</td>
</tr>
<tr>
<td>Treatment is already started</td>
<td>25 (31)</td>
</tr>
<tr>
<td>Collecting blood samples in children is too stressful</td>
<td>25 (31)</td>
</tr>
<tr>
<td>Child is fully vaccinated</td>
<td>17 (21)</td>
</tr>
<tr>
<td>Time to receive test result</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Referral to specialist</td>
<td>16 (19)</td>
</tr>
</tbody>
</table>

### Intention to test*

<table>
<thead>
<tr>
<th>Intention to testa</th>
<th>mean (SD)</th>
<th>% (n) likely or would definitely test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical pertussis case vignette:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A patient with two weeks of persistent coughing who had direct contact with a person diagnosed with pertussis</td>
<td>0.7 (1.1)</td>
<td>64 (78)</td>
</tr>
<tr>
<td>Symptom based vignettes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient with a cough and a ‘whooping’ inhalation</td>
<td>0.6 (1.1)</td>
<td>57 (70)</td>
</tr>
<tr>
<td>Patient with frequent expiratory coughing</td>
<td>0.6 (1.0)</td>
<td>53 (65)</td>
</tr>
<tr>
<td>Patient who vomits after coughing</td>
<td>0.5 (1.1)</td>
<td>49 (60)</td>
</tr>
<tr>
<td>Patient with a persistent cough of more than six weeks without other symptoms</td>
<td>0.3 (1.0)</td>
<td>46 (56)</td>
</tr>
<tr>
<td>Vulnerable groups at risk vignettes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infants of 3 months to 1 year old with two weeks of persistent coughing</td>
<td>-0.1 (0.9)</td>
<td>25 (30)</td>
</tr>
<tr>
<td>A pregnant woman (third trimester) with two weeks of persistent coughing</td>
<td>-0.1 (1.0)</td>
<td>29 (35)</td>
</tr>
<tr>
<td>An adult with two weeks of persistent coughing who has a baby of two months old</td>
<td>-0.2 (1.1)</td>
<td>26 (32)</td>
</tr>
</tbody>
</table>

*a Likelihood to test case ranging from -2=definitely not, -1=probably not, 0=possibly, 1=likely, 2 =definitely.
Acknowledgements

We wish to thank Vivian Claessen for her input in the development of the questionnaire. This work was supported by the National Institute for Public Health and the Environment – Centre for Infectious Disease Control (RIVM-CId), the Netherlands (Grant number: 3910007379).
Reasons for testing and non-testing for pertussis

References

esten in de huisartspraktijk: huidig gebruik en behoefte aan testen in de toekomst.

Part 2

Hepatitis
Chapter 5

The Spatial Distribution of Hepatitis C Virus Infections and Associated Determinants—An Application of a Geographically Weighted Poisson Regression for Evidence-Based Screening Interventions in Hotspots

Boris Kauhl, Jeanne Heil, Christian J.P.A. Hoebe, Jürgen Schweikart, Thomas Krafft, Nicole H.T.M. Dukers-Muijres

*PloS one 2015;10(9)*
Abstract

Background
Hepatitis C Virus (HCV) infections are a major cause for liver diseases. A large proportion of these infections remain hidden to care due to its mostly asymptomatic nature. Population-based screening and screening targeted on behavioural risk groups had not proven to be effective in revealing these hidden infections. Therefore, more practically applicable approaches to target screenings are necessary. Geographic Information Systems (GIS) and spatial epidemiological methods may provide a more feasible basis for screening interventions through the identification of hotspots as well as demographic and socio-economic determinants.

Methods
Analysed data included all HCV tests (n = 23,800) performed in the southern area of the Netherlands between 2002–2008. HCV positivity was defined as a positive immunoblot or polymerase chain reaction test. Population data were matched to the geocoded HCV test data. The spatial scan statistic was applied to detect areas with elevated HCV risk. We applied global regression models to determine associations between population-based determinants and HCV risk. Geographically weighted Poisson regression models were then constructed to determine local differences of the association between HCV risk and population-based determinants.

Results
HCV prevalence varied geographically and clustered in urban areas. The main population at risk were middle-aged males, non-western immigrants and divorced persons. Socio-economic determinants consisted of one-person households, persons with low income and mean property value. However, the association between HCV risk and demographic as well as socio-economic determinants displayed strong regional and intra-urban differences.

Discussion
The detection of local hotspots in our study may serve as a basis for prioritization of areas for future targeted interventions. Demographic and socio-economic determinants associated with HCV risk show regional differences underlining that a one-size-fits-all approach even within small geographic areas may not be appropriate. Future screening interventions need to consider the spatially varying association between HCV risk and associated demographic and socio-economic determinants.
Introduction

Hepatitis C virus (HCV) infections are a major cause of liver diseases and are the leading cause for liver cirrhosis worldwide [1]. The World Health Organization estimates that 123 million people globally are infected with HCV [2]. A major challenge for public health response to HCV is its mostly asymptomatic nature and therefore the limited number of HCV positive individuals aware of their HCV status. Infected, but undiagnosed persons are an important source for further transmission [3]. Several studies estimated the proportion of asymptomatic infections to account for 70% [4,5] to 90% [6] of acute infections, leading to only a small proportion of infected individuals seeking medical attention for symptoms related to HCV infection [7]. It is estimated that less than one-third of HCV infected individuals are aware of their HCV status [8–10]. Many infections are either undetected or are detected at a late stage. Highly effective therapeutic options for HCV are becoming available, [11,12] but logically only to persons, who’s HCV infection is diagnosed.

To provide an opportunity for treatment of HCV positive persons, which are yet undiagnosed and therefore currently hidden to care, preventive screening is necessary.

The HCV prevalence and its associated risk factors varies considerable between countries [13,14]. Past interventions focused on the population in general were not very cost-effective, especially in countries where the overall HCV prevalence is low. In the Netherlands, the HCV prevalence in the Dutch adult population is estimated to be relatively low with 0.2%, although estimates vary between 0.1 and 0.4%, depending largely on the study design and population studied [15,16]. A meta analysis on the effectiveness of screening interventions suggests that for low HCV prevalence populations, pre-screening selection criteria should be used to increase efficiency [17]. The World Health Organisation (WHO) recommends in its new guidelines to offer HCV tests to people with high risk behaviour and to people from high risk populations [18]. These target populations include transmission risk groups such as injecting drug users (IDU) [5,10,11], blood transfusion recipients [3], surgery and dialysis patients [13], professionals in patient care [5], immigrants from endemic countries [13], persons with low socio-economic status [5] and HIV positive men who have sex with men (MSM) [3]. However, screening approaches to target these risk groups have not been shown to be effective in revealing the totality of hidden cases as the identification of people who belong to such risk groups in the first place appeared to be quite challenging. Furthermore, in the Netherlands it had been shown that a substantial part (25%) of all HCV infections is not attributable to any of the aforementioned risk groups and is therefore not included in screening interventions targeted at risk groups [16]. Although the prevalence of HCV in the US is higher with an estimated 2% [19], the Centre for Disease Control (CDC) similar to the WHO advises screening of persons in risk groups (IDU, blood transfusion or organ transplant recipients before July 1992, health care personnel with history of exposure and born to an HCV-positive mother) [20]. However, these criteria appeared also in the US difficult to include in practical screening interventions [10]. As a result, future screening interventions need to find characteristics of HCV that are more practically applicable than the risk groups and behavioural factors outlined above.
Other relevant factors than behavioural and demographic risk factors associated with HCV are socio-economic characteristics. As for many infectious diseases, including HCV, lower socio-economic status tends to be associated with higher prevalence [1,13,21,22]. The identification of socio-economic determinants provides a more practically applicable basis for screening interventions [10], as population characteristics are typically available within population data [23]. The application of Geographic Information Systems (GIS) is essential to display the spatial heterogeneity of disease risk and to quantify the impact of socio-economic determinants on the incidence of infectious diseases [22,24].

Exploratory disease mapping and local cluster tests have shown to help identifying areas with statistically significant high risks (often referred to as hotspots or clusters) for prioritizing future interventions for Hepatitis C in the mainland of China [25] as well as many other infectious diseases including HIV [26], *Chlamydia trachomatis* and *Neisseria gonorrhoea* [27].

The increasing availability of a wide range of population-based variables allows a detailed analysis of demographic and socio-economic determinants of disease risk using spatial regression models at the ecological level [24,28,29].

With respect to HCV, it has been shown that not only prevalence varies between and within countries, but also the association between risk factors and HCV prevalence [13], highlighting the necessity to account for local variation in spatial regression models for HCV.

In settings where strong local variation of the association between disease risk and possible determinants can be expected, geographically weighted Poisson regression models (GWPR) have proven to be very effective to measure the spatially varying association between possible determinants and disease risk. This in turn, often led to the conclusion that the determinants of a specific disease depend largely where infected populations live, allowing public health preventions to be targeted directly at those population groups, that are most at risk in a specific location [30–32].

The aim of this paper is therefore to (i) determine hotspots for future screening interventions using the spatial scan statistic and (ii) to assess demographic and socio-economic determinants of HCV risk within these hotspots using GWPR to facilitate targeted, evidence-based screening interventions aimed directly at risk-groups.

Data and Methods

**Ethics Statement**

The medical ethics committee of the Maastricht University Medical Centre (Maastricht, the Netherlands) approved the study (11-4-136) and waived the need for consent to be collected from participants. Since retrospective data originated from standard care (in which one can opt-out for the use of their data for scientific research) and were analysed anonymously, no further informed consent for data analysis was obtained.
Dependent Variable
The dependent variable consisted of the HCV diagnoses that were made in the southern part of the province Limburg, the Netherlands between January 1st, 2002 and December 31st, 2008, comprising an adult population of 500,955 in 2008 [10,33]. The diagnoses were retrieved from HCV test data that were provided by three hospital laboratories, which perform tests on HCV upon request of nearly all care providers serving the area. In total 23,800 HCV tests were conducted of which 823 unique patients were tested positive. According to screening procedures in the Netherlands, HCV antibodies were detected with an ELISA. Confirmation was performed with an immunoblot and/or polymerase chain reaction (PCR). When an acute infection was suspected or when the patient was HIV positive or on haemodialysis, only PCR was used for screening. In the current study, we defined a positive confirmation test or PCR as a positive case. Of these 823 unique positive individuals, 781 had valid postal codes assigned and were included in the analysis. Next to postal code and HCV test result, the laboratory dataset included sex and age [10].

Explanatory Variables
We assessed several demographic and socio-economic variables for their association with HCV risk. The data for these variables were downloaded from the Central Bureau for Statistics Netherlands. In this study, we used data and map sources from the Statline database 2009 [33] (Table 1). The data were available on neighbourhood level and had to be matched to the four-digits postal codes of the HCV data. A neighbourhood is a part of a municipality with a homogenous socio-economic structure [33,34]. Due to privacy restrictions, socio-economic data on neighbourhood level is only available for neighbourhoods with more than 50 persons, 200 persons, 10 households and 70 households, depending on the respective variable [33]. We therefore aggregated to the four-digits postal codes based on those neighbourhoods, for which socio-economic data was made available.

Table 1. Explanatory variables
<table>
<thead>
<tr>
<th>Variable</th>
<th>Average</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Married (%)</td>
<td>44.7</td>
<td>1.0</td>
<td>62.0</td>
</tr>
<tr>
<td>Unmarried (%)</td>
<td>40.9</td>
<td>15.0</td>
<td>96.0</td>
</tr>
<tr>
<td>Divorced (%)</td>
<td>3.8</td>
<td>0.0</td>
<td>10.6</td>
</tr>
<tr>
<td>Widowed (%)</td>
<td>6.8</td>
<td>0.0</td>
<td>48.0</td>
</tr>
<tr>
<td>Non-western immigrants (%)</td>
<td>4.8</td>
<td>0.0</td>
<td>14.2</td>
</tr>
<tr>
<td>One-person households (%)</td>
<td>36.4</td>
<td>7.0</td>
<td>71.8</td>
</tr>
<tr>
<td>Households w/o children (%)</td>
<td>32.1</td>
<td>20.7</td>
<td>57.0</td>
</tr>
<tr>
<td>Social welfare recipients (%)</td>
<td>1.2</td>
<td>0.5</td>
<td>14.6</td>
</tr>
<tr>
<td>Average income (in 1000 Euro)</td>
<td>20.5</td>
<td>14.2</td>
<td>29.1</td>
</tr>
<tr>
<td>Persons with low income (%)</td>
<td>2.1</td>
<td>0.5</td>
<td>15.5</td>
</tr>
<tr>
<td>HH with low purch. power (%)</td>
<td>8.9</td>
<td>0.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Households with low income (%)</td>
<td>45.9</td>
<td>17.0</td>
<td>67.0</td>
</tr>
<tr>
<td>HH below social minimum (%)</td>
<td>9.3</td>
<td>0.0</td>
<td>22.0</td>
</tr>
<tr>
<td>Mean prop. value (in 1000 Euro)</td>
<td>213.6</td>
<td>116.7</td>
<td>433</td>
</tr>
<tr>
<td>Males aged 36–45 (%)</td>
<td>6.4</td>
<td>0.0</td>
<td>12.2</td>
</tr>
<tr>
<td>Males aged 46–55 (%)</td>
<td>8.2</td>
<td>0.0</td>
<td>11.2</td>
</tr>
</tbody>
</table>
Demographic variables included stratified population data for 2012 on four-digits postal code level [10,16]. The population data was extracted from customised data by Statistics Netherlands (Extraction date: 20/02/2013).

Socio-economic variables included marital status (proportion of residents that were married, unmarried, divorced, or widowed) [35], proportion of non-western immigrants [16], proportion of one-person households, proportion of households without children, average income [10,36], proportion of persons having low income [36] (defined as an income below 19,200 Euro per year [33]), households having low purchasing power (defined as households having less than 9,250 Euro available per year [33]), households having low income [36] (defined as households with an annual income below 25,100 Euro [33]), households below social minimum and mean property value as indicator for potential area deprivation [10,33].

Exploratory Disease Mapping
We calculated the prevalence rate of HCV and the relative risk (RR) for the adult population aged between 16 and 65.

The RR estimates provide useful information how common HCV infection in a specific location is as compared to the global baseline [37]. We additionally applied spatial empirical Bayes smoothing since the population at risk displayed strong regional variation. This leads to a large variance of the prevalence rate and the relative risk especially in areas where the underlying population is small [38]. Due to strong regional variation in the HCV prevalence, we applied a local smoothing approach. The prevalence rates and the RR were therefore smoothed towards a local mean where the neighbours were defined as areas sharing a common edge and a common boundary [39]. The calculation of the spatial empirical Bayes smoothing was carried out using OpenGeoDa 1.2.0 [40] and the results were then imported in ESRI ArcGIS 10.1.

Global Cluster Detection
To test whether there is spatial autocorrelation of the HCV prevalence, we used Moran`s I. Moran`s I is a widely used global cluster test, which determines the degree of clustering or dispersion within a data set. The resulting values may range from 1 (perfect correlation), 0 (complete spatial randomness) to -1 (perfect dispersed) [41]. For the HCV data, a positive spatial autocorrelation means that postal code areas with high HCV prevalence are close to other postal code areas with high HCV prevalence. For this study, we defined adjacency as postal code areas sharing a common edge or corner. The presence of global clustering justified the subsequent local cluster analysis. The computation of Moran`s I was carried out in OpenGeoDa 1.2.0 [40]

Local Cluster Detection
The spatial scan statistic has been widely applied in several spatial-epidemiological studies to detect local clusters with statistically significant elevated risk of infectious diseases [22,26,42,43]. The spatial
The spatial distribution of hepatitis C virus infections and associated determinants

scan statistic is a local cluster test, which identifies the location and the statistical significance of local clusters [26]. We applied a Poisson purely spatial model where the number of HCV cases follows an inhomogeneous Poisson process [44]. The input data for this model consisted of the number of positive individuals per postal code, the number of adults aged between 16 and 65 and the centroid coordinates for each area. The spatial scan statistic imposes a circular scanning window, which is flexibly in size and position and gradually moves over all coordinates, evaluating all potential cluster locations and sizes up to either a user-defined maximum radius, a user-defined maximum percentage of the population at risk or the default value of up to 50% of the population at risk [45].

In our study, the purpose of the spatial scan statistic was to detect areas with significantly elevated risk of diagnosed HCV, which can serve as a basis for the prioritization of future screening interventions [46,47]. We set the maximum population at risk to not exceed 5% of the adult population. This was done to detect local clusters as precisely as possible since the default settings of 50% of the population at risk are more likely to produce clusters of no practical use [48]. The computation was carried out using the SaTScan software version 9.2 [45].

Spatial Regression

Ordinary Least Squares Regression. To specify a meaningful geographically weighted Poisson regression model, we conducted several steps: First, we performed a natural log-transformation of the dependent variable. We then used a data-mining tool called Exploratory Regression in ESRI ArcGIS 10.1 to determine potential candidate explanatory variables. This tool evaluates all possible variable combinations that form a properly specified ordinary least squares (OLS) regression model. Exploratory regression is comparable to a step-wise regression [31]. However, it evaluates all possible variable combinations based on following criteria: (i) the coefficients are statistically significant, (ii): the explanatory variables are free from multicollinearity, (iii): the residuals are normally distributed and (iv): the residuals do not display spatial autocorrelation [31,49,50].

Based on the results of the exploratory regression, we determined overall model significance, the presence of heteroscedasticity and a wide range of diagnostics by creating an OLS regression model in OpenGeoDa 1.2.0 [40] with the same dependent and explanatory variables as suggested by the exploratory regression.

Geographically Weighted Regression. Since the OLS regression is a global regression model, it estimates the strength of the relationship between the dependent variable and the explanatory variables averaged over the whole study area. However, the larger the study area, the more unlikely it is that one single coefficient per explanatory variable reflects the true underlying spatial relationship between the dependent variable and the explanatory variable since spatial data tend to vary over space. Global statistics tend to lead to the conclusion that relationships between variables are equal across the entire study area whereas local statistics can show the falsity of this assumption by displaying how the relationships vary across space [51]. The geographically weighted regression (GWR) method is therefore an extension to the traditional standard regression methodology and estimates a
wide range of local parameters and diagnostics.

The Poisson distribution within the GWR framework is currently the most suitable for disease data, especially if observed counts of cases are low in specific areas [52–54]. The dependent variable was specified within the geographically weighted Poisson regression (GWPR) as the observed number of HCV cases per postal code and the offset variable was specified as the number of adult persons per postal code. The GWPR model calculates an additional global Poisson regression model, which can be compared to the results of the global OLS model to test the hypothesis that a Poisson regression is more suitable for HCV than the traditional OLS regression. The explanatory variables for the global and local Poisson regression models were the same variables that were found to be significant as specified by the OLS model. The centroids of each postal code were used as input coordinates. The GWPR model then uses a kernel and fits for each coordinate a regression equation where the coordinate in the centre of the kernel is the regression point. The data points inside the kernel are weighted from the centre of the kernel towards the edge of the kernel. Data points outside the kernel receive a weight of zero and are not included in the regression equation. For each coordinate, the data points are weighted differently so that each regression point has a unique regression equation. We used an adaptive kernel size so that in rural areas where data points are sparse, the kernel bandwidth will increase in size and will decrease in urban areas where data points are plentiful. The size of the bandwidth for each kernel and regression point is optimized using Akaike's Information Criterion (AIC) [51]. To facilitate interpretation of the regression coefficients of the GWPR, the coefficients were exponentiated to show an increase or decrease of the relative risk of the dependent variable per one-unit change in the respective explanatory variable [52]. Statistical significance for each coefficient per postal code was calculated using pseudo t-values [51]. The statistic behind the GWPR method is described in detail elsewhere [52]. The computation of the GWPR was carried out using the GWR4 software [55].

Results

Spatial Distribution of Hepatitis C Prevalence among Adults

The prevalence and the risk estimates between the postal code areas varied widely, ranging from 0 to 1.02% of the adult population per postal code. The overall prevalence rate among adults was 0.19% of the total adult population. There was a clear urban–rural divide within the study area. Areas with higher risks were strongly concentrated within the urban areas of Heerlen, Maastricht and to a lower extent in Sittard-Geleen (Fig 1). Moran’s I revealed significant positive global autocorrelation of the HCV prevalence (Moran’s I = 0.43, p<0.001), indicating that postal codes with higher risks are close to each other.

The spatial scan statistic detected five significant local clusters (Fig 1). These are postal codes with statistically significant elevated risk of diagnosed HCV. All clusters could be observed within the three urban areas of the study area (Table 2). In total, these clusters contain 268 (34%) of all observed HCV infections in the study area.
The spatial distribution of hepatitis C virus infections and associated determinants

Figure 1. Spatial Distribution of HCV prevalence in % of adult population and RR, 2002–2008

Table 2. Significant clusters with high HCV risk as determined by the spatial scan statistic

<table>
<thead>
<tr>
<th>Cluster nr.</th>
<th>Location</th>
<th>RR</th>
<th>Cases</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Southern part of Heerlen (3 postal codes)</td>
<td>4.30</td>
<td>91</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2</td>
<td>Northern part of Heerlen (2 postal codes)</td>
<td>2.83</td>
<td>60</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>3</td>
<td>Northern part of Maastricht (1 postal code)</td>
<td>4.03</td>
<td>31</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>4</td>
<td>Centre of Maastricht (3 postal codes)</td>
<td>1.91</td>
<td>71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>5</td>
<td>Eastern part of Sittard-Geleen (1 postal code)</td>
<td>3.29</td>
<td>15</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
Demographic and Socio-economic Determinants of HCV

As our analysis was exploratory in nature, we were interested in determining variable combinations based on population data that delivered a plausible explanation of HCV risk. We therefore identified two models that met all requirements for a properly specified OLS model that delivered a plausible explanation of the HCV prevalence. The AICc value of both OLS models differed only by 3, justifying a comparison of both models [56]. The first model consisted of the following explanatory variables that were overall positively associated with HCV risk: (i) proportion of divorced persons, (ii) proportion of one-person households, (iii) proportion of non-western immigrants and (iv) proportion of males aged 36–45. The second model consisted of the variables (i) average income per person, (ii) one-person households, (iii) mean property value and (iv) males aged 36–45. Variables for the second model were overall positively associated with HCV risk except for average income and mean property value, that both showed an inverse association. The same variables that were found to be significant in the OLS models were then used for further analysis in the global and local Poisson models.

By comparing model performance in terms of the goodness-of-fit AICc statistic (Table 3), the model with the lowest AICc value is the model with the best fit [24]. Based on this criterion, for both Model 1 and Model 2 the AICc value suggests that the global Poisson regression had a better fit than the OLS regression. However, the local Poisson regression outperformed both global regression approaches. The local Poisson regression of model 2 was the overall best-fitting regression model in terms of the AICc value as well as the percentage of local deviance explained.

Table 3. Comparison of global and local models

<table>
<thead>
<tr>
<th></th>
<th>Local Deviance Explained</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Model 1</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLS</td>
<td>0.50</td>
<td>495</td>
</tr>
<tr>
<td>Global Poisson</td>
<td>0.47</td>
<td>372</td>
</tr>
<tr>
<td>Local Poisson</td>
<td>0.53</td>
<td>334</td>
</tr>
<tr>
<td><strong>Model 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OLS</td>
<td>0.50</td>
<td>498</td>
</tr>
<tr>
<td>Global Poisson</td>
<td>0.48</td>
<td>360</td>
</tr>
<tr>
<td>Local Poisson</td>
<td>0.55</td>
<td>323</td>
</tr>
</tbody>
</table>

Results of the Geographically Weighted Poisson Regression

Model 1. The results of the local Poisson model revealed strong local differences of the regression coefficients within the local clusters of elevated HCV risk (Table 4). The impact of the proportion of divorced persons on HCV risk was strongest in cluster 3 and 4 in Maastricht and cluster 5 in the northern part of Sittard-Geleen. In Heerlen, the impact of divorced persons was lowest (Fig 2). The impact of one-person households displayed intra-urban differences as well as regional differences. The association between the proportion of one-person households and HCV risk was strongest in the northern part of Heerlen (cluster 2) and the southern part of Sittard-Geleen (cluster 5). In cluster 3 and 4 in Maas-
The spatial distribution of hepatitis C virus infections and associated determinants

tricht, the impact of one-person households was overall lower than in the other urban areas and clusters. However, the northern part of Maastricht displayed a stronger association of one-person households to HCV risk than the southern part (Fig 2). The association between the proportion of non-western immigrants and HCV risk was only significant in cluster 3 and 4 in Maastricht and surrounding areas (Fig 2). Also, the association between the proportion of males aged 36–45 years and HCV risk displayed large regional differences; its impact was only significant in cluster 5 in Sittard-Geleen, followed by clusters 3 and 4 in Maastricht and the rural areas in between (Fig 2).

Table 4. Significant (p<0.05) coefficients per HCV cluster for model 1

<table>
<thead>
<tr>
<th>HCV Cluster nr.</th>
<th>Determinants</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Divorced persons</td>
<td>1.102</td>
</tr>
<tr>
<td></td>
<td>One-person households</td>
<td>1.034</td>
</tr>
<tr>
<td>2</td>
<td>Divorced persons</td>
<td>1.096</td>
</tr>
<tr>
<td></td>
<td>One-person households</td>
<td>1.036</td>
</tr>
<tr>
<td>3</td>
<td>Divorced persons</td>
<td>1.161</td>
</tr>
<tr>
<td></td>
<td>One-person households</td>
<td>1.035</td>
</tr>
<tr>
<td></td>
<td>Non-western immigrants</td>
<td>1.036</td>
</tr>
<tr>
<td></td>
<td>Males aged 36–45</td>
<td>1.157</td>
</tr>
<tr>
<td>4</td>
<td>Divorced persons</td>
<td>1.164</td>
</tr>
<tr>
<td></td>
<td>One-person households</td>
<td>1.034</td>
</tr>
<tr>
<td></td>
<td>Non-western immigrants</td>
<td>1.035</td>
</tr>
<tr>
<td></td>
<td>Males aged 36–45</td>
<td>1.146</td>
</tr>
<tr>
<td>5</td>
<td>Divorced persons</td>
<td>1.159</td>
</tr>
<tr>
<td></td>
<td>One-person households</td>
<td>1.036</td>
</tr>
<tr>
<td></td>
<td>Males aged 36–45</td>
<td>1.161</td>
</tr>
</tbody>
</table>

Model 2. Comparable to the first model, the second model revealed strong local differences of the coefficients within the HCV clusters (Table 5). The association of HCV risk to average income was overall negative, indicating that a lower income is associated with a higher HCV risk. The local coefficients however, revealed that this association is not in the whole study area significant and negative. Average income is only significant inversely associated with HCV risk in cluster 5 in Sittard-Geleen and one postal code area in Maastricht (Fig 3). The proportion of one-person households was positively associated with HCV risk in cluster 5 in Sittard-Geleen and the northern postal codes of Maastricht in cluster 3. This association decreased in strength towards cluster 1 and 2 in Heerlen (Fig 3). Mean property value was negatively associated to HCV risk in all areas but the association displayed strong regional and intra-urban differences and was strongest in the southern postal codes of Heerlen in cluster 1 (Fig 3). The association between the proportion of males aged 36–45 and HCV risk displayed a similar pattern as observed in model 1. The association was only significant in the northern parts of Maastricht in cluster 3 and 4, the southwestern parts of Sittard-Geleen in cluster 5 and areas in between (Fig 3).
Figure 2. Local coefficients for model 1
The spatial distribution of hepatitis C virus infections and associated determinants.

Figure 3. Local coefficients for model 2
### Table 5. Significant (p<0.05) coefficients per HCV cluster for model 2

<table>
<thead>
<tr>
<th>HCV Cluster nr.</th>
<th>Determinants</th>
<th>Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>One-person households</td>
<td>1.038</td>
</tr>
<tr>
<td></td>
<td>Mean property value</td>
<td>-1.009</td>
</tr>
<tr>
<td>2</td>
<td>One-person households</td>
<td>1.039</td>
</tr>
<tr>
<td></td>
<td>Mean property value</td>
<td>-1.008</td>
</tr>
<tr>
<td>3</td>
<td>One-person households</td>
<td>1.047</td>
</tr>
<tr>
<td></td>
<td>Mean property value</td>
<td>-1.006</td>
</tr>
<tr>
<td></td>
<td>Males aged 36–45</td>
<td>1.213</td>
</tr>
<tr>
<td>4</td>
<td>One-person households</td>
<td>1.045</td>
</tr>
<tr>
<td></td>
<td>Mean property value</td>
<td>-1.006</td>
</tr>
<tr>
<td></td>
<td>Males aged 36–45</td>
<td>1.186</td>
</tr>
<tr>
<td>5</td>
<td>Average income per person</td>
<td>-1.070</td>
</tr>
<tr>
<td></td>
<td>One-person households</td>
<td>1.046</td>
</tr>
<tr>
<td></td>
<td>Mean property value</td>
<td>-1.006</td>
</tr>
<tr>
<td></td>
<td>Males aged 36–45</td>
<td>1.161</td>
</tr>
</tbody>
</table>

### Discussion

The prevalence of HCV varies geographically within the province of South Limburg and clusters were located in urban areas. The main population at risk were divorced persons, male residents aged 36–45 and non-western immigrants residing in the area. Socio-economic determinants associated with HCV risk included one-person households, low income at individual level and areas with low mean property value. The associations between these determinants and HCV risk displayed strong regional and intra-urban differences.

The overall prevalence of diagnosed HCV cases was 0.19%, which is in the range of previous overall estimations of the HCV prevalence within the Dutch population [7,57]. However, the prevalence showed strong local variations with prevalence ranging between 0 and 1.023%.

Five local clusters of significantly elevated HCV risk were detected. These clusters were located in the three urban areas in the region. These results suggest that HCV risk is higher in urban areas than in rural areas and clusters geographically. Thereby, HCV prevalence does not only vary between countries, as was noted before [13,14] but also on small geographic scales such as postal code areas. The small-scale variation of HCV prevalence corresponds with findings of another spatial analysis of HCV in a higher prevalence country [58]. Local clustering of HCV prevalence in urban areas is typical for a wide range of infectious diseases, including HIV [26], Neisseria gonorrhoea [42] and Chlamydia trachomatis [27]. The detection of local clusters in our study may serve as a basis for prioritization of areas for future targeted and evidence-based screening interventions [26,42]. However, it should be noted that only a third of all HCV cases were detected in these clusters. The other cases showed a more random distribution over the region.
To what extent would these demographic and socio-economic determinants be of additional value to focus prevention strategies? When assuming that the population-based determinants represent the actual individual-based risk factors, then all determinants revealed here may indicate who are the key populations for HCV. Targeting these risk factors in the areas identified as clusters could serve as a practically applicable basis for prioritization of future screening interventions.

While there is a wide range of literature available about the prevalence of HCV infections and its associated risk factors [13,14], only a local analysis as employed here may help to understand the patterns of HCV infections and its associations to socio-economic determinants to effectively use available financial resources for targeted screening efforts.

The proportion of residents that were divorced was found to be associated with HCV risk over the complete study region. Marital status had been previously associated with HCV risk, yet findings were inconsistent [59–62]. Being divorced could be a proxy for sexual and economic instability. The found association between HCV risk and divorced persons may therefore serve as basis for future research on the role of marital status and potential high-risk sexual behaviour on HCV transmission in the study area. Non-western immigrants were identified as ethnic risk group in our study. Although this association corresponds well to previous studies focusing on risk factors of HCV in the Netherlands [15,16], the association of non-western immigrants to HCV risk was only significant in Maastricht. Potentially, in the other cities, immigrants from eastern-European countries might be more relevant as ethnic risk group [13,15].

Males aged 36–45 were another main demographic risk group identified in our analysis confirming US findings [3]. It is considered unlikely that this association can be for a large part explained by HIV positive MSM, as they comprise an important but only small part of the HCV cases in the Netherlands [63]. However, the association between males aged 36–45 and HCV risk was only significant in the western part of the study area. One-person households were identified as a risk factor relating to household size. Although this association to HCV may not be obvious at first, it is in line with our findings that divorced persons are an overall risk factor for HCV and could be a potential additional proxy for sexual and economic instability. This finding may additionally serve as a basis for future research on the role of one-persons households and HCV transmission. Mean property value and low income at personal level were important socio-economic determinants associated with HCV risk [35] and are in line with other studies showing that low socio-economic status is an important risk factor for HCV [10,13,36]. However, our study demonstrated that low income at personal level was only significant in the urban area of Sittard-Geleen, while mean property value was found to be overall significant within the study area. Although this corresponds well to previous findings [10,13,36], it highlights the importance of including several markers for low socio-economic status on personal, household and area level to understand how these different measures of low socio-economic status impact the prevalence of HCV infections.

Several determinants were associated with HCV risk in the complete study region while others were only associated in certain regions; but all associations showed regional variance. The strong spa-
tional differences observed suggest that the importance of demographic and socio-economic determinants to characterize the HCV key population may depend largely on the area where the HCV infected individual lives. Our findings are therefore in line with other studies applying GWR for infectious diseases [24,30,64].

In all clusters, an association was observed between HCV risk and divorced persons, one-person households and low mean property value. The proportion of middle-aged males were only associated to HCV in the clusters 3–5, and the proportion of non-western immigrants were only associated in the clusters 3 and 4. Income at personal level was only inversely associated in cluster 5. Thus, the impact of demographic and socio-economic determinants differed across the study area for the identified clusters.

Limitations
First, the spatial analysis of this study was based on the four-digits postal code areas of the Netherlands. Although this spatial aggregation may be considered as a fine geographic scale [34], the prevalence rate of HCV follows the potentially arbitrary administrative boundaries of these postal codes. The results of our analysis might differ if a different level of aggregation had been chosen. This problem is often referred to as the modifiable areal unit problem (MAUP) and has not only an impact on the spatial distribution of HCV risk and the location of the detected clusters, but also on the results of the ecological regression analysis [65]. For our study, it would have been favourable to use street-level addresses of the HCV positive persons and underlying population at risk to analyse the spatial distribution of HCV without the limitation of arbitrary administrative boundaries [26]. This would not only allow a precise localization of HCV clusters, but could offer the chance to perform a geographically weighted logistic regression to provide more detailed insights on the spatially varying association between HCV risk and associated socio-economic and demographic determinants [51]. However, the HCV laboratory data as well as the population data used in this study were not available on this scale.

Second, it is unknown whether testing was motivated by the individuals due to symptoms related to HCV infection or was advised by a general practitioner due to prior knowledge of potential exposure factors of the tested individual. It is also unknown whether geographical, demographic or socio-economic determinants may have been associated with access to testing services (e.g. by distance, lack of knowledge, illiteracy) hence may have influenced the observed associations. The tested persons might therefore differ from the general population. During the initial data analysis, we tested the association of tested persons to demographic or socio-economic population characteristics through an additional exploratory regression model with the log-transformed percentage of tested persons as dependent variable. However, the exploratory regression analysis could not find demographic or socio-economic population characteristics that delivered a properly specified OLS regression model.

Additionally, we compared the spatial pattern of the ratio of HCV positive persons to tested persons with the ratio of positive persons to the adult population. Both approaches displayed a similar spatial pattern. An additional cluster analysis using a Bernoulli model in SaTScan with the number of negative
tested persons as controls [45] could be used to test whether the location of spatial clusters will change when using the negative tested persons as denominator. This might additionally indicate, whether testing is performed randomly or follows different spatial patterns that cannot yet be explained by population or demographic characteristics that were available for this study. However, we applied only a Poisson model as our goal was to compare the HCV prevalence within our study area to previous estimates of the HCV prevalence in the Netherlands, which would not be possible when applying a case-control study design.

In our study, we consider the geographical spread of diagnosed HCV as a realistic representation of the diagnosed HCV prevalence among the adult population since the proportion of tested persons could not be properly explained by demographic or socio-economic population characteristics and the two compared ratios displayed a similar spatial pattern.

Third, the demographic and socio-economic determinants examined are practically applicable but are hampered by lack of precision as they are based on population data and not on an individual level. Population data provide population characteristics per neighbourhood. Therefore, additional research is needed to study whether the population-based determinants for key populations actually capture the individuals comprising such key population.

Fourth, we previously estimated that up to 66% of all HCV-positive patients in the study region were hidden to current screening practices [10]. As a result, cases that were diagnosed may differ from the cases that were still hidden with respect to the variables studied here.

Given the limitations outlined above, it is unknown to what extent the clusters and the demographic and socio-economic determinants really reflect the hidden population. A proof-of-principle intervention targeting postal codes in a detected cluster is currently being set up to reveal whether the hidden HCV infected individuals are appropriately addressed by our detected clusters and determinants. Additionally, we may have missed associations of potential determinants not captured in our analyses, as these were unavailable in the population databases such as educational level.

Also, the population-based determinants used in this study were taken from the Statline database 2009 as this was the earliest population data to include socio-economic variables and the customized stratified demographic data on sex and age were only available for 2012 but not for the years between 2002–2008. Although this might influence the results, it is unlikely that this has a strong impact as the demographic composition in the Netherlands remained relatively stable within the last few years [66].

The application of a Geographically Weighted Poisson regression clearly demonstrated spatial variability of the coefficients and underlined that future screening interventions for HCV clearly have to take into account the spatially varying association between demographic and socio-economic determinants. However, Paéz et al. point out that the use of GWPR delivers more robust results when applied on large datasets containing more than 160 administrative units [67]. Therefore, future research applying GWPR for HCV should focus on larger areas such as whole countries to gain more robust insights on the spatial variation of determinants for HCV [23,29,30]. The reproducibility of our study would allow a similar analysis for the whole of the Netherlands.
Conclusions

In this study, we used spatial epidemiological methods to analyse the spatial distribution of HCV and its associated demographic and socio-economic determinants. Our results revealed strong regional differences not only of the HCV prevalence but also of the association between demographic and socio-economic determinants and HCV risk. These findings underline that a one-size-fits-all approach is not appropriate and that future screening interventions need to take into account the spatially varying demographic and socio-economic determinants for HCV. Our approach may not only be useful for South-Limburg, but may be useful in other countries as well.

Acknowledgments

The authors acknowledge the three medical microbiology laboratories for providing the laboratory data: Dick van Dam and Monique Manders (Orbis Medical Centre, Sittard, the Netherlands), Frans Stals and Jos Bus (Atrium Medical Center Parkstad, Heerlen, The Netherlands), and Inge van Loo and Gert Grauls (Maastricht University Medical Centre, Maastricht, The Netherlands). The authors would also like to thank Jennifer Ilius for enhancing the maps using Adobe Illustrator. The authors received no specific funding for this work.
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The spatial distribution of hepatitis C virus infections and associated determinants


Chapter 6

Detecting Hepatitis B and C by Combined Public Health and Primary Care Birth Cohort Testing


Ann Fam Med 2018;16(1)
Abstract

Purpose
Both chronic hepatitis C (HCV) and B virus (HBV) infections are generally asymptomatic, and many remain undetected or are diagnosed at a late stage. Studies that evaluate best practice hepatitis testing strategies are needed to better detect this hidden population.

Methods
In this prospective cohort study, we aimed to determine the diagnostic yield (test uptake and rate of positive test results) of a combined public health and primary care birth cohort testing strategy in detecting hidden cases of HCV and HBV infections. We invited all patients aged between 40 and 70 years (n = 6,743) registered with 11 family practices serving 2 higher prevalence areas, or hotspots (i.e., estimated HCV prevalence of 1%; national estimated prevalence is 0.1-0.4%), in the south of the Netherlands.

Results
Test uptake was 50.9% (n = 3,434 patients). No active or chronic HCV infection was detected: 0.00% (95% CI, 0.00%-0.11%). Positive test rates were 0.20% (95% CI, 0.08%-0.42%) for anti-HCV (n = 7), 0.26% (95% CI, 0.12%-0.50%) for hepatitis B surface antigen (n = 9), and 4.14% (95% CI, 3.49%-4.86%) for anti-hepatitis B core (n = 142).

Conclusions
This best practice testing strategy was effective in achieving a high test uptake. It completely failed, however, to detect hidden chronic HCV infections and is not recommended for countries with a low prevalence of the disease.
Introduction

Chronic hepatitis C virus (HCV) and B virus (HBV) infections, major causes of chronic liver disease worldwide, are generally asymptomatic, and many infections remain hidden to care or are diagnosed at a late stage.[1-5] Because treatment for HCV infection has greatly improved, identification of HCV-infected individuals has become even more imperative.[6] Yet, strategies to detect cases of HCV infection hidden in the general population have been disappointing. Currently, it is universally recommended to offer testing to individuals who are part of a population with a high prevalence of HCV or HBV infection or who have a history of risk exposure or behavior.[7-10] Risk groups include injection drug users, recipients of infected blood products, children of HCV-positive mothers, and human immunodeficiency virus (HIV)-infected persons.[11-14] Past drug users or recipients of blood transfusions will remain hidden, however, as they may not remember or report these behaviors.[15]

To capture all persons who have HCV infection, prospective studies are needed that evaluate the diagnostic yield of HCV testing strategies not based on exposure risk factors.[16] Birth cohort screening, as currently recommended in the United States (HCV prevalence of 2.0%), has also been suggested for European countries.[9,17,18] In simulation models, onetime HCV testing in the 1945-1965 birth cohort with subsequent treatment was likely cost-effective in the United States.[19] Yet again, primary care clinicians faced with the increasing societal need to screen for HCV infections encounter practical challenges in diagnosis and linking patients to appropriate care.[20] There is also debate on whether expanded screening will lead to net clinical benefit or harm, given the lack of evidence regarding screening efficacy.[21] Whether the birth cohort approach is a useful strategy for countries with low estimated HCV prevalence, such as the United Kingdom (0.7%) and the Netherlands (0.1-0.4%), needs to be demonstrated in practice.[15,22-25]

In countries with a low prevalence of HCV infection, a high test uptake is crucial to increase the number of diagnoses and thereby the diagnostic yield of a birth cohort approach. According to a meta-analysis, targeted practitioner-based studies are most effective in increasing test uptake and cases detected.[26] Diagnostic yield could be further optimized by focusing on areas of higher prevalence. Based on spatial-epidemiological analyses of laboratory data, the estimated prevalence of HCV infection in the south of the Netherlands is 0.5%, and HCV infections are found to cluster in urban areas, with a prevalence of up to 1%.[27,28] These so-called hotspots are postal codes where there is a statistically significant elevated risk of diagnosed HCV infection (relative risk >4). In addition, an estimated 66% of HCV-infected persons are estimated to be untested and thus hidden to care.[27]

We developed a best practice approach by combining public health and primary care in a birth cohort testing strategy. We applied this approach in 2 identified Dutch hotspots to detect cases of HCV and HBV infection that are hidden in the general population. Our current study describes the development of this strategy and an evaluation of its diagnostic yield to provide insights on possible implementation in low-prevalence countries.
Methods

Testing Strategy Development

The strategy was developed and implemented in a collaboration between public health and primary care.

All family physicians located in the hotspots were approached by a peer family physician. Family physicians agreeing to take part personally invited their eligible patients by means of a letter, which was developed by a multidisciplinary team of family physicians, behavioural scientists, epidemiologists, and language ambassadors. Testing was advertised in local newspapers and through waiting room posters.

The regional public health service (PHS) facilitated the practical organization of the testing by distributing the letters of invitation and reminders, arranging the testing location, and planning and collecting measurements. They organized 3 testing days with different time slots (September 2014 to April 2015) at 2 local community centres. Alternatively, patients could be tested at their family practice, the PHS clinic, at home, or at the hospital. Tests were free of charge. Patients not tested received a reminder letter for testing 1 week later. Both participants tested and patients who declined to be tested were invited to complete a short questionnaire. Participants provided written informed consent, and the study was approved by the university medical ethics committee (MUMC;14-4-042).

Testing Strategy Implementation

Two hotspots were targeted because of their highest regional estimated prevalence of HCV infection. [28] The family practices agreeing to participate invited patients aged between 40 and 70 years for a HCV and HBV test. Blood samples were transported within 3 hours of collection to the laboratory. Initial screening included anti-HCV and anti-hepatitis B core (anti-HBc) (Anti-HCV II and Anti-HBc, Roche Diagnostics). When a screening test was positive, subsequent tests included the following: HCV ribonucleic acid (RNA) (COBAS, Ampliprep/COBAS, Taqman HCV Quantitative Test, version 2.0, Roche Diagnostics) and/or immunoblot (Mikrogen), a hepatitis B surface antigen (HBsAg) test, and an anti-hepatitis B surface (anti-HBs) test (HBsAg II and Anti-HBs, Roche Diagnostics) (see Figure 1).

In HBsAg-positive samples additional tests were performed, including hepatitis B e antigen (HBeAg), anti-HBeAg, anti-HBc immunoglobulin M (IgM), HBV deoxyribonucleic acid (DNA), and alanine aminotransferase (ALT) (HBeAg, Anti-HBe, Anti-HBc IgM, COBAS, Ampliprep/COBAS, Taqman HBV Test, ALT, Roche Diagnostics). In solitary anti-HBc-positive samples, HBeAg, anti-HBeAg, and HBV DNA tests were performed (Roche Diagnostics). Samples with weak positive anti-HBc, HBsAg, or anti-HCV test results (between 1 and 2x cut-off value) were confirmed with a second screening test (BioMerieux).

Within 3 weeks after testing, the family physician informed the patient about the test results. In the case of an active HCV or HBV infection, the family physician referred the patient to a gastroenterologist at the academic hospital, who ensured routine care and treatment when needed. For those with HBsAg-positive tests, follow-up testing was conducted after 6 months to discriminate between acute
and chronic infections. The regional public health service performed contact tracing for all cases of active infections and offered hepatitis A virus (HAV) vaccination for the patient and HBV vaccination for close contacts to prevent further spread.

**Data Collection**

For this prospective cohort study, coded data on sex, age, postal code, and referral to a gastroenterologist were retrieved from the family physician registry, and test results were obtained from the laboratory. Questionnaires included the following determinants: country of birth (participant, mother, and father), educational level, working situation, living situation, and previous HCV or HBV infection. Education was categorized into low (no, primary, and lower vocational education), intermediate (intermediate vocational, higher secondary, and vocational education), and high (higher professional education and university). The participants questionnaire also included HCV exposure risk factors (i.e., surgery abroad, blood transfusion or organ transplant before 1992, diagnosis of HIV infection, or a history of intravenous drug use), as well as reasons for participating. The questionnaire for those who declined testing included reasons for not participating. The PHS data included the number of close contacts of all active infections and the number of vaccinations of both the patients (for HAV) and their contacts (for HBV).

**Statistical Analysis**

The diagnostic yield of the testing strategy was evaluated in terms of achieved rates of test uptake and positive test results. A priori, we expected a test uptake of 40% based on international targeted-testing studies and Dutch studies using personal letter invitations.[29-34] A positive anti-HCV test rate higher than the estimated Dutch prevalence of 0.1%-0.4% would be considered promising to explore further assessment and implementation of the testing strategy.[23-25] Test uptake and rates of positive test results were calculated among the patients invited and participants, and associated determinants
were assessed using multivariate logistic regression analyses; determinants included hotspot, sex, age, educational level, and hepatitis exposure risk factors as non-Western migrant, surgery abroad, blood transfusion or organ transplant before 1992, HIV positive, or a history of intravenous drug use. Analyses were performed using SPSS 21.0 (IBM Inc).

Results

All 11 family practices in the targeted areas agreed to take part, and 6,743 patients (excluding failed delivery) between the ages of 40 and 70 years were sent invitations to participate in the study.

Diagnostic Yield: Test Uptake
In total, 3,434 of the 6,743 patients invited were tested for HCV and HBV infection. The test uptake was 50.9% (95% CI, 49.7%–52.1%) (Figure 2). Of those participating, 54.3% were women, the mean age was 57.3 years (SD = 8.1 years), 47.4% had a low education level, and 17.2% had at least 1 hepatitis exposure risk factor.

Diagnostic Yield: Rate of Positive Test Results
No active HCV infection was diagnosed (95% CI, 0.00%–0.11%). The rate of positive tests for anti-HCV, confirmed by immunoblot, was 0.20% (95% CI, 0.08%–0.42%) (n = 7), for HBsAg was 0.26% (95% CI, 0.12%–0.50%) (n = 9), and for anti-HBc was 4.14% (95% CI, 3.49%–4.86%) (n = 142) (Figure 2). The numbers screened needed to detect 1 case of a positive anti-HCV, HBsAg, or anti-HBc test were 491, 382, and 24, respectively. Seventy-one percent (n = 5) of the anti-HCV positive tests results, 22% (n = 2) of the HBsAg positive tests results, and 9% (n = 13) of the anti-HBc positive findings were associated with a history of HCV or HBV infection. All 9 HBsAg-positive participants had negative HBeAg and IgM anti-HBc findings and had an ALT level of <45 U/L. All were referred to a gastroenterologist. Seven of 9 active HBV infections were undiagnosed and were further assessed by the hospital and the PHS. Six participants were chronically infected with HBV, i.e., their HBsAg tests remained positive after 6 months. Treatment was not indicated for any of these patients according to their serum ALT levels, HBV DNA load, or signs of liver fibrosis and status on hepatocellular carcinoma. The PHS traced 13 close contacts of HBsAg-positive participants, and HBV vaccination was needed and given in 9 (Figure 2).

Determinants of Test Uptake
No differences in test uptake between the 2 hotspots were observed. Test uptake was higher among women (54.4%) and older participants, with an uptake of 61.2% among those aged 60 to 70 years (Table 1). Participants had a lower educational level than those not tested (Supplemental Appendix 1). The reported reasons to get tested were because of the invitation by the family physician (62%, n = 2,120) or
**Figure 2. Flow diagram of the birth cohort testing for hepatitis C virus and hepatitis B virus infections**

ALT = alanine aminotransferase; DNA = deoxyribonucleic acid; HAV = hepatitis A virus; HBV = hepatitis B virus, HCV = hepatitis C virus.

* Medical treatment is not indicated in active HBV infections. In chronic HBV infections, treatment is indicated if the HBV DNA level is >1.0 x 10^5 copies/mL combined with an elevated ALT level or major liver fibrosis.

To rule out hepatitis (33%, n = 1,132). Patients declining to be tested reported reasons for not testing as a low risk perception (33%, n = 133), practical reasons (24%, n = 94), and being vaccinated or tested in the past (14%, n = 55). Reasons based on stigma or fear were rare (3%, n = 12).
Table 1. Sociodemographic Determinants of Test Uptake of the Public Health and Primary Care Birth Cohort Testing Strategy

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Test Uptake</th>
<th>Not Tested</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (No.)</td>
<td>% (No.)</td>
<td></td>
</tr>
<tr>
<td>Higher prevalence area</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hotspot A (n = 2,891)</td>
<td>50.2 (1,450)</td>
<td>49.8 (1,441)</td>
<td>1.05 (0.95-1.16)</td>
</tr>
<tr>
<td>Hotspot B (n = 3,949)</td>
<td>50.2 (1,984)</td>
<td>49.8 (1,965)</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n = 3,417)</td>
<td>46.0 (1,571)</td>
<td>54.0 (1,846)</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>Female (n = 3,423)</td>
<td>54.4 (1,863)</td>
<td>45.6 (1,560)</td>
<td>1.41 (1.28-1.55)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40-49(^a) (n = 1,891)</td>
<td>35.0 (662)</td>
<td>65.0 (1,229)</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td>50-59 (n = 2,531)</td>
<td>51.6 (1,292)</td>
<td>49.0 (1,239)</td>
<td>1.93 (1.71-2.18)</td>
</tr>
<tr>
<td>60-70 (n = 2,418)</td>
<td>61.2 (1,680)</td>
<td>38.8 (938)</td>
<td>2.95 (2.60-3.34)</td>
</tr>
</tbody>
</table>

OR = odds ratio.
\(^a\) Adjusted for higher prevalence area, sex, and age.
\(^b\) P ≤.001.
\(^c\) Includes 2 aged <40 years.
\(^d\) Includes 9 aged >70 years.

Determinants of Positive Test Results
Rates of positive test results were higher in those individuals with an exposure risk factor for anti-HCV, HBsAg, and anti-HBc (0.68%, 1.19%, and 11.02%, respectively) than in those without such a risk factor (0.11%, 0.07%, and 2.71%, respectively). Logistic regression analyses assessing additional determinants for past HBV infection found the highest positive anti-HBc test rate among non-Western migrants (27.6%, n = 48 of 174) (OR [odds ratio] = 16.1; 95% CI, 10.4-24.9), HIV-positive patients (22.2%, n = 2 of 9) (OR = 6.7; 95% CI, 1.4-33.3), and individuals with a history of intravenous drug use (28.6%, n = 2 of 7) (OR = 8.2; 95% CI, 1.5-43.2), when adjusted for a higher prevalence area, sex, and age.

Discussion
A combined public health and primary care birth cohort testing strategy to detect hidden HCV and HBV infections in higher prevalence areas in the Netherlands had a limited diagnostic yield. This best practice approach was able to gain a higher than expected test uptake of 51%, but it completely failed to detect hidden HCV infections.

The current test uptake of 51% was higher compared with international studies targeted to risk factors (10% to 49%) and other Dutch studies with a personal invitation (18% to 42%).[29-36] The family physicians’ personal invitation appeared key for achieving this high uptake, as confirmed by the reported main reason to get tested. Test uptake in specific settings, such as the emergency department, may be even higher (up to 65%),[37] as here tests can be offered and performed within the routine diagnostic work-up.
The main result is that no active HCV infections were detected. A higher prevalence was expected based on previous estimations in these hotspots (0.5% to 1%),[27,28] or other Dutch studies focusing on risk groups (0.3% to 4.8%).[32,34,38,39] and on (inter)national estimations that a substantial population is untested.[2-5] Estimates of the hidden HCV-infected population, however, are largely derived from mathematical models (43% to 72%). The number of hidden cases of chronic HCV infection seems to be overestimated compared with a real-life setting. The detected rate of tests positive for anti-HCV is 0.2% and for HBsAg is 0.3%, which is comparable to the general prevalence in the Netherlands (0.1% to 0.4%).[23-25,40,41] The rate of positive anti-HBc test results (4.1%) was higher compared with the Dutch estimated prevalence of 2.1%.[41] Yet, this finding has no implications for patient care. Higher positive rates and lower numbers needed to screen to detect 1 case have been found in birth cohort studies in higher prevalence settings and countries, such as the United States.[15,26,37,42]

Detected positive rates were higher in those with a hepatitis exposure risk factor, a finding in line with studies targeting risk factors.[15,26] Using a risk group instead of the birth cohort, however, would have missed 22% to 54% of all positive cases in this study (3 of 7 anti-HCV-positive cases, 2 of 9 HBsAg-positive cases, and 77 of 142 anti-HBc-positive cases).[7-10] For chronic HBV infections specifically, several studies state that systematic screening among migrants is likely to be cost-effective or at least increases its efficiency.[43,44] This is in line with family physician guidelines in the United Kingdom and the Netherlands, as well as the recommendations of the Dutch Health Council to target HBV testing, such as to non-Western migrants.[7,8,45]

Our study is the first in Europe to develop a public health and primary care birth cohort testing strategy to detect hidden cases of HCV and HBV infection in identified hotspots and evaluate its diagnostic yield. Our study has some limitations. First, selection bias cannot be ruled out, as many HCV-infected patients are at a disadvantage in terms of health literacy.[46] In the Netherlands, health insurance is obligatory, and family physicians function as a gatekeeper to specialist care, but some individuals might have been less likely to get tested. Nevertheless, those who were tested and those who were not did not differ in assessed characteristics, such as working or living situation, or migrant status. Moreover, study participants had an even lower educational level compared with those declining to be tested, and national estimates support that we were able to reach intravenous drug users.[47,48]

Second, behavioural data are self-reported and could be influenced by recall bias and social desirability bias. Third, questions on sexual exposure risk were not included, based on feasible questionnaire length. Fourth, the data from those not tested might be less generalizable to the general population because of low response (23%). Fifth, hotspots were determined based on laboratory data of the tested population. We assumed that the hidden, untested population follows the same geographical pattern. Finally, as no active HCV infections and only small numbers of past HCV and active HBV infections were detected, we were able to assess more detailed determinants only for past HBV infections.

Because no active HCV infections were found in the identified hotspots, it is likely that the strategy taken would not be effective in other areas of the Netherlands and other low-prevalence countries. Our testing strategy can be applied to detecting HCV and HBV infections, as well as other infectious
diseases, in high-endemic countries, such as the United States. If the test is not offered for free, however, a lack of health insurance could be a limiting factor in the United States. Considering the different health care system in the United States, the test uptake of a similar testing strategy might be lower in certain groups at risk, such as intravenous drug users or patients co-infected with HIV. Lessons learned can also be applied to future studies aimed at retracing patients previously diagnosed with HCV and HBV. The success factor of our high test uptake is the combination of a personal invitation accompanied by reminders, and the cooperation between public health care and primary care. Moreover, our findings informed the Dutch Health Council in their recommendation not to screen the general population for HCV and HBV infection.[45]

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References


Combined public health and primary care birth cohort testing in hotspots


Supplemental material

**Supplemental Appendix 1. Comparing the distribution of socio-demographic characteristics of the testers and non-testers**

<table>
<thead>
<tr>
<th>Educational level</th>
<th>Testers (N=3396)</th>
<th>Non-testers (N=568)</th>
<th>OR*</th>
<th>95%CI</th>
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<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>47.4 (1609)</td>
<td>30.8 (175)</td>
<td>1.67</td>
<td>1.33-2.10</td>
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<tr>
<td>Medium</td>
<td>23.1 (783)</td>
<td>24.3 (138)</td>
<td>1.28</td>
<td>1.01-1.61</td>
</tr>
<tr>
<td>High</td>
<td>28.5 (969)</td>
<td>41.4 (235)</td>
<td>ref</td>
<td>***</td>
</tr>
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</table>

*Adjusted for higher prevalence area, gender, and age.

<table>
<thead>
<tr>
<th>Working situation</th>
<th>Testers (N=3396)</th>
<th>Non-testers (N=568)</th>
<th>OR*</th>
<th>95%CI</th>
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<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Working (part-time or full-time)</td>
<td>47.8 (1623)</td>
<td>54.2 (308)</td>
<td>ref</td>
<td></td>
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<tr>
<td>Retired*b</td>
<td>26.5 (899)</td>
<td>22.2 (126)</td>
<td>1.21</td>
<td>0.89-1.65</td>
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<tr>
<td>Not working (unemployed/ receiving social welfare)c</td>
<td>24.6 (836)</td>
<td>19.7 (112)</td>
<td>1.24</td>
<td>0.98-1.58</td>
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<table>
<thead>
<tr>
<th>Living situation</th>
<th>Testers (N=3396)</th>
<th>Non-testers (N=568)</th>
<th>OR*</th>
<th>95%CI</th>
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<tbody>
<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alone</td>
<td>24.1 (819)</td>
<td>25.4 (144)</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>With partner/ children/ parents</td>
<td>75.3 (2557)</td>
<td>71.1 (404)</td>
<td>1.04</td>
<td>0.85-1.29</td>
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<table>
<thead>
<tr>
<th>Migrant</th>
<th>Testers (N=3396)</th>
<th>Non-testers (N=568)</th>
<th>OR*</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-western migrant</td>
<td>5.1 (174)</td>
<td>5.8 (33)</td>
<td>ref</td>
<td></td>
</tr>
<tr>
<td>Autochthonous</td>
<td>80.4 (2732)</td>
<td>78.2 (444)</td>
<td>1.15</td>
<td>0.77-1.71</td>
</tr>
<tr>
<td>Western migrant</td>
<td>13.1 (444)</td>
<td>13.6 (75)</td>
<td>1.14</td>
<td>0.72-1.79</td>
</tr>
</tbody>
</table>

Abbreviation: n, number of participants; OR, odds ratio; CI, confidence interval.
***p≤0.001
*a Adjusted for higher prevalence area, gender, and age.
b Including all retired persons (with/without work).
c Including housewives.
Chapter 7

Hepatitis E prevalence in a sexual high-risk population compared to the general population


PloS one 2018; 13(1)
Abstract

Transmission routes of the hepatitis E virus (HEV) are under debate. Here, we studied possible sexual transmission by comparing HEV prevalence in a Dutch sexual high-risk population (n = 1,482) with that in a general population (n = 1,487) while assessing sociodemographic and sexual risk factors. Overall prevalence of anti-HEV IgG of 18.9% (n = 562) was, adjusting for confounders, similar between the two populations (p = 0.44). Prevalence was higher with each year’s increase in age (adjusted OR: 1.03, 95%CI: 1.02-1.04, p<0.01), among men (adjusted OR: 1.24, 95%CI: 1.02-1.50, p = 0.03) and among individuals diagnosed with sexually transmitted infections (adjusted OR: 1.60, 95%CI: 1.02-2.49, p = 0.04). Our results only hint at the possibility of a sexual transmission route for HEV given higher rates in those with chlamydia and/or gonorrhoeal infections. Sexual transmission is not a dominant transmission route, as its prevalence was not higher for the sexual high-risk population than for the general population.
Hepatitis E prevalence in a sexual high-risk population

Introduction

Recently, the hepatitis E virus (HEV) has become a global public health concern, as an increase of autochthonous HEV infections was observed in developed countries. HEV is considered to be endemic in developing countries, where it is transmitted mainly through contaminated water and the faecal-oral route, causing epidemic outbreaks of genotypes 1 and 2 [1, 2]. Traditionally, HEV was considered to be a travel-related disease in developed countries, associated with genotypes 1 and 2. For genotypes 1 and 2, the mortality rate is usually low (0.07-0.6%); it is particularly severe among pregnant women, with mortality rates between 15-25% [3]. However, reported cases of patients that have not travelled to endemic areas - so-called autochthonous hepatitis infections - have increased in developed countries [4-10]. These infections are mainly caused by HEV genotype 3, symptomatic HEV genotype 3 infections are more common among middle-aged and older individuals as well as among men [10-12]. HEV infections are usually asymptomatic, but genotype 3 infections may cause chronic hepatitis in immunocompromised patients. This group includes patients who have received an organ transplant, patients receiving chemotherapy and HIV-infected individuals [13-16].

Transmission routes continue to be one of the most debated aspects of HEV. The most recent evidence suggest that HEV caused by genotypes 3 and 4 is transmitted zoonotically. In Europe, North America and Japan, HEV genotype 3 is widespread among pigs [17-19]. In these countries, domestic pigs and wild boars act as a reservoir [20]. It is proven that HEV is transmitted by ingesting uncooked or poorly cooked pork or game meat [21-23].

Studies are needed to identify all transmission routes of HEV, so appropriate preventive and control measures can be taken. The hepatitis A virus (HAV) and HEV genotypes 1 and 2 are both single-stranded RNA viruses with similar incubation periods and a faecal-oral transmission route. Sexual activity, including oral-anal contact, as the major transmission route among men who have sex with men (MSM) is another widely documented aspect of HAV [24, 25], but only a limited number of studies have focused on the possible sexual transmission route of HEV infections [26-30]. This study could contribute to policy on the prevention and control of HEV infections, to be targeted at vulnerable individuals who are at highest risk. To explore the possible role of sexual transmission, we compared the HEV prevalence of a population with higher sexual risk to the general population. In addition, we assessed potential risk factors of sexual transmission through a cross-sectional study in the south of the Netherlands.

Methods

Study population

The STI clinic study and the GP study were approved by the Maastricht University Medical Centre Medical Ethical Committee (11-4-108 for the STI clinic cohort and 14-4-042 for the GP cohort). No informed consent was needed because of prevailing laws in the Netherlands, as it concerns an observational
study using anonymous data only. Our study population consisted of two populations: a sexual high-risk population from a STI clinic cohort and the general population from a GP cohort. Venous blood samples were tested on HEV IgG. All individuals from the two study populations lived in urban areas in South Limburg. In the study period (December 2011 to November 2015), no clusters or outbreaks of HEV were reported in the study region.

First, the sexual high-risk study population (STI clinic cohort; n = 1,482) was compiled as a sample from a cohort of individuals aged 20-70, all of whom were tested for a sexually transmitted infection (STI) at the STI clinic between December 2011 and November 2015 (original cohort size about 24,500 individuals). This sample included women (n = 350); female swingers, heterosexuals who as a couple practise partner-swapping or group sex, or who visit sex clubs for couples [31] (n = 184); heterosexual men (n = 480); and men who have sex with men (MSM; n = 468). This STI clinic cohort represented a high-risk population, as 75.2% reported anal sex, had three or more sexual partners in the past six months or tested positive for *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), syphilis or HIV (see S1 Table).

Second, the general study population (GP cohort; n = 1,487) was compiled as a sample from a cohort of individuals aged 40-70, all of whom were tested by general practitioners (GPs) for a study of the hepatitis B virus (HBV) and the hepatitis C virus (HCV) between September 2014 and April 2015 (original study size n = 3,434). The prevalence of past HCV infections was 0.27% (4/1,487). This is comparable to the prevalence in the general population [32-34]. No active HCV infections were diagnosed in the GP cohort. The prevalence of active and past HBV infections was 0.5% (7/1,487) and 8.9% (132/1,487) respectively. Overall, the prevalence of active and past HBV infections is 0.2-0.3% [35, 36] and 2.1% [36] in the Netherlands. This GP cohort represented a general population of both man and women from two urban areas, without any further exclusion criteria.

**Data collection**

Data on the test year, age, gender, ethnicity, HIV positivity and anti-HEV test results were available for all samples. In the STI clinic cohort, HIV and syphilis positivity was based on the testing of high-risk groups such as MSM. In the GP cohort, HIV positivity was based on self-reported data.

Additional data on sexual preference, reported anal sex, number of sexual partners in the past six months and diagnosis of CT/NG/syphilis were only available for the STI clinic cohort. Additional data on educational level, HBV and HCV positivity were only available for the GP cohort. Reporting anal sex, having three or more sexual partners in the past six months and being CT positive, NG positive, Syphilis positive or HIV positive were categorised as risk factors of sexual transmission.

**Serological testing**

Stored sera from the participants of the two study populations were tested for HEV IgG (recomWell hepatitis E virus IgG, Mikrogen GmbH, Neuried, Germany) at the Department of Medical Microbiology at Maastricht University Medical Centre. HEV IgG was measured in U/ml: results below 20 U/ml were
reported as negative, 20–24 U/ml (n = 41) were reported as grey areas (considered as negative in current analyses) and results above 24 U/ml were reported as positive. In a recent study, the Wantai assay detected more positives (prevalence of 48.2% compared to 17.3% with the Mikrogen assay) and had a lower detection limit. However, it is likely that this included more false positives and the Mikrogen assay had the highest correlation to the estimated overall IgG (prevalence of 18.7%). Therefore, we chose for the Mikrogen assay.

Statistical analysis
The prevalence of HEV was based on the number of positive HEV test results, divided by the total number of HEV tests and multiplied by 100. Three different models were constructed, including an overall model, a model for the STI clinic cohort and a model for the GP cohort. Univariate logistic regression analyses were used to assess the unadjusted associations between potential risk factors and HEV prevalence. All previously described variables (see the Data collection section) were included in the analyses as potential risk factors. However, we identified several known confounders on the basis of the literature, which led to adjustments in the multivariate logistic regression analyses. These confounders include the test year, age and gender. We used the Enter method to assess the OR and the 95% confidence intervals (p ≤ 0.05). The age curves in Fig 1 were designed on the basis of a second-degree polynomial fit.

Results
A total of 2,969 individuals were tested on HEV IgG antibodies: 1,482 from the STI clinic cohort and 1,487 from the GP cohort. The study population had a median age of 44 (IQR 30–54), 54.9% were men (n = 1,630) and 11.0% (n = 326) had a non-western ethnicity (see S1 Table). Anti-HEV IgG was detected in 562 samples (18.9%, 95%CI: 17.5–20.4).

Univariate risk factors
In univariate analyses, the overall HEV prevalence was higher with each year’s increase in age (p<0.01) as well as for individuals from the GP cohort (p<0.01; see Table 1). For the STI clinic cohort, HEV prevalence was higher with each year’s increase in age (p<0.01), for MSM (p = 0.04), for individuals who reported anal sex (p = 0.02), for individuals who had three or more sexual partners (p = 0.05) and with a diagnosis of CT or NG (p = 0.05). For the GP cohort, the HEV prevalence was higher with each year’s increase in age (p<0.01) and borderline significant for men (p = 0.10).
Figure 1. Anti-HEV IgG prevalence in five-year age groups (n = 2,969), south Netherlands, Dec 2011 to Nov 2015

Independent risk factors

In multivariate analyses, the overall HEV prevalence was higher with each year’s increase in age (adjusted OR: 1.03, 95%CI: 1.02-1.04, p<0.01) and for men as compared to women (adjusted OR: 1.24, 95%CI: 1.02-1.50, p = 0.03; see Table 1 and Fig 1). HEV prevalence was similar among the STI clinic cohort and the GP cohort, after adjusting for confounding factors (see Table 1).

For the STI clinic cohort, most univariate sexual risk factors attenuated and became statistically non-significant after adjusting for test year, age and sexual preference. Independent predictors for HEV prevalence were older age (adjusted OR: 1.02, 95%CI: 1.01-1.04, p<0.01) and a diagnosis of CT or NG (adjusted OR: 1.60, 95%CI: 1.02-2.49, p = 0.04).

For the GP cohort, HEV prevalence was higher with each year’s increase in age (adjusted OR: 1.04, 95%CI: 1.03-1.05, p<0.01).
Hepatitis E prevalence in a sexual high-risk population

Table 1. Determinants of anti-HEV IgG prevalence, south Netherlands, Dec 2011 to Nov 2015

<table>
<thead>
<tr>
<th>Total study population (n=2,969)</th>
<th>Univariate anti-HEV IgG prevalence</th>
<th>Multivariate OR (95%CI) adjusted for test year*, age, gender</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (median=44, IQR=30-54)</strong></td>
<td>***</td>
<td>1.03*** (1.02-1.04)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Men</td>
<td>309/1,630</td>
<td>19.0%</td>
</tr>
<tr>
<td>• Women</td>
<td>253/1,339</td>
<td>18.9% (ref)</td>
</tr>
<tr>
<td><strong>Population</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• High sexual risk (STI clinic cohort)</td>
<td>176/1,482</td>
<td>11.9% (ref)</td>
</tr>
<tr>
<td>• General (GP cohort)</td>
<td>386/1,487</td>
<td>26.0%***</td>
</tr>
<tr>
<td><strong>STI clinic cohort (n=1,482)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Age (median=30, IQR=24-39)</strong></td>
<td>***</td>
<td>1.02** (1.01-1.04)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Men</td>
<td>118/948</td>
<td>12.4%</td>
</tr>
<tr>
<td>• Women</td>
<td>58/534</td>
<td>10.9% (ref)</td>
</tr>
<tr>
<td><strong>Sexual preference</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Women</td>
<td>33/350</td>
<td>9.6% (ref)</td>
</tr>
<tr>
<td>• Female swinger</td>
<td>25/184</td>
<td>13.6%</td>
</tr>
<tr>
<td>• Heterosexual men</td>
<td>52/480</td>
<td>10.8%</td>
</tr>
<tr>
<td>• MSM</td>
<td>66/468</td>
<td>14.1%*</td>
</tr>
<tr>
<td><strong>Reported anal sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>87/629</td>
<td>13.8%</td>
</tr>
<tr>
<td>• No</td>
<td>81/812</td>
<td>10.0% (ref)</td>
</tr>
<tr>
<td>• Unknown</td>
<td>8/41</td>
<td>19.5%*</td>
</tr>
<tr>
<td><strong>Number of sexual partners (median=3, IQR=2-5)</strong></td>
<td>61/617</td>
<td>9.9% (ref)</td>
</tr>
<tr>
<td>• &lt;3 in past 6 months</td>
<td>114/856</td>
<td>13.3%*</td>
</tr>
<tr>
<td>• ≥3 in past 6 months</td>
<td>1/9</td>
<td>11.1%</td>
</tr>
<tr>
<td>• Unknown</td>
<td>1/9</td>
<td>10.9%</td>
</tr>
<tr>
<td><strong>Chlamydia and/or gonorrhoeae positive</strong></td>
<td>28/171</td>
<td>16.4%*</td>
</tr>
<tr>
<td>• Yes</td>
<td>148/1,311</td>
<td>11.3% (ref)</td>
</tr>
<tr>
<td>• No</td>
<td>1/4</td>
<td>25.0%</td>
</tr>
<tr>
<td>• Untested</td>
<td>161/1,364</td>
<td>11.8%</td>
</tr>
<tr>
<td><strong>Syphilis positive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>6/37</td>
<td>16.2%</td>
</tr>
<tr>
<td>• No</td>
<td>167/1,427</td>
<td>11.7% (ref)</td>
</tr>
<tr>
<td>• Untested</td>
<td>3/18</td>
<td>16.7%</td>
</tr>
<tr>
<td><strong>HIV positive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>185/676</td>
<td>27.4%</td>
</tr>
<tr>
<td>• No</td>
<td>94/371</td>
<td>25.3%</td>
</tr>
<tr>
<td>• Untested</td>
<td>102/427</td>
<td>23.9% (ref)</td>
</tr>
<tr>
<td><strong>HBV positive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes (active or past infection)</td>
<td>43/139</td>
<td>30.9%</td>
</tr>
<tr>
<td>• No (no infection or vaccinated)</td>
<td>343/1,348</td>
<td>25.4% (ref)</td>
</tr>
<tr>
<td><strong>HCV positive</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes (past infection)</td>
<td>7/18</td>
<td>75.0%*</td>
</tr>
<tr>
<td>• No (no infection)</td>
<td>383/1,483</td>
<td>25.8% (ref)</td>
</tr>
</tbody>
</table>

a Test year of 2012 includes two tests from December 2011.

b Including 14 individuals not tested for CT and 13 individuals not tested for NG.

*p ≤ 0.05

**p ≤ 0.01

***p ≤ 0.001

0.05 ≤ p ≤ 0.10
Discussion

No firm conclusions can be drawn about sexual transmission of HEV from this study given that the overall prevalence of anti-HEV IgG was the same between the sexual high-risk population and the general population. Our results only hint at the possibility of sexual transmission of HEV as having a sexually transmitted infection (CT and/or NG) was found to be associated with a higher HEV prevalence. Though this may lead to occasional HEV outbreaks, as is the case for HAV, sexual HEV transmission is unlikely to influence the prevalence at the population level. More research is needed on possible sexual transmission of HEV.

Anti-HEV IgG was detected in 18.9% of the 2,969 blood samples from individuals either attending an STI clinic or belonging to the general population. The adjusted HEV prevalence was similar among the two cohorts. This observed prevalence is similar to the figure of 17% found in a German study among persons aged 18–79 years, which used the same HEV assay [37]. The observed prevalence is lower than a study among Dutch blood donors with a prevalence of 27% [11]. This difference in HEV prevalence is unlikely only explained by the use of the Wantai assay with a slightly higher sensitivity [38], it is more likely explained by the older study population. The identification of age and gender factors with relation to HEV prevalence confirms the results from several previous studies [10-12, 39]. HEV prevalence is thought to be related to age, because of the cumulative effect of lifetime exposure. However, no clear and thorough explanation has as yet been found for its connection with gender.

Interestingly, HEV prevalence was higher for individuals who tested positive for CT and/or NG. This higher prevalence is unlikely explained by the zoonotic transmission route of HEV. The ingestion of HEV-infected food is not believed to be more common among individuals who tested positive for CT and/or NG. Furthermore, a similar trend is seen in other risk factors of sexual transmission such as being an MSM, reporting anal sex, having three or more sexual partners in the past six months and being tested positive for syphilis. This may suggest that sex may be a possible transmission route for HEV. Whether HEV could be transmitted via the faecal-oral route, via blood contact during risky sexual behaviour or via person-to-person contact remains unknown and should be the subject of further study.

To our knowledge, this is the first study to explore the possible sexual transmission of HEV and its impact at the population level in the Netherlands. However, the following limitations must be considered. First, no data on sexual risk behaviour were available for individuals from the GP cohort and data on parenteral risk behaviour, injective drug use, were not included due to its low prevalence. However, we compared the sexual risk behaviour of the STI clinic cohort to the general population in the Netherlands. In our study 40% of the individuals reported anal sex in the past six months compared to 7-18% in the general population [40, 41]. Overall more sexual risk behaviour was reported in the STI clinic cohort compared to the general population. Second, the age distribution differs between the STI clinic cohort and the GP cohort. Age was included as confounder in the multivariate logistic regression analyses. Additionally, we assessed the HEV prevalence in the 40-50 year olds. No difference was detected between the STI clinic cohort and the GP cohort in this age group too, p = 0.678. Matching cases and
controls on age and/or sex is desirable for future studies[26, 27]. Third, the comparison to HEV prevalence in other studies is difficult to make, as sensitivity and specificity are highly variable between the different serological assays [42, 43]. Fourth, no data on occupational exposure to swine, as a known risk factor of HEV, were available. Finally, using this cross-sectional design we were unable to assess causal associations, a longitudinal study including genotyping would have been the ideal design. Our results only hint at the possibility of a sexual transmission route for HEV given higher rates in those with chlamydia and/or gonorrhoeal infections. Sexual transmission is not a dominant transmission route, as its prevalence was not higher for the STI clinic cohort than for the GP cohort. Whether sexual transmission of HEV occurs, and whether it could consequently lead to outbreaks in the same vein as HAV, should preferably be studied at the individual level in future prospective research. Considering the limited clinical impact of HEV infections, we do not recommend increased HEV testing in sexual high risk clinical settings, with the possible exception of patients who are immunocompromised.
References


32. Kok A, Zuure FR, Weegink CJ. Hepatitis C in Nederland: schaarse gegevens over actuele prevalentie en de noodzaak van epidemiolo-


### Supporting information

**S1 Table. Characteristics of total samples tested for HEV (n = 2,969), south Netherlands, Dec 2011 to Nov 2015**

<table>
<thead>
<tr>
<th></th>
<th>Sexual high-risk population (N=1,482)</th>
<th>General population (N=1,487)</th>
<th>Total (N=2,969)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>median=30, IQR=24-39</td>
<td>median=53, IQR=46-62</td>
<td>median=44, IQR=30-54</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Men</td>
<td>64.0 (948)</td>
<td>45.9 (682)</td>
<td>54.9 (1,630)</td>
</tr>
<tr>
<td>• Women</td>
<td>36.0 (534)</td>
<td>54.1 (805)</td>
<td>45.1 (1,339)</td>
</tr>
<tr>
<td><strong>Non-western ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>13.4 (199)</td>
<td>8.5 (127)</td>
<td>11.0 (326)</td>
</tr>
<tr>
<td>• No</td>
<td>86.6 (1,283)</td>
<td>91.5 (1360)</td>
<td>89.0 (2,643)</td>
</tr>
<tr>
<td><strong>Additional variables only available for STI clinic cohort</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sexual preference</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Women</td>
<td>23.6 (350)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>• Female swinger</td>
<td>12.4 (184)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>• Heterosexual men</td>
<td>32.4 (480)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>• MSM</td>
<td>31.6 (468)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Sexual transmission risk factor</strong></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>• Yes</td>
<td>75.2 (1,114)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>• No</td>
<td>24.8 (368)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>Additional variables only available for GP cohort</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Educational level</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Low</td>
<td>-</td>
<td>45.5 (676)</td>
<td></td>
</tr>
<tr>
<td>• Medium</td>
<td>-</td>
<td>24.9 (371)</td>
<td></td>
</tr>
<tr>
<td>• High</td>
<td>-</td>
<td>28.7 (427)</td>
<td></td>
</tr>
<tr>
<td>• Unknown</td>
<td>-</td>
<td>0.9 (13)</td>
<td></td>
</tr>
</tbody>
</table>

*Test year of 2012 includes two tests from December 2011.*

*Individuals who were born in or who had at least one parent born in Africa, Asia (excluding Indonesia and Japan) or Latin-America.*

*Including reporting anal sex, having three or more sexual partners in the past six months and being CT positive, NG positive, Syphilis positive or HIV positive.*

*Including five tests for which the risk factor was unknown.*
Chapter 8

General discussion
The studies presented in this thesis aimed to identify and address hidden infections and evaluate current infectious disease prevention, surveillance and control. Better insight into hidden infections allows for example more targeted and effective testing practices in order to eventually protect the most vulnerable and susceptible groups at risk. In this chapter, I will discuss the main findings of this thesis and provide recommendations for future policy and research on hidden infections, by reflecting on recent literature and research on pertussis and hepatitis.

8.1 Hidden pertussis infections

Pertussis surveillance aims to monitor the impact of the vaccination programme, identify high-risk areas, detect outbreaks and monitor case management in order to take timely preventive measures [1]. To avoid morbidity and mortality in young infants, pertussis surveillance data based on notifications is currently used to guide pertussis control measures. However, there is a discrepancy between the number of pertussis notifications and the number of pertussis infections. In sero-surveys antibody levels against pertussis toxin (PT) are used to estimate the prevalence of pertussis infections in the population [2]. The Dutch seroprevalence of PT-IgG levels ≥ 50 IU/mL increased from 4% in 1995-1996 to 9% in 2006-2007 [3,4]. Results of the third Dutch pertussis seroprevalence study conducted in 2016-2017 are expected this year. In 2006–2007, the estimated seroprevalence of pertussis infections was 100-fold higher than the reported notifications at that time [5,6].

Study aim and methods

Therefore, more insight was needed into the accuracy of pertussis surveillance and control, including the identification of spatial clusters of infections (Chapter 3) and studying impeding factors in the medical care (healthcare provider and laboratory) and public healthcare (Chapter 2 and 4). We studied a unique combination of data sources from one region (1.1 million residents), including online GPs questionnaires, laboratory registries and public health care registries. In this thesis we used geographical analyses and a surveillance study as methods to give more insight into the factors that hamper the identification of hidden pertussis infections.

Main findings

Pertussis surveillance and control is hampered by, for example, under-ascertainment as individuals with mild symptoms or who are asymptomatic may not present to healthcare for diagnosis [7,8]. Clinically, the disease resembles other respiratory diseases, particularly in the early catarrhal phase when an individual is already highly contagious [7]. Also, the classical pertussis symptoms are often absent in adolescents and adults, therefore these cases may not be recognized by HCPs [7,9,10]. Clinical recognition, perceived barriers in testing, and a lack of being familiar with testing protocols influence the testing behaviour of physicians [11]. In contrast to national guidelines, in our study GPs reported to
perform laboratory diagnostic tests for pertussis mainly in patients with clinical symptoms. This practice is in accordance with expressed facilitators and barriers to test for pertussis among GPs [12]. Laboratory diagnostic procedures, especially interpretation of serology, are complicated because of changing cut-off value recommendations, in cases of recent immunisation and because of limitations of the available diagnostic tests [8,11,13]. As the pertussis diagnostic process is quite challenging, not all cases presenting to healthcare are diagnosed (underdiagnosis) or notified (under-notification) [14]. Our studies revealed that the current pertussis incidence is largely the result of testing, possible misdiagnosis, substantial under-notification (18%) and a large delay between patient symptoms and notification to the local public health services (median 34 days) precluding timely preventive actions [15,16]. Hidden pertussis infections are likely not unique for this area of the Netherlands and are generalizable to the rest of Europe. These countries are likely to encounter varying guidelines, difficulties in pertussis diagnostics and unexplained local differences in notification data too. It is not feasible and desirable to identify all pertussis infections. Ideally, the accuracy of surveillance would be improved by focusing on the factors identified in this thesis. This contributes to optimisation of the surveillance system and more importantly, to improved pertussis control by preventing young infants from being infected with pertussis and avoiding morbidity and mortality in this risk group.

**Recommendations for practice and future research**

Based upon this thesis and recent literature we have several recommendations on how to improve current practice and policy. We were able to give more insight into the factors that hamper the identification of hidden pertussis infections. These findings function as an important input for the discussion on how to reduce the workload of PHS and the debate on how to reduce the overall delay until notification to improve pertussis control [17,22].

First, to reduce misdiagnosis and the variation in pertussis diagnostics, we recommend laboratories and HCPs to improve adherence to national guidelines concerning timing and choice of test. For laboratories, a national uniform and standardised guideline on cut-off values in serology and the use of IgA and/or IgG is desirable. Single serological testing is not suitable to detect recent pertussis infection in individuals vaccinated with an acellular pertussis vaccine within the past year. Multiple serology is also recommended when the first titre is below the cut-off value specific for a pertussis infection. Among infants younger than 1 year of age and individuals with less than three weeks of coughing, PCR testing is recommended [23-25]. Testing all patients presenting with cough is not feasible, as only 3% of adult patients presenting with acute cough in primary care had evidence of an acute pertussis infection in Europe [26]. Moreover, testing for pertussis follows to an extent the general healthcare seeking behaviour for common respiratory infections, whereas the current pertussis incidence is largely the result of testing. Increased testing would thus not necessarily improve pertussis control. Therefore, HCPs should focus pertussis testing on family members surrounding incompletely vaccinated infants <1 year of age, women >34 weeks pregnant and children with severe comorbidities such as heart or lung failure. In addition, they are advised of whooping cough have vulnerable groups at risk in their
household. As such, GPs could play a more prominent role in contact tracing (now carried out by the public health authorities in the Netherlands) in order to minimise the disease burden in infants.

Second, to reduce under-notification, laboratories and HCPs could benefit from introducing automated notification systems [15]. In an endemic situation with severe underreporting, the obligatory notification of pertussis is not effective for the protection of unvaccinated children [22]. On the other hand, the notification of pertussis infections is of great value to national policy on pertussis surveillance and control [17,18] although we showed the limitations of pertussis surveillance [15]. Pertussis has to remain a notifiable disease as it is necessary to prohibit a pertussis-positive patient to go to work when he/she works with vulnerable young infants. The detection of outbreaks using space-time cluster detection is feasible and could help to facilitate an early and appropriate public health response. However, this approach has to be adjusted for the strong dependency to testing and is probably most efficient when using test-positivity as indicator [16].

Third, as public health is almost always too late to intervene, delays need to be reduced and we need earlier preventive measures by healthcare providers [19]. Patient delays due to late presentation to healthcare may be unavoidable, however delays in reporting once a patient presents can be reduced [21]. GPs, midwives and day nursery employees could play a major role here. Creating awareness among these professionals as well as patients themselves about taking timely preventive measures could lead to lower individual disease burden and to a higher cost-effectiveness of pertussis care.

At last, we also need additional preventive measures for the most vulnerable and susceptible group of unvaccinated infants, as the Dutch national vaccination program starts at the age of two months. In the first few months young infants are unprotected as vaccination is only effective after at least three doses. The efficacy of pertussis vaccines has been debated because of waning immunity, incomplete protection of infants below 5 months, genetic changes in *Bordetella pertussis* and limited duration of protection. Currently, maternal immunisation has been recommended by the WHO and the ECDC. In England, maternal immunisation was found to provide 91% protection against infant disease (vaccine-effectiveness), whilst no important side effects were observed. The Dutch health council advised the introduction of maternal vaccination to the national vaccination program (December 2015) and pertussis vaccinations for healthcare workers (June 2017). The practical implementation of these recommendations into the Dutch healthcare system is undecided yet.
8.2 Hidden hepatitis infections

Chronic hepatitis C virus (HCV) and B virus (HBV) infections, major causes of chronic liver disease worldwide, are generally asymptomatic and many infections remain hidden to care or are diagnosed at a late stage [27-31]. A national serosurvey in 2006-2007 in the Netherlands estimated a weighted HBsAg seroprevalence of 0.2% and 0.3% of anti-HCV antibodies in the general Dutch population [32,33]. However, because HBV and HCV infections are concentrated within high-risk groups such as migrants, men who have sex with men (MSM), and people who inject drugs (PWID), groups which are often underrepresented in (voluntary) national serosurveys, these estimations are possibly an underestimation. Three studies using a risk group approach estimated a prevalence of chronic HBV of 0.36-0.53% in 2006 [34], a prevalence of anti-HCV antibodies of 0.22% in 2009 [35], and a prevalence of chronic HCV infection of 0.11% in 2015 [36]. The chronic HBV and HCV prevalence estimates were updated in 2016, stratified by country-of-origin for first-generation migrants. The estimated chronic HBV infection prevalence is 0.34% and the estimated chronic HCV infection prevalence is 0.16% [37]. Approximately 20% of people with untreated chronic HBV and 15-30% of people with chronic HCV infection are estimated to develop cirrhosis within 20 years [38,39]. In the Netherlands approximately 500 people died annually between 2002 and 2015 due to the consequences of chronic HBV or HCV infection [40]. These deaths are to a large extent avoidable as effective antiviral treatment is available for chronic HBV infections and direct-acting antivirals (DAA’s) are accessible for all patients with chronic HCV infection in the Netherlands [41-43].

Unlike HBV and HCV infections, transmission routes of hepatitis E virus (HEV) are under debate. Recently, HEV has become a global public health concern, as an increase of autochthonous HEV infections was observed in developed countries. The most recent evidence suggests HEV caused by genotypes 3 and 4 is transmitted zoonotically. HEV is proven to be transmitted by ingesting uncooked or poorly cooked pork or game meat [44-46]. The hepatitis A virus (HAV) and HEV genotypes 1 and 2 are both single-stranded RNA viruses with similar incubation periods and a faecal-oral transmission route. Sexual activity, including oral-anal contact, as the major transmission route among men who have sex with men (MSM) is another widely documented aspect of HAV [47,48], but only a limited number of studies have focused on the possible sexual transmission route of HEV infections [49-53].

Study aim and methods

Because treatment for HBV and HCV infection has greatly improved, identification of infected individuals has become even more important. Yet, strategies to detect cases of HCV infection hidden in the general population have been disappointing. To capture all persons who have HCV infection, prospective studies are needed to evaluate the diagnostic yield of HCV testing strategies not based on exposure risk factors [54]. Birth cohort screening, as currently recommended in the United States (HCV prevalence of 2.0%), has also been suggested for European countries [55-57]. In simulation models, onetime HCV testing in the 1945-1965 birth cohort with subsequent treatment was likely cost-effective
in the United States [58]. According to a meta-analysis, targeted practitioner-based studies are most effective in increasing test uptake and cases detected [59]. Diagnostic yield could be further optimized by focusing on areas of higher prevalence. In this thesis we used geographical analyses and we examined this model and estimates in a real-life setting to identify hidden hepatitis B and C infections.

To explore the possible role of sexual transmission, in Chapter 7 we compared the HEV prevalence in a Dutch sexual high-risk population with the prevalence in a general population while assessing sociodemographic and sexual risk factors. In this thesis we used a seroprevalence study as method to identify hidden hepatitis E infections.

Main findings

Based on spatial-epidemiological analyses of laboratory data, the estimated prevalence of HCV infection in the south of the Netherlands is 0.5%, and we identified HCV clusters in urban areas, with a prevalence of up to 1%, as described in Chapter 5 [60,61]. In addition, an estimated 66% of HCV-infected persons were estimated to be untested and thus hidden to care [60]. All of this, made us develop a best practice approach by combining public health and primary care efforts in a birth cohort testing strategy as described in Chapter 6. We applied this approach in two identified hotspots in the Netherlands to detect hidden HCV and HBV infections in the general population. However, this best practice approach had a limited diagnostic yield. We were able to gain a higher than expected test uptake of 51%, but our approach completely failed to detect hidden HCV infections [62].

A higher prevalence was expected based on previous estimations in these hotspots (0.5% to 1%) [60,61], other Dutch studies focusing on risk groups (0.3% to 4.8%) [63-66], and (inter)national estimations of a substantial untested population (43% to 72%) [28-31]. The low positivity rate can be due to the possibility of selection bias. This cannot be ruled out, as many HCV-infected patients are at a disadvantage in terms of health literacy [67]. In the Netherlands, health insurance is obligatory, and general practitioners function as a gatekeeper to specialist care, but some individuals might have been less likely to get tested. Nevertheless, those who were tested and those who were not did not differ in assessed characteristics, such as working or living situation, or migrant status. Moreover, study participants had an lower educational level compared with those declining to be tested, and national estimates support our ability to reach intravenous drug users [68,69]. Another explanation for the low positivity rate can be found in the fact that estimates of the hidden HCV-infected population are largely derived from mathematical models. Additionally, the hotspots were determined based on laboratory data of the tested population. We assumed the hidden, untested population to follow the same geographical pattern as the tested population. Therefore, the number of hidden cases of chronic HCV infection seems to be overestimated within a real-life setting.

Recommendations for practice and future research

Lessons learned from this evidence-based practice can be applied to future studies. At first, as no active HCV infections were found in the identified hotspots, the hotspot strategy would probably not work
in other areas of the Netherlands as well and this also account for other countries with low HCV prevalence. Our findings partly informed the Dutch Health Council in their advice against screening of the general Dutch population for HCV and HBV (November 2016). This advice is part of the national hepatitis elimination plan, developed by specialists, doctors of the public health service and GPs, aimed at the prevention of transmission and reduction of hepatitis related disease burden and deaths. The committee recommends case finding by GPs for people in risk groups and by institutions who are responsible for delivering care to people in risk groups or for accommodating these people. The GP guidelines in the UK and the Netherlands advice to focus more on targeted HBV testing, especially among non-western migrants. This is in line with the recommendation of the Dutch Health Council on regional HBV screening programmes in areas with relatively large groups of first-generation migrants from high-endemic countries (≥2% chronic HBV infection). In the Netherlands, PWID and HIV-positive MSM are tested for HBV and/or HCV as part of comprehensive health care programs (i.e., HIV treatment, HBV vaccination, and harm reduction programs for PWID) [70-72]. However, for migrants who account for a relatively large proportion of HBV and HCV infections [73], there are no structural screening programs. This may partly be caused by the heterogeneity of this population, in terms of ethnic origin, risk for infection and awareness of infection status. Some screening interventions in the Netherlands have targeted different migrant groups [63,64,66,74-76]. These interventions, however, were limited in geographical coverage, and uptake was often low/moderate depending on the screening model. Structural screening programs for (subgroups of) migrants are needed to substantially reduce chronic viral hepatitis related morbidity and mortality among migrants, and the sustainability, linkage to care and access to treatment has to be ensured. Several interventions to further optimize the continuum of care have been described [77]. In addition, the feasibility and cost-effectiveness of introducing HCV to the prenatal screening program among first generation migrants could be studied. Future studies could also focus at the knowledge gap on the effectiveness of HBV/HCV screening among asylum seekers.

Additionally, our testing strategy could be applied to detecting HCV and HBV in high-endemic countries, to other infectious diseases and high-endemic settings. Higher positivity rates have been found in studies of countries with higher prevalence and in settings other than the general population with higher prevalence, such as the emergency department. An American study showed that HCV-infected individuals are more likely to use the emergency department (ED) for care than any other health care setting [78]. HCV testing in the ED was found acceptable by 63% [79]. Testing in this setting reveals hidden infections as this testing could reach those who do not respond to screening projects, do not feel at risk or avoid health care in general. Furthermore, test-uptake in this setting may be very high as tests can be offered and performed within routine diagnostic work-up. Several studies in the UK and US support routine testing on HCV in the ED setting with test-uptake varying from 27 to 66% and anti-HCV prevalence from 2 to 11% [80-83]. In the context of applicability, it is shown that routinely offering HCV testing to patients who are undergoing laboratory testing had no effect on length of stay at the emergency department [82].
Consciousness of health care providers, for example to prompt viral hepatitis testing when people present with abnormal liver function, is needed to identify those infected who do not belong to one of the risk groups. Automated screening alerts for ALAT elevation at the GP clinic can be a tool to detect hidden HCV infections, although this is a challenging process. The risk of HCV infection is substantially elevated in Dutch primary care patients with an ALAT elevation between 50 IU/l and 100 IU/l, whereas the risk of HBV infection is not [84]. Therefore, diagnostic follow-up for HCV is recommended in all these patients, particularly in those for whom no clear explanation for the ALAT elevation is found [84,85]. Among HBV patients without an indication for referral, ALAT should be checked during three years, both the GP and the patient should be reminded for these check-ups [86].

Future projects aimed at detecting chronic HBV and HCV infections hidden to care should focus on retrieval projects. This includes retracing and evaluating patients previously diagnosed with HBV or HCV who are eligible for (new) treatment. In the Netherlands, there is evidence for patients diagnosed with HBV or HCV who are eligible for treatment still remain untreated [87]. These patients were lost to follow-up as they stopped treatment because of the side effects, were not able to start treatment because of contra-indications, or did not attend their follow-up appointments. The Health Council of the Netherlands recommend retracing people who have been diagnosed with HBV or HCV in the past but are no longer receiving care [88]. This retracing should focus on giving patients the opportunity to make use of the new treatment options. Once they have been retraced, they must promptly be given appropriate care. Studies on detection of chronic hepatitis B and C patients who were lost to follow up, enables evaluation of the indication for current treatment options [89,90].

Currently, a national registry of all HBV and HCV infected patients is in development and will include patients characteristics and treatment data. HBV infections and recent HCV infections are notifiable diseases. Until now, chronic HCV infections are not notifiable. Therefore, there is no national surveillance system nor a database for chronic HCV infections. The surveillance of HCV could be improved by introducing chronic HCV infections into the notification policy. This would also make the current HBV and HCV estimated prevalence more precise.

At last, our results only hint at the possibility of a sexual transmission route for HEV given higher rates in those with chlamydia and/or gonorrhoeal infections. Sexual transmission is not a dominant transmission route, however, as its prevalence was not higher for the sexual high-risk population than for the general population. Whether sexual transmission of HEV occurs, and whether it could consequently lead to outbreaks such as HAV in MSM, should preferably be studied at the individual level in future prospective research. Considering the limited clinical impact of HEV infections, we do not recommend increased HEV testing in sexual high-risk clinical settings, with the possible exception of patients who are immunocompromised.
8.3 Concluding remarks

In conclusion, several methodologies can be used to detect hidden infections. This thesis showed the value of the surveillance pyramid as a tool to determine the different levels in infectious disease control and factors influencing its accuracy. Both epidemiological and geographical analyses compliment each other in identifying risk groups and estimating the prevalence of infectious diseases including hidden infections. However, there is no one-size-fits-all approach which can be applied to every infectious disease. Furthermore, this thesis demonstrates the importance of examining models and estimates in a real-life setting.
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Chapter 9

Appendices
The studies presented in this thesis aimed to identify and address hidden infections and evaluate current prevention, surveillance and control of infectious diseases.

In order to avoid pertussis-related infant morbidity and mortality, pertussis surveillance data are used to guide pertussis control measures. **Chapter 2** provides more insight into the accuracy of pertussis surveillance and control, and into the range of healthcare and public health-related factors that hamper the identification of hidden pertussis infections. We analysed a unique combination of data sources from one Dutch region of 1.1 million residents, including data from laboratory databases and local public health notifications. This large surveillance study (12,090 pertussis tests) reveals possible misdiagnoses, substantial under-notification (18%) and a delay between patient symptoms and notification to the local public health services (median 34 days, IQR: 27–54). In addition to the introduction of maternal immunisation, based on current findings, we further recommend greater adherence to testing guidelines, standardisation of test interpretation guidelines, use of automatic notification systems and earlier preventive measures.

In **Chapter 3** we used geographical analyses to identify clusters of pertussis infections. We analysed laboratory registry data including all geocoded pertussis tests in the southern area of the Netherlands. Socio-demographic and infrastructure related population data were matched to the geo-coded laboratory data. Testing for pertussis follows to an extent the general healthcare seeking behaviour for common respiratory infections, whereas the current pertussis incidence is largely the result of testing. More testing would thus not necessarily improve pertussis control. Detecting outbreaks using space-time cluster detection is feasible but needs to be adjusted for the strong impact of testing on the detection of pertussis cases.

**Chapter 4** describes, additional to Chapter 2, healthcare-related factors that hamper the identification of hidden pertussis infections. A total of 122 GPs (21%) completed an online questionnaire on their diagnostic practices and perceived facilitators, barriers and intention towards pertussis testing. In contrast to national guidelines, that advise to perform laboratory testing for pertussis in patients at risk for (causing) complications from pertussis infections, general practitioners report to mainly perform laboratory diagnostic tests for pertussis in patients with clinical symptoms. This is in accordance with GPs’ expressed facilitators and barriers to test for pertussis. We recommend GPs to focus pertussis testing more on family members surrounding incompletely vaccinated infants <1 year of age, women >34 weeks pregnant and children with severe comorbidities such as heart or lung failure.

In **Chapter 5** hotspots of HCV infections and risk factors within these hotspots were identified to facilitate targeted, evidence-based testing strategies. We analysed 23,800 HCV tests performed in the southern area of the Netherlands. Population data were matched to the geocoded HCV test data. Based on spatial-epidemiological analyses of laboratory data, the estimated prevalence of HCV infection in the south of the Netherlands is 0.5%, and we identified HCV hotspots in urban areas, with a prevalence of up to 1%. The main population at risk were middle-aged males, non-western immigrants and divorced persons. Socio-economic determinants consisted of one-person households, persons with low income and mean property value. However, the association between HCV risk and demo-
graphic as well as socio-economic determinants displayed strong regional and intra-urban differences. These results underline that a one-size-fits-all approach may not be appropriate.

In Chapter 6 we determined the diagnostic yield (test uptake and rate of positive test results) of a combined public health and primary care birth cohort testing strategy in detecting hidden cases of HCV and HBV infections. We invited all patients aged between 40 and 70 years (n=6,743) registered with 11 family practices serving 2 HCV hotspots, in the south of the Netherlands. This best practice testing strategy was effective in achieving a high test uptake of 50.9%. It completely failed, however, to detect hidden chronic HCV infections and is not recommended for countries with a low prevalence of the disease. The success factor of our high test uptake is the combination of a personal invitation accompanied by reminders, and the cooperation between public health care and primary care. Moreover, our findings informed the Dutch Health Council in their recommendation not to screen the general population for HCV and HBV infection.

In Chapter 7 we studied possible sexual transmission of HEV using a seroprevalence study as method to identify hidden hepatitis E infections. We compared the HEV prevalence in a Dutch sexual high-risk population with the prevalence in a general population while assessing sociodemographic and sexual risk factors. Overall prevalence of anti-HEV IgG of 18.9% (n=562) was, adjusting for confounders, similar between the two populations. Prevalence was higher with each year's increase in age, among men and among individuals diagnosed with sexually transmitted infections. Our results only hint at the possibility of a sexual transmission route for HEV given higher rates in those with chlamydia and/or gonorrhoeal infections. Sexual transmission is not a dominant transmission route, as its prevalence was not higher for the sexual high-risk population than for the general population. Considering the limited clinical impact of HEV infections, we do not recommend increased HEV testing in sexual high-risk clinical settings, with the possible exception of patients who are immunocompromised.

In Chapter 8 the main findings of this thesis and recommendations for future policy and research on hidden infections are discussed. This is illustrated by recent studies on pertussis and hepatitis. In conclusion, several methodologies can be used to detect hidden infections. This thesis showed the value of the surveillance pyramid as a tool to determine the different levels in infectious disease control and factors influencing its accuracy. Epidemiological and geographical analyses compliment each other in identifying risk groups and estimating the prevalence of infectious diseases including hidden infections. However, there is no one-size-fits-all approach which can be applied to every infectious disease. Furthermore, this thesis demonstrates the importance of examining models and estimates in a real-life setting.
Samenvatting
De studies zoals gepresenteerd in dit proefschrift zijn gericht op het identificeren van verborgen infecties en evalueren de huidige preventie, surveillance en bestrijding van infectieziekten.

Surveillancedata zijn leidend voor het nemen van de juiste maatregelen en het voorkomen van morbiditeit en mortaliteit door kinkhoest bij zuigelingen. **Hoofdstuk 2** geeft meer inzicht in de nauwkeurigheid van kinkhoest surveillance en de verschillende factoren in de (volks-)gezondheidszorg die het opsporen van verborgen infecties belemmeren. We analyseerden een unieke combinatie van databronnen in een regio met 1.1 miljoen inwoners, bestaande uit laboratoria data en GGD-meldingen. Deze grote surveillance studie (12,090 kinkhoest testen) onthulde mogelijke misdiagnoses, veel onderrapportage (18%) en een grote vertraging tussen eerste ziektedag en de uiteindelijk melding aan de GGD (mediaan 34 dagen, IQR: 27–54). Aanvullend op het introduceren van maternale vaccinatie, adviseren we een hogere naleving van test richtlijnen, het standaardiseren van test interpretatie richtlijnen, het gebruik van gaautomatiseerde meldingssystemen en het eerder nemen van preventieve maatregelen.

**Hoofdstuk 3** gebruikten we geografische analyses om clusters van kinkhoest infecties te identificeren. We analyseerden alle kinkhoest testen met postcodes van de laboratoria in Limburg. Sociaal-demografische kenmerken en gegevens over de infrastructuur werden gekoppeld aan deze geografisch-gecodeerde testdata. Het testen op kinkhoest wordt vooral bepaald door het hulpzoekgedrag van de patiënt, terwijl de kinkhoest incidentie vooral het resultaat is van testen. Meer testen verbeterd dus waarschijnlijk niet de kinkhoest bestrijding. Het detecteren van uitbraken is mogelijk met deze geografische analyses, maar er moet dan wel gecorrigeerd worden voor het testgedrag per regio.

**Hoofdstuk 4** beschrijft, aanvullend op Hoofdstuk 2, factoren in de gezondheidszorg die het opsporen van verborgen kinkhoest infecties belemmeren. In totaal vulden 122 huisartsen (21%) een online vragenlijst in over hun kinkhoest diagnostiek, bevorderende factoren, belemmerende factoren en de intentie om op kinkhoest te testen. In tegenstelling tot nationale richtlijnen rapporteren huisartsen vooral diagnostiek in te zetten voor kinkhoest bij patiënten met symptomen. Dit aanvraaggedrag komt overeen met de door de huisarts gerapporteerde bevorderende en belemmerende factoren om te testen op kinkhoest. Huisartsen zouden kinkhoest testen meer moeten richten op gezinsleden met niet- of onvolledig gevaccineerde kinderen <1 jaar, vrouwen die >34 weken zwanger zijn en kinderen met ernstig onderliggend hart- of longlijden.

**Hoofdstuk 5** werden hotspots voor hepatitis C infecties en risicofactoren in kaart gebracht om gerichte, evidence-based test strategieën mogelijk te maken. We analyseerden 23,800 HCV testen in Zuid-Limburg. Bevolkingsgegevens werden aan deze testdata gekoppeld. Gebaseerd op geografische en epidemiologische analyses van deze laboratoria data is de geschatte HCV prevalentie 0.5% in Zuid-Limburg met een oplopende prevalentie tot 1% in de gedetecteerde hotspots. De risicopopulatie bestond uit mannen van middelbare leeftijd, niet-westse migranten en gescheiden personen. Sociaaleconomische determinanten waren éénpersoons huishoudens, personen met een laag inkomen en de gemiddelde woningwaarde. De associatie tussen deze demografische, sociaaleconomische determinanten en het risico op HCV verschilde echter sterk per regio en stadsdeel. Deze resultaten onderstrepen dat een one-size-fits-all aanpak niet van toepassing is.
In Hoofdstuk 6 bepaalden we de diagnostische opbrengst (respons en percentage positief getest) van een geboortecohort test strategie. Dit was een samenwerking van publieke gezondheidszorg en eerstelijnszorg om verborgen HCV en HBV infecties te detecteren. Alle patiënten tussen de 40 en 70 jaar oud (n=6,743) van 11 huisartspraktijken in twee HCV hotspots in Zuid-Limburg werden uitgenodigd. Deze teststrategie was effectief in het bereiken van een hoge respons van 50.9%. Er werden daarentegen geen verborgen chronische HCV infecties opgespoord. Daarom wordt deze strategie niet aangeraden in andere landen met een lage HCV prevalentie. Succesfactoren van de hoge respons zijn de combinatie van een persoonlijke uitnodiging met herinneringen en de samenwerking van publieke gezondheidszorg en eerstelijnszorg. Onze bevindingen informeerden bovendien de Nederlandse gezondheidsraad in hun advies om niet de algemene bevolking te screenen op HCV en HBV infecties.

In Hoofdstuk 7 onderzochten we de mogelijke seksuele transmissie van HEV in een seroprevalentie studie om verborgen hepatitis E infecties te identificeren. We vergeleken de HEV prevalentie tussen een populatie met een verhoogd seksueel risicogedrag en de algemene populatie en bepaalden sociodemografische en seksuele risicofactoren. De prevalentie van anti-HEV IgG (18.9%, n=562) was gelijk in de twee onderzochte populaties na het corrigeren voor confounders. De prevalentie was wel hoger bij een hogere leeftijd, onder mannen en bij mensen met een chlamydia en/of gonorroe infectie. Onze resultaten geven daarom slechts een kleine indicatie voor de mogelijke seksuele transmissie route van HEV. Seksuele transmissie is geen dominante transmissieroute omdat er geen verschillende prevalentie werd gevonden tussen de twee onderzochte populaties. Gezien de beperkte klinische impact van HEV infecties raden we niet aan om meer te testen op HEV bij mensen met verhoogd seksueel risicogedrag, met uitzondering van immuun gecomprimeerde patiënten.

In Hoofdstuk 8 worden de belangrijkste resultaten van dit proefschrift en aanbevelingen voor toekomstig beleid en onderzoek naar verborgen infecties bediscussieerd. Dit is geïllustreerd aan de hand van studies naar kinkhoest en hepatitis. Samenvattend kunnen er verschillende methodes worden gebruikt om verborgen infecties op te sporen. Dit proefschrift laat zien dat de meldingspiramide een waardevolle tool is om de verschillende niveaus en beïnvloedende factoren van de infectieziekten bestrijding in kaart te brengen. Epidemiologische en geografische analyses vullen elkaar aan in het identificeren van risicogroepen en het schatten van de prevalentie van verborgen infecties. Er bestaat echter geen one-size-fits-all aanpak die kan worden toegepast op alle infectieziektes. Dit proefschrift toont bovendien het belang aan van het toetsen van modellen en schattingen in de praktijk.
Valorisation
Besides its scientific relevance this thesis also has implications for society as we identified and addressed hidden infections and evaluated current infectious disease prevention, surveillance and control. Based upon this thesis and recent literature we have several recommendations on how to improve current practice and policy. Overall, better insight into hidden infections allows for more targeted and effective testing practices in order to eventually protect the most vulnerable groups at risk. Several methodologies can be used to detect hidden infections. This thesis showed the value of the surveillance pyramid as a tool to determine the different levels in infectious disease control and factors influencing its accuracy. Epidemiological and geographical analyses compliment each other in identifying risk groups and estimating the prevalence of infectious diseases including hidden infections. However, there is no one-size-fits-all approach which can be applied to every infectious disease. Furthermore, this thesis demonstrates the importance of examining models and estimates in a real-life setting.

Hidden pertussis infections

We were able to give more insight into the factors that hamper the identification of hidden pertussis infections. These findings function as an important input for the discussion on how to reduce the workload of Public Health Service (PHS) and the debate on how to reduce the overall delay until notification to improve pertussis control. Overall, it is likely that the identified misdiagnosis, under-notification and overall delay in surveillance data are not unique for this area of the Netherlands and are generalizable to the rest of Europe. These countries are likely to encounter varying guidelines, difficulties in pertussis diagnostics and unexplained local differences in notification data too. Ideally, the accuracy of surveillance would be improved by focusing on the factors identified here. This contributes to optimisation of the surveillance system and more importantly, to improved pertussis control by preventing young infants from being infected with pertussis and avoiding morbidity and mortality in this young group.

First, to reduce misdiagnosis and the variation in pertussis diagnostics, we recommend laboratories and healthcare providers (HCPs) to improve adherence to national guidelines concerning timing and choice of test. For laboratories, a national uniform and standardised guideline on cut-off values in serology and the use of IgA and/or IgG is desirable. Testing all patients presenting with cough is not feasible, as only 3% of adult patients presenting with acute cough in primary care had evidence of an acute pertussis infection in Europe. Moreover, testing for pertussis follows to an extent the general healthcare seeking behaviour for common respiratory infections, whereas the current pertussis incidence is largely the result of testing. Increased testing would thus not necessarily improve pertussis control. Therefore, HCPs should focus pertussis testing on family members surrounding incompletely vaccinated infants <1 year of age, women >34 weeks pregnant and children with severe comorbidities such as heart or lung failure. In addition, they are advised to check whether patients with symptoms
suggestive of whooping cough have vulnerable groups at risk in their household. As such, general practitioners (GPs) could play a more prominent role in contact tracing (now carried out by the PHS in the Netherlands) in order to minimise the disease burden in infants. We informed the GPs and laboratories in our region about our study results to remind them of the pertussis testing guidelines and to advise on possible preventive measures.

Second, to reduce under-notification, medical microbiology laboratories and HCPs could benefit from introducing automated notification systems. In an endemic situation with severe underreporting, the obligatory notification of pertussis is not effective for the protection of unvaccinated children. On the other hand, the notification of pertussis infections is of great value to national policy on pertussis surveillance and control although we showed the limitations of pertussis surveillance. Pertussis has to remain a notifiable disease as it is necessary to prohibit a pertussis-positive patient to go to work when he/she works with vulnerable young infants. In this thesis, we showed that the detection of pertussis outbreaks using space-time cluster detection is feasible and could help to facilitate an early and appropriate public health response. However, this approach has to be adjusted for the strong dependency to testing and is probably most efficient when using test-positivity as indicator.

Third, as public health is almost always too late to intervene, delays need to be reduced and we need earlier preventive measures by healthcare providers. Patient delays due to late presentation to healthcare may be unavoidable, however delays in reporting once a patient presents can be reduced. GPs, midwives and day nursery employees could play a major role here. Creating awareness among these professionals as well as patients themselves about taking timely preventive measures could lead to lower individual disease burden and to a higher cost-effectiveness of pertussis care.

At last, we also need additional preventive measures for the most vulnerable and susceptible group of unvaccinated infants, as the Dutch national vaccination program starts at the age of two months. In the first few months, young infants are unprotected as vaccination is only effective after at least three doses. The efficacy of pertussis vaccines has been debated because of waning immunity, incomplete protection of infants below 5 months, genetic changes in *Bordetella pertussis* and limited duration of protection. Currently, maternal immunisation provides good protection against infant disease, whilst no important side effects were observed, and has been recommended by the World Health Organisation and the European Centre for Disease Prevention and Control. The Dutch health council advised the introduction of maternal vaccination to the national vaccination program (December 2015) and pertussis vaccinations for healthcare workers (June 2017). Maternal vaccination will be executed at the child healthcare centres and introduced to the national vaccination program in 2019.

**Hidden hepatitis infections**

Lessons learned from our evidence-based practice to detect hidden hepatitis infections can be applied to future testing strategies and policy. The success factors of our high test uptake were the combina-
tion of a personal invitation letter accompanied by reminder letters, the birth cohort testing, the hotspot approach and the cooperation between public health care and primary care.

At first, as no active hepatitis C virus (HCV) infections were found in the identified hotspots, the hotspot strategy would probably not work in other areas of the Netherlands as well as other countries with low HCV prevalence. Our findings partly informed the Dutch Health Council in their advice against screening of the general Dutch population for HCV and HBV (November 2016). This advice is part of the national hepatitis elimination plan, developed by specialists, doctors of the public health service and GPs, aimed at the prevention of transmission and reduction of hepatitis related disease burden and deaths. The committee recommends case finding by GPs for people in risk groups and by institutions who are responsible for delivering care to people in risk groups or for accommodating these people. The GP guidelines in the UK and the Netherlands advice to focus more on targeted HBV testing, especially among non-western migrants as they account for the largest proportion of HBV and HCV infections. This is in line with the recommendation of the Dutch Health Council on regional HBV screening programmes in areas with relatively large groups of first-generation migrants from high-endemic countries (≥2% chronic HBV infection). In the Netherlands, people who inject drugs (PWID) and HIV-positive men who have sex with men are tested for HBV and/or HCV as part of comprehensive health care programs (i.e., HIV treatment, HBV vaccination, and harm reduction programs for PWID). However, for migrants there are no structural screening programs. This may partly be caused by the heterogeneity of this population, in terms of ethnic origin, risk for infection and awareness of infection status. Some screening interventions in the Netherlands have targeted different migrant groups. These interventions, however, were limited in geographical coverage, and uptake was often low/moderate depending on the screening model. Structural screening programs for (subgroups of) migrants are needed to substantially reduce chronic viral hepatitis related morbidity and mortality among migrants, and the sustainability, linkage to care and access to treatment has to be ensured.

Additionally, our testing strategy could be applied to detecting HCV and HBV in high-endemic countries, to other infectious diseases and high-endemic settings. Higher positivity rates have been found in studies of countries with higher prevalence and in settings other than the general population with higher prevalence, such as the emergency department. An American study showed that HCV-infected individuals are more likely to use the emergency department (ED) for care than any other health care setting. Testing in this setting reveals hidden infections as this testing could reach those who do not respond to screening projects, do not feel at risk or avoid health care in general. Furthermore, test-uptake in this setting may be very high as tests can be offered and performed within routine diagnostic work-up. Several studies in the UK and US support routine testing on HCV in the ED setting with high test-uptakes and anti-HCV prevalence. In the context of applicability, it is shown that routinely offering HCV testing to patients who are undergoing laboratory testing had no effect on length of stay at the emergency department.

Consciousness of health care providers, for example to prompt viral hepatitis testing when people present with abnormal liver function, is needed to identify those infected who do not belong to one of
the risk groups. Automated screening alerts for ALAT elevation (diagnostic liver enzyme) at the GP clinic can be a tool to detect hidden HCV infections, although this is a challenging process. The risk of HCV infection is substantially elevated in Dutch primary care patients with an ALAT elevation between 50 IU/l and 100 IU/l, whereas the risk of HBV infection is not. Therefore, diagnostic follow-up for HCV is recommended in all these patients, particularly in those for whom no clear explanation for the ALAT elevation is found. Among HBV patients without an indication for referral, ALAT should be checked, both the GP and the patient should be reminded for these check-ups.

Future projects aimed at detecting chronic HBV and HCV infections hidden to care in the Netherlands should focus on retrieval projects. This includes retracing and evaluating patients previously diagnosed with HBV or HCV who are eligible for treatment. In the Netherlands, there is evidence for patients diagnosed with HBV or HCV who are eligible for treatment still remain untreated. These patients were lost to follow-up as they stopped treatment because of the side effects, were not able to start treatment because of contra-indications, or did not attend their follow-up appointments. The Health Council of the Netherlands recommend retracing people who have been diagnosed with HBV or HCV in the past but are no longer receiving care. This retracing should focus on giving patients the opportunity to make use of the new treatment options. Once they have been retraced, they must promptly be given appropriate care. Studies on detection of chronic hepatitis B and C patients who were lost to follow up, enables evaluation of the indication for current treatment options.

Currently, a national registry of all HBV and HCV infected patients is in development and will include patients characteristics and treatment data. HBV infections and recent HCV infections are notifiable diseases. Until now, chronic HCV infections are not notifiable. Therefore, there is no national surveillance system nor a database for chronic HCV infections. The surveillance of HCV could be improved by introducing chronic HCV infections into the notification policy. This would also make the current HBV and HCV estimated prevalence more precise.

We have provided scientific and societal relevance in the work presented. We identified and addressed hidden infections and evaluated current infectious disease prevention, surveillance and control. Based upon this work and recent literature we suggested on improvements of current practice and policy in hepatitis and pertussis control. Overall, better insight into hidden infections allows for more targeted and effective testing practices in order to eventually protect the most vulnerable groups at risk. Although, there is no one-size-fits-all approach we showed several methodologies that can be applied to the assessment of hidden infections in infectious diseases.
Dankwoord
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Chapter 9

Courses and training

2016
Analytic Story Telling
by Evert-Jan Kamerbeek

Workshop “Crafting the Valorisation Paragraph for your Doctoral Thesis”
by Ivo George

Presentation Skills
by John Rouvroye and Mabel Frumau

2015
Online Academic Writing Skills (English for PHD Students)
by Neill Wylie

Heuvellandcursus (cursus schrijven en publiceren)
by Jochen Cats and Daniel Kotz

Presentations

International
European Scientific Conference on Applied Infectious Disease Epidemiology
Saint Julian’s, Malta (2018)

European Scientific Conference on Applied Infectious Disease Epidemiology
Stockholm, Sweden (2017)

26th European Congress of Clinical Microbiology and Infectious Diseases
Amsterdam, the Netherlands (2016)

National

Annual Research Day Medical Microbiology, Maastricht (2016)

Nationale Hepatitis Dag, Amsterdam (2016)
Annual CAPHRI researchday, Maastricht (2016)

Nederlandse Huisartsen Genootschap Wetenschapsdag, Amsterdam (2016)

26e Transmissiedag Rijks Instituut Volksgezondheid en Milieu, Amersfoort (2016)

Annual Research Day Medical Microbiology, Maastricht (2015)

Nationale Hepatitis Dag, Amsterdam (2015)

Nationale Hepatitis Dag, Amsterdam (2014)

Grants

Travel grants
Personal travel grant to the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE), 6-8 November 2017, Stockholm, Sweden

Personal travel grant for the 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), 9-12 April 2016, Amsterdam, the Netherlands

Funding from industry
HCV retrieval project in South Limburg. Retrieval of patients diagnosed with chronic hepatitis C in the past who were lost to follow-up
Period: October 2017 – September 2018
Funded by: Gilead Sciences Netherlands B.V. and MSD B.V.

Memberships

RIVM expert board on HCV/HBV prevalence estimations

Workgroup on health literacy at Public Health Service of South-Limburg
List of publications
2018


Heil J, Cals JWL, ter Waarbeek HLG, Hoebe CJPA, Dukers-Muijrers NHTM. Reasons for testing and non-testing for pertussis are not well know. Fam Pract 2018 (submitted)


2017


2015

“Winners... are not those who never fail,
but... those who never quit”
Banksy
CURRICULUM VITAE

Jeanne Heil was born on the 27th of November in 1991. In 2009, she graduated from high school at Carboon-college St. Jan in Hoensbroek. Following this she studied Health Sciences at Maastricht University - Health Promotion and Education and Movement Sciences. In 2013, she obtained her master degree in Health Sciences - Prevention and Public Health at Vrije Universiteit Amsterdam. In the summer of 2013, Jeanne worked as a volunteer in Masaka, Uganda. Thereafter, she started working at the Department of Sexual Health, Infectious Diseases and Environmental Health at the Public Health Service of South Limburg. Under the supervision of Prof. dr. Christian Hoebe, Prof. dr. Jochen Cals and dr. Nicole Dukers-Muijers, she did her PhD at the Department of Medical Microbiology of the Maastricht University Medical Centre (MUMC+) and the Care and Public Health Research Institute (CAPHRI). Currently, she is involved in several research projects and will continue to work at the Public Health Service South Limburg.

UITNODIGING

Voor het bijwonen van de openbare verdediging van het proefschrift:

Optimizing infectious disease control: how to detect hidden infections? Illustrated by studies on pertussis and hepatitis

Op donderdag 25 oktober 2018 om 12.00 uur in de aula van de Universiteit Maastricht, Minderbroedersberg 4-6 te Maastricht.

Na afloop bent u van harte welkom op de receptie ter plaatse.

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