SEROEPIDEMIOLOGY IN PREVENTION AND CONTROL:

VACCINE-PREVENTABLE DISEASES IN CARIBBEAN NETHERLANDS AND SARS-COV-2 IN THE NETHERLANDS

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Voor mijn ouders

'Het is een krankzinnig avontuur'
Hans van Mierlo

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CHAPTER 1

General introduction

A brief history of public health surveillance

The 'great pestilence' was the first recorded epidemic in human history and dates back to ancient Egypt (3180 B.C.) [1]. Hippocrates (460–370 B.C.), the 'father of medicine' and 'first epidemiologist', started to regularly collect and analyze data for determination of illness, and created terms as 'endemic' and 'epidemic' [2, 3]. After the 'great pestilence', several major epidemics have been documented. The most devastating are considered the 'plague of Justinian' (541–549, followed by multiple major waves up until the 8th century) and 'Black Death' (1348–1351), both caused by *Yersinia pestis*; and the 'Spanish flu' (1918-1920), attributable to influenza H1N1 [1, 4]. After introduction of vaccinations and antibiotics in the preceding centuries, infectious diseases were believed to have been problems of the past [5]. Nonetheless, pathogens adapted, re-emerged, and novel ones with pandemic potential arose. In the two previous decades alone, zoonotic spillover events caused the emergence of influenza type A(H1N1)pdm09 ('swine flu') and novel coronaviruses: severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle-Eastern respiratory syndrome coronavirus (MERS). The most recent pandemic causing a global crisis was SARS-CoV-2, causative agent of coronavirus disease 2019 (COVID-19), first detected in China in late 2019 [6].

Three types of information are generally documented in historical records of epidemics: the health outcome, risk factors and interventions. Health outcomes measure the state of public health, risk factors (or determinants) denote the features that skew the health outcome, and interventions apply the information on risk factors to control the disease [7]. Effectively using surveillance has been documented for the first time during the 'Black Death' when ships were prohibited from docking at the port for forty days, which gave rise to the term 'quarantine' – a control measure still considered one of the most effective during outbreaks [8, 9]. The concept of ongoing collection of data started in the 16th century when the town council of London kept count of mortality due to the plague, albeit not systematically and without use for actual surveillance purposes [10]. The concept of modern surveillance was created two centuries later by William Far, who practiced systematic ongoing collection and comprehensive analysis of statistics to describe the impact of diseases. Findings were reported to responsible authorities and the general public, and surveillance efforts were used to develop legislation and social change. A modern surveillance system was established in which the state was deemed responsible for the health of its people [11]. Nowadays, the World Health Organization (WHO) defines public health surveillance as 'the continuous and systematic collection, orderly consolidation and evaluation of pertinent data with prompt dissemination of results to those who need to know, particularly those who are in a position to take action' [12]. Effective surveillance and response systems are essential in controlling infectious diseases. Primarily to recognize

cases and clusters to halt transmission, and on a longer time scale: to identify specific risk factors within populations to enable targeted interventions; to address the need for (preventive) evidence-based interventions and resources; to monitor trends; and to facilitate research to demonstrate the impact of interventions and test hypotheses in newly developed research designs [13].

Vaccination: a cornerstone of public health

Vaccination is among the most effective preventive measures in history, and therefore often regarded as a cornerstone of public health [14, 15]. In the late 18th century, Edward Jenner demonstrated that his practice of 'variolation' induced protection against smallpox [5, 16]. Two centuries later, smallpox was the first infectious disease to be declared eradicated by the World Health Assembly in 1980 [17].

Vaccines and immune response

Vaccines generally contain (purified) antigens derived from a pathogen, produced synthetically to mimic components, or comprise the whole pathogen. This safely induces an immune response, (preferably) before encountering the pathogen, aiming to protect against infection and disease upon subsequent exposure due to swift activation of the immune system [16]. Principally, when initially encountering an antigen – either via vaccination or infection – an immune response is induced through production of molecules and activation of cells. These responses are generally divided into non-specific: mediated by the innate system, which is fast (minutes to days) and present from birth; and specific: via recognition of specific antigens, mediated by T- and B-lymphocytes (adaptive immunity), which is acquired and takes longer to develop (few days to weeks) but usually maintained over a prolonged time (via immune memory) [5]. An effective response is greatly influenced by communication between these two systems. T-cells differentiate into T-effector- and memory-cells, prepared to eliminate infected cells, and can drive B-cell development. B-cells differentiate into B-memory-cells and plasma-cells that produce and secrete specific antibodies belonging to the immunoglobulin (Ig) family [18]. Antibodies are capable of binding specific extracellular pathogens in order to neutralize them directly, or activate other immune responses via agglutination and opsonization, such as the complement system or other cells with cytotoxic mechanism or those capable of phagocytosis [19, 20]. A rapid rise in serum antibody levels (humoral immunity) is observed in the first weeks, which usually starts with isotype IgM (and IgA). Whilst IgM levels wane after a few weeks to months, isotype switching due to B-cell maturation causes IgG levels to rise somewhat later but sustain over longer periods [18]. Most persons seroconvert after initial exposure, however the height of the antibody level and endurance of the response depend on multiple factors,

such as age, sex, immunocompetence, comorbidities, vaccine-platform, -dosage and -schedule, or – if infected – severity of disease (local/systemic) [16, 21]. A potential encounter hereafter, i.e., with the pathogen itself or via a booster dose, generally quickly results in multifold higher levels of IgG due to immune memory. These antibodies are capable of binding antigens with higher affinity and avidity, reflective of a matured immune response, facilitating to prevent infection and/or disease [5, 18].

Traditionally, vaccines have been characterized as live – containing attenuated replicating strains of the organism – or non-live (or inactivated) – comprising only components of a pathogen often combined with an adjuvant [16]. A century after Jenner, Louis Pasteur's work on the rabies vaccine led to development of new vaccines [22]. The first vaccines against typhoid, diphtheria and tetanus, as inactivated pathogen-products or toxoids, were approved in the beginning of the 20th century. Alongside the development of viral vaccines in the 1930s, the number of vaccines increased and urged the need for development of combination vaccines comprising multiple targets. The first licensed combinations were those against diphtheria and tetanus (DT) in 1947, two years later pertussis was added (DTP), and several others followed, e.g., measles-mumps-rubella (MMR) in the 1980s [18]. Other platforms have been developed more recently, e.g., viral-like particles, viral vectors, and nucleic acid-based ((m)RNA and DNA); vaccines focused on providing the genetic code needed to assemble the immunogenic antigen to induce an immune response [16].

The National Immunization Program (NIP)

Public health outcomes transformed tremendously after vaccines were combined with comprehensive coordinated National Immunization Programs (NIP). Since the 1950s, the first programs became established and properly coordinated [16]. In countries with high vaccination coverage, many of the diseases that had been responsible for the vast majority of childhood deaths and long term sequelae reduced dramatically [14, 15], of which pertussis, diphtheria and polio are striking examples in the Netherlands [23]. In 1974, the WHO initiated the Expanded Program on Immunization ensuing that children across the world would benefit from vaccines. The goal was set to make vaccination against DTP, poliomyelitis, measles and tuberculosis available for every child globally by 1990 [24]. Unfortunately that goal was not reached, but stimulated by the WHO's Sustainable Development Goals – particularly those regarding reducing under-5-years death rates – nearly all countries around the world have a (childhood) program in place nowadays [25]. This has currently resulted in an estimated 2–3 million lives saved per annum, and these global efforts contributed to a reduction from 93 deaths per 1,000 livebirths in 1990 to 39 in 2018 [26]. Global coverage of three doses of the DTP-vaccine has risen to 84% in 2022, however nearly 22 million children missed their first dose

of measles (which is almost 3 million more than in 2019). Hence, a lot of work is still required, especially in low-income countries that lag behind greatly [27].

Goals and strategies of the NIP

Decisions around the NIP, such as optimalization of the program or introduction of new vaccines, are based on evidence-based recommendations made by National Immunization Technical Advisory Groups (NITAG) or expert committees in most countries. Immunization policies might vary reflected by differences in healthcare structure, respective financing and epidemiology [5]. In the Netherlands, like in any other sovereign state, the government is responsible for protecting the people and society against serious infectious diseases whilst achieving a fair distribution of care. The NIP was established in 1957. The minister of Health, Welfare and Sport (VWS) decides which vaccinations should be included in the program, and the Health Council is requested to provide advice [28]. Strategies to support the primary objective of the NIP – i.e., protecting the population by means of vaccination – are multifold and follow a distinct hierarchy [5, 29]:

- to eliminate or eradicate serious infectious diseases;
- to reach and maintain herd immunity where possible;
- to protect as many individuals in the vulnerable group(s) as possible.

Reaching these objectives first and foremost depend on the vaccination coverage, vaccine type and specific pathogen. High coverage usually results in reduced morbidity and mortality of the target population due to direct protection and prevention of severe clinical manifestation; e.g., the primary objective for diphtheria and (cancer due to) human papillomavirus (HPV); particularly when introduced just recently in the program. Besides prevention of disease, some vaccines also protect against infection which reduces or even blocks the acquisition of a pathogen and onward transmission [29]. Disrupting transmission combined with a high vaccination coverage relative to the basic reproductive number (R0) – i.e., the expected number of secondary cases produced by one index case in a completely susceptible population – could result in herd immunity [16, 18]; the main objective for mumps vaccination for instance [29]. Individuals who are not immunized – e.g., due to age, contraindications or refusal – or those who lack a sufficient immune response due to immunodeficiency or waning over time, benefit from this indirect effect [16]. Highly transmissible pathogens, e.g., measles or varicella-zoster virus (VZV), require a high number of immun(iz)e(d) persons (> 95%) to prevent outbreaks, and reach and maintain herd immunity. For some vaccines immunity might wane, for instance due to a lack of natural boosting of vaccinated individuals, and these require booster doses to maintain herd immunity [18]. Moreover, targeting specific risk groups for vaccination, e.g., because they have contacts frequently and are accountable for the

largest part of transmission, can also eliminate a pathogen and might therefore be a highly effective strategy to protect the whole community [5]. To illustrate, after introduction of the meningococcal vaccination in adolescents in the Netherlands in 2018 incidence has reduced greatly in all age groups [30]. Measles and Congenital Rubella Syndrome (CRS) are examples of candidates for elimination in several WHO Regions in the future; eradication of these diseases would however require tremendous additional global efforts [31].

Surveillance of the NIP

Evaluating the effectiveness and impact of the NIP on the short and long term is essential. Epidemiology might change, e.g., due to reduced coverage, waning immunity, or adaptation (variants) or emergence of novel pathogens. This requires continued vigilance and optimization while taking into account the benefit/risk balance for the individual and population, which in turn will enhance trust in the program and thus overall success [28-30]. In the Netherlands, the Centre for Infectious Disease Control (CIb) at the National Institute for Public Health and Environment (RIVM), commissioned by the Ministry of VWS, coordinates the control and surveillance of infectious diseases.

Five pillars can be distinguished within the Dutch surveillance system of the NIP [30]. These pillars are intertwined and together provide integrated understanding of the effectiveness of the program. Firstly, vaccination coverage [32]. Nationwide coverage is assessed via electronic national vaccination registries ('Praeventis' and 'CIMS') which can shed light on potential changes over time and groups with lower uptake. The second pillar is safety surveillance. Every individual can report (severe) adverse events following vaccination to the Dutch Pharmacovigilance ('Lareb'), and reports are published yearly [33]. Thirdly, disease surveillance. Care providers are obliged to notify NIP-diseases (via 'Osiris'), which provides understanding into changes in incidence – between groups – over time [30, 34]. Disease-specific hospitalization and deaths provide additional understanding (of alterations) in severity of disease. The fourth area of interest is pathogen surveillance. Possible strain variations in circulating pathogens are evaluated and give insights into potential changes in disease severity [35]. And finally, the fifth pillar is serosurveillance which provides understanding of vaccine-induced immunity and/or previous exposure to a pathogen through assessment of the presence of specific antibodies [9, 36-39].

Serosurveillance: a key tool in the toolbox

Serosurveillance is often called immunosurveillance, serological surveillance, a study of seroprevalence or a serosurvey, as the specimen mostly obtained is serum (from a blood sample) [40]. Serosurveillance is essential in guiding vaccination policy in terms of planning and shaping programs, and, once established, in monitoring and adjusting if needed [41-45]. When serology is linked to sociodemographic- and in-depth questionnaire data, risk factors can be assessed to identify susceptible pockets that may require specific attention; this field is referred to as seroepidemiology [46, 47]. This can complement potential poor systematic reporting of proof of vaccination or, when studied periodically, shed light on shifts in vaccination uptake, or waning immunity after vaccination and/or infection [41, 46, 48]. Additionally, in (partly) vaccination-naïve populations, serosurveillance can monitor prevalence, seroincidence (between timepoints) and dissemination of pathogens [49-52]. This allows estimation of cumulative incidence as – to a great extent – both asymptomatic and symptomatic cases are covered, and assessment of disease severity when linked to hospitalization and (excess) deaths [53]. Seroprevalence data are also important input for modelling, e.g., when used in transmission models to project the course of an epidemic or impact of future vaccination [54, 55].

Assessment of antibodies

Seroprevalence represents the proportion of a population above a defined serum antibody concentration – which, to note, does not represent a correlate of protection per se [49, 56, 57]. Serum antibody testing can be standardized enabling high-throughput which is preferred in large studies. Particularly the fluorescent bead-based multiplex immunoassay (MIA), i.e., a derivative of an enzyme-linked immunosorbent assay (ELISA), is valuable in this regard as it is capable of quickly quantifying multiple biomarkers simultaneously, and has a wide range of detection whilst necessitating low volumes [58, 59]. Each antigen of interest is coupled to differently color-coded/labeled beads and incubated with diluted serum samples. Adding conjugate (R-phycoerythrin anti-human IgG) enables assessment of the median fluorescent intensity, and these concentrations are often calibrated to international (or in-house) standards to maximize alignment of results. Assessment of multiple antigens from the same pathogen as well as the height of the antibody response can be useful trying to distinguish infection-from vaccine-induced antibodies [45, 60, 61].

Seroepidemiological studies in the (kingdom of the) Netherlands

Serosurveillance in the Netherlands is assessed periodically via the cross-sectional population-based PIENTER studies (in Dutch: 'Peiling Immunisatie Effect Nederland Ter Evaluatie van het Rijksvaccinatieprogramma'), in which participants aged 0–90

years across the Netherlands provide biological samples and fill out questionnaires on risk factors and vaccination status. The first study was conducted in 1995–96 [38], which was followed by a second study in 2006–07 [36], and more recently by a third study in 2016-17 (PIENTER-3) [37]. As part of the PIENTER-3-study, serosurveillance was performed in Caribbean Netherlands (CN) for the first time through the establishment of the Health Study CN in 2017 (**part 1** of this thesis) [37]. At the start of the SARS-CoV-2 pandemic in early 2020, PIENTER-3 was the foundation for the PIENTER-Corona (PICO) study: a prospective serosurveillance study to monitor SARS-CoV-2 in the Netherlands on a population- and immunological level (**part 2**).

Application of the seroepidemiological tool

Evaluation of population immunity of vaccine-preventable diseases in Caribbean Netherlands

In 1954, the former colonial Dutch Caribbean islands Aruba, Bonaire, Curaçao (ABC-islands), St. Maarten, St. Eustatius and Saba were united as a single country within the kingdom of the Netherlands: the Netherlands Antilles [62]. As Aruba did in 1986, Curaçao and St. Maarten voted for '*status aparte*' in a referendum and became autonomous countries on 10 October 2010. The Netherlands Antilles dissolved, and Bonaire, St. Eustatius and Saba became special municipalities/public bodies within the (European) Netherlands, and are since then referred to as CN or BES-islands [63]. The public bodies carry many functions performed by municipalities in the Netherlands, however since they are not part of a Dutch province, power is not exercised by a provincial council, but division is made between island governments and the central government via the National Office for CN (in Dutch: 'Rijksdienst CN') [64].

Public health in CN falls under the direct responsibility of the Dutch government. Each island harbors a public health department (in Dutch: 'Gemeentelijke Gezondheidsdienst', GGD) that executes policy [65]. There are multiple general practitioners active, and a hospital relative to the size of the island (in 2017, Bonaire had ~19,000 inhabitants, St. Eustatius ~3,250, and Saba ~2,000) [66, 67]. Implemented by Curaçao and designed in close collaboration with the Netherlands, the former Netherlands Antilles mainly followed a similar NIP with some slight alterations depending on the epidemiological situation and availability of vaccines in the region [68, 69]. More specifically, DTP-containing vaccines had already been administered since the 1940s, polio since the 1950s and some islands administered BCG-vaccination. Monovalent rubella was first introduced in 1975 via school-based programs for 10-year-old girls, and was replaced by routine vaccination for all infants in the beginning of the 1980s. Monovalent measles vaccination was administered to all infants in the mid-1970s. The MMR-vaccine was routinely given in the late 1980s, and in the beginning of the 1990s a booster dose (MMR-2) was offered.

In the mid-1990s, some islands introduced vaccines against *Haemophilus influenzae* and hepatitis B. Since 2010, the Dutch government has been responsible for the supply, execution and monitoring of the NIP in CN [65, 69]. The program has been harmonized with that in the Netherlands, and vaccines against hepatitis B (if not already implemented), pneumococcal disease, meningococcal disease, and HPV were added following advice from the Health Council [70]. Vaccinations for children aged < 4 years are provided at the Child/Youth Health Centers, and for school-aged children mostly at primary schools offered per school-year [69]. Vaccination coverage has been monitored routinely since harmonization with the Netherlands and is generally high [71]. An overview of the NIP in CN in 2017 (the year of the data collection of the Health Study CN) is shown in *Figure 1*.

Surveillance of infectious diseases in CN relies greatly on symptom reporting. Syndromic surveillance by selected general practitioners on the former Dutch Antilles was initiated in 2007, and acts as early warning system. CN has a notification obligation for NIP-diseases [65], however a fair number of cases may remain undetected due to a lack of facilities [72]. No population-based serosurveillance study on protection against and susceptibility to vaccine-preventable diseases has been conducted thus far [73], complicating evidence-based policy, which has mostly relied on limited data and experiences from the field. The Health Study CN was set up to fill this knowledge gap and can provide valuable information for the whole region as studies are scarce [37]. Specific focus for this thesis will be on seroepidemiology of MMR, diphtheria, HPV and VZV (see *Table 1* for detailed information on these diseases regarding the pathogen, transmission, symptoms and potential (severe) complications, and vaccination coverage).

Bonaire

Fase 1	Inenting 1	Inenting 2
2 maanden (7-9 weken)	DKTP HepB Hib	Pneu
3 maanden	DKTP HepB Hib	
4 maanden	DKTP HepB Hib	Pneu
vanaf 11 maanden	DKTP HepB Hib	Pneu
14 maanden	BMR	MenC

Fase 2	Inenting 1	Inenting 2
4 jaar	DKTP	

Fase 3	Inenting 1	Inenting 2
9 jaar	HPV* DTP**	BMR

Fase 4	Inenting 1	Inenting 2
9,5 jaar	HPV*	DTP*

Betekenis afkortingen

D	Difterie	HepB	Hepatitis B	MenC	Meningokokken C
K	Kinkhoest	Pneu	Pneumokokken	HPV	Humaan Papillomavirus
T	Tetanus	B	Bof		
P	Polio	M	Mazelen	*	Alleen voor meisjes
Hib	Haemophilus influenzae type b	R	Rodehond	**	Alleen voor jongens

St. Eustatius

Fase 1	Inenting 1	Inenting 2
2 maanden	DKTP HepB Hib	Pneu
3 maanden	DKTP HepB Hib	
4 maanden	DKTP HepB Hib	Pneu
11 maanden	DKTP HepB Hib	Pneu
12 maanden	BMR	MenC

Fase 2	Inenting 1	Inenting 2
4 jaar	DKTP	BMR

Fase 3	Inenting 1	Inenting 2
9 jaar	DTP	

Fase 4	Inenting 1	Inenting 2
10 jaar	HPV*	HPV* (na 6 maanden)

Betekenis afkortingen

D	Difterie
K	Kinkhoest
T	Tetanus
P	Polio
Hib	Haemophilus influenzae type b
HepB	Hepatitis B
Pneu	Pneumokokken
B	Bof
M	Mazelen
R	Rodehond
MenC	Meningokokken C
HPV	Humaan Papillomavirus
*	Alleen voor meisjes

Saba

Fase 1	Inenting 1	Inenting 2
2 maanden	DKTP HepB Hib	Pneu
3 maanden	DKTP HepB Hib	
4 maanden	DKTP HepB Hib	Pneu
11 maanden	DKTP HepB Hib	Pneu
14 maanden	BMR	MenC

Fase 2	Inenting 1	Inenting 2
4 jaar	DKTP	BMR

Fase 3	Inenting 1	Inenting 2
9 jaar	HPV* DTP**	BMR (indien nodig)

Fase 4	Inenting 1	Inenting 2
9,5 jaar	HPV*	DTP*

Betekenis afkortingen

D	Difterie
K	Kinkhoest
T	Tetanus
P	Polio
Hib	Haemophilus influenzae type b
HepB	Hepatitis B
Pneu	Pneumokokken
B	Bof
M	Mazelen
R	Rodehond
MenC	Meningokokken C
HPV	Humaan Papillomavirus
*	Alleen voor meisjes
**	Alleen voor jongens

Figure 1. Vaccination schedules (in Dutch) on Bonaire, St. Eustatius and Saba in 2017 *(adapted from [72]). Note: up-to-date schedules can be found at www.rijksvaccinatieprogramma.nl.*

Table 1. Diseases studied in this thesis.

Pathogen	Transmission	General symptoms	Potential (severe) complications	Vaccination coverage in CN in 2017 [71]
Diphtheria [74, 75]				
Toxin-producing strains of bacteria *Corynebacterium diphtheriae* (sometimes also *C. ulcerans and C. pseudotuberculosis*).	Spreads by coughs and sneezes, direct contact (with skin lesions), contaminated objects, or raw dairy products (to note: *C. ulcerans and C. pseudotuberculosis* are zoonotic); Infectious up to four weeks; R0 is around 5.	Short incubation period (2-5 days); The majority develops a respiratory tract infection with flu-like symptoms in the first few days (some remain asymptomatic). Cutaneous diphtheria may present as a (scaling) rash or ulcers (with a membrane), and rarely ends in severe disease.	Release of the cytotoxin can cause kidney problems, myocarditis or inflammation of nerves resulting in paralysis, and necrotizing of the respiratory tract where a dense grey pseudomembrane can form blocking the airway. (Non-vaccinated) children and elderly are mostly affected. Case-fatality rate is high (5-17%) even with treatment (antitoxin and antibiotics).	Diphtheria-containing vaccines on Bonaire: -1-year-olds: 93% -5-year-olds: 87% -9/10-year-olds: 54%
Measles [76-80]				
Measles virus is a single-stranded RNA virus of the genus *Morbillivirus* in the family *Paramyxoviridae.*	Among the most contagious airborne infectious diseases; Main route through coughing, sneezing and via breathing of contaminated air, direct contact and infected surface (transplacental is very rare); Humans are the only reservoir; Infectious four days pre- and post-symptoms; R0 usually between 12-18, but setting-specific.	Incubation period is around 10-14 days; Symptoms include high fever, cough, a typical rash across the body and spots in the mouth (Koplik spots), and conjunctivitis.	Case-hospitalization rate ~5-10% in the Netherlands (in some high-income countries up to ~20%). Complications (more frequently in those undernourished) can include otitis media, pneumonia, (severe) diarrhea, encephalitis, and premature birth in pregnant women. Case fatality rates vary from <0.01% in high-income countries to >5% in low- and middle-income countries; Enhanced susceptibility to other respiratory infections due to reduced humoral immune memory is observed up to two years after infection; A rare long-term complication is subacute sclerosing panencephalitis (SSPE) years after infection and has a fatal outcome.	Measles-mumps-rubella (MMR), dose 1: -Bonaire: 90% -St. Eustatius: 97% -Saba: 100% MMR, dose 2: -Bonaire: 67% -St. Eustatius: 85% -Saba: 100%
Mumps [81-83]				
Mumps is a RNA virus of the genus *Rubulavirus* in the family *Paramyxoviridae.*	Transmission (restricted to humans) by aerosols and respiratory droplets, and potentially direct contact (in densely populated settings), or transplacental; Infectious a few day before to a week after onset of symptoms; R0 varies between 3-10.	Generally respiratory and non-specific (one third may be asymptomatic, men more frequently symptomatic), and followed by acute parotitis (in 95% of symptomatic cases) (uni- or bilateral) 2-3 weeks after exposure; Orchitis in 15-30% of males, and oophoritis or mastitis in 5% of women.	Could include sensorineural deafness, pneumonia, subfertility (rarely), meningitis (1-10%, usually mild), myocarditis, pancreatitis, and encephalitis (0.1%); Fetal death can occur in women who contract mumps in the first trimester.	

Table 1. (Continued)

Pathogen	Transmission	General symptoms	Potential (severe) complications	Vaccination coverage in CN in 2017 [71]
Rubella [84-86]				
Rubella is a RNA virus of the genus *Rubivirus* in the family *Matonaviridae*.	Airborne and spreads through inhalation from coughs or sneezes, direct contact, or transplacental; Humans are the sole reservoir; Infectious one week before the rash till one week after; R0 between 3-8.	Incubation period of 2-3 weeks. Disease is usually self-limiting, mild or even asymptomatic (25-50%) when contracted by children and adults (especially men); First sign is a rash (in the face) combined with non-specific symptoms; Majority of women may experience arthritis.	Very rarely pneumonia or encephalitis; High risk of miscarriage or stillbirth when contracted by a pregnant women in the first trimester, and risk of Congenital Rubella Syndrome (CRS) (90%), which is characterized by a constellation of ophthalmologic-, neurologic-, cardiac- and auditory anomalies and cognitive disabilities in the developing baby.	
Human papillomavirus (HPV) [87-91]				
HPV are non-enveloped double-stranded DNA viruses, of which the *Alpha* genus (in the *Papillomaviridae* family) are linked to mucosal infections.	Around 40 types can infect the anogenital epithelium (through microtraumas) via sexual contact (or direct skin-to-skin contact).	Around 90% of infections are transient and cleared by the immune system within 2 years.	At least 12 types (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59) have oncogenic potential and are considered high-risk types, of which HPV16 and -18 are responsible for 70% of cervical cancers. ~1-3% of persistent infections can develop into pre-cancerous states and are marked by low- or high-grade squamous intraepithelial lesions (HSIL). Transition from infection to HSIL can occur within 5 years, of which a small part progress into invasive cancer.	-Bonaire (one-dose coverage): 41% -St. Eustatius: 88% -Saba: 100%
Varicella (chickenpox) and herpes zoster (shingles) [92-95]				
Varicella-zoster virus (VZV), also referred to as *Human alpha herpesvirus (type 3)*, is a double-stranded DNA virus of the genus *Varicellovirus* in the family *Herpesviridae*.	Spreads very easily by air through inhalation of droplets or aerosols origination from the oropharynx or derived from skin lesions. Direct contact and placental transfer are other routes; Humans are the only reservoir; Infectious two days prior to symptoms up till crusting of the blisters; R0 up to 17.	The incubation period is 10-21 days; Primary infection with VZV results in varicella (chickenpox), 80-90% is symptomatic with flu-like symptoms and an itchy blister-like rash across the body; Illness is mild and self-limiting in immunocompetent cases, particularly in young childhood; VZV establishes a latent infection in neuronal cells in the sensory nerve ganglia. Reactivation leads to herpes zoster (shingles), causing a rash of blisters of which the pain can last for a prolonged time.	Complications increase with older age. Pneumonia, secondary bacterial infections, and in rare cases acute cerebellar ataxia or encephalitis. Risks for pregnant women are highest in the third semester and include pneumonia (with relative high death rates) and premature delivery. Intrauterine death is possible at any stage during pregnancy. Congenital Varicella Syndrome (CVS) can develop when infected before 20 weeks of gestation (2%), resulting in skin-, eye- and other neural deficiencies. Neonatal varicella may develop if mother contracted VZV in the last three weeks of pregnancy and can include severe pneumonia, meningitis, encephalitis and hepatitis.	Had not yet been implemented in 2017.

Table 1. (Continued)

Pathogen	Transmission	General symptoms	Potential (severe) complications	Vaccination coverage in CN in 2017 [71]
Coronavirus disease 2019 (COVID-19) [96-106]				
Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is single-stranded RNA virus of the genus *Betacoronavirus* (subgenus *Sarbecovirus*) within the family *Coronaviridae*.	Transmission through respiratory droplets, including smaller-sized (aerosols, particularly important in non-ventilated crowded rooms); Infectious from two days prior to symptoms up to ten days; R0 of the wildtype virus was 2.5 (yet substantially higher for later emerging variants (of concern)).	Incubation period is generally 3-5 days; In the majority, disease starts with respiratory- and flu-like symptoms, and typical is the change in the sense of smell/taste in around half of cases (pre-Omicron variant).	Moderate illness develops in ~2% after a week, including dyspnea, hypoxemia and pneumonia. Severity of disease is related to increased age, male sex and comorbidities, e.g., kidney disease or obesity. Strong and dysregulated pro-inflammatory responses (cytokine storm) can cause micro-blood clots and tissue damage with respiratory failure, septic shock and multiple organ dysfunction (high case fatality). Immune modulators can increase the survival rate (as well as vaccination, applicable later into the pandemic); Multisystemic inflammatory syndrome in children (MIS-C), a rare but severe Kawasaki-like syndrome, can appear a few weeks after infection; ~5-10% of cases have sustained long-term symptoms, referred to as post-COVID syndrome, and include prolonged fatigue, shortness of breath, heart palpitations, loss of smell/taste, and memory problems.	Not applicable for this thesis.

Measles-mumps-rubella (MMR) and diphtheria

The live-attenuated MMR-vaccine is safe, cheap and very effective at protecting against disease and severe complications, and onward transmission among those vaccinated is rarely observed [76, 107]. An estimated 25.5 million deaths due to measles were averted by the vaccine during the last two decades. However, still ~10 million infections and over 200,000 deaths are projected annually, with rising numbers worldwide since 2016 [108]. The Americas was the first WHO Region to reach measles elimination in 2016. Nevertheless, endemic transmission has officially been reestablished in Venezuela in August 2018 due to an ongoing humanitarian crisis that disrupts the NIP heavily resulting in large outbreaks including fatalities [109-111]. Likewise, although diphtheria is rare in developed countries with NIPs in place, relatively large outbreaks with high case fatality (~10%) were reported from Venezuela (~2,000 cases vs. ~5,000 worldwide) [112]. Millions of Venezuelans have been displaced and fled the country to surrounding countries, potentially unvaccinated and infected, causing outbreaks and fatalities elsewhere. Venezuela is the nearest country off the coast of the ABC-islands, and over ~25,000 refugees have arrived in 2018 [110]. This causes substantial pressure on these small islands, and potential risk of introduction of vaccine-preventable diseases, particularly measles and diphtheria. Although no cases have been reported on Bonaire, insight into the population immunity and potentially susceptible pockets is needed.

Given the unstable situation in Venezuela, increased circulation of other viral diseases, such as rubella and mumps, can be expected. Pre-vaccination, up to 150,000 cases of rubella were reported annually in Latin America and the Caribbean (LAC), causing stillbirths, death of newborns and over 20,000 CRS cases. Due to enormous efforts of mass vaccination campaigns in the WHO region of the Americas in the late 1990s, no autochthonous case of rubella or CRS has been reported since 2009 [84, 113], and in 2015 the Region was the first to be declared free of endemic rubella transmission [114]. Mumps cases also dropped significantly after introduction of vaccination [81]. However, since two decades there has been a resurgence globally, particularly among adolescents and young adults engaged in behaviors involving close contact. This could be due to waning vaccine effectiveness, absence of boosting due to reduced natural exposure, or a mismatch between the vaccine type and circulating strain; however, it should be noted that severe cases and sequelae among those vaccinated are rare [115-120]. No MMR cases have been reported in CN since the introduction of MMR-vaccination (1988), while it should be noted that only few suspected cases undergo laboratory confirmation due to a lack of facilities. Some mumps cases are confirmed annually on Aruba, hence circulation is expected on the other Dutch Caribbean islands given the tight bonds and frequent exchange [72]. In-depth evaluation of the NIP concerning MMR in terms of seropositivity, susceptible pockets and potential waning is warranted to inform policy.

Human papillomavirus (HPV)

A viral pathogen that also requires specific attention in CN is HPV. HPV is considered the most common sexually transmitted infection worldwide, and ~80% of the sexually-active population will be infected with an oncogenic/high-risk (hr)-HPV type at some point [87]. The majority of infections of the anogenital epithelium are cleared by the immune system, but persistent infections have the potential to cause cancer over a prolonged time [121]. Worldwide, 680,000 HPV-related cancers are estimated to occur in women and men yearly, with cervical cancer being the fourth leading cause of cancer [122]. Incidence of and mortality due to HPV-related cancers differs greatly geographically, with the majority (85%) in low- and middle-income countries, including LAC [123]. Data from Curaçao and Suriname are illustrative of these higher incidences, with 13.4 and 22.4 per 100,000, respectively [124, 125] vs. Western Europe with 6.8 and 2.1 per 100,000 [123], respectively. Population-based cervical cancer screening programs have only been introduced in the minority (30%) of Caribbean countries. If current trends continue, 90% of cervical cancer deaths in the Americas are predicted to occur in LAC [126]. Although cervical screening has been absent in CN, girls-only HPV-vaccination has recently been included in the NIP (2013-2015), yet uptake remains low [71]. Seroprevalence provides insight into lifetime cumulative hr-HPV infections in vaccination-naive (sub)populations, however such data are lacking for CN, and few population-based studies have been conducted in the Caribbean region [127-129]. Linking seroprevalence estimates to risk factors will provide useful insights for targeted preventive programs and can serve as a baseline for post-implementation investigation.

Varicella-zoster virus (VZV)

VZV is a highly-transmissible viral pathogen causing a substantial health burden [130, 131]. Clinical varicella (chickenpox) is usually self-limiting when contracted in childhood and confers lifelong immunity. Severity of disease is associated with older age at infection, being immunocompromised, including pregnant women and their (unborn) offspring in whom Congenital Varicella Syndrome (CVS) can develop [94]. Moreover, VZV establishes a latent infection in neuronal cells and if reactivated (mainly in elderly) this leads to herpes zoster (shingles) [93]. The annual global disease burden of VZV is substantial, with conservative estimates of 140 million cases, 4.2 million severe complications and 4,200 deaths [130]. Seasonality is less pronounced in tropical regions potentially due to factors such as climate, risk of exposure, and population density [132-137]. Less endemic circulation results in higher proportions of susceptible adolescents and adults – in contrast to for instance the Netherlands where 95% of the population has contracted varicella at the age of six years [94, 138-140]. Serosurveillance in hospital workers on Curaçao showed that 40% of 20-year-olds were seronegative and 30% of those 50 years and older [141]. In CN, hospital admissions due to varicella are

reported regularly and Saba experienced a large outbreak in 2017 affecting ~12.5% of the population, including severe cases [142]. Forty percent of LAC-countries, and 20% of countries from Central America and the Caribbean, had implemented universal childhood VZV-vaccination (one or two doses) with high coverage, causing disease burden to decline dramatically [131, 143, 144]. In line with the Netherlands, VZV-vaccination has not been part of the NIP in CN at that time. Serosurveillance can provide insights into VZV population dynamics that will be key for decision-making regarding future vaccination.

Sero-monitoring the SARS-CoV-2 epidemic in the Netherlands

At the end of 2019, the world became acquainted with a new coronavirus: SARS-CoV-2 (*Table 1*) [98, 145, 146]. This highly-transmissible virus initiated a pandemic (declared on 11 March 2020 by the WHO [147]) and global crisis due to its swift spread in completely immunity-naïve populations causing (severe) respiratory illness: COVID-19 [148-151]. Strict lockdown measures were implemented across the world [152], and in the Netherlands these included, for instance, closure of schools, elderly homes, restaurants/bar/cafes, cultural institutions and sport facilities, cancelation of gatherings and working remotely [153]. Also, social distancing-, hygienic-, and control measures, such as isolation and quarantine, are applied to curb transmission and prevent health systems from collapsing due to an enormous inflow of patients [154]. Particularly those from 60 years of age and with comorbidities are at substantially higher risk of hospitalization and fatal outcome [96, 97]. In addition to monitoring (NIP-)pathogens in less acute epidemiological phases, seroepidemiology can be a useful tool after the emergence of a novel pathogen when a more rapid response is required [155, 156]. Hence, the nationwide longitudinal PICO-study was set up swiftly in the Netherlands at the start of the SARS-CoV-2 pandemic.

During the first wave of infections in the Netherlands, a lack of capacity and resources restricts testing to moderate-severely-ill patients and groups at high risk of exposure [153, 157]. To complement other surveillance tools [158], population-based serosurveillance – using seropositivity against the spike S1 antigen as a measure of previous infection [58] – can provide insights into the extent of the epidemic, affected groups and symptomatology (frequency and types) in the general population that can guide researchers and policymakers [155, 159]. Moreover, the first lockdown in the Netherlands lasted from mid-March–May 2020, and over 11,000 hospitalizations, 3,000 intensive care unit admissions and 10,000 fatalities were reported after the first wave in June 2020 [154, 160]. To inform and support decisionmakers for the potential waves to come, in-depth analyses on the effects of social distancing measures on infection in the general population are desired. Finally, whereas observations of reinfection are rare the first half year into the pandemic, some reports (from small studies) address

potential waning of the humoral response (particularly against the Nucleocapsid antigen) shortly after infection [161-164]. The longitudinal character of the PICO-study allows population-based investigation of persistence and functionality (avidity) of infection-induced antibodies relative to severity, which will be important in shedding light on future protection and also on informing vaccination trials that have started recently [165].

Scope and outline

Taken together, seroepidemiology is a key tool in the prevention and control of infectious diseases. Its multifaceted applicability will be explored in this thesis covering different pathogens (those already implemented in the NIP or candidates vs. emerging), geographical settings (Caribbean Netherlands vs. the (European) Netherlands), and epidemiological phases (control, alert, and more acute pandemic) in order to provide valuable input for public health policy. Large serosurveillance studies have therefore been set up to evaluate population immunity for vaccine-preventable diseases in CN (**part 1**), and to sero-monitor SARS-CoV-2 in the first year of the pandemic in the Netherlands (**part 2**).

Part 1 begins with outlining the design, (experience with the) set up and participant characteristics of the serosurveys conducted in the Netherlands (PIENTER-3) and CN (Health Study CN) in **chapter 2**. Given the unstable humanitarian situation and outbreaks of vaccine-preventable diseases in Venezuela, assessment of specific groups at-risk for measles and diphtheria on Bonaire was the first priority, and is outlined in **chapter 3**. Further in-depth evaluation of the NIP regarding MMR on all CN-islands in terms of seropositivity and exposure (recently and in pre-vaccination era), susceptible pockets, and potential waning immunity is described in **chapter 4**. Another viral pathogen that causes a large burden in the Caribbean region is HPV, yet vaccination has only recently been introduced in CN. To provide insight for future preventive programs, lifetime cumulative exposure (in the vaccination-naïve population) to seven hr-HPV types and risk factors that contribute to seropositivity are assessed in **chapter 5**. Part 1 ends with studying the seroepidemiological dynamics of VZV in the CN-island populations as these most likely differ from the (European) Netherlands, and will thus be key in the decision-making process on introducing vaccination (**chapter 6**).

Besides evaluation of the NIP and gaining insights into groups at-risk in the Caribbean part of the kingdom, **part 2** outlines the significant role of seroepidemiology in the Netherlands in a more acute phase, during emergence and dissemination of SARS-CoV-2. In **chapter 7**, using seropositivity as a marker of past infection, the extent of the SARS-CoV-2 epidemic at the peak of the first wave (1st PICO-study round) is investigated. Specific focus is on exposed groups and symptomatology (in relation to the humoral

response) in the general population. Moreover, social distancing measures have been implemented to curb transmission during this first wave. Data covering the entire first wave (2nd PICO-study round) are used in **chapter 8** to investigate associations of these measures with infection in order to guide decisionmakers. Finally, persistence and maturation of antibodies following SARS-CoV-2 infection might be a proxy for protection (against severe disease) and could mimic the response after vaccination. The kinetics of IgA, IgM and IgG antibodies, targeted against the spike S1 antigen, relative to symptomatology are studied up to seven months after infection (combining the first three PICO-study rounds) in **chapter 9**.

The main findings of part 1 and 2 are summarized in **chapter 10**. The public health implications of these results are further discussed in **chapter 11**, and a reflection of future perspectives as well as recommendations for areas of research are provided, including the role of seroepidemiology.

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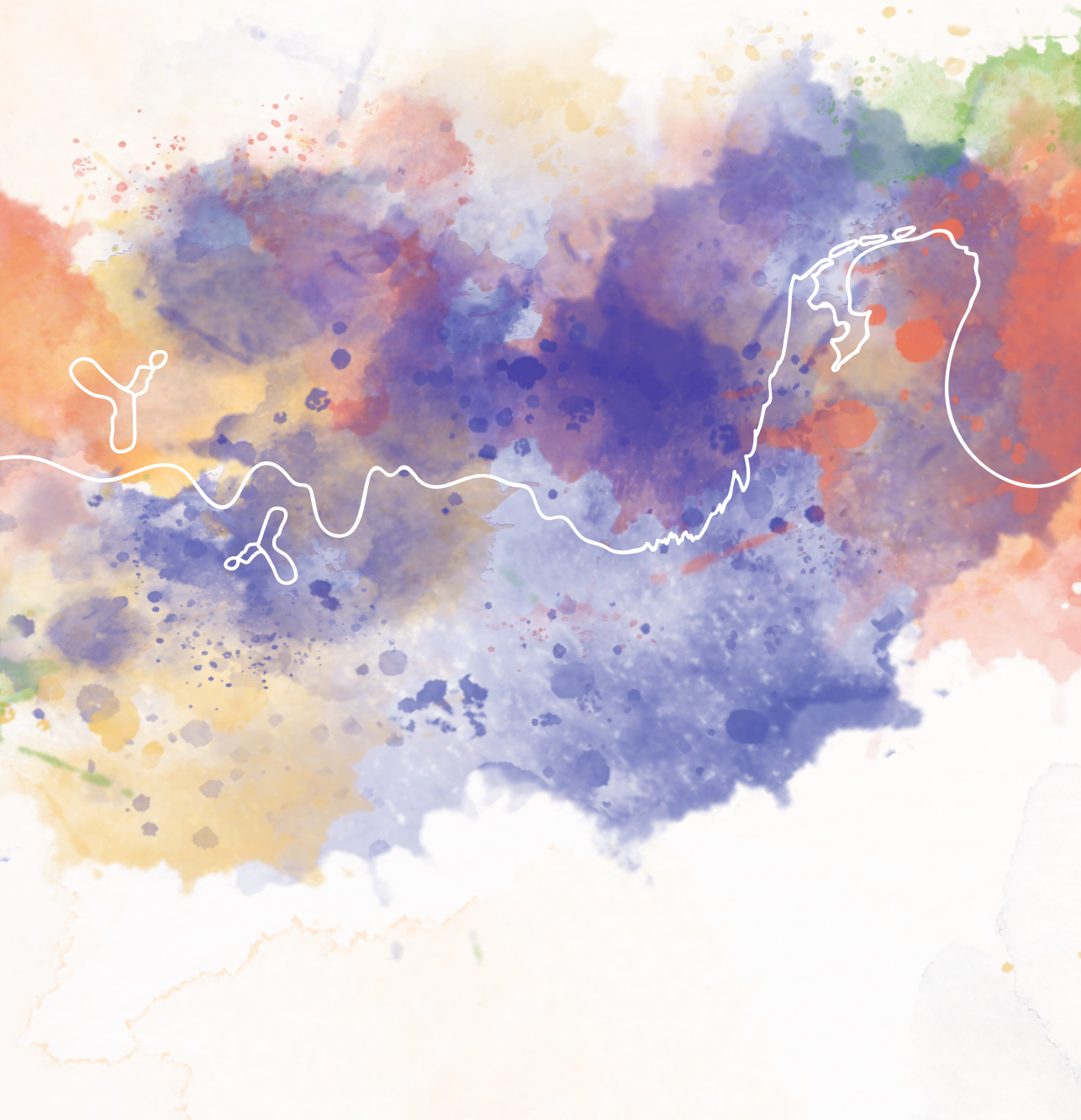

CHAPTER 2

Third national biobank for population-based seroprevalence studies in the Netherlands, including the Caribbean Netherlands

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ABSTRACT

Background

This paper outlines the methodology, study population and response rate of a third large Dutch population-based cross-sectional serosurvey carried-out in 2016/2017, primarily aiming to obtain insight into age-specific seroprevalence of vaccine-preventable diseases to evaluate the National Immunization Programme (NIP). In addition, Caribbean Netherlands (CN) was included, which enables additional research into tropical pathogens.

Methods

A two-stage cluster sampling technique was used to draw a sample of Dutch residents (0–89 years) (NS), including an oversampling of non-Western migrants, persons living in low vaccination coverage (LVC) areas, and an extra sample of persons born in Suriname, Aruba and the former Dutch Antilles (SAN). A separate sample was drawn for each Caribbean island. At the consultation hours, questionnaires, blood samples, oro- and nasopharyngeal swabs, faeces − and only in the Netherlands (NL) saliva and a diary about contact patterns – were obtained from participants. Vaccination- and medical history was retrieved, and in CN anthropometric measurements were taken.

Results

In total, blood samples and questionnaires were collected from 9,415 persons: 5,745 (14.4%) in the NS (including the non-Western migrants), 1,354 (19.8%) in LVC areas, 501 (6.9%) SAN, and 1,815 (23.4%) in CN.

Conclusions

This study will give insight into protection of the population against infectious diseases included in the NIP. Research based on this large biobank will contribute to public health (policy) in NL and CN, e.g., regarding outbreak management and emerging pathogens. Further, we will be able to extend our knowledge on infectious diseases and its changing dynamics by linking serological data to results from additional materials collected, environmental- and pharmacological data.

INTRODUCTION

The seroprevalence of National Immunization Programme (NIP)-targeted diseases is periodically monitored in the Netherlands (NL) by national seroepidemiological (PIENTER) studies, executed by the National Institute for Public Health and the Environment (RIVM) in collaboration with Public Health Services and municipalities. The first serosurvey was performed in 1995/1996 [1] and the second in 2006/2007 [2].

Gathering seroepidemiological data forms an important tool for the evaluation and optimization of the NIP, and gives insight into the protection against infectious diseases in different (sub)groups in the population. The results of previous Dutch serosurveys have contributed to vaccine policy, e.g., during the measles epidemic in 2013/2014, data on seroprevalence (particular the maternal antibody levels in infants) from the second Dutch serosurvey in 2006/2007 were used to advise on measles-mumps-rubella (MMR)-vaccination at an earlier age [3]; tetanus seroprevalence data led to an advice regarding a more restricted use of tetanus prophylaxis [4]; and the decision to revaccinate against meningococcal C disease at an adolescent age with a tetravalent vaccine were partly based on these data [5].

Since the last serosurvey in 2006/2007 several adaptations in de NIP were applied (*Table 1*) and some (small) outbreaks occurred, e.g., measles outbreak in the Dutch Bible Belt in 2013/2014 [6], mumps among vaccinated young adults [7] and increased incidence of meningococcal serogroup W disease since 2015 [8]. Events like these will have impact on the immune status in the population and justify close investigation. In addition, monitoring the seroprotection of the population is required at regular time intervals as vaccination can affect the dynamics of infectious diseases on the long term, for instance leading to an increasing age of infection or waning antibody levels, e.g., against diphtheria and measles [3, 9]. For these reasons, a third seroepidemiological study (PIENTER-3) was performed [10] to identify (new) population groups at risk for infectious diseases and to evaluate the adaptations made in the vaccination scheme in order to improve the overall quality of the programme.

This third study has been extended with the collection of saliva and faecal samples, as well as oro- and nasopharyngeal swabs, creating a more comprehensive biobank for the Dutch population. With the collection of these diverse human materials, accompanied by extensive individual information from questionnaires and the linkage with other data sources (medication histories and environmental exposures), this biobank harbours a wealth of information.

Importantly, this serosurvey has been expanded to include the Caribbean Netherlands (CN) for the first time (Health Study Caribbean Netherlands, HSCN). The Caribbean islands Bonaire, St. Eustatius and Saba (together CN) are officially part of NL and considered public entities under Dutch law since October 10, 2010. Hence, public

health falls under the direct responsibility of the Dutch government, e.g., the supply, execution and monitoring of the NIP. Strikingly, no data on protection against infectious diseases and associated risk factors are available. The need for knowledge is underlined by outbreaks of measles and diphtheria in neighboring countries in Latin America [11, 12] and epidemics of vector-borne diseases in the Caribbean region (e.g., zika, dengue and chikungunya) [13-15].

This paper outlines the design of population-based cross-sectional serosurveys in NL and CN, its study population and response rates. Subsequently, future research possibilities of this extensive data collection and experience with conducting large population-based public health research in CN will be described.

Table 1. Adaptations in the National Immunization Programme (NIP) in the Netherlands from 2006 to 2018.

Year	Vaccination	Adaptation in the Dutch NIP
2018	Meningococcal ACWY vaccination	Change from MenC conjugate vaccine administered at 14 months of age to MenACWY conjugate vaccine.
2014	Human Papillomavirus vaccination	Change from 3 vaccinations to 2 vaccinations administered at 12 years of age.
2013	Pneumococcal vaccination	Change from 4 vaccinations administrated at 2, 3, 4 and 11 months of age to 3 vaccinations at 2, 4 and 11 months.
2011	Hepatitis B vaccination	Change from vaccination offered to infants at risk to vaccination for all children administered at 2, 3, 4 and 11 months of age, via DTaP-IPV-Hib-HepB.
2011	Pneumococcal vaccination	Change from vaccination against 7 serotypes to vaccination against 10 serotypes.
2009	Human Papillomavirus vaccination	Introduction of vaccination for girls 12 years of age with catch-up for girls up to 17 years of age.
2006	Pneumococcal vaccination	Introduction of vaccination administered at 2, 3, 4 and 11 months of age, simultaneously with DTaP-IPV-Hib-(HepB).

METHODS

Study population and sample design

Similar to the previous serum banks, a two-stage cluster sampling technique was used to draw a national sample (NS) in NL [1, 2]. Forty municipalities were sampled within five regions proportional to size (*Figure 1*). Within each of these municipalities, an age-stratified sample was drawn from the population register. As life expectancy

is increasing, the maximum age in this study was extended from 79 (in the previous surveys) to 89 years, resulting in age strata 0, 1–4, 5–9, ..., up to 75–79, 80–89 years of age. A detailed description of the sample size calculations and total number of invitees per study sample can be found in *Supplement Table S1*. In total, we aimed for 158 participants per municipality, resulting in 6,320 participants. Therefore, initially, a sample of in principal 494 individuals per municipality was drawn in the first 11 municipalities, however during the study this was adjusted for the age strata 0–54 years because of lower response rate than expected, which resulted in a total of 818 persons invited in the next 13 municipalities. Finally, in the last 16 municipalities 193 extra men (in total 1,011 persons) were invited in the age range of 20–54 year since women responded predominantly. In total 32,244 individuals were planned to receive an invitation in the Dutch national sample.

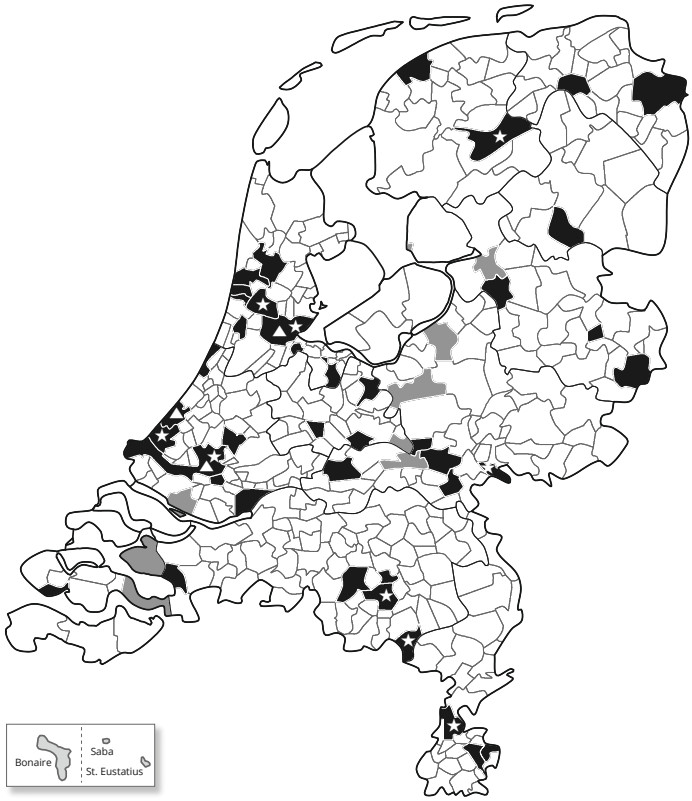

Figure 1. Overview of the selected municipalities. Municipalities depicted in black are included in the national sample and municipalities in grey are low vaccination coverage areas. * indicate a municipality with oversampling of non-Western migrants, ▲ indicate a municipality with oversampling of non-Western migrants and oversampling of people with migration background from Suriname, Aruba and the former Dutch Antilles. The Caribbean Netherlands sample, taken from the Dutch Caribbean islands Bonaire, St. Eustatius and Saba, are shown at the bottom left.

Oversampling of subpopulations

First, people with a migration background from non-Western countries living in NL (i.e., migrants) were oversampled using age strata 0–9, 10–34, 35–59, 60–89 years. Age-stratification was based on the NIP-ages, i.e., NIP-vaccines (except for human papillomavirus) are administered before the age of 10 years and those below the age of 59 were eligible for the routine NIP (introduced in 1957). A random sample of migrants from Turkey/Morocco, Suriname/Aruba/former Dutch Antilles, and other non-Western countries was drawn within nine municipalities of the NS (*Supplement Table S1*). In total, 8,259 migrant individuals were planned to receive an invitation in this sample. Note, migrants were also invited in the NS as we sampled at random regardless country of birth.

Second, an additional sample of 7,328 individuals was drawn from persons with a migration background from Suriname, Aruba and the former Dutch Antilles (SAN), living in the largest municipalities in the NS sample, to be able to compare them with participants from CN (as described at the end of this section) (*Supplement Table S1*).

Third, persons living in low vaccination coverage (LVC) areas in NL were oversampled. In these areas Orthodox Reformed individuals (ORI), who (partly) refuse vaccination based on religious grounds, live socio-geographically clustered. Eight areas were selected from which the vaccination coverage for diphtheria-tetanus-pertussis-polio (DTaP-IPV) and MMR was below 85% in the years between 2010 and 2014 (nationwide coverage > 90%) [16]. A sample was drawn in similar age strata as for migrants (0–9, 10–34, 35–59 and 60–89 years) (*Supplement Table S1*). An extra municipality was added halfway the study to reach a sufficient number of participants living in LVC areas. In total, 6,864 persons living in LVC areas were planned to be invited.

In summary, we planned to invite 54,695 persons in total for NL, however, due to incorrect data from the municipal health register, eventually the number of invited individuals was 54,170.

Last, for CN, a sample was drawn from the population registry of the Dutch overseas territories (PIVA-V, January 1, 2017). Of each island, an age-stratified sample with age strata 0–11, 12–17, 18–34, 35–59 and 60–89 years was drawn (*Supplement Table S1*). All children aged < 18 years on St. Eustatius (n = 744) and Saba (n = 339) were approached to take part in the study in order to meet the power requirements. Students from Saba University School of Medicine were a priori excluded from the total population (n = 245) as they are non-permanent residents of Saba. In total, 8,068 individuals were invited (Bonaire n = 4,798; St. Eustatius n = 2,135; and Saba n = 1,135).

Data collection

In NL, data collection took place from February 1, 2016 to October 16, 2017 and on CN from May 2, 2017 to June 19, 2017. Each person received an invitation letter by mail

along with a brochure containing information on the study and an informed consent form. For the Dutch migrant populations, the invitation letter contained a reference to the translated invitation letter on the website and a supplementary instruction flyer was sent in English, Turkish, Arabian and French. On CN, all research material was available in the four most common languages on the islands: Papiamentu, English, Dutch and Spanish.

The invitees in NL were asked to fill in an online questionnaire and to visit a consultation hour in their municipality. A paper questionnaire was sent to people above 60 years of age. At the consultation hour, a shortened translated version of the questionnaire was available in English, Turkish, Arabian and French in case the invitee was not able to fill in the Dutch questionnaire. The invitees in CN received a paper questionnaire in their preferred language (Papiamentu, English, Dutch or Spanish) at the consultation hour and were guided by a trained interviewer in case of illiteracy or on request by the participant. The questionnaire of CN had some minor differences and was longer as compared to NL. Paper questionnaires and completed diaries were registered and digitized by trained data typists.

All invitees received a pre-made appointment for a consultation hour (to control the flow of visitors), however it was clarified in the letter that they were able to visit at any moment. Invitees in NL received a reminder by mail and were contacted via telephone by a call centre a few days prior to the visit. Invitees who did not show up were contacted by phone again. The call centre conducted a non-response questionnaire if a person was not willing to participate.

Several communication tools were applied to promote the study, such as twitter, radio- and television interviews and newspapers. Websites were operational for information and invitees could e-mail or call the research team with questions and/or consult an independent general practitioner if they preferred. Especially for CN, an extensive communication plan was composed in collaboration with all relevant stakeholders and was tailor-made for each island. At the start of the study a press conference and official kick-off was organised on each island.

At the consultation hours several body materials were collected. In NL, the collection consisted of saliva and venous blood samples. For babies a heel prick was offered and for young children or people who were dreading blood donation a finger prick was available. A small subset of the participants (n = 338) was asked to donate an extra blood sample for cellular immunity analyses and a subset (n = 1,939) was asked to fill in a dairy about contact patterns. Moreover, participants could optionally donate additional materials for which they received an extra incentive. These materials included oro- and nasopharyngeal swabs (for children below the age of 8 years solely one swab was required, preferably a nasopharyngeal swab), and a faecal sample including an additional questionnaire. These participants were also asked for their consent regarding

retrieval of their complete medication history – through collaboration with Dutch pharmacies (the UPPER-network) and the Foundation for Pharmaceutical Statistics ('Stichting Farmaceutische Kerngetallen'). In CN, anthropometric measurements height, weight and blood pressure (the latter from 4 years of age) were taken via calibrated instruments (SECA 214, SECA 877, and Omron M3, respectively) and standardized methods. If height and weight were not able to be assessed this was acquired from the latest measurement (growth booklet) at the Public Health Services or estimated by the participant. Thereafter, blood samples – via a finger- or heel prick using the dried blood spot (DBS) method – and oro- and nasopharyngeal swabs were collected. Participants were asked to collect a faecal sample at home and were offered a gift voucher after returning their sample. Permission was asked for retrieval of their medication history of the preceding year from the local health insurance office ('Zorgverzekeringskantoor'). All participants in NL and CN were asked for their consent regarding participation in a possible follow-up research (nested cohort).

The vaccination history of the participants was either checked by copying the vaccination certificates brought by the individuals to the consultation hour or retrieved via Praeventis: the Dutch electronic (web-based) vaccination register of the NIP for birth cohorts from 1990 on. For participants born before 1990 in NL, vaccination histories were retrieved via former local authority for registration of vaccinations. In CN, the vaccination statuses were additionally obtained via Public Health Services, consultation offices and hospitals.

Invitees older than 6 years of age who were not able to visit the consultation hour or did not show up at the clinic in NL, yet filled in (part) of the (online) questionnaire, were sent a kit to self-administer a finger prick sample at home. Likewise, people who were willing to participate but were not able to come to the clinic were sent a finger prick-kit in NL or were visited at home in CN.

Information on environmental exposure at the address level (e.g., different parameters for air pollution (PM10, PM2.5, livestock-associated air pollution) and green space) were retrieved from national databases in NL [17-19]. The linkage to these environmental parameters and the medication histories enables investigation of effects on the microbiome and antibiotic resistance at varies sites. In combination with results from sera and oro- and nasopharyngeal swabs the association of vaccination responses and carriage of pathogens, microbiome, environmental and lifestyle factors can be investigated.

The study proposal was approved by the Medical Ethics Committee Noord-Holland (METC number: M015–022) and written informed consent was obtained from all adult participants, and parents or legal guardians of minors included in the study.

Processing and storage of body materials

In NL, collected materials were transported to the RIVM's laboratory at the end of each consultation day. In adults, blood was drawn in two 8.5 mL vacutainer tubes (Becton and Dickenson SST II) and one 2 mL EDTA blood tube (total of max. 19 mL), and in children until the age of five years a maximum of 10 mL of blood was drawn. Heel- or finger pricks were collected in 300 µL cups. In the subset of participants who also donated blood for cellular immunity analyses, additional blood was drawn in two 9 mL heparin tubes. The blood samples were stored in a cold room (4 °C) overnight at the RIVM. The next day, the 2 mL EDTA blood tube was registered and stored in the freezer (−20 °C). The blood collected in vacutainers was centrifuged and divided into portions up to 4.5 mL serum. One tube of serum was stored at −20 °C (for further aliquoting) and the remaining serum, if available, was stored at −80 °C. Heel- and finger prick blood was centrifuged, aliquoted, and stored in −20 °C. The blood collected in heparin tubes was used for whole blood phenotyping by FACS analyses, the plasma was stored in aliquots in −20 °C and PBMCs were isolated and stored in vials at −135 °C. Saliva samples were collected using a sponge that was swabbed through the mouth for one minute. Saliva was immediately harvested from the swab by squeezing fluids from the sponge and dividing the sample into a cryovial® tube and a tube containing a glycerol solution (50% glycerol in DEPC water, for culture). These two tubes were immediately frozen on dry-ice at the consultation hour and stored the next day at −80 °C. The remaining saliva was collected into a 2 mL spray dried EDTA tube and stored at room temperature. The next morning the samples were centrifuged, aliquoted and stored at −80 °C.

Oro- and nasopharyngeal swabs were taken and stored in liquid Amies medium. The swabs were immediately frozen at the consultation hour on dry-ice and transported at the end of the consultation day to the RIVM, where they were stored the next day at −80 °C. For the collection of faecal material, subjects were requested to donate a small amount of faeces in three separate containers at home, of which one contained 15% glycerol-physiological salt solution. The samples were then packed in a plastic bag directly after collection and kept in the freezer at home until they were delivered by the subject in cold packs to the mobile study team. Detailed instructions and all materials needed were supplied at the first visit at the consultation hour. Faecal samples were kept frozen on dry-ice during transport to the RIVM and stored at −80 °C the next day.

All samples collected in CN were stored at, preferably air-conditioned, room temperature at the consultation hour. Blood samples were collected via a finger -or heel prick using the DBS method via air-dried filter paper (Whatman® 903 protein saver cards), removing barriers related to sample collection and transportation. These were dried for a minimum of two hours before storage in plastic bags with silica pads. Oro- and nasopharyngeal swabs were collected and stored in a 1 mL MMB tube (DNA

Genotek Inc., Ottawa, Canada) and faecal samples in a 1 mL OMNIgene®-gut (OMR-200) (DNA Genotek Inc.) tube, both containing stabilizing liquid for the microbiome. Directly after the fieldwork, samples were air shipped to the laboratory of the RIVM where the material was stored instantly at −80 °C pending analyses.

RESULTS

The Netherlands

From the 54,170 people invited, 195 (0.4%) persons were excluded from the sample for not having received the invitation (due to rehousing, no delivery or other reasons, *n* = 186) or due to mental disability (*n* = 9). This resulted in 53,975 (99.6%) invitees: 39,898 within the NS, including 8,184 oversampled non-Western migrants, 6,825 from LVC areas and 7,252 oversampled people with SAN background.

In total, 7,600 (14.1%) sera and questionnaires were collected in NL: 5,745 (response rate 14.4%, range 4.9–21.9 per municipality) in the NS including the extra sample of migrants (*n* = 601, response 7.3%), 1,354 in the LVC sample (response rate 19.8%, range 15.0–26.3 per municipality) and 501 (6.9%) persons in the SAN sample. A detailed description of response per study sample, stratified by age groups can be found in *Supplement Table S1*. An overview of collected materials per sample is shown in *Table 2*. Moreover, 5,105 (82.1%) participants with any material in the NS gave consent to be approached for a follow-up study if applicable. Of all non-responders, 15,141 (77.0%) answered the question concerning their reason not to participate: 36.0% indicated that they did not have time to participate, 12.0% was dreading blood donation and 45.0% gave a reason other than the above mentioned answer categories and 7.0% did not answer this question.

Supplement Table S2 shows the frequencies on a set of sociodemographic characteristics for responders (participants with any material) versus non-responders for the NS sample (including the oversampling of non-Western migrants) and the CN sample. Among the responders there are relatively more persons aged between 10 and 19 years and between 40 and 79 years, more women, more indigenous Dutch people, and less people living in areas with the highest degree of urbanisation compared to non-responders.

Overall, more females (54.7%) responded than males (45.3%) (*Table 3*), however in the youngest and highest age classes more males participated. In both the NS (*Figure 2A*) and LVC sample (*Figure 2B*) the highest response rate was seen in women aged 55–59 years. The high number of invited 20–54-year-olds resulted in a high inclusion of females from this age class.

Table 2. Overview of number of participants and collected materials in the study, by sample (*n* (%)).

	NS & oversampling of migrants	LVC sample	SAN sample	CN sample
Sample invited	40,065	6,830	7,275	8,068
Net sample size[a]	39,898 (99.6%)	6,825 (99.9%)	7,252 (99.7%)	7,768 (96.3%)
Information from population register only	19,638 (49.0%)	2,436 (35.7%)	4,040 (55.5%)	NA
Non-response questionnaire	14,043 (35.2%)	2,964 (43.4%)	2,652 (36.6%)	NA
Participant with any material	6,217 (15.6%)	1,425 (20.9%)	560 (7.7%)	1,900 (24.5%)
Full participant (all materials)	2,682 (6.7%)	674 (9.9%)	280 (3.7%)	1,515 (19.5%)
Participant with both blood sample and questionnaire	5,745 (14.4%)	1,354 (19.8%)	501 (6.9%)	1,815 (23.4%)
Materials[b]				
Blood[c]	5,762 (92.7%)	1,358 (95.3%)	503 (89.8%)	1,829 (96.3%)
Venous blood sample	4,977 (86.4%)	1,174 (86.5%)	454 (90.3%)	NA
Finger/heel prick	581 (10.1%)	162 (11.9%)	25 (5.0%)	NA
Dried blood spot sample	208 (3.6%)	23 (1.7%)	26 (5.2%)	1,829 (100%)
Questionnaire	6,200 (99.7%)	1,421 (99.7%)	558 (99.6%)	1,885 (99.2%)
Saliva sample	5,544 (89.2%)	1,319 (92.6%)	477 (85.2%)	NA
Nasopharyngeal swab	3,849 (61.9%)	939 (65.9%)	369 (65.9%)	1,752 (92.2%)
Oropharyngeal swab	3,319 (53.4%)	791 (55.5%)	326 (58.2%)	1,502 (79.1%)
Faeces	2,765 (44.5%)	704 (49.4%)	285 (50.9%)	1,547 (81.4%)
Additional questionnaire	2,775 (44.6%)	702 (49.3%)	284 (50.7%)	NA
Vaccination status[d]	3,819 (71.1%)	970 (76.3%)	263 (57.9%)	974 (51.3%)
Diary contact patterns[e]	1,310 (72.7%)	NA	67 (48.9%)	NA
Consent to approach for follow-up[d]	5,105 (82.1%)	1,171 (82.2%)	436 (77.9%)	1,762 (92.7%)

[a] Reasons for exclusion included mentally disabled, died, rehousing or other reasons why mail could not be delivered.

[b] Percentages were calculated with participants with any material as denominator.

[c] Four, one and two person(s) with both finger prick and venous blood in NS, LVC sample and SAN sample, respectively.

[d] Percentages were calculated for participants with any material and eligible for the NIP programme (≤ 65 years), 5,374, 1,271, and 454 for NS, LVC, and SAN sample, respectively. For the CN sample, percentage was calculated for all participants with any material.

[e] Percentages were calculated with number of diaries handed out as denominator, 1,802 and 137 in NS and SAN sample, respectively.

Abbreviations: *CN* sample, Caribbean Netherlands: sample from the Dutch Caribbean islands Bonaire, St. Eustatius and Saba; *LVC* sample, Low vaccination coverage sample; *NA*, Not applicable; *NS* sample, National sample; *SAN* sample, Sample from persons with a migration background from Suriname, Aruba and the former Dutch Antilles.

Table 3. Overview of sociodemographic characteristics for participants with both a blood sample and questionnaire, by sample (*n* (%)).

	NS & oversampling of migrants ***n* = 5,745 (14.4%)**	**LVC sample** ***n* = 1,354 (19.8%)**	**CN sample** ***n* = 1,815 (23.4%)**
Sex	5,745 (100.0%)	1,354 (100.0%)	1,815 (100.0%)
Male	2,629 (45.8%)	594 (43.9%)	814 (44.8%)
Female	3,116 (54.2%)	760 (56.1%)	1,001 (55.2%)
Ethnic background	5,744 (99.9%)	1,354(100.0%)	1,804 (99.4%)
Indigenous Dutch	4,490 (78.1%)	1,299 (96.0%)	146 (8.1%)
Morocco and Turkey	142 (2.5%)	3 (0.2%)	1 (0.1%)
Suriname, Aruba and former Dutch Antilles	285 (5.0%)	4 (0.3%)	1,301 (72.1%)
Other non-Western countries	445 (7.7%)	15 (1.1%)	280 (15.5%)[a]
Other Western countries	382 (6.7%)	33 (2.4%)	76 (4.2%)
Religion	5,330 (92.8%)	1,200 (88.6%)	1,784 (98.3%)
Protestant	841 (15.8%)	843 (70.3%)	44 (2.5%)
Orthodox-Reformed	79 (9.4%)	299 (35.5%)	NA
Roman Catholic	1,279 (24.0%)	29 (2.4%)	892 (50.0%)
Other religion	598 (11.2%)	44 (3.7%)	625 (35.0%)
No religion	2,612 (49.0%)	284 (23.7%)	223 (12.5%)
Educational level[b]	5,407 (94.1%)	1,280 (94.5%)	1,574 (86.7%)
High educational level	2,087 (38.6%)	443 (34.6%)	319 (20.3%)
Middle educational level	1,855 (34.3%)	540 (42.2%)	401 (25.5%)
Low educational level	1,465 (27.1%)	297 (23.2%)	854 (54.2%)
Urbanisation degree[b]	5,745 (100.0%)	1,354 (100.0%)	NA
1. Highly urbanized	1,246 (21.7%)	NA	NA
2. Urbanised	1,873 (32.6%)	NA	NA
3. Moderate urbanised	1,090 (19.0%)	159 (11.7%)	NA
4. Little urbanized	1,041 (18.1%)	753 (55.6%)	NA
5. Countryside	495 (8.6%)	442 (32.6%)	NA

[a] In the CN sample *n* = 260 of 280 (93%) participants from ethnic background group 'other non-Western countries' had a Latin American background. Total proportion of Latin Americans in the CN sample is 14.4%.

[b] Definitions according to Statistics Netherlands (CBS). Urbanisation degree is based on the environmental address density municipalities are divided into five classes of urbanity. The environmental address density is the average value of the address density of a municipality. The address density is based on an area with a radius of 1 km around an address.

Abbreviations: *CN* sample, Caribbean Netherlands: sample from the Dutch Caribbean islands Bonaire, St. Eustatius and Saba; *LVC* sample, Low vaccination coverage sample; *NA*, Not applicable; *NS* sample, National sample.

Nearly half of the people (49.0%) in the NS were not religious, 24.0% considered themselves Roman Catholic, 15.8% Protestant, and 11.2% reported to have another religion, such as Islamic, Jewish, Buddhism or Hinduism (*Table 3*). Most participants in the NS were high educated (38.6%), followed by middle (34.3%) and low (27.1%). Moreover, the highest response was seen in indigenous Dutch and the lowest in individuals with a Moroccan or Turkish background, in which the anticipated 70 persons per age strata were not reached. Regarding the other migrant groups, over 70 participants per age group were included, except for the 0–34-year-olds with a migration background from Suriname/Aruba/former Dutch Antilles (0–9 year: *n* = 44; 10–34 year: *n* = 62). Nevertheless, as the total number of participants with a SAN background (including *n* = 501 in the SAN sample) was 786, sufficient participants were included in each age group.

In the LVC sample, the largest amount of people considered themselves Protestant (*n* = 844, 70.3%) and among them 299 (35.4%) were ORI (*Table 3*). Together with those included in the NS, a total of 378 ORIs were included, in which all age strata included over 70 persons, except for 60–89-years-old (*n* = 49). Mainly middle- and high-educated people were included in the LVC sample; 23% of the LVC sample was low educated.

Caribbean Netherlands

Of 8,068 invitees, 300 (3.7%) were excluded from the sample for not having received the invitation (due to rehousing or because they were unknown on the address (*n* = 196), or because of delivery issues (*n* = 84)), mental disability (*n* = 17) or death (*n* = 3). *Table 2* shows the participants and collected materials in CN. In total, 1,815 persons (23.4%) completed the questionnaire and donated a blood sample (Bonaire: 1,122 (24.0%); St. Eustatius: 473 (22.9%); Saba: 220 (21.2%)), and 1,762 (92.7%) participants with any material gave consent to be approached for a follow-up study. A detailed description of response per island, stratified by age groups, can be found in *Supplement Table S1*. Among the responders there are relatively more women, more persons aged 0–11 years old and fewer people aged 18–34 years old compared to the non-responders (see *Supplement Table S2*).

In total, more females (55.2%) were included than males (44.8%) (*Table 3*). Females responded better than males on each island, with 27.1% vs. 20.0% on average in CN, respectively (*Figure 2C* and *Figure 2D*). More specifically, the highest response was seen in females aged 35–59 years (30.0%) and the lowest in males aged 18–34 years (11.0%). As for country of birth, invitees born in Aruba and the former Dutch Antilles (24.4%) as well as in NL (28.3%) responded significantly better compared to participants born in another country (18.9%), especially on St. Eustatius and Saba (data not shown).

Half of the people included considered themselves as Roman Catholic, a small portion as Protestant (2.5%), 35% as other than the previous two, such as Methodist or Adventist, and 12.5% indicated not to be religious. Moreover, more than half of the participants (54.2%) indicated to be lower educated, followed by middle (25.5%) and high (20.3%).

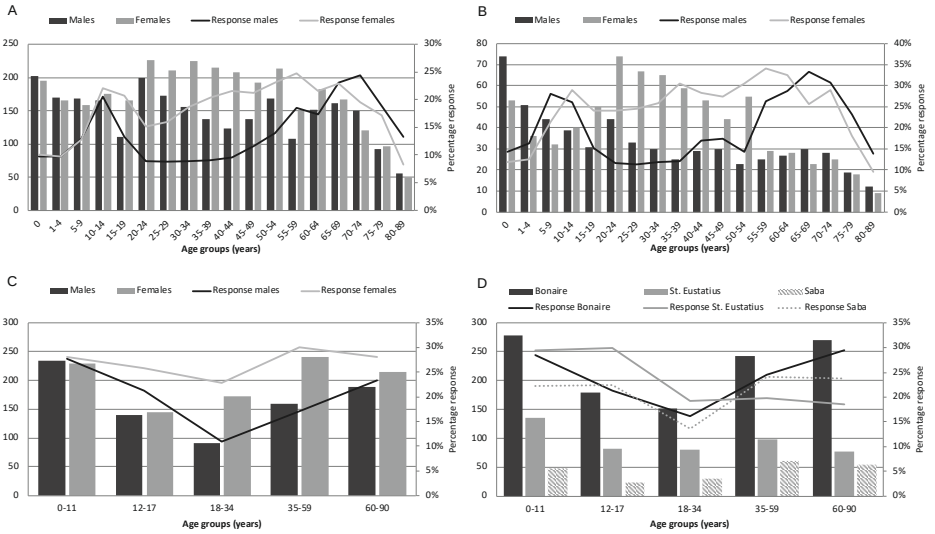

Figure 2. Overview of participants with both a blood sample and a questionnaire and corresponding response rates (n (%)). **A**. Overview of number of participants of the national sample and oversampling of non-Western migrants of the PIENTER-3 study, stratified by sex and age class. **B**. Overview of number of participants of the low vaccination coverage sample of the PIENTER-3 study, stratified by sex and age class. **C**. Overview of number of participants of the Caribbean Netherlands sample, stratified by sex and age class. **D**. Overview of number of participants of the Caribbean Netherlands sample, stratified by island and age class.

DISCUSSION

A third national biobank among the general population of NL has been generated and will be an important tool to evaluate infectious disease control in NL and contribute to public health policy. The seroprevalence data will provide insight into the effectiveness of the Dutch NIP and direction for improvement. These results will also be of value for future outbreak management. In addition, data collection has been extended to CN for the first time, which resulted in an extensive amount of information that will enable us to support future public health policy on these Caribbean islands, e.g., regarding tropical pathogens. Moreover, besides serum collection, a large number of additional materials have been collected, which allows us to look into relevant emerging topics, such as antibiotic resistance and the microbiome. Hence, this biobank offers unique opportunities to investigate infectious diseases in a much broader sense.

A high number of persons participated in this study, which enables us to perform most seroepidemiological (sub)analyses with sufficient power as calculated beforehand. Response rate (blood and questionnaire) in the NS sample was 14.4% and in CN

23.4% and, in line with other international studies [20-22], females and elderly as well as natives were highest responders among the invitees. Overall, the NS sample (including the oversampling of non-Western migrants) is rather comparable to the Dutch population, especially regarding urbanisation degree and religion [23, 24]. Religion is an important variable with regard to seroprevalence as vaccination coverage among several denominations in NL is much lower compared to the rest of NL [16]. Females as well as indigenous Dutch, people from SAN and other non-Western countries – other than Morocco and Turkey – were slightly overrepresented (e.g., 54.2% females and 78.1% indigenous Dutch in our sample vs. 50.4% and 73.2%, respectively, in the Dutch population) [25]. Overrepresentation of SAN and non-Western migrants was due to the oversampling. Further, participants had a higher educational level as compared to the general Dutch population (31.4% is low-educated in the Dutch population vs. 27.1% in our sample) [26]. Generally, for each age strata in the NS sample, sufficient participants were included, although overall we reached marginally lower numbers per age strata than in the 2006/2007 study, except for 0-year-olds. For the LVC sample, sufficient ORI-participants were included in each age strata, except for the oldest age group (60–89 years).

The CN sample in this study is generally a good reflection of the total population on the islands concerning religion and educational level [27, 28]. People born in non-Western countries (which consists of 93% Latin Americans in our CN sample) and males are slightly less represented in our study population though (namely, 14.4% Latin Americans and 44.8% males in our sample vs. 19.3% and 51.5%, respectively, in the CN population) [29].

Sociodemographic dissimilarities compared with the general populations of NL and CN, due to selectivity in response and the sample design, will be taken into account by weighting the participants on a set of variables (age, sex, ethnicity and degree of urbanisation). An in-depth non-response analysis for NL will be carried-out and published in the near future. Moreover, having applied an identical robust design for the third time ensures maximum comparison with previous studies and opens opportunities for changes over time and modelling analyses. A less costly and less extensive design of sample collection, for instance via residual sera, is more prone to selective response and lacks the opportunity to collect additional materials as well as data on various characteristics of participants to perform risk factor analyses.

The response rate in the NS sample (14.4%) was lower as compared to the two previous serosurveys performed in NL (50% in 1995/1996 and 32% in 2006/2007). Nonetheless, low(er) response rates have also been reported in other recently conducted large population-based studies in NL (e.g., 'NL de Maat genomen', phase 1: < 20% [30] and 'Lifelines': 24.5% [31]) as well as abroad, for instance in the United Kingdom ('UK biobank' 5.5% [32]). Likewise, participation in health examination studies

in other European countries (e.g., 'FINRISK', Finland, 'HSE', England, 'DEGS', Germany, etc.) all show a decrease in responses over the past decades [21] and this declining trend is also observed in a large serosurveillance study in America ('NHANES') [20]. Although the underlying reasons for this worldwide decreasing trend in study participation is not exactly known and may differ per study and country, our non-response questionnaire indicated that most persons did not have time to come to the clinic or were dreading blood collection. It has been previously reported that participation in a population-based survey including collection of blood, especially in children, is likely to be low [33, 34]. Although we tried to organize several consultation hours at centralized locations, travel time might have also been a conflicting factor for some people, especially in larger municipalities where response rates were lowest. Other sampling options, such as self-sampling finger pricks or house visits, could therefore be considered in the future to overcome some of the hurdles of reluctance to participate. This implies, however, that laboratory techniques should be suitable for analyses in low volumes of blood or other sources of material, like DBS or saliva. Last, as participants often do not receive any personal results when participating in health studies, we suspect that, in nowadays more individualized societies, there might be less willingness to contribute without direct personal benefit; hence, future studies should consider such incentives if feasible.

The response rate in CN (23.4%) was higher compared to NL. Possible explanations might be a high awareness of the study as a relatively high proportion of island residents were invited to participate, the extensive local media attention, and the presumably less individualistic island culture. Previous studies on the islands reported higher participation rates (e.g., 'Omnibusenquête' (2013) [35]: 40–62%; 'Kon Salu ta…' ('How healthy is…') (2002): 80–86%) [36-38], however a completely different approach was used in these studies: house visits with multiple contact efforts, the design (solely questionnaire), and longer duration of the study, i.e., our efficient time planning limited adjustment during the study in order to increase response. Further, the population registry was not fully up-to-date and the delivery of the invitations by mail was challenging for the local postal department, which both could have negatively affected our net response. This reaffirms that logistical matters are unavoidable for these small islands and thus future studies should consider building in large(r) time margins. Moreover, population-based studies including sample collection are unfamiliar among the CN population. In order to increase awareness and response, an extensive communication plan was made tailored per island using all communication tools available. We experienced that personal appeal, repetition and promotion via key figures was most beneficial in term of response. Nevertheless, initially we might have missed some participants due to taboos towards collection of faecal material. In line with our study in NL, future studies in CN could consider introducing additional collection with ditto incentive regarding such samples to increase overall response.

CONCLUSIONS

In conclusion, this third Dutch biobank offers unique future research possibilities. Over 9,000 blood samples and questionnaires as well as additional materials, such as saliva, oro- and nasopharyngeal swabs and faeces, have been collected. This offers the opportunity to perform thorough seroprevalence studies assessing immunity against and risk factors for (candidate) NIP-targeted diseases, taking into account different infection dynamics in the Caribbean region. Furthermore, for the first time, these data will inform us on the occurrence of and risk factors for tropical pathogens in the Caribbean region, such as zika, dengue, chikungunya, West Nile virus and yellow fever. Moreover, we will be able to connect the serological data to results from additional materials collected (via molecular typing and bacterial cultures) and environmental- and pharmacological data. Hence, we are able to gain relevant new insights into the emerging fields of the microbiome, antibiotic resistance and carriage of pathogens in relation to vaccination responses, allergies, environmental- and lifestyle factors [39]. Besides the extensive data collection, the vast majority of participants gave consent for participation in a potential follow-up study enabling a nested-case cohort study in the future. Summarized, this large biobank forms a great base for research in the field of infectious disease epidemiology and its changing dynamics and consequently, this knowledge can guide future public health policy in NL and CN.

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SUPPLEMENTARY MATERIALS

Supplement Table S1. Supplementary information regarding sample size calculations, number of invitees and response of the PIENTER-3 study, stratified by study sample.

Age-specific prevalence	Precision (one-way test)	Alpha error	Anticipated response rate	Nr. of invitees per age group in years (anticipated nr. of participants)	Nr. of participants with a questionnaire and blood sample (response %)[a]	Remarks
National sample (NS)						
50%	2.5% overall seroprevalence and 10-15% age-specific seroprevalence	5%	The starting point was a similar number of invitees per municipality as in the former study and response rates of NS sample in previous study (PIENTER-2)[1] thereby oversampling 0-4-year-olds and 20-39-year-olds because of lower response rates	0: 4,059 (400) 1–4: 2,557 (400) 5–9: 1,410 (480) 10–14: 1,308 (480) 15–19: 1,348 (360) 20–24: 3,441 (360) 25–29: 2,883 (360) 30–34: 2,528 (320) 35–39: 2,129 (360) 40–44: 1,841 (320) 45–49: 1,693 (360) 50–54: 1,767 (360) 55–59: 880 (360) 60–64: 880 (400) 65–69: 880 (400) 70–74: 880 (320) 75–79: 880 (280) 80–89: 880 (280) Total: 32,244 (6,320)	393 (10%) 288 (11%) 240 (17%) 314 (24%) 256 (19%) 408 (12%) 362 (13%) 350 (14%) 321 (15%) 303 (17%) 294 (18%) 348 (20%) 221 (26%) 261 (30%) 271 (31%) 239 (28%) 178 (21%) 97 (11%) 5,144 (16%)	In the first 11 municipalities a sample of in principal 494 individuals per municipality was drawn, thereby oversampling 0-year-olds and 5-19-year-olds to calculate seroprevalence for each four months in the first year of life and per year until the age of 19 years. However, during the study this was adjusted for the age strata 0-54 years because of lower response rate than expected, which resulted in a total of in principal 818 persons invited in the next 13 municipalities based on the actual inclusion rates. Finally, in the last 16 municipalities 193 extra men (a total of in principal 1011) were invited in the age range of 20-54 year since women responded predominantly.

Supplement Table S1. (Continued)

Age-specific prevalence	Precision (one-way test)	Alpha error	Anticipated response rate	Nr. of invitees per age group in years (anticipated nr. of participants)	Nr. of participants with a questionnaire and blood sample (response %)[a]	Remarks
Oversampling non-Western migrants (migrants)						
75%	10% age-specific seroprevalence	5%	Response rates of migrant sample in previous study (PIENTER-2)[1]	0–9: 2,167 (199) 10–34: 1,815 (167) 35–59: 2,017 (187) 60–89: 2,260 (207) Total: 8,259 (760)	138 (6.6%) 116 (6.2%) 166 (8.1%) 181 (8.4%) 601 (7.3%)	The number of invited migrants per municipality was in line with the distribution of the migrant groups per urbanisation degree in the Dutch population. We aimed for 70 participants per migrant group and age group. Note, that for the calculation of the number of extra non-Western migrants to invite in this sample we took into account the number of expected non-Western migrant participants in the NS sample based on response rates per migrant group and age group of the previous study (PIENTER-2).
Oversampling people in low vaccination coverage areas (LVC)						
75%	10% age-specific seroprevalence	5%	Response rates of LVC sample in previous study (PIENTER-2)[1]	0–9: 1,879 (70 ORIs) 10–34: 2,460 (70 ORIs) 35–59: 1,625 (70 ORIs) 60–89: 900 (70 ORIs) Total: 6,864 (280 ORIs)	84 ORIs (4.5%) 117 ORIs (4.8%) 65 ORIs (4.0%) 33 ORIs (3.7%) 299 ORIs (4.4%)	An oversampling of 0-year-olds took place, with the aim to include 50 Orthodox Reformed infants in total. For each of the four age strata we aimed to include 70 Orthodox Reformed Individuals (ORIs). An extra municipality was added halfway the study to reach a sufficient number of participants living in LVC areas. In the last municipality, the number of invited individuals was increased in the 20-54 year-old men.
Oversampling people with migration background from Suriname, Aruba and the former Dutch Antilles (SAN)						
50%	10% age-specific seroprevalence	5%	7%, based on response rate SAN people first 10 municipalities NS	0–9: 1,833 (95) 10–34: 1,833 (95) 35–59: 1,832 (94) 60–89: 1,830 (94) Total: 7,328 (378)	96 (5.3%) 79 (4.4%) 137 (7.6%) 189 (10.4%) 501 (6.9%)	The SAN group will be part of a specific serosurvey analysis.

Supplement Table S1. (Continued)

Age-specific prevalence	Precision (one-way test)	Alpha error	Anticipated response rate	Nr. of invitees per age group in years (anticipated nr. of participants)	Nr. of participants with a questionnaire and blood sample (response %)[a]	Remarks
Sample of Caribbean Netherlands (CN)						
				Total: 7,768 (2,442)	Total: 1,815 (23%)	
Bonaire						
50%	5.5% age-specific seroprevalence	5%	30%, based on response rate NS sample in previous study (PIENTER-2)[1]	0–11: 982 (284) 12–17: 839 (258) 18–34: 940 (296) 35–59: 990 (305) 60–89: 916 (289) Total: 4,667 (1432)	279 (28%) 179 (21%) 152 (16%) 242 (24%) 270 (29%) Total: 1,122 (24%)	Samples were drawn using PIVA-V of January 1st, 2017. An additional sample of Bonaire was drawn using PIVA-V of April 1, 2017 to include all registered new-borns from January 1 till March 31, 2017 (*n* = 42).
St. Eustatius						
50%	7.5% age-specific seroprevalence	5%	30%, based on response rate NS sample in previous study (PIENTER-2)[1]	0–11: 461 (126) 12–17: 274 (107) 18–34: 417 (136) 35–59: 495 (151) 60–89: 415 (132) Total: 2,062 (652)	136 (30%) 82 (30%) 80 (19%) 98 (20%) 77 (19%) Total: 473 (23%)	
Saba						
50%	10% age-specific seroprevalence	5%	30%, based on response rate NS sample in previous study (PIENTER-2)[1]	0–11: 220 (69) 12–17: 107 (51) 18–34: 227 (78) 35–59: 253 (84) 60–89: 232 (76) Total: 1,039 (358)	49 (22%) 24 (22%) 31 (14%) 61 (21%) 55 (24%) Total: 220 (21%)	

[a] For the response (%) percentages were calculated based on net response, i.e. excluding non-eligible invitees.

[1] van der Klis FR, Mollema L, Berbers GA, de Melker HE, Coutinho RA. Second national serum bank for population-based seroprevalence studies in the Netherlands. The Netherlands journal of medicine. 2009;67(7):301-8.

Supplement Table S2. Supplementary information regarding response of the PIENTER-3 study, stratified by study sample.

National sample (including the oversampling of non-Western migrants) (n (%))

	Nr. of responders with any material (%)	**Nr. of non-responders (%)**
Age groups (years)	6,217 (100%)	33,681 (100%)
0	451 (7.3%)	3,548 (10.5%)
1–4	382 (6.1%)	3,068 (9.1%)
5–9	354 (5.7%)	2,182 (6.5%)
10–14	367 (5.9%)	1,233 (3.7%)
15–19	313 (5.0%)	1,331 (4.0%)
20–24	469 (7.5%)	3,269 (9.7%)
25–29	429 (6.9%)	2,843 (8.4%)
30–34	422 (6.8%)	2,541 (7.5%)
35–39	380 (6.1%)	2,188 (6.5%)
40–44	361 (5.8%)	1,894 (5.6%)
45–49	351 (5.7%)	1,735 (5.2%)
50–54	405 (6.5%)	1,734 (5.2%)
55–59	273 (4.4%)	915 (2.7%)
60–64	342 (5.5%)	1,372 (4.1%)
65–69	334 (5.4%)	1,096 (3.3%)
70–74	277 (4.5%)	957 (2.8%)
75–79	193 (3.1%)	862 (2.6%)
80–89	114 (1.8%)	913 (2.7%)
Sex	6,217 (100%)	33,681 (100%)
Males	2,859 (46.0%)	18,756 (55.7%)
Females	3,358 (54.0%)	14,925 (44.3%)
Country of birth	6,217 (100%)	33,681 (100%)
The Netherlands	4,827 (77.2%)	19,586 (58.2%)
Morocco and Turkey	162 (2.6%)	3,901 (11.6%)
Suriname, Aruba and (former) Dutch Antilles	323 (5.2%)	3,334 (9.9%)
Other non-Western countries	489 (7.9%)	3,954 (11.7%)
Other Western countries	415 (6.7%)	2,884 (8.6%)
Unknown	1 (0.1%)	22 (0.1%)
Degree of urbanisation	6,217 (100%)	33,681 (100%)
1. Highly urbanised	1,369 (22.0%)	11,475 (34.1%)
2. Urbanised	2,027 (32.6%)	10,142 (30.1%)
3. Moderate urbanised	1,179 (19.0%)	5,523 (16.4%)
4. Little urbanised	1,116 (18.0%)	4,156 (12.3%)
5. Countryside	526 (8.5%)	2,385 (7.1%)

Sample of Caribbean Netherlands (CN) (n (%))

	Nr. of responders with any material (%)	**Nr. of non-responders (%)**
Island	1,815 (100%)	5,953 (100%)
Bonaire	1,122 (61.8%)	3,545 (59.5%)
St. Eustatius	473 (26.1%)	1,589 (26.7%)
Saba	220 (12.1%)	819 (13.8%)
Sex	1,815 (100%)	5,953 (100%)
Males	813 (44.8%)	3,260 (54.8%)
Females	1,002 (55.2%)	2,693 (45.2%)
Age groups (years)	1,815 (100%)	5,953 (100%)
0–11	464 (25.6%)	1,199 (20.1%)
12–17	285 (15.7%)	935 (15.7%)
18–34	263 (14.5%)	1,321 (22.2%)
35–59	401 (22.1%)	1,337 (22.5%)
60–89	402 (22.1%)	1,161 (19.5%)
Sex, by age groups (years)	1,815 (100%)	5,953 (100%)
Males	813 (100%)	3,260 (100%)
0–11	234 (28.8%)	610 (18.7%)
12–17	140 (17.2%)	519 (15.9%)
18–34	91 (11.2%)	740 (22.7%)
35–59	160 (19.7%)	776 (23.8%)
60–89	188 (23.1%)	615 (18.9%)
Females	1,002 (100%)	2,693 (100%)
0–11	230 (23.0%)	589 (21.9%)
12–17	145 (14.5%)	416 (15.4%)
18–34	172 (17.2%)	581 (21.6%)
35–59	241 (24.1%)	561 (20.8%)
60–89	214 (21.4%)	546 (20.3%)
Country of birth	1,815 (100%)	5,953 (100%)
Aruba and (former) Dutch Antilles	1,170 (64.5%)	3,627 (60.9%)
the Netherlands	49 (13.7%)	631 (10.6%)
Other	396 (21.8%)	1,695 (28.5%)

PART I

Evaluation of population immunity of vaccine-preventable diseases in Caribbean Netherlands

CHAPTER 3

Risk of measles and diphtheria introduction and transmission on Bonaire, Caribbean Netherlands, 2018

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ABSTRACT

Endemic transmission of measles has been reestablished in Venezuela, and outbreaks of diphtheria remain ongoing across Latin America (LA). Hence, a large cross-sectional population-based serosurveillance study was conducted on Bonaire, one of the Dutch Leeward Antilles, to assess specific age and population groups at risk. Participants (aged 0–90 years) donated a blood sample and completed a questionnaire (*n* = 1,129). Antibodies against measles and diphtheria were tested using bead-based multiplex immunoassays. Our data revealed that immunity against measles is suboptimal, especially for those aged less than 5 years from Suriname, Aruba, and former Dutch Antilles (SADA), and adolescents from LA; and against diphtheria for persons aged more than 30 years, particularly among females and residents from SADA and LA. As refugees arrive persistently, health authorities on the Dutch Leeward Antilles should be on alert to detect early cases and prevent subsequent transmission. Ultimately, there is an urgent need for serosurveillance studies in the Caribbean region.

Whereas in 2016 the Americas was the first WHO Region to have reached measles elimination, endemic transmission has been reestablished in Venezuela as of August 2018 [1]. Concurrently, diphtheria is emerging rapidly as large outbreaks have been ongoing since mid-2016 [2]. Venezuela is facing a profound humanitarian crisis with the outflow of millions of its inhabitants into neighboring countries [3]. Because of political developments and socioeconomic depression, the country faces lack of funding for public health activities. Together with shortages of supply of medicine, including vaccines, this resulted in a disrupted National Immunization Program (NIP) [4]. As of August 2018, 8,544 confirmed measles cases had been reported across the country, resulting in 62 deaths, and 1,992 suspected diphtheria cases, with 168 deaths [1, 2]. The massive outflow of unvaccinated and possibly infected Venezuelans to surrounding countries cause a substantial risk of introduction of vaccine-preventable diseases (VPDs) [3]. Neighboring countries in Latin America (LA) have already reported imported and autochthonous measles and diphtheria cases (e.g., Brazil (measles) and Colombia (both)), and corresponding deaths [1, 2].

The Dutch Leeward Antilles Aruba, Bonaire, and Curaçao are located in the southern Caribbean Sea nearby the northern coast of Venezuela. More than 25,000 Venezuelan refugees have arrived on these islands and this number is growing [3]. Hence, considering the small size and limited capacity of these Antilles, large numbers of arrival — which account for ~10% of the total combined population — have great impact on the community and could potentially introduce measles and diphtheria in a population with possible susceptible pockets.

Vaccination is a highly effective method of preventing measles and diphtheria. On the Dutch Leeward Antilles, monovalent measles vaccination (one dose) for children aged 15 months was introduced in 1977 and was replaced by the measles–mumps–rubella (MMR) vaccine in 1988 for infants aged 14 months. A booster for 9-year-olds followed in 1991 [5]. Diphtheria-containing vaccines have been administered from the 1940s. The present NIP [5] recommends five doses of diphtheria-tetanus-acellular pertussis–inactivated poliovirus vaccine (DTaP-IPV, at the ages of 2, 3, 4, and 11 months, and 4 years) and one dose of diphtheria-tetanus–inactivated poliovirus vaccine (DT-IPV) (at 9 years). On Bonaire, the early childhood vaccination coverage is 90% (at the age of 2 years); however, the coverage is below 70% at the age of 10 years. Fortunately, no cases of measles or diphtheria have been reported in the last decade [6].

Supported by our cross-sectional population-based serosurveillance study ('Health Study Caribbean Netherlands', for a brief description [7]) conducted on Bonaire in mid-2017, we present the population seroprevalence underpinning the potential emerging risk of measles and diphtheria introduction and transmission and discuss the corresponding preventive measures.

The study proposal was approved by the Medical Ethics Committee Noord-Holland, the Netherlands (METC-number: M015-022), and informed consent was obtained from all adult participants and parents or legal guardians of minors included in the study. From the population registry (*n* = 19,203), an age-stratified sample of 4,798 inhabitants (with age strata 0–11, 12–17, 18–34, 35–59, and 60–90 years) was drawn, of which *n* = 1,197 responded (net response rate: 26%). At the clinic, participants were requested to donate a fingerstick blood sample — which was collected via the dried blood spot method — and to complete a questionnaire on infectious diseases and other health-related factors (*n* = 1,129). Samples were air-shipped to the laboratory of the National Institute for Health and the Environment (RIVM), Bilthoven, the Netherlands, directly after the fieldwork period. IgG antibodies against measles and diphtheria were analyzed using bead-based multiplex immunoassays, as described previously [8, 9]. For measles, IgG antibody levels ≥ 0.120 international units per mL (IU/mL) were considered seropositive [10], and for diphtheria, 0.01 IU/mL was considered the minimum protective level [11].

In this study, among those eligible for the NIP (i.e., until 41 and 64 years for measles and diphtheria, respectively), the vaccination registry showed that 463 participants (68.9%) received at least one dose of a measles-containing vaccine (more specifically, one dose: 248 (36.9%); two or more doses: 215 (32.0%)) and 530 (55.8%) participants had been administered at least once with a diphtheria-containing vaccine (more precisely, one dose: 39 (4.1%); two to five doses: 313 (32.9%); six or more doses: 178 (18.7%)). From NIP-eligible participants without vaccination registry, 164 (78.5%) self-reported to have (partly) joined the NIP and 304 (73.1%) self-reported to have been administered with a diphtheria-containing vaccine as a child. The vaccination coverage (i.e., at least one dose based on registry or self-reporting) for measles was 93.4%, 93.9%, and 86.9% in age groups 0–11, 12–17, and 18–34 years, respectively, and for diphtheria, the vaccination coverage was 99.6%, 92.8%, 83.1%, and 74.4% in age groups 0–11, 12–17, 18–34, and 35–59 years, respectively.

Population-based estimates showed that the overall measles seroprevalence was 93.7% (95% confidence interval (CI): 91.9–95.4) with an overall geometric mean concentration (GMC) of 0.918 IU/mL (95% CI: 0.829–1.016). None of the infants aged less than 1 year in our sample (*n* = 15; all unvaccinated) had (maternal) antibodies above the cutoff (*Figure 1).*

The seroprevalence for the age group 1–4 years — i.e., after the first dose of the MMR vaccine at 14 months — was 85.2% (95% CI: 76.6–93.9) and steadily increased to 94.5% (95% CI: 90.8–98.3) for the age group 10–14 years, which most probably reflects the vaccine response after the (second) dose (mostly) administered at 9 years, also demonstrated by a slightly elevated GMC. Thereafter, the seroprevalence was below 95% — a level considered necessary for herd immunity [12] — until the age group

40–44 years. After the first MMR vaccination, GMCs remain above the protective cutoff and steeply increased from the age group 40–44 years onward, reflecting the people naturally exposed to the virus. The overall seropositivity was lowest for people from LA (91.0%), especially at the age group 12–17 years (64.0% (95% CI: 45.6–82.4) (data not shown)), and from Suriname, Aruba, and former Dutch Antilles (SADA) (93.8%) (*Table 1*), particularly at the age group 1–4 years (86.2% (95% CI: 77.1–95.2)) (data not shown).

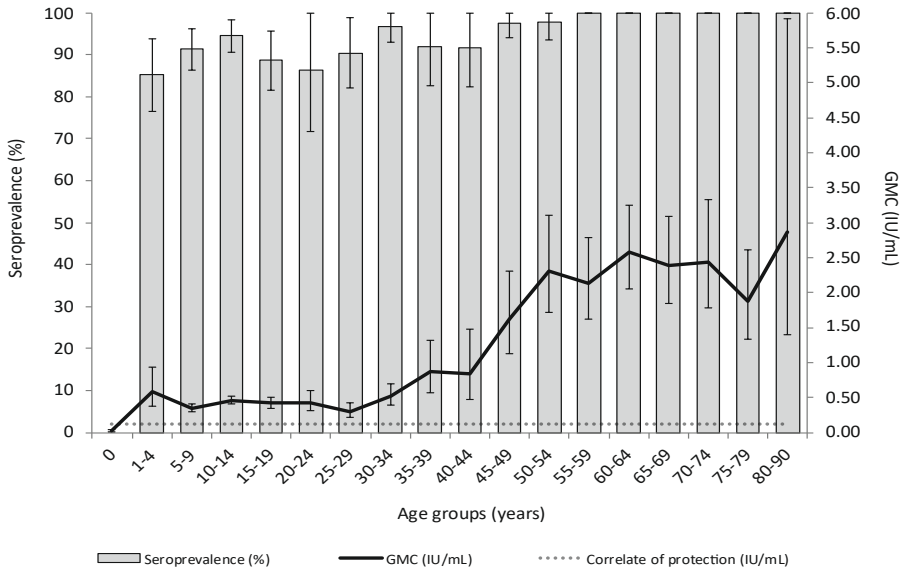

Figure 1. Weighted age-specific seroprevalence and geometric mean concentration (GMC) (with 95% CIs) of measles IgG antibodies in the general population of Bonaire, Caribbean Netherlands, 2017. Note: Antibody concentration ≥ 0.120 IU/mL was considered protective, i.e., the correlate of protection. A seroprevalence of 95% is considered necessary for herd immunity.

For diphtheria, 78.3% (95% CI: 75.2–81.3) of the overall antibody levels was above the minimum protective level (of 0.01 IU/mL), with a GMC of 0.047 IU/mL (95% CI: 0.042–0.053). After the last DT-IPV vaccine administered at the age of 9 years, the GMC rapidly declined and remained just above the minimum protective level from the age of 30 years onward. From 30 years onward, the overall seropositivity was below 75% — a level considered important for herd protection in adults [13] — namely, 69.3% (95%: CI: 65.0–73.7), aside from the age group 60–64 years (82.1%) (*Figure 2*).

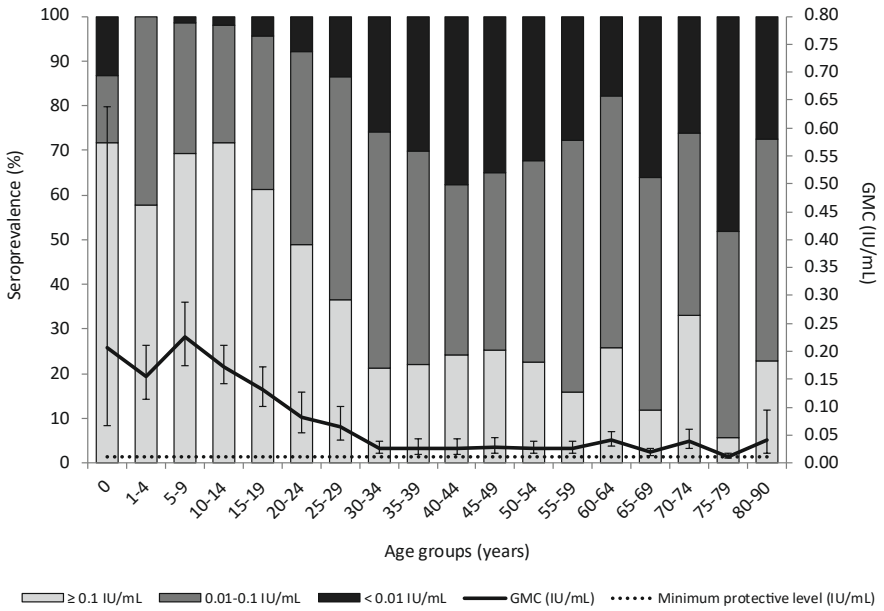

Figure 2. Weighted age-specific seroprevalence and geometric mean concentration (GMC) (with 95% CIs) of diphtheria IgG antibodies in the general population of Bonaire, Caribbean Netherlands, 2017. Note: Antibody concentration below 0.01 IU/mL was considered non-protective, 0.01–0.1 IU/mL provides basic protection (i.e., 0.01 IU/mL is the minimum protective level), and ≥ 0.1 IU/mL provides full protection. A seroprevalence of 75% is considered necessary for herd immunity in adults.

Notably, the seropositivity for females was significantly lower than that for males (73.5% versus 82.7%; $p < 0.005$) (*Table 1*), particularly at the age group 50–54 years (45.6% versus 88.0%; $p < 0.001$) (data not shown). Males slightly more often self-reported to be vaccinated because of their profession, a trip abroad, or military service in the past (29.6% versus 24.6%). Furthermore, the seropositivity was lowest among residents from SADA (78.1%) (*Table 1*), LA (72.9% (95% CI: 65.1–80.7)), and other non-Western countries (79.2% (95% CI: 56.7–100.0)) (data not shown) — who all self-reported to be less vaccinated than indigenous Dutch and people from other Western countries — especially at the age group 35–59 years (SADA: 60.0% (*Table 1*); and LA: 67.2% (95% CI: 55.2–79.3) (data not shown)).

Table 1. Weighted age-specific seroprevalence (with 95% confidence interval (CI)) of measles and diphtheria IgG antibodies in the general population of Bonaire, Caribbean Netherlands, 2017, by sex and age stratum, and ethnic background and age stratum.

	***n* (%)** ***n* = 1,129**	**Measles seroprevalence (95% CI)**	**Diphtheria seroprevalence (95% CI)**
Sex and age stratum (years)			
Males	506 (44.8%)	93.4% (90.7–96.0)	82.7% (78.2–87.2)
0–11	141	83.6% (76.9–90.2)	99.1% (97.4–100.0)
12–17	85	87.9% (80.3–95.5)	95.5% (90.9–100.0)
18–34	51	90.2% (81.8–98.7)	83.9% (73.1–94.8)
35–59	96	96.5% (92.5–100.0)	76.7% (67.8–85.5)
60–90	133	100.0% (100.0–100.0)	77.0% (69.6–84.3)
Females	623 (55.2%)	94.0% (91.9–96.0)	73.5% (69.5–77.6)
0–11	130	83.6% (76.5–90.7)	97.6% (94.8–100.0)
12–17	96	93.8% (88.9–98.7)	94.6% (89.7–99.5)
18–34	109	92.8% (87.9–97.7)	86.0% (79.5–92.5)
35–59	146	95.6% (92.1–99.1)	57.5% (49.3–65.6)
60–90	142	100.0% (100.0–100.0)	65.4% (57.2–73.7)
Ethnic background[a] and age stratum (years)			
Indigenous Dutch and other Western countries[b]	143 (14.2%)	95.9% (92.1–99.7)	86.0% (79.5–92.4)
0–11	22	96.7% (90.4–100.0)	100.0% (100.0–100.0)
12–17	9	100.0% (100.0–100.0)	75.0% (45.1–100.0)
18–34	14	83.7% (62.8–100.0)	86.4% (68.8–100.0)
35–59	44	95.9% (90.4–100.0)	86.7% (75.3–96.1)
60–90	54	100.0% (100.0–100.0)	83.2% (73.4–93.0)
Suriname, Aruba, and former Dutch Antilles[c]	803 (64.5%)	93.8% (91.7–95.8)	78.1% (74.3–81.9)
0–11	236	83.2% (77.9–88.5)	98.5% (96.7–100.0)
12–17	142	96.3% (93.0–99.5)	98.2% (96.0–100.0)
18–34	110	93.5% (88.1–98.8)	86.2% (78.3–94.1)
35–59	128	96.1% (92.4–99.7)	60.0% (51.1–69.0)
60–90	187	100.0% (100.0–100.0)	68.3% (61.2–75.3)
Latin America and other non-Western countries[d]	182 (21.3%)	91.8% (87.2–96.4)	73.5% (66.1–80.9)
0–11	13	68.5% (42.6–94.5)	93.9% (82.2–100.0)
12–17	30	65.4% (47.5–83.3)	87.4% (75.2–99.6)
18–34	36	88.2% (76.0–100.0)	81.1% (67.8–94.5)
35–59	69	96.1% (90.8–100.0)	68.1% (56.7–79.4)
60–90	34	100.0% (100.0–100.0)	68.6% (52.9–84.3)

[a] Ethnic background was unknown for one male in the age group 35–59 years.

[b] *n* = 41 (29%) participants from Western countries other than indigenous Dutch.

[c] Former Dutch Antilles includes the islands Bonaire, Curaçao, Saba, St. Eustatius, and St. Maarten.

[d] *n* = 171 (94%) participants from Latin American countries within the group Latin America and other non-Western countries.

This is the first ever conducted serosurveillance study on Bonaire, providing important data demonstrating that immunity against measles and diphtheria is insufficient. As outbreaks of these VPDs are ongoing in surrounding countries and Venezuelan refugees are arriving constantly, the risk of introduction and subsequent transmission is present. The overall seroprevalence for measles was high (93.7%), however, not reaching the level considered necessary for herd immunity (i.e., 95%) [12]. Subgroups with the lowest seroprevalence are those aged less than 44 years, more specifically adolescents (aged 12–17 years) from LA and, most strikingly, infants aged less than 5 years from SADA. To decrease susceptibility in this vulnerable group and in line with countries in the region, the public health department on Bonaire has lowered the age for the second MMR vaccine from 9 years to 18 months as of January 1, 2019. Furthermore, the diphtheria overall seroprevalence (i.e., proportion of people with a minimum protective level) was rather low (78.3%). Waning immunity, indicated by declining GMCs with age, has been reported previously by others [14-17], and because of the increased exposure, this could be a potential risk on Bonaire. Risk groups include people aged more than 30 years, especially females and people from SADA and LA. Importantly, because measles and diphtheria are highly contagious, the probability of introduction and transmission is more likely when individuals who lack protection cluster together, for example, children at schools or people from the same cultural background or religion [18].

Taken together, there is an urgent need for increased awareness on Bonaire, one of the Dutch Leeward Antilles, considering potential introduction of measles and diphtheria cases amid groups with lower seroprotection. Surrounding islands facing an ongoing influx of refugees should be on the alert too. The vaccination status of refugees remains to be verified on arrival if possible, with vaccinations offered to those who are eligible to ensure full protection. (Re)vaccination of risk groups who lack protection and people who are in close contact with refugees should be considered. In addition, early detection, rapid treatment, and well-coordinated source- and contact tracing (according to ring principle) are of great importance to prevent transmission and disease. Health-care workers, who should be well vaccinated themselves, must be aware of the control measures according to applicable guidelines. Diphtheria can cause severe complications (e.g., myocarditis), and the case fatality rate without treatment is 50% [19]; hence, a rapid supply of antitoxins (and antibiotics) should be facilitated. This, together with confirmation by laboratory diagnostics and notification of cases, is essential to control subsequent transmission. Last, serosurveillance studies in the Caribbean region are scarce. The present study enables us to carry out representative epidemiological (sub) analyses as we chose a robust design to diminish selective response, for example, instead of using residual sera, and weighted our sample on a set of sociodemographic factors to correctly represent the population of Bonaire. Ultimately, there is a need for data across the region to detect gaps in terms of population immunity and to further

decrease the risk of imported and autochthonous transmission of VPDs. Preventive measurements as described here should be considered across the region in the meantime.

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CHAPTER 4

Seroepidemiology of measles, mumps and rubella on Bonaire, St. Eustatius and Saba: the first population-based serosurveillance study in Caribbean Netherlands

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ABSTRACT

The National Immunization Program (NIP) on Bonaire, St. Eustatius and Saba (i.e., Caribbean Netherlands (CN)) includes the measles-mumps-rubella (MMR) vaccine since 1988/89. Seroepidemiological data is an important tool to evaluate the NIP, hence a cross-sectional representative population-based serosurveillance study was conducted for the first time in CN in mid-2017. Participants (*n* = 1,829, aged 0–90 years) donated a blood sample and completed a health-related questionnaire. MMR-specific IgG antibodies were determined using a bead-based multiplex immunoassay and risk factors were analyzed using logistic regression models. Overall seroprevalence was high for measles (94%), but lower for mumps and rubella (both 85%). In NIP eligibles, including women of childbearing age, rubella seroprevalence (88%) exceeded the threshold for protection (85%); however, for measles (89%) this protective level (95%) was not met. MMR seropositivity was lowest in children who became CN resident at 11–17 years of age (especially for measles (72%)), mostly originating from Latin America and other non-Western countries. Interestingly, rubella seroprevalence was lowest in non-NIP eligible adults from Dutch overseas territories and Suriname (75%). Taken together, MMR immunity is generally good in CN, nonetheless some risk groups were identified. Additionally, we found evidence for a unique island epidemiology. In light of recent regional measles outbreaks, disease monitoring remains of utmost importance.

INTRODUCTION

Measles, mumps and rubella (MMR) are highly contagious viral diseases. Vaccination with trivalent MMR vaccine is safe and very effective at protecting against disease and severe complications [1]. Although incidence of MMR has declined drastically since the introduction of routine vaccination in the 1980s, elimination remains challenging, particularly for measles [2]. In fact, recent global resurgence of measles, involving large outbreaks in the World Health Organization (WHO) Region of the Americas, is of great concern as vaccination coverage is frequently insufficient to achieve herd protection in most countries [3, 4].

Caribbean Netherlands (CN), situated in the Caribbean Sea, consists of three Dutch special municipalities: Bonaire (one of the Dutch Leeward Antilles together with Aruba and Curaçao), St. Eustatius and Saba (both 800 km to the northeast). MMR vaccinations have been administered routinely in CN for decades. *Supplement Figure S1* gives an overview of introduction of MMR vaccinations and adaptations since 1975. Currently, the National Immunization Program (NIP) recommends two doses against MMR: On Bonaire, the first dose (MMR-1) is administered at 14 months and MMR-2 at 18 months (MMR-2 before 2019 at nine years of age); on St. Eustatius and Saba, MMR-1 is given at 12 months and MMR-2 at four years of age (MMR-2 before 2007 and 2016, respectively, at nine years of age) [5]. Vaccination coverage in CN has been registered routinely since a few years: In 2017, MMR-1-coverage was 92% (range 90–100%) and MMR-2 70% (range 67–100%) [6].

Since the implementation of syndromic surveillance in 2007, no imported or endemic MMR cases have been detected in CN. Additionally, registers on St. Eustatius and Saba indicated that no confirmed cases of measles or (Congenital) Rubella (Syndrome) have occurred since the introduction of the MMR vaccination (1988). However, it should be noted that only few suspected cases undergo laboratory confirmation due to a lack of facilities. On Curaçao, outbreaks of rubella have been reported in 1977 and 1985/1986; however, its scale and dissemination to Bonaire remains unspecified. Moreover, one imported case of measles was confirmed in May 2019 on Curaçao, and Aruba reports a few confirmed cases of mumps every year [7].

Seroepidemiological data play a crucial role in profiling population immunity, and is an important tool to evaluate the NIP and, if needed, adapt its policy [8]. The recent large measles outbreak across the Americas emphasizes the urgent need for information on protection against vaccine-preventable diseases, which is lacking for CN [3]. The aim of this cross-sectional population-based seroepidemiological study was to investigate the humoral immunity against MMR in the general population of CN, which enables identification of possible gaps in immunity (seronegativity) and risk factors associated with these gaps.

METHODS

Study design and study population

From May–June 2017, a biobank was established in CN: Health Study Caribbean Netherlands. Details on study design and data collection have been described elsewhere [9]. Briefly, on each island an age-stratified sample, with age strata 0–11, 12–17, 18–34, 35–59 and 60–89 years, was randomly drawn from the population registry of the Dutch overseas territories (PIVA-V, January 1, 2017). In total, 7,768 eligible individuals were invited (Bonaire *n* = 4,667; St. Eustatius *n* = 2,062 and Saba *n* = 1,039; see *Supplement Figure S2* for a flowchart of the study). Prior to participation, signed informed consent was obtained (from: < 12 years of age: Parent/legal guardian; 12–17 years of age: Participant and parent/legal guardian and ≥ 18 years of age: Participant). The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Medical Ethics Committee Noord-Holland (METC-number: M015-022). At the clinic, participants were requested to donate a blood sample — via a finger or heel prick using the dried blood spot method (DBS) on air-dried filter paper (Whatman® 903 protein saver cards)—to complete a questionnaire, and to bring their vaccination certificate. If the latter was not available, vaccination status was retrieved from the local public health department if obtainable.

Laboratory analyses

After the fieldwork, blood samples were air-shipped to the laboratory of the National Institute for Public Health and the Environment the Netherlands (RIVM) and stored instantly at −80 °C until analyses. MMR-specific IgG antibodies were determined with a fluorescent bead-based multiplex immunoassay using Luminex technology, as described previously [10]. In short, following standard protocol, a 3.2 mm (1/8 inch) punch was taken from the DBS and incubated in 300 µL phosphate-buffered saline containing 0.1% Tween-20 and 3% bovine serum albumin (i.e., assay buffer) at 4 °C overnight on a shaker to release serum (resulting in a 1:200 dilution) [11, 12]. Sera were further diluted to 1:4,000 in assay buffer. Controls, blanks and the international standard for rubella (RUBI-1-94), which was calibrated against the international standard for measles and an in-house standard for mumps, were included on each plate. Antibody concentrations were obtained by interpolation of the mean fluorescent intensity in the reference serum curve using a logistic-5PL regression type and expressed in international units per mL (IU/mL) for measles and rubella and RIVM units per mL (RU/mL) for mumps — as no international standard is available. An antibody concentration of ≥ 0.120 IU/mL for measles [13] and ≥ 10.0 IU/mL for rubella [14] was considered protective and used as

cut-off for seropositivity. For mumps, no correlate of protection is available: an antibody concentration of ≥ 45.0 RU/mL was used as arbitrary criterion for seroprevalence, upon agreement by the European Sero-Epidemiology Network [15].

Data analyses

Seroprevalence and GMC

Data were analyzed in SAS v.9.4 (SAS Institute Inc., USA) and R v.3.6. Analyses took account of the survey design. To match the population distribution on each island as of January 1, 2017, overall seroprevalence and geometric mean concentrations (GMC) for IgG antibodies were estimated by linear weighting, taking into account sex, age group and country of birth (and neighborhood on Bonaire). Differences in seroprevalence of MMR-specific antibodies between islands and gender were determined by estimating the parameters of the beta distribution for these seroprevalence rates using the methods of moments [16]. Risk ratios, their corresponding 95% confidence intervals (CI) and *p* values were estimated by Monte Carlo simulations of these seroprevalence estimates. Dissimilarities in GMC between islands and gender were identified by calculating the difference in natural logarithmic (ln) concentrations and tested using a t-test. Age-specific seroprevalence, GMC and 95% CIs were calculated for CN and per island. *P* values of < 0.05 were considered statistically significant.

Waning immunity after MMR vaccination

Linear regression analyses of MMR ln-antibody concentrations were conducted to study the persistence of antibodies after one and two MMR vaccination(s) received in the Dutch overseas territories. Analyses were restricted to participants who had received MMR-1 between 13–16 months of age (as vaccine response and waning of antibodies is shown to be different in children up to 12 months of age [17]) and MMR-2 between 8–10 years of age, both given at least 2 months before inclusion in the study. Additionally, maximum age at study inclusion was 9 and 30 years for one and two dose(s), respectively. For mumps, those with self-reported mumps symptoms in the preceding year were excluded.

Risk factors for seronegativity

Risk factors for MMR seronegativity were identified using separate logistic regression models. A complete case analysis was conducted for both mumps and rubella (*n* = 1,816; *Supplement Figure S2*). Allowing the measles model to converge, those born before introduction of routine vaccination were exclude (n_{total} = 1,075), i.e., a period characterized by widespread measles circulation causing nearly all participants to be seropositive (on Bonaire from 42 years of age, and on St. Eustatius and Saba from 36

years). Information on the history of MMR vaccinations on Bonaire before 1988 was derived from neighboring Dutch Leeward Antilles island Curaçao as the same NIP was applied. Studied risk factors included sociodemographics, vaccination history and other health-related factors. Aside from age and sex, variables with $p < 0.10$ in univariate analyses were included in the multivariate analyses. Backward selection was used to identify independent determinants in which $p < 0.05$ was considered statistically significant associated. Crude and adjusted odds ratios (OR) and 95% CIs were estimated as well as unadjusted seroprevalence and 95% CIs for all studied factors.

RESULTS

Study characteristics

In the present study, 1,900 participants (response rate 24.5%) were included, of which 1,829 donated a blood sample (*Supplement Figure S2*; 824 (45%) men and 1,005 (55%) women; aged 3 months to 90 years), with equal distribution over the islands according to population size (Bonaire: 1,129 (62%); St. Eustatius: 477 (26%); Saba: 223 (12%); *Table 1*). Most participants originated from the Dutch overseas territories (comprising CN, Aruba, Curaçao and St. Maarten) and Suriname (DOT-Sur; *n* = 1,312, 72%), followed by Latin America and other non-Western countries (LA-nonW; *n* = 281, 16%), and indigenous Dutch and other Western countries (iD-Wes; *n* = 223, 12%). Almost half of the participants reported to be low educated (*n* = 883), compared to 26% middle and 18% high (8% unknown). On Saba, relatively more iD-Wes, LA-nonW and those with a high educational level participated — consistent with its population composition [18, 19] — as compared to Bonaire and St. Eustatius. Among NIP eligible participants, i.e., those born in the MMR vaccination era, registered vaccination coverage with at least one dose against MMR ranged between 69–76% among the islands, and 8–9% were unvaccinated (and the remainder self-reported to have (partly) participated in the NIP).

Table 1. Sociodemographic characteristics and vaccination history of participants with a blood sample in the Health Study Caribbean Netherlands, by island (*n* (%)).

Sociodemographic characteristics and vaccination history	Bonaire *n* = 1,129 (61.7%)	St. Eustatius *n* = 477 (26.1%)	Saba *n* = 223 (12.2%)	Total *n* = 1,829
Sex				
Men	506 (44.8%)	221 (46.3%)	97 (43.5%)	824 (45.1%)
Women	623 (55.2%)	256 (53.7%)	126 (56.5%)	1,005 (54.9%)
Age, mean (sd)	34.6 (25.0%)	30.8 (23.7%)	37.5 (25.3%)	34.0 (24.8%)
Age groups (years)				
0–11	271 (24.0%)	128 (26.8%)	50 (22.4%)	449 (24.6%)
12–17	181 (16.0%)	86 (18.0%)	24 (10.8%)	291 (15.9%)
18–34	160 (14.2%)	83 (17.4%)	32 (14.3%)	275 (15.0%)
35–59	242 (21.4%)	99 (20.8%)	60 (26.9%)	401 (21.9%)
60–90	275 (23.4%)	81 (17.0%)	57 (25.6%)	413 (22.6%)
Ethnic background[a]				
Dutch overseas territories and Suriname	803 (71.2%)	383 (82.0%)	126 (57.0%)	1,312 (72.2%)
Indigenous Dutch and other Western countries	143 (12.7%)	30 (6.4%)	50 (22.6%)	223 (12.3%)
Latin America and other non-Western countries	182 (16.1%)	54 (11.6%)	45 (20.4%)	281 (15.5%)
(Maternal) educational level[b]				
High	172 (15.2%)	68 (14.3%)	87 (39.0%)	327 (17.9%)
Middle	298 (26.4%)	125 (26.2%)	45 (20.2%)	468 (25.6%)
Low	571 (50.6%)	232 (48.6%)	80 (35.9%)	883 (48.3%)
Unknown	88 (7.8%)	52 (10.9%)	11 (4.9%)	151 (8.2%)
Monthly gross income				
High (≥ $3001)	197 (17.4%)	91 (19.1%)	60 (26.9%)	348 (19.0%)
Middle ($1501–3000)	328 (29.1%)	88 (18.5%)	60 (26.9%)	476 (26.0%)
Low (< $1500)	329 (29.1%)	133 (27.8%)	56 (25.1%)	518 (28.3%)
Does not want to answer	106 (9.4%)	73 (15.3%)	23 (10.3%)	202 (11.1%)
Unknown	169 (15.0%)	92 (19.3%)	24 (10.8%)	285 (15.6%)
Vaccination history among National Immunization Program (NIP) eligible participants[c]				
Measles, total	672 (59.5%)	302 (63.3%)	107 (48.0%)	1,081 (59.1%)
2 or more doses	215 (32.0%)	106 (35.1%)	29 (27.1%)	350 (32.4%)
1 dose	248 (36.9%)	118 (39.1%)	51 (47.7%)	417 (38.6%)
(Partly) participated in the NIP (self-reported)	148 (22.0%)	47 (15.5%)	20 (18.7%)	215 (19.9%)
Not vaccinated	61 (9.1%)	31 (10.3%)	7 (6.5%)	99 (9.1%)

Table 1. (Continued)

Sociodemographic characteristics and vaccination history	Bonaire *n* = 1,129 (61.7%)	St. Eustatius *n* = 477 (26.1%)	Saba *n* = 223 (12.2%)	Total *n* = 1,829
Mumps, total	624 (55.3%)	263 (55.1%)	106 (47.5%)	993 (54.3%)
2 or more doses	213 (34.1%)	99 (37.6%)	29 (27.4%)	341 (34.3%)
1 dose	245 (39.3%)	113 (43.0%)	51 (48.1%)	409 (41.2%)
(Partly) participated in the NIP (self-reported)	115 (18.4%)	30 (11.4%)	19 (17.9%)	164 (16.5%)
Not vaccinated	51 (8.2%)	21 (8.0%)	7 (6.6%)	79 (8.0%)
Rubella, total	736 (65.2%)	263 (55.1%)	106 (47.5%)	1,105 (60.4%)
2 or more doses	216 (29.3%)	100 (38.0%)	29 (27.4%)	345 (31.2%)
1 dose	249 (33.8%)	112 (42.6%)	51 (48.1%)	412 (37.3%)
(Partly) participated in the NIP (self-reported)	197 (26.8%)	30 (11.4%)	19 (17.9%)	246 (22.3%)
Not vaccinated	74 (10.0%)	21 (8.0%)	7 (6.6%)	102 (9.2%)

[a] Dutch overseas territories include: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten. Within the ethnic group of indigenous Dutch and other Western countries, *n* = 147 (66%) were indigenous Dutch. Within Latin America and other non-Western countries, *n* = 261 (93%) were born in Latin America.

[b] Maternal educational level was used for participants 0–11y, active education was used for participants 12–25y and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1) and lower general secondary education (MAVO/VMBO); Middle = intermediate/secondary vocational education (MBO-2-4), higher/senior vocational education (HAVO) and pre-university education (VWO/Gymnasium); High = higher professional education (HBO), university BSc., university MSc. and doctorate.

[c] On Bonaire, NIP eligible participants for measles include those until 41y, for mumps 36y and for rubella 52y for women and 44y for men (in accordance with data from Curaçao). On St. Eustatius NIP eligible participants for measles include those until 35y, and for mumps and rubella 29y. On Saba NIP eligible participants for measles include those until 35y, and for mumps and rubella 34y. The self-reported variable on NIP participation was used if a vaccination certificate was unavailable. A participant was categorized as 'not vaccinated' if both a vaccination certificate was unavailable as well as if they self-reported about no participation in the NIP or did not know whether they participated.

Missing: ethnic background *n* = 13.

Age-specific seroprevalence and GMC

Table 2 shows the overall weighted IgG seroprevalence and GMC of MMR in the total CN population, stratified by island and sex, and among NIP eligibles and non-NIP eligible adults. In total, 72.0% (*n* = 1,337) were seropositive for all three pathogens, and 2.5% seronegative (*n* = 47; of which *n* = 26 had not reached the NIP eligible age, including all infants between 3–5 months of age *(n* = 6) for whom protective maternal antibody concentrations could be expected). There was no difference in overall seroprevalence for

MMR between islands (all $p > 0.05$). Weighted MMR seroprevalence and GMC, stratified by age groups, for CN are depicted in *Figure 1A–C*, and per island in *Supplement Figure S3A–C*. The possible effect of storage and transportation on antibody concentrations of DBS samples in this study was investigated: No significant difference (all $p > 0.05$, one-way ANOVA) was found between MMR antibody concentrations of samples obtained at the start of the study (having the longest storage period (4 weeks)) compared to samples stored shorter (1, 2 and 3 weeks) — while displaying a similar age distribution.

Measles

Overall weighted measles IgG seroprevalence was 93.8% (95% CI 92.3–95.2), with an overall GMC of 0.93 IU/mL (95% CI 0.86–1.01; *Table 2*). Seroprevalence in CN did not differ significantly between men and women (93.1% vs. 94.5%, respectively, $p = 0.31$); however overall GMC was lower for men (0.87 vs. women: 1.00 IU/mL, $p = 0.03$). On St. Eustatius a sex difference was observed in both the total population (seroprevalence men: 91.0% vs. women: 97.0%, $p = 0.02$; and GMC men: 0.75 vs. women: 1.27 IU/mL, $p < 0.0001$) and among NIP eligibles (seroprevalence men: 81.4% vs. women: 93.3%, $p = 0.01$; data not shown).

Seroprevalence in CN increased rapidly from 64.7% at one year of age to 94.2% at two years, with a corresponding upsurge of GMC (from 0.27 to 1.39 IU/mL, respectively) reflecting the vaccine response to MMR-1 at 12/14 months of age (*Figure 1A*). Seroprevalence fluctuated between 85–100% for children up to 18 years of age, with the lowest seropositivity among residents from LA-nonW (e.g., 65.5% in age group 12–17 years; *Figure 2A*). GMC declined to 0.37 IU/mL at seven years of age, from where it remained range bound between 0.30–0.56 IU/mL until 35 years (*Figure 1A*). In adults aged 18–35 years, seroprevalence ranged between 80–95%. Seroprevalence and GMC were lower for NIP-eligible participants (89.2% and 0.46 IU/mL, respectively) as compared to non-NIP eligible adults (98.6% and 1.96 IU/mL, respectively; *Table 2*). From 35 years of age, GMC rapidly inclined to 2.5 IU/mL at 60 years, and seroprevalence remained 100% from 55 years onward (*Figure 1A*).

Table 2. Weighted seroprevalence (%) and geometric mean concentration (GMC) (with 95% confidence intervals (CI)) of measles, mumps and rubella IgG antibodies in the national population of Caribbean Netherlands.

	Measles				Mumps				Rubella			
	Seroprevalence ≥ 0.120 IU/mL		GMC		Seroprevalence ≥ 45 RU/mL		GMC		Seroprevalence ≥ 10.0 IU/mL		GMC	
	%	*(95% CI)*	*IU/mL*	*(95% CI)*	*%*	*(95% CI)*	*RU/mL*	*(95% CI)*	*%*	*(95% CI)*	*IU/mL*	*(95% CI)*
Total Caribbean Netherlands population												
Overall	93.8	(92.3–95.2)	0.93	(0.86–1.01)	85.0	(83.0–87.0)	125	(133–188)	84.5	(82.4–86.6)	31.2	(28.5–34.2)
Island												
Bonaire	93.7	(91.9–95.4)	0.92	(0.83–1.02)	86.0	(83.7–88.3)	129	(120–138)	85.1	(82.6–87.6)	32.0	(28.7–35.6)
St. Eustatius	93.9	(91.2–96.5)	0.97	(0.83–1.14)	81.0	(76.2–85.8)	104	(92–118)	82.3	(77.8–86.8)	24.8	(20.3–30.2)
Saba	94.9	(91.4–98.4)	1.01	(0.81–1.24)	81.4	(75.4–87.4)	135	(113–161)	82.7	(76.8–88.6)	36.6	(27.5–48.7)
Sex												
Men	93.1	(90.8–95.3)	0.87	(0.76–0.99)	84.9	(81.8–87.9)	120	(110–131)	86.0	(82.8–89.1)	33.6	(29.2–38.6)
Women	94.5	(92.8–96.2)	1.00	(0.90–1.12)	85.1	(82.5–87.7)	131	(121–142)	83.0	(80.2–85.8)	28.8	(25.6–32.5)
Among NIP eligibles[a]												
Overall	89.2	(86.7–91.8)	0.46	(0.42–0.51)	83.9	(81.0–86.8)	116	(107–125)	87.5	(84.8–90.1)	30.6	(27.5–34.0)
Among non-NIP eligible adults												
Overall	98.6	(97.6–99.7)	1.96	(1.77–2.18)	85.6	(83.0–88.7)	133	(123–145)	80.6	(77.1–84.1)	32.1	(27.3–37.7)

[a] On Bonaire, NIP eligible participants for measles include those until 41y, for mumps 36y and for rubella 52y for women and 44y for men (in accordance with data from Curaçao). On St. Eustatius, NIP eligible participants for measles include those until 35y, and for mumps and rubella 29y. On Saba, NIP eligible participants for measles include those until 35y, and for mumps and rubella 34y.

Abbreviations: *IU/mL*, international units per mL; *RU/mL*, RIVM units per mL.

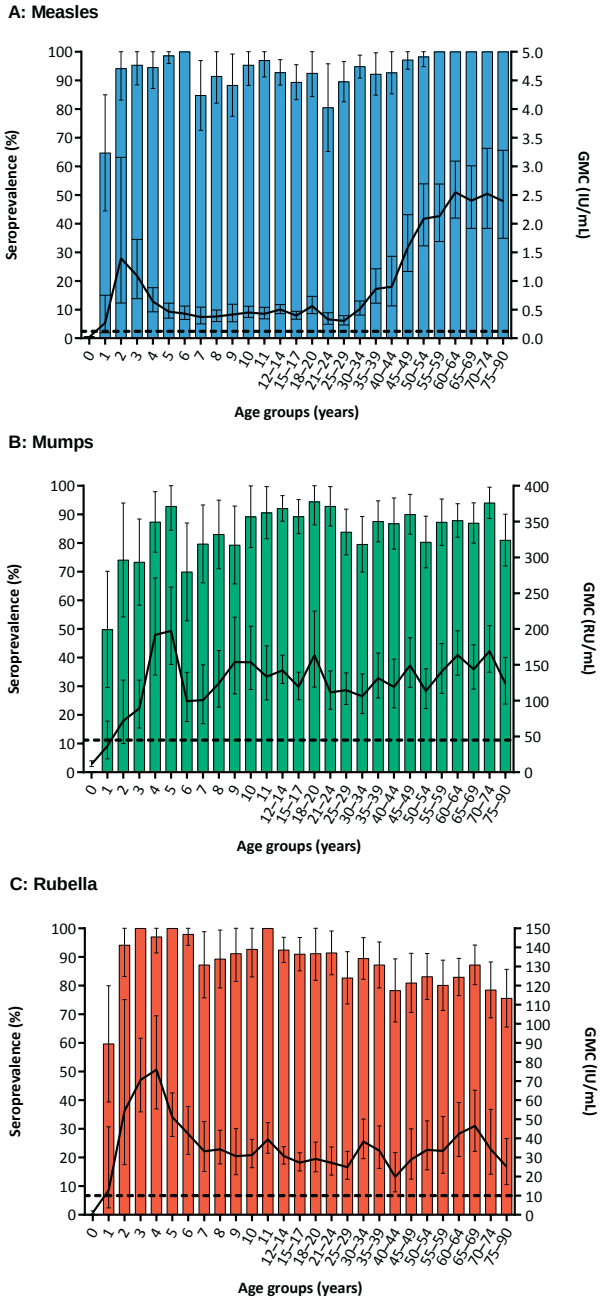

Figure 1. Age-specific seroprevalence (%) and geometric mean concentration (GMC) (with 95% confidence intervals) of measles (**A**), mumps (**B**) and rubella (**C**) IgG antibodies in the general population of Caribbean Netherlands, 2017. Note: Antibody concentrations ≥ 0.120 international units (IU)/mL for measles, ≥ 45.0 RIVM units (RU)/mL for mumps and ≥ 10.0 IU/mL for rubella were considered seropositive (dashed lines).

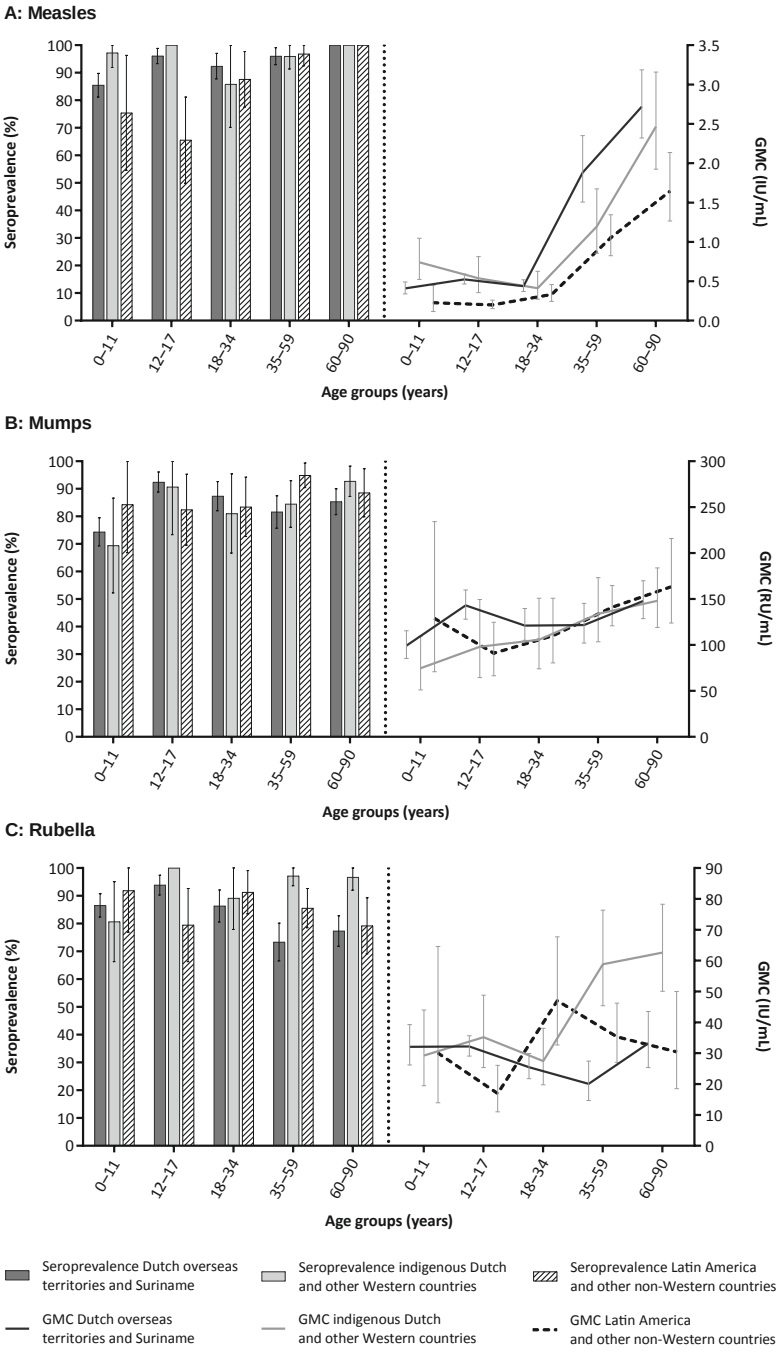

Figure 2. Age-specific seroprevalence (%) and geometric mean concentration (GMC) (with 95% confidence intervals) of measles (**A**), mumps (**B**) and rubella (**C**) IgG antibodies in the general population of Caribbean Netherlands, 2017, by ethnic background.

Mumps

Overall weighted mumps IgG seroprevalence was 85.0% (95% CI 83.0–87.0) — with an overall GMC of 125 RU/mL (95% CI 133–188) — and was similar between NIP eligibles and non-NIP eligible adults (*Table 2*). GMC was lower on St. Eustatius (104 RU/mL) as compared to Bonaire and Saba (both $p < 0.005$). Among sexes, overall seroprevalence and GMC were similar ($p > 0.05$; *Table 2*); however, on St. Eustatius seroprevalence (men: 76.2% vs. women: 86.1%, $p = 0.03$) and GMC (men: 85.8 vs. women: 127 RU/mL, $p < 0.0001$) were higher in women (data not shown). Symptoms of mumps in the preceding year (n total = 27) were most frequently self-reported by 13–34 year-olds ($n = 16$), and their GMC was higher than those 13–34 years without symptoms (202 vs. 116 RU/mL, respectively, $p = 0.02$).

Seroprevalence of mumps in CN was 74.1% at two years of age (after MMR-1) and rose to 93.2% at 5 years, with a corresponding increase in GMC from 37 to 197 RU/mL, respectively, reflecting the response to MMR-2 at 4 years on St. Eustatius and Saba (*Figure 1B* and *Supplement Figure S3B*). At 10 years of age (after MMR-2 on Bonaire), seroprevalence in CN was 89.2% and GMC 154 RU/mL. Thereafter, seroprevalence and GMC fluctuated between 80–94% and 106–169 RU/ml, respectively, with age group 18–20 years displaying the highest prevalence (94.4%). All islands showed a similar trend in seroprevalence with age, except for age group 18–34 years in which seroprevalence was considerably higher on Bonaire (90.2%) than the other islands (< 70%; *Supplement Figure S3B*). Overall seroprevalence in non-NIP eligible adults was lowest in residents from DOT-Sur (82.2%, and, e.g., in age group 60–90 years: 85.3% vs. 92.8% in iD-Wes and 88.6% in LA-nonW; *Figure 2B*)).

Rubella

Overall weighted rubella IgG seroprevalence was 84.5% (95% CI 82.4–86.6)–with a GMC of 31.2 IU/mL (95% CI 34.2–28.5)—and was higher among NIP eligibles (87.5%) as compared to non-NIP eligible adults (80.6%, $p = 0.002$; *Table 2*). GMC on St. Eustatius was lower than on the other islands (24.8; Bonaire: 32.0 and Saba: 36.6 IU/mL (St. Eustatius vs. Bonaire: $p = 0.02$ and St. Eustatius vs. Saba: $p = 0.004$)). Between sexes, no difference in overall seroprevalence was observed in CN (men: 86.0% vs. women 83.0%, $p = 0.17$) and on each island (*Table 2*); yet, on St. Eustatius, GMC was higher in women (29.9 vs. men: 20.6 IU/mL, $p = 0.01$; data not shown). Notably, on Bonaire, seroprevalence in non-NIP eligible men (86.6%) was higher than in women (73.9%, $p = 0.005$; data not shown), also reflected by a higher overall GMC in men (36.0 vs. women: 28.2 IU/mL, $p = 0.007$; *Table 2*).

Seroprevalence of rubella showed a similar age pattern as measles among NIP eligibles (*Figure 1C*) and was consistent across the islands (*Supplement Figure S3C*). After MMR-1, seroprevalence in CN was 94.2% for two-year-olds and fluctuated between

87–100% until 18 years of age. Participants from LA-nonW aged 12–17 years were least seropositive (79.5%; *Figure 2C*). GMC reached its highest peak at four years of age at 76.0 IU/mL and declined to 33.3 IU/mL at seven years (*Figure 1C*). From there it steadily declined towards 40 years of age (ranging between 25–39 IU/mL), reaching its lowest concentration at age group 40–44 years (19.8 IU/mL), but still above the cut-off for protection. Seroprevalence varied between 83–91% in adults aged 18–40 years. Unlike measles, seroprevalence remained indifferent after 40 years of age, varying between 76–87% and GMC between 25–47 IU/mL. Interestingly, seroprevalence and GMC in non-NIP eligible adults from DOT-Sur and LA-nonW were substantially lower than in iD-Wes (e.g., overall seroprevalence was 75.4%, 81.8% and 97.0%, respectively), and this also applied to the different age groups among them (*Figure 2C*)).

Waning immunity after MMR vaccination

For measles and rubella, waning of IgG antibody concentration after MMR-1 (n = 128) was significantly faster than after MMR-2 (n = 126; all $p < 0.05$), namely for measles with a slope of –0.25 and –0.04 ln-IU/mL per year, respectively; and for rubella with a slope of –0.19 and –0.04 ln-IU/mL per year, respectively (*Supplement Figure S4A–D*). For mumps, no decline in antibody concentration was observed after MMR-1 (n = 125; slope: 0.03 ln-RU/mL per year, p = 0.55,) and MMR-2 (n = 124; slope: –0.02, p = 0.31; *Supplement Figure S4E & 4F*).

Risk factors for seronegativity

Risk factors for measles were solely studied among NIP eligible participants as non-NIP eligible adults were nearly all seropositive. In multivariate analysis, men (vs. women), infants aged 0–1 years (vs. 2–10), those who have been resident of CN since age group 11–17 years (vs. 0–1) and participants who self-reported to have (partly) followed the NIP and who were unvaccinated (vs. two or more doses) had significantly higher odds of being seronegative (*Table 3*). For mumps, participants aged 0–1 and 2–10 years (vs. 11–17), those who have been resident of CN since age group 11–17 years (vs. 0–1), individuals who were vaccinated once, those self-reported to have (partly) followed the NIP, those who were unvaccinated and who were not eligible for the NIP (vs. two or more doses) were found to be significant risk factors for seronegativity in multivariate analysis (*Table 4*). The multivariate model for rubella revealed that all age groups except 11–17 years (vs. 2–10), those who have been resident of CN since age 0–17 years (vs. 40–59), people who self-reported to have (partly) followed the NIP, those unvaccinated and who were not eligible for the NIP (vs. two or more doses) were significantly associated with seronegativity (*Table 5*).

Table 3. Risk factor analysis for measles IgG seronegativity among the Health Study Caribbean Netherlands population without non-National Immunization Program (NIP) eligible adults[a].

Potential risk factor for measles seronegativity	*n* (%) *n* = 1,075	% Measles seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Island				**0.04**		
Bonaire	671 (62.4%)	89.4 (87.1–97.7)	Ref.			
St. Eustatius	297 (27.6%)	92.3 (89.2–95.3)	0.62 (0.37–1.06)			
Saba	107 (10.0%)	94.4 (90.0–98.8)	**0.38 (0.15–0.95)**			
Sex				**0.01**		**0.003**
Men	492 (45.8%)	88.6 (85.8–91.4)	**1.78 (1.14–2.78)**		**2.06 (1.29–3.30)**	
Women	583 (54.2%)	92.5 (90.3–94.6)	Ref.		Ref.	
Age group, years				**< 0.0001**		**< 0.0001**
0–1	49 (4.6%)	49.0 (35.0–63.0)	**17.94 (8.71–36.99)**		**8.78 (3.80–20.27)**	
2–10	356 (33.1%)	94.1 (91.6–96.6)	Ref.		Ref.	
11–17	335 (31.1%)	93.4 (90.8–96.1)	1.13 (0.61–2.11)		0.54 (0.26–1.15)	
18–29	172 (16.0%)	88.4 (83.6–93.2)	**2.29 (1.20–4.37)**		1.08 (0.50–2.32)	
30–41	163 (15.1%)	92.6 (88.6–96.7)	**1.78 (1.14–2.78)**		0.40 (0.16–1.00)	
Ethnic background						
Dutch overseas territoriesd and Suriname	857 (79.7%)	91.9 (90.1–93.8)	Ref.	**0.0001**		
Indigenous Dutch and other Western countries	80 (7.4%)	92.5 (86.7–98.3)	1.19 (0.47–3.00)			
Latin America and other non-Western countries	138 (12.8%)	81.9 (75.4–88.3)	**3.38 (1.93–5.90)**			
(Maternal) educational level[e]				**0.097**		
High	171 (15.9%)	90.6 (86.3–95.0)	Ref.			
Middle	358 (33.3%)	92.5 (89.7–95.2)	1.02 (0.50–2.10)			
Low	479 (44.6%)	89.4 (86.6–92.1)	1.89 (0.95–3.75)			
Unknown	67 (6.2%)	91.0 (84.2–97.9)	1.49 (0.52–4.29)			

Table 3. (Continued)

Potential risk factor for measles seronegativity	*n* (%) *n* = 1,075	% Measles seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Monthly gross income household				0.74		
High (≥ $3,001)	187 (17.4%)	91.4 (87.4–95.5)	Ref.			
Middle ($1501–3000)	272 (25.3%)	91.2 (87.8–94.6)	1.16 (0.57–2.36)			
Low (≤ $1500)	219 (20.4%)	89.0 (84.9–93.2)	1.37 (0.67–2.81)			
Does not want to answer	144 (13.4%)	88.9 (83.7–94.0)	1.70 (0.77–3.75)			
Unknown	253 (23.5%)	92.1 (88.8–95.4)	1.26 (0.56–2.85)			
Resident of Caribbean Netherlands since, years of age				**< 0.0001**		**0.005**
0–1	703 (65.4%)	91.8 (89.7–93.8)	Ref.		Ref.	
2–10	144 (13.4%)	93.1 (88.9–97.2)	1.45 (0.69–3.02)		1.11 (0.52–12.25)	
11–17	47 (4.4%)	72.3 (59.5–85.1)	**9.17 (4.07–20.7)**		**5.12 (2.13–12.30)**	
18–41	128 (11.9%)	89.8 (84.6–95.1)	1.89 (0.84–4.27)		1.03 (0.45–2.37)	
Unknown	53 (4.9%)	88.7 (80.1–97.2)	1.85 (0.70–4.90)		1.46 (0.54–3.97)	
Number of vaccinations against measles[f]				**< 0.0001**		**< 0.0001**
2 or more doses	349 (32.5%)	95.1 (92.9–97.4)	Ref.		Ref.	
1 dose	416 (38.7%)	94.7 (92.6–96.9)	0.79 (0.38–1.61)		0.74 (0.36–1.52)	
(Partly) followed NIP (as a child) (self-reported)	213 (19.8%)	85.9 (81.2–90.6)	**4.29 (2.13–8.63)**		**3.24 (1.55–6.75)**	
Not vaccinated	97 (9.0%)	68.0 (58.7–77.3)	**6.82 (3.21–14.49)**		**5.67 (2.62–12.25)**	
(Parent/caregiver) influenced by beliefs about vaccination[g]				0.83		
Yes	118 (11.0%)	89.0 (83.3–94.6)	Ref.			
No	820 (76.3%)	90.7 (88.7–92.7)	0.83 (0.43–1.60)			
Unknown	137 (12.7%)	92.0 (87.4–96.5)	0.79 (0.32–1.92)			

Table 3. (Continued)

Potential risk factor for measles seronegativity	*n* (%) *n* = 1,075	% Measles seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Household size, persons				0.97		
Single-person household	48 (4.5%)	89.6 (80.9–98.2)	Ref.			
2–5	868 (80.7%)	90.9 (89.0–92.8)	0.86 (0.31–2.35)			
≥ 6	150 (14.0%)	90.0 (85.2–94.8)	0.98 (0.31–3.08)			
Unknown	9 (0.8%)	88.9 (8.3–100.0)				
Contact yesterday, persons				0.31		
0–8	415 (38.6%)	89.6 (86.7–92.6)	Ref.			
≥ 9	537 (50.0%)	92.2 (89.9–94.5)	0.89 (0.55–1.44)			
Unknown	123 (11.4%)	87.8 (82.0–93.6)	1.49 (0.77–2.90)			

[a] On Bonaire, NIP eligible participants for measles include those until 41y (in accordance with data from Curaçao), and on St. Eustatius and Saba until 35y.
[b] Crude odds ratios were adjusted for sex and age, and significant (a)odds ratios (ORs) were marked in bold type.
[c] *p* values were determined by means of Wald tests for logistic regression, and significant *p* values (< 0.1 in univariate and < 0.05 in multivariate analysis) were marked in bold type.
[d] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten.
[e] Maternal educational level was used for participants 0–11y, active education was used for participants 12–25y and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1) and lower general secondary education (MAVO/VMBO); Middle = intermediate/secondary vocational education (MBO-2-4), higher/senior vocational education (HAVO) and pre-university education (VWO/Gymnasium); and High = higher professional education (HBO), university BSc., university MSc. and doctorate.
[f] The self-reported variable on NIP participation was used if a vaccination certificate was unavailable. Participants were categorized as 'not vaccinated' if both a vaccination certificate was unavailable as well as if they self-reported about no participation in the NIP or did not know whether they participated.
[g] Beliefs include anthroposophy and natural healing, religion, social media, and other.
Abbreviations: *aOR*, adjusted odds ratio; *CI*, confidence interval; *OR*, odds ratio; *Ref.*, reference category.

Table 4. Risk factor analysis for mumps IgG seronegativity among the total Health Study Caribbean Netherlands population with a blood sample and questionnaire data.

Potential risk factor for mumps seronegativity	*n* (%) *n* = 1,816	% Mumps seropositive (95% CI)	Univariate Crude OR[a] (95% CI)	*p* value[b]	Multivariate aOR [a] (95% CI)	*p* value[b]
Island				0.29		
Bonaire	1,128 (62.1%)	85.2 (83.1–87.3)	Ref.			
St. Eustatius	467 (25.7%)	84.2 (80.8–87.5)	1.04 (0.77–1.42)			
Saba	221 (12.2%)	80.5 (75.3–85.8)	1.36 (0.93–1.99)			
Sex				0.25		0.19
Men	818 (45.0%)	83.5 (81.0–86.0)	1.17 (0.90–1.52)		1.20 (0.92–1.56)	
Women	998 (55.0%)	85.1 (82.9–87.3)	Ref.		Ref.	
Age group, years				**< 0.0001**		**< 0.0001**
0–1	49 (2.7%)	36.7 (23.3–50.2)	**17.07 (8.57–34.00)**		**10.15 (4.72–21.80)**	
2–10	356 (19.6%)	83.1 (79.3–87.0)	**1.98 (1.25–3.15)**		**1.87 (1.11–3.17)**	
11–17	335 (18.5%)	90.7 (87.6–93.9)	Ref.		Ref.	
18–29	172 (9.5%)	84.3 (78.9–89.7)	**1.86 (1.07–3.24)**		1.81 (0.99–3.32)	
30–59	493 (27.1%)	83.2 (79.9–86.5)	**2.02 (1.30–3.14)**		2.02 (0.97–4.21)	
60–90	411 (22.6%)	87.3 (84.1–90.6)	1.43 (0.89–2.29)		1.44 (0.62–4.86)	
Ethnic background				0.13		
Dutch overseas territories[c] and Suriname	1,312 (72.2%)	83.5 (81.5–85.5)	Ref.			
Indigenous Dutch and other Western countries	223 (12.3%)	83.4 (78.5–88.3)	1.03 (0.69–1.53)			
Latin America and other non-Western countries	281 (15.5%)	89.0 (85.3–92.6)	0.66 (0.44–1.00)			
(Maternal) educational level[d]				0.39		
High	326 (18.0%)	81.9 (77.7–86.1)	Ref.			
Middle	466 (25.7%)	85.6 (82.4–88.8)	0.83 (0.55–1.24)			
Low	877 (48.3%)	84.3 (81.9–86.7)	1.10 (0.77–1.57)			
Unknown	147 (8.1%)	86.4 (80.8–91.9)	0.88 (0.50–1.54)			

Table 4. (Continued)

Potential risk factor for mumps seronegativity	*n* (%) *n* = 1,816	% Mumps seropositive (95% CI)	Univariate Crude OR[a] (95% CI)	*p* value[b]	Multivariate aOR [a] (95% CI)	*p* value[b]
Monthly gross income				0.61		
High (≥ $3001)	346 (19.0%)	81.5 (77.4–85.6)	Ref.			
Middle ($1501–3000)	475 (26.2%)	84.6 (81.4-87.9)	0.81 (0.55-1.18)			
Low (≤ $1500)	513 (28.2%)	85.2 (82.1–88.3)	0.84 (0.57–1.22)			
Does not want to answer	199 (11.0%)	83.4 (78.2–88.6)	1.08 (0.67–1.75)			
Unknown	283 (15.6%)	86.6 (82.6–90.5)	1.04 (0.62–1.73)			
Resident of Caribbean Netherlands since, years of age				**0.009**		**0.02**
0–1	1,034 (56.9%)	83.7 (81.4–85.9)	Ref.		Ref.	
2–10	161 (8.9%)	82.0 (76.0–87.9)	1.54 (0.98–2.43)		1.47 (0.92–2.34)	
11–17	54 (3.0%)	78.9 (64.5–87.3)	**2.85 (1.42–5.71)**		**2.12 (1.02–4.41)**	
18–39	294 (16.2%)	86.7 (82.9–90.6)	0.73 (0.48–1.11)		0.63 (0.41–0.97)	
40–59	163 (9.0%)	89.0 (84.1–93.8)	0.63 (0.36–1.09)		0.61 (0.35–1.06)	
≥ 60	28 (1.5%)	89.3 (77.8–100.0)	0.70 (0.20–2.44)		0.68 (0.20–2.35)	
Unknown	82 (4.5%)	84.1 (76.2–92.1)	1.00 (0.53–1.89)		0.95 (0.50–1.81)	
Number of vaccinations against mumps[e]				**< 0.0001**		**< 0.0001**
2 or more doses	349 (19.1%)	94.3 (91.8–96.7)	Ref.		Ref.	
1 dose	421 (23.0%)	81.0 (77.2–84.7)	**2.81 (1.63–4.86)**		**2.82 (1.63–4.86)**	
(Partly) followed NIP (as a child) (self-reported)	163 (8.9%)	78.5 (72.2–84.8)	**3.93 (2.08–7.42)**		**3.75 (1.96–7.15)**	
Not vaccinated	79 (4.3%)	58.2 (47.3–69.1)	**7.17 (3.56–14.44)**		**7.00 (3.45–14.18)**	
Not eligible for NIP	817 (44.7%)	85.7 (83.3–88.1)	**3.06 (1.39–6.76)**		**2.93 (1.32–6.49)**	
(Parent/caregiver) influenced by beliefs about vaccination[f]				0.35		
Yes	195 (10.7%)	84.1 (79.0–89.2)	Ref.			
No	1,369 (75.4%)	84.7 (82.8–86.6)	1.00 (0.66–1.54)			
Unknown	252 (13.9%)	82.5 (77.8–87.2)	1.31 (0.78–2.21)			

Table 4. (Continued)

Potential risk factor for mumps seronegativity	*n* (%) *n* = 1,816	% Mumps seropositive (95% CI)	Univariate Crude OR[a] (95% CI)	*p* value[b]	Multivariate aOR [a] (95% CI)	*p* value[b]
Household size, persons				0.96		
Single-person household	218 (12.0%)	85.5 (80.9–90.2)	Ref.			
2–5	1,382 (76.1%)	84.2 (82.3–86.2)	1.01 (0.66–1.54)			
≥ 6	204 (11.2%)	84.8 (79.9–89.7)	0.95 (0.54–1.68)			
Unknown	12 (0.7%)	75.0 (50.5–99.5)	1.40 (0.33–5.92)			
Contact yesterday, persons				0.52		
0–8	810 (44.6%)	84.4 (81.9–86.9)	Ref.			
≥ 9	794 (43.7%)	84.8 (82.3–87.3)	0.99 (0.75–1.32)			
Unknown	212 (11.7%)	82.5 (77.4–87.7)	1.25 (0.82–1.89)			
Mumps symptoms in preceding year[g]				0.43		
Yes	27 (1.5%)	81.5 (66.8–96.1)	Ref.			
No	1,622 (89.3%)	84.6 (82.9–86.4)	0.68 (0.25–1.82)			
Unknown	167 (9.2%)	82.0 (76.2–87.9)	0.85 (0.30–2.47)			

[a] Crude odds ratios were adjusted for sex and age, and significant (a)ORs are marked in bold type.
[b] *p* values were determined by means of Wald tests for logistic regression, and significant *p* values (< 0.1 in univariate and < 0.05 in multivariate analysis) were marked in bold type.
[c] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten.
[d] Maternal educational level was used for participants 0–11y, active education was used for participants 12–25y and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1) and lower general secondary education (MAVO/VMBO); Middle = intermediate/secondary vocational education (MBO-2-4), higher/senior vocational education (HAVO) and pre-university education (VWO/Gymnasium); and High = higher professional education (HBO), university BSc., university MSc. and doctorate.
[e] The self-reported variable on NIP participation was used if a vaccination certificate was unavailable. Participants were categorized as 'not vaccinated' if both a vaccination certificate was unavailable as well as if they self-reported about no participation in the NIP or did not know whether they participated. On Bonaire, NIP eligible participants for mumps include those until 36y (in accordance with data from Curaçao), on St. Eustatius until 29y and on Saba until 34y.
[f] Beliefs include anthroposophy and natural healing, religion, social media and other.
[g] Whether or not diagnosed by a physician.
Abbreviations: *aOR*, adjusted odds ratio; *CI*, confidence interval; *OR*, odds ratio; *Ref.*, reference category.

Table 5. Risk factor analysis for rubella IgG seronegativity among the total Health Study Caribbean Netherlands population with a blood sample and questionnaire data.

Potential risk factor for rubella seronegativity	*n* (%) *n* = 1,816	% Rubella seropositive (95% CI)	Univariate Crude OR[a] (95% CI)	*p* value[b]	Multivariate aOR [a] (95% CI)	*p* value[b]
Island				0.48		
Bonaire	1,128 (62.1%)	86.1 (84.1–88.1)	Ref.			
St. Eustatius	467 (25.7%)	85.9 (82.7–89.0)	1.07 (0.77–1.48)			
Saba	221 (12.2%)	81.9 (76.8–87.0)	1.28 (0.86–1.90)			
Sex				0.23		0.34
Men	818 (45.0%)	87.0 (84.7–89.3)	Ref.		0.87 (0.65–1.16)	
Women	998 (55.0%)	84.3 (82.0–86.5)	1.18 (0.90–1.56)		Ref.	
Age group, years				**< 0.0001**		**< 0.0001**
0–1	49 (2.7%)	44.9 (31.0–58.8)	**24.28 (11.53–51.14)**		**14.60 (6.50–32.81)**	
2–10	356 (19.6%)	95.2 (93.0–97.4)	Ref.		Ref.	
11–17	335 (18.4%)	93.7 (91.1–96.3)	1.33 (0.69–2.57)		1.12 (0.54–2.31)	
18–39	337 (18.6%)	85.5 (81.7–89.2)	**3.30 (1.86–5.87)**		**2.61 (1.33–5.10)**	
40–59	328 (18.1%)	79.6 (75.2–83.9)	**5.04 (2.89–8.79)**		**3.34 (1.52–7.35)**	
60–90	411 (22.6%)	80.0 (76.2–83.9)	**4.93 (2.86–8.50)**		**2.89 (1.25–6.64)**	
Ethnic background				**0.0002**		
Dutch overseas territories[c] and Suriname	1,312 (72.2%)	84.8 (82.8–86.7)	**3.16 (1.83–5.43)**			
Indigenous Dutch and other Western countries	223 (12.3%)	92.8 (89.4–96.2)	Ref.			
Latin America and other non-Western countries	281 (15.5%)	83.3 (78.9–87.6)	**2.94 (1.60–5.39)**			
(Maternal) educational level[d]				**0.053**		
High	326 (18.0%)	86.2 (82.4–89.9)	Ref.			
Middle	466 (25.7%)	87.8 (84.8–90.7)	1.18 (0.76–1.84)			
Low	877 (48.3%)	83.8 (81.4–86.2)	**1.59 (1.08–2.35)**			
Unknown	147 (8.1%)	87.1 (81.6–92.5)	1.04 (0.57–1.89)			

Table 5. (Continued)

Potential risk factor for rubella seronegativity	*n* (%) *n* = 1,816	% Rubella seropositive (95% CI)	Univariate Crude OR[a] (95% CI)	*p* value[b]	Multivariate aOR [a] (95% CI)	*p* value[b]
Monthly gross income				0.22		
High (≥ $3001)	346 (19.0%)	87.6 (84.1–91.1)	Ref.			
Middle ($1501–3000)	475 (26.2%)	85.5 (82.3–88.6)	1.28 (0.84–1.96)			
Low (≤ $1,501)	513 (28.2%)	80.9 (77.5–84.3)	**1.61 (1.07–2.41)**			
Does not want to answer	199 (11.0%)	85.9 (81.1–90.8)	1.55 (0.90–2.65)			
Missing	283 (15.6%)	91.2 (87.9–94.5)	1.27 (0.69–2.33)			
Resident of Caribbean Netherlands since, years of age				**< 0.0001**		**< 0.0001**
0–1	1,034 (56.9%)	84.0 (81.8–86.3)	**3.44 (1.96–6.05)**		**3.58 (2.03–6.29)**	
2–10	161 (8.9%)	87.0 (81.7–92.2)	**5.68 (2.68–12.03)**		**5.48 (2.57–11.69)**	
11–17	54 (3.0%)	79.6 (68.9–90.4)	**8.70 (3.46–21.91)**		**6.83 (2.66–17.52)**	
18–39	294 (16.2%)	86.1 (82.1–90.0)	**1.88 (1.00–3.51)**		1.72 (0.91–3.23)	
40–59	163 (9.0%)	90.2 (85.6–94.8)	Ref.		Ref.	
≥ 60	28 (1.5%)	96.4 (89.5–100.0)	0.36 (0.05–2.83)		0.37 (0.05–2.91)	
Unknown	82 (4.55)	90.2 (83.8–96.7)	1.76 (0.70–4.43)		1.63 (0.64–4.11)	
Number of vaccinations against rubella[e]				**< 0.0001**		**< 0.0001**
2 or more doses	349 (19.2%)	95.1 (92.9–97.4)	Ref.		Ref.	
1 dose	425 (23.4%)	92.7 (90.2–95.2)	1.27 (0.65–2.48)		1.30 (0.67–2.53)	
(Partly) followed NIP (as a child) (self-reported)	245 (13.55)	84.9 (80.4–89.4)	**2.37 (1.21–4.65)**		**2.45 (1.24–4.84)**	
Not vaccinated	101 (5.6%)	66.3 (57.1–75.6)	**4.76 (2.32–9.75)**		**5.15 (2.50–10.62)**	
Not eligible for NIP	696 (38.3%)	79.3 (76.3–82.3)	**3.68 (1.74–7.79)**		**3.75 (1.77–7.96)**	
(Parent/caregiver) influenced by beliefs about vaccination[f]				0.99		
Yes	195 (10.7%)	85.1 (80.1–90.1)	Ref.			
No	1,369 (75.4%)	85.5 (83.7–87.4)	1.02 (0.65–1.58)			
Unknown	252 (13.9%)	85.7 (81.4–90.0)	1.04 (0.60–1.81)			

Table 5. (Continued)

Potential risk factor for rubella seronegativity	*n* (%) *n* = 1,816	% Rubella seropositive (95% CI)	Univariate Crude OR[a] (95% CI)	*p* value[b]	Multivariate aOR [a] (95% CI)	*p* value[b]
Household size, persons				0.52		
Single-person household	218 (12.0%)	78.4 (73.0–83.9)	Ref.			
2–5	1382 (76.1%)	86.5 (84.7–88.3)	0.78 (0.53–1.13)			
≥ 6	204 (11.2%)	86.3 (81.5–91.0)	0.88 (0.51–1.52)			
Unknown	12 (0.7%)	91.7(76.0–100.0)	0.40 (0.04–3.74)			
Contact yesterday, persons				**0.003**		
0–8	810 (44.6%)	84.3 (81.8–86.8)	Ref.			
≥ 9	794 (43.7%)	88.4 (86.2–90.6)	0.85 (0.63–1.15)			
Unknown	212 (11.7%)	79.2 (73.8–84.7)	**1.76 (1.17–2.63)**			

[a] Crude odds ratios were adjusted for sex and age, and significant (a)ORs are marked in bold type.

[b] *p* values were determined by means of Wald tests for logistic regression, and significant *p* values (< 0.1 in univariate and < 0.05 in multivariate analysis) were marked in bold type.

[c] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten.

[d] Maternal educational level was used for participants 0–11y, active education was used for participants 12–25y and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1) and lower general secondary education (MAVO/VMBO); Middle = intermediate/secondary vocational education (MBO-2-4), higher/senior vocational education (HAVO) and pre-university education (VWO/Gymnasium); and High = higher professional education (HBO), university BSc., university MSc. and doctorate.

[e] The self-reported variable on NIP participation was used if a vaccination certificate was unavailable. Participants were categorized as 'not vaccinated' if both a vaccination certificate was unavailable as well as if they self-reported about no participation in the NIP or did not know whether they participated. On Bonaire, NIP eligible participants for mumps include those until 36y (in accordance with data from Curaçao), on St. Eustatius until 29y and on Saba until 34y.

[f] Beliefs include anthroposophy and natural healing, religion, social media and other.

Abbreviations: *aOR*, adjusted odds ratio; *CI*, confidence interval; *OR*, odds ratio; *Ref.*, reference category.

DISCUSSION

This cross-sectional population-based serosurveillance study estimated the level of humoral immunity against MMR and risk factors associated with seronegativity in CN. Overall seroprevalence was high for measles (94%), but lower for mumps and rubella (both 85%). In NIP eligibles, including women of childbearing age, rubella seroprevalence (88%) exceeded the threshold for protection (85%); however, for measles (89%) this level (95%) was not met [20, 21]. MMR seropositivity was lowest in children who became CN resident at 11–17 years of age (especially for measles (72%)), mostly originating from Latin America and other non-Western countries. MMR vaccinations elicited good antibody responses and receiving two doses of MMR (vs. one) indicated prolonged humoral immunity. Interestingly, rubella seroprevalence was lowest in non-NIP eligible adults from DOT-Sur (75%), illustrative of a specific island epidemiology in the pre-vaccination era.

Overall seroprevalence for measles in CN (94%) was consistent with our previously reported estimate for Bonaire [22]. As reviewed by Dimech and colleagues [23], other large population studies reported measles seroprevalence rates between 54–96% (e.g., Italy: 74%, USA: 93%), of which the Netherlands was among the highest (96%). Seroprevalence for mumps in CN (85%) was rather similar to the USA (88%) [24], but somewhat lower than the Netherlands (91%) [25]. Rubella seroprevalence in CN (85%) was mostly lower than studies performed elsewhere, e.g., Colombia (89%), the Netherlands (95%) and Thailand (98%) [23]. Main drivers coinciding with differences in seroprevalence and antibody responses between and within populations can be attributed to vaccination status (and vaccine effectivity in general) as well as (past) natural exposure to these pathogens.

Children who reside in CN since age 11 years, i.e., after the regular NIP, had a high likelihood of being MMR seronegative. Indeed, lowest seroprevalence was observed in LA-nonW residents aged 12–17 years (e.g., for measles 66%) as they were less vaccinated. Hence, they probably missed vaccination opportunities in their country of birth due to lack of goods or migration — as their beliefs on vaccination (e.g., anti-vaccination) were indifferent from their peers (data not shown) — and did not catch up on missed vaccinations upon arrival to CN (which is regular policy). Based on these findings and in light of recent dissemination of measles across the region and influx of refugees [4], vaccination policy with respect to eligible immigrants aged < 18 years was tightened where possible.

Moreover, male sex was an independent determinant for measles seronegativity among NIP eligibles (note: Additional risk factor analyses for rubella and mumps among NIP eligibles revealed a similar — although non-significant — association with sex (data not shown)). This sex difference was most prominent on St. Eustatius, while according to our registry vaccination coverage was even somewhat higher in men. To

note, although vaccination status was available from a large proportion of participants, not all records could be retrieved. In that case, we used a self-reported variable on overall NIP attendance as a surrogate, which could not differentiate between (number of) vaccines unfortunately, and recall bias might have played a role too. Hence, we could not exclude that vaccination coverage against MMR on St. Eustatius was slightly higher in women as compared to men. Conversely, given the higher GMC in women too, women might serologically respond better to the vaccine components, as postulated by others [26, 27]. Nonetheless, as cellular immunity is assumed to be an essential part of protection [28], higher risk of susceptibility among vaccinated men remains questionable. Future research in outbreak settings might provide clarification on this potential sex difference in vaccinated cases.

We detected significant dissimilarities between NIP eligibles and non-NIP eligible adults. Generally, the latter have (frequently) been naturally exposed to MMR during their life, elucidating high GMCs, indicative of lifelong protection [25, 29, 30]. This concept is best underlined by measles, a highly infectious agent that is capable of disseminating throughout susceptible populations [31]. Hence, nearly all non-NIP eligible adults in our study were seropositive for measles displaying high antibody concentrations, i.e., were infected, possible boosted regularly and thus protected. However, this was different for the less infectious pathogens mumps and, in particular, rubella. Interestingly, adult participants who were born on the islands or resided there since childhood were more likely to be seronegative. This was confirmed by a lower seroprevalence and GMC in adults from DOT-Sur and LA-nonW descent when compared to iD-Wes who were born in rubella endemic countries (mostly the Netherlands) prior to introduction of MMR vaccination. Principally for rubella, differences in seroprevalence between countries in the pre-vaccination period have been described [32]. Hence, as CN was even more remote and isolated during the pre-globalization/vaccine era, we hypothesize that introduction and transmission of rubella occurred less often due to its lesser infectious character, causing less circulating and exposure, affecting less people. Whilst a proportion of these inhabitants might still be susceptible currently and future cases cannot be ruled-out completely, disease in elderly is expected to be mostly mild, and yet sufficient herd immunity should prevent transmission. Fortunately, seroprotection for rubella was above the threshold for protection in NIP eligibles, including women of childbearing age who are at risk of developing Congenital Rubella Syndrome — resulting in serious birth defects or miscarriage — via infection with rubella during pregnancy [20].

Consistent with literature, waning immunity of measles and rubella specific IgG antibodies after vaccination was present, but much slower after a second dose, staying well above the cut-off for seropositivity [29, 30, 33]. This indicates long lasting immunological humoral memory —when extended with similar rate of waning. Although this underlines the purpose of booster vaccination — besides preventing

primary vaccine failure—we could not draw firm conclusions on the persistence of these antibodies as these data were cross-sectional. Similarly, the non-prospective character of our data was most likely the reason why mumps antibody levels were indifferent eight years after the first dose. Furthermore, seroprevalence rates for mumps should be interpreted with caution as a defined correlate of protection — albeit recent research endeavored [34] — is still lacking. While two doses of MMR (vs. one) indicated a lower risk of mumps seronegativity in our study, outbreaks among twice vaccinated students — with intensive and homogenous contact — have been reported [35]. In fact, in contrast to St. Eustatius and Saba, we observed a high seroprevalence and GMC in young adults on Bonaire and self-reporting of mumps symptoms was highest among this group too. This, together with confirmed cases from nearby Dutch Leeward Antilles island Aruba and recurrent traffic between these islands, could suggest possible exposure to mumps on Bonaire, whereas this likelihood might be lower on St. Eustatius and Saba, which are more isolated.

All infants too young to be vaccinated with MMR were seronegative in our study; even among the infants 3–5 months of age for whom protective maternal antibody concentrations could be expected. This phenomenon is well-known among babies from mothers who have not been naturally infected as antibody concentrations from vaccination are significantly lower and thus reach cutoffs for seropositivity earlier [36]. Timely vaccination and close monitoring remains of great importance, especially in light of recent regional circulation of the measles virus and migration of large populations at risk (e.g., from Venezuela) [3, 4]. While considering an optimal age for vaccination, health authorities should take into account several factors, including immunological response, vaccine coverage, herd immunity thresholds and risk of infection [37]. Although no cases of measles have been detected via the surveillance systems in CN recently, the public health department on Bonaire decided to lower the age of MMR-2 from 9 years to 18 months of age (as of January 1, 2019), in order to timely and adequately protect young infants and reduce the risk of viral introduction and transmission.

This study has some additional limitations. Due to the overall response rate of 25%, the possibility of non-response bias cannot be excluded (as described earlier [9]). However, we partly corrected for this by weighting our sample on important sociodemographic characteristics. Further, to overcome logistical hurdles, we used DBS to collect our blood samples. Whilst this is a widely used and validated method for measuring antibodies, we could not exclude that storage and transportation might have had some effect on the antibody levels. We have investigated this and found little overall effect that has not affected our results.

CONCLUSIONS

In conclusion, this is the first large-scale serosurveillance study in CN providing evidence on humoral immunity against MMR. The CN population is overall well protected against MMR, albeit some groups were identified that could be at risk of infection. Our data also indicate that infectious disease epidemiology on these islands might have been different in the pre-vaccination era as compared to past MMR endemic countries, such as the Netherlands. Particularly in light of recent outbreaks in the region, it is important to have sensitive disease surveillance in place and to sustain high vaccination coverage in order to meet herd immunity thresholds and, ultimately, reach the WHO measles and rubella elimination goals [20]. Lastly, it is highly recommended to conduct serosurveillance studies in CN on a regular basis in the future in order to monitor the protection against vaccine-preventable diseases and timely detect (additional) gaps in population immunity [8].

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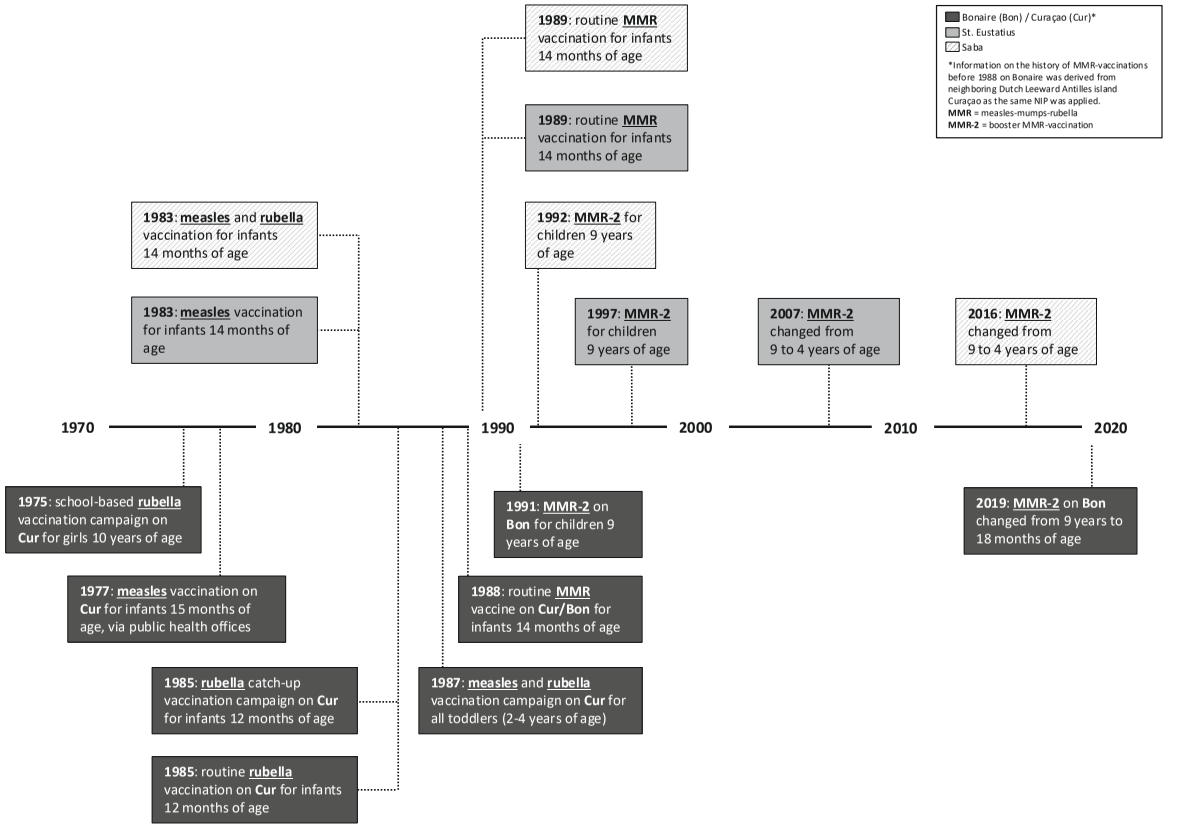
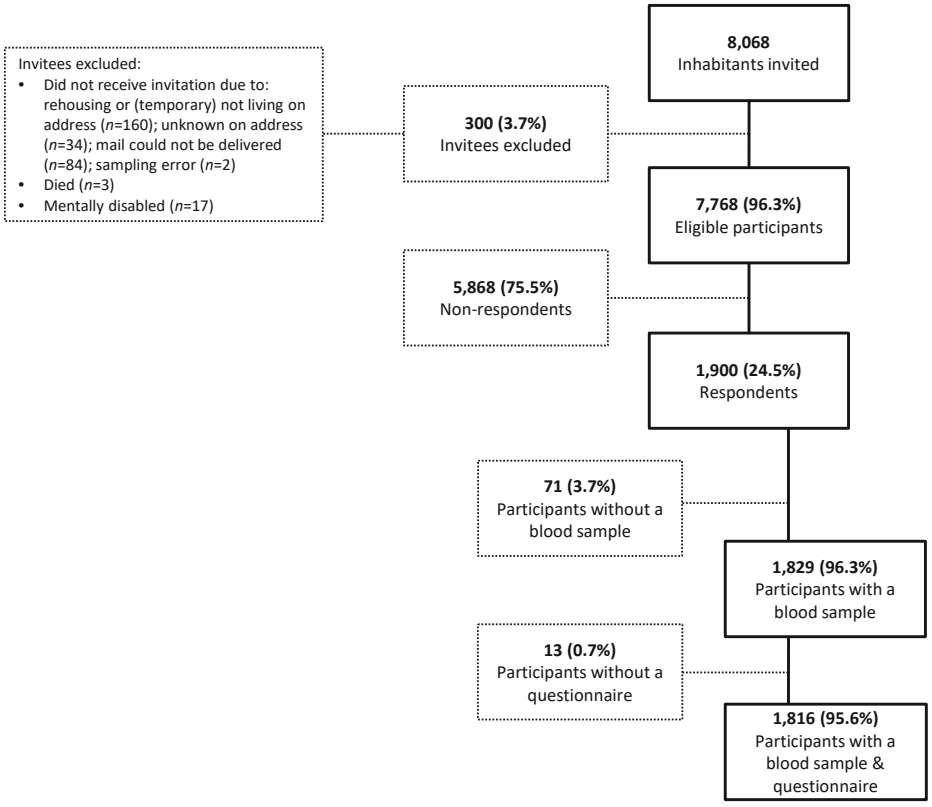
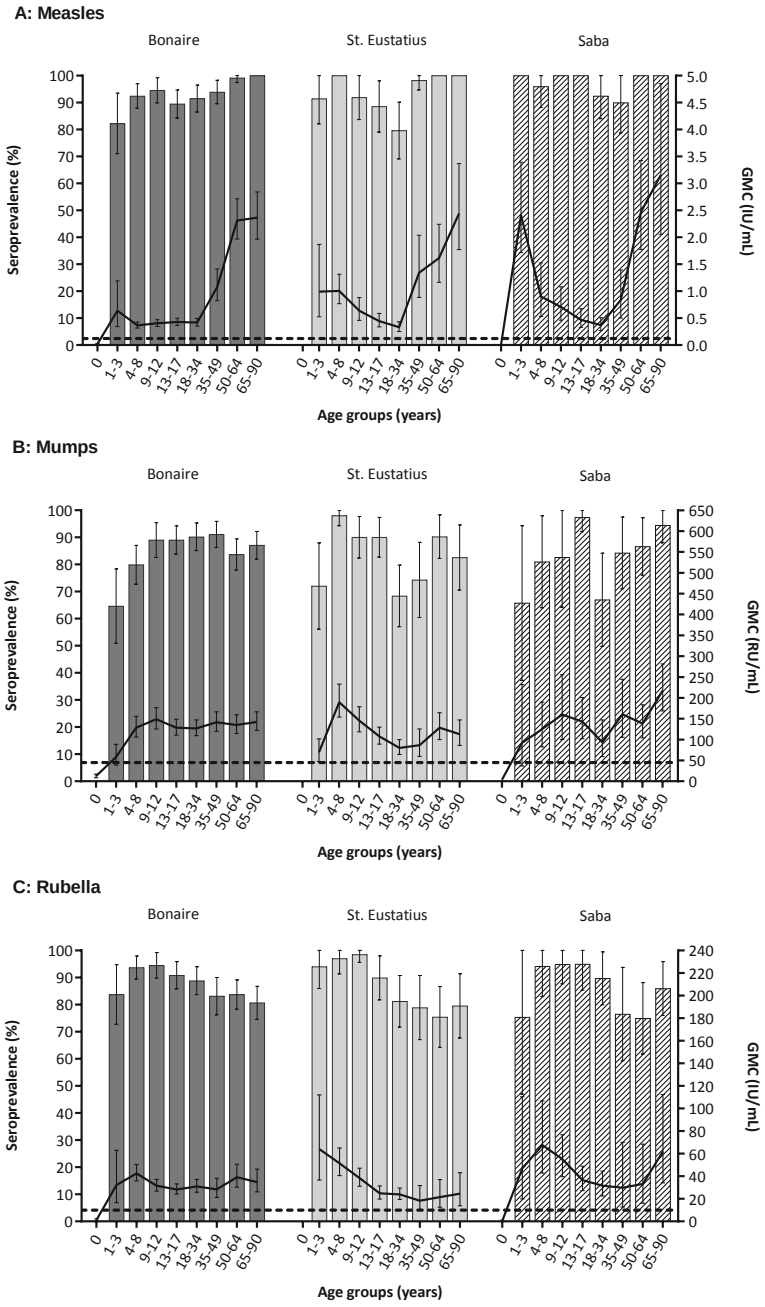
SUPPLEMENTARY MATERIALS



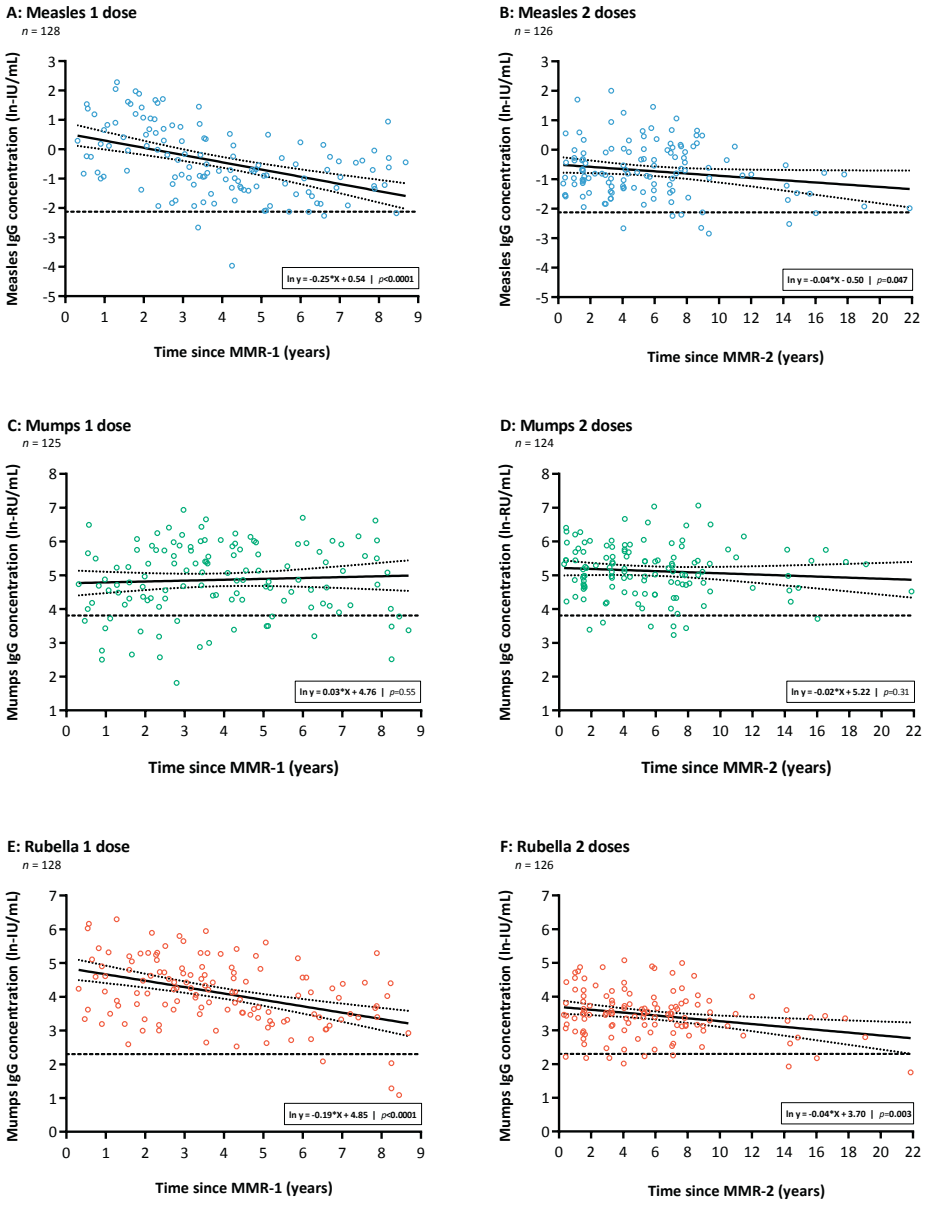
Supplement Figure S1. Overview of introduction and adaptations of measles, mumps and rubella (MMR)-vaccinations in Caribbean Netherlands from 1975–present.



Supplement Figure S2. Flowchart of the Health Study Caribbean Netherlands.



Supplement Figure S3. Age-specific seroprevalence (%) and geometric mean concentration (GMC) (with 95% confidence intervals) of measles (**A**), mumps (**B**), and rubella (**C**) IgG antibodies in the general population of Bonaire, St. Eustatius and Saba, 2017. Note: antibody concentrations ≥ 0.120 international units (IU)/mL for measles, ≥ 45.0 RIVM units (RU)/mL for mumps, and ≥ 10.0 IU/mL for rubella were considered seropositive (dashed lines).



Supplement Figure S4. Persistence of measles (**A** & **B**), mumps (**C** & **D**), and rubella (**E** & **F**) IgG antibodies (ln-international units (IU) or RIVM units (RU)/mL)) after one and two doses of MMR-vaccination among participants from the Dutch overseas territories. Note: the solid line represents the fitted model via linear regression analyses, the small dotted lines the 95% confidence intervals, and the dashed lines the ln-cutoff for seropositivity.

CHAPTER 5

High seroprevalence of multiple high-risk human papillomavirus types among the general population of Bonaire, St. Eustatius and Saba, Caribbean Netherlands

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ABSTRACT

Background

Incidence and mortality of human papillomavirus (HPV)-related cancers differs geographically, with high rates in Caribbean countries. Seroepidemiological data provide information on lifetime cumulative HPV exposure and contributing risk factors, but has not been available yet for Caribbean Netherlands (CN), comprising the islands Bonaire, St. Eustatius and Saba. Therefore, a cross-sectional population-based serosurveillance study was performed in this (recently girls-only HPV-vaccinated) population in 2017.

Methods

Blood samples from participants (*n* = 1,823, 0–90 years) were tested for seven high-risk (hr)-HPV-specific IgG-antibodies using a VLP-based multiplex-immunoassay. Risk factors for HPV-seropositivity were analysed among persons unvaccinated aged ≥ 15 years who ever had sex (*n* = 1,080).

Results

Among unvaccinated individuals aged ≥ 15 years, overall seropositivity was high (34%), with over half of them being seropositive for ≥ 2 hr-HPV types, and HPV16 and 52 being most prevalent (13%). Seroprevalence was substantial higher in unvaccinated women (51%) than men (18%), predominantly peaking in women aged 20–59 years, and was highest on St. Eustatius (38%). Besides age and sex, sexual risk factors were associated with HPV-seropositivity.

Conclusions

In accordance with the Caribbean region, seroprevalence of multiple hr-HPV types was high in CN. These data corroborate the decision regarding introduction of a sex-neutral HPV-vaccination program and the relevance for considering a population-based cervical cancer screening program.

INTRODUCTION

Human papillomavirus (HPV) is the most common sexually transmitted pathogen in men and women worldwide, approximately infecting 80% of people at some time. Over 200 different HPV genotypes have been identified, of which 40 can infect the genital tract [1]. Persistent infection with high-risk (hr)-HPV types can lead to anogenital- and oropharyngeal cancers, of which cervical cancer is the most prevalent. Annually, 680,000 HPV-related cancers are estimated to occur worldwide, including 570,000 cervical cancer cases [2]. Hr-HPV types 16 and 18 are mostly detected in women and thereby responsible for 70% of all cervical cancer cases [3].

Incidence and mortality of HPV-related diseases differ geographically. For cervical cancer this can largely be explained by presence of organized prevention programs. Caribbean countries, that mostly lack vaccination and cervical cancer screening programs, have a higher than world average incidence and mortality rate with 15.2 and 8.5 per 100,000, respectively, whereas, e.g., in Western Europe this is below average with 6.8 and 2.1 per 100,000, respectively [2, 4, 5]. In Caribbean Netherlands (CN) – consisting of the three Dutch overseas municipalities Bonaire, St. Eustatius and Saba, comprising a diverse ethic population of ~ 25,000 people – HPV-vaccination has been included in the National Immunization Program since 2013. The quadrivalent vaccine was introduced on St. Eustatius and Saba in 2013, and bivalent vaccine on all three islands in 2015 (two doses for girls aged 9/10 years of age), with coverage in 2018 ranging between 28 and 67% across islands [6]. A population-based cervical cancer screening program, however, has not been introduced in CN thus far.

Insight into the population-based HPV serostatus provides information on age- and sex-specific lifetime cumulative HPV exposure and past infections of (vaccine-relevant) circulating genotypes, and can be linked to contributing risk factors. Moreover, these insights can serve as a guide for policymakers in their development of future HPV preventive programs, such as consideration of a population-based cervical cancer screening or as a baseline for future evaluation of the vaccination program. For instance, by estimating vaccine uptake (since vaccine-induced antibody levels are far higher than after natural infection), and monitoring changes in epidemiological dynamics of HPV infection after vaccination, including (indirect) herd effects in those ineligible for vaccination (by comparing age- and gender-specific serological profiles pre- and post-vaccination) as well as impact on other HPV types by the vaccine used (cross-protection/replacement) [7]. However, such data have not been available for CN yet; hence, by means of a representative serosurveillance study conducted in this (recently girls-only HPV-vaccinated) population in 2017 for the first time, we describe the seroprevalence of seven hr-HPV types (16, 18, 31, 33, 45, 52, 58) and associated risk factors for HPV-seropositivity.

METHODS

Study design and sample collection

A cross-sectional population-based serosurveillance study (Health Study Caribbean Netherlands) was conducted by the National Institute for Public Health and the Environment of the Netherlands (RIVM) in mid-2017. Details of the survey methods, data collection and inclusion have been described previously [8]. Briefly, on Bonaire, St. Eustatius and Saba, an age-stratified sample, with age strata 0–11, 12–17, 18–34, 35–59 and 60–89 years, was randomly drawn from the population registry (PIVA-V, January 1, 2017). A total of 7,768 eligible individuals were invited. All procedures performed were in accordance with the 1964 Declaration of Helsinki and its later amendments. The Medical Ethics Committee Noord-Holland in the Netherlands approved the study (METC number: M015-022), and, prior to participation, signed informed consent was obtained from all participants aged ≥ 12 years and, if < 18 years of age, also from their parents or legal guardians. In total, 1,900 participants were included in this study (response rate 24.5%).

Participants donated a blood sample – via a finger- or heel prick using the dried blood spot (DBS) method on air-dried filter paper (Whatman® 903 protein saver cards) – and completed a questionnaire on sociodemographics, sexual behaviour (from 15 years of age) and other factors possibly related to HPV infection. Information on HPV-vaccination was collected via vaccination certificates or, if unavailable, retrieved from the local public health department if obtainable. Women up till 30 years of age without any documented vaccination record were considered vaccinated if their antibody concentration was within a range of vaccinated adolescent girls from a large cohort measured at the same laboratory (i.e., HPV16 ≥ 100 Luminex units (LU)/mL and HPV18 ≥ 50 LU/mL (Hoes *et al.*; submitted)).

Serological measurements

Blood samples were air-shipped to the laboratory of the RIVM and stored instantly at −80 °C awaiting analyses. For the detection of HPV-specific IgG-antibodies levels against HPV L1 virus-like-particle (VLP) 16, 18, 31, 33, 45, 52, 58, a VLP-based multiplex-immunoassay was used, as previously described [9] (VLPs were kindly donated by MSD (Merck & Co, Inc, Kenilworth, NJ)). In short, following standard protocol, a 3.2 mm (1/8 in.) punch was taken from the DBS and incubated in phosphate-buffered-saline containing 0.2% Tween-20 and 1% bovine serum albumin (i.e., assay buffer) at 4 °C overnight on a shaker to release serum, resulting in a 1:200 dilution [10, 11]. If detection was out of range, samples were further diluted to 1:20,000 in assay buffer. HPV-specific antibodies

were detected using R-phycoerythrin conjugated goat anti-human IgG after incubation with VLP-conjugated beads (Bio-Rad Laboratories, Hercules, CA). Blanks, four in-house controls and a standard were used consistently. HPV-specific IgG-antibodies were analyzed using the Bioplex200 system and software (Bio-Rad Laboratories, Hercules, CA), measured in arbitrary LU/mL (and for HPV16 and 18 converted to international units (IU)/mL by dividing LU/mL by 2.8 and 3.3, respectively). Samples were assumed to be seropositive above cutoffs determined via a method by Frey *et al.* [12] (with 99% one-sided t-values based on n = 215 controls, aged 1–6 years from the present study), namely: HPV16: ≥ 9 LU/mL, HPV18: ≥ 15 LU/mL, HPV31: ≥ 9 LU/mL, HPV33: ≥ 11 LU/mL, HPV45: ≥ 27 LU/mL, HPV52: ≥ 19 LU/mL, HPV58: ≥ 17 LU/mL.

Statistical analyses

Data were analysed in SAS v.9.4 (SAS Institute Inc., Cary, NC) and R v.3.6. Overall seroprevalence and geometric mean concentrations (GMC) for IgG-antibodies against the seven hr-HPV types among the total population were estimated. These data were weighted, taking into account island, sex, age group, country of birth (and for Bonaire neighbourhood too), in order to match the population distribution of each island as of January 1, 2017. Differences in seroprevalence of HPV-specific antibodies between islands, sex and age were determined by estimating the parameters of the beta distribution of these seroprevalence rates using the methods of moments [13]. Risk ratios, their corresponding 95% confidence intervals (CI) and p values were estimated by Monte Carlo simulations of both seroprevalence estimates. Differences in the GMC between islands, sex and age were identified by calculating the differences in logarithmic (ln)-concentrations and tested via a t-test. Age-specific seroprevalence, GMC and 95% CI were determined for CN, per islands and sex, and stratified for HPV-vaccination. Seroprevalence for 'any' or 'all' hr-HPV-type(s) refer to the seven hr-serotypes that have been measured in this study. Statistically significance was set at $p < 0.05$.

Risk factors were determined for hr-HPV-seropositivity among sexual active and HPV-unvaccinated participants from 15 years of age. Generalized estimating equations with an exchangeable correlation structure was used. Each hr-HPV type was treated as a separate endpoint accounting for multiple antibodies against hr-HPV types per person and ultimately estimating the exposure effect on hr-HPV-seropositivity as a whole. Risk factors included in the model were: island, sex, age group, ethnicity, residency in CN, educational level, smoking, alcohol consumption, body mass index (BMI), having a steady partner, age at sexual debut, sexual partners, sexual preference, condom use, oral contraceptive use, history of sexual transmittable disease(s) (STD) (note: participants with missing values for a specific variable were allocated to a missing category). In univariate analyses, all variables were adjusted for multiple hr-HPV types,

and sex and age group thereby taking into account the survey design. Variables in univariate analyses with a $p < 0.10$ were included in the multivariate analysis and backward selection (dropping variables one-by-one manually) was then used to identify risk factors in which a $p < 0.05$ was considered statistically significant associated. Crude and adjusted odds ratios (ORs) and 95% CIs were estimated as well as unweighted seroprevalence and 95% CI for all studied factors.

RESULTS

Study characteristics

Sociodemographic study characteristics have been described in-depth elsewhere [8]. Shortly, 1,823 persons, aged 3 months to 90 years, donated a blood sample from which HPV-specific IgG-antibodies could be determined and filled-out the questionnaire (*Table 1*). There were 820 (45%) men and 1,003 (55%) women, and in accordance with the sampling, the largest part resided on Bonaire (*n* = 1,124 (62%), followed by St. Eustatius (*n* = 478 (26%)) and Saba (*n* = 221 (12%)). Most people originated from the Dutch overseas territories (comprising CN, Aruba, Curaçao and St. Maarten) and Suriname (*n* = 1,309, 72%), followed by Latin America and other non-Western countries (*n* = 280, 16%), and indigenous Dutch & other Western countries (*n* = 221, 12%). People from the Dutch overseas territories and Suriname were relatively often present in the study sample of St. Eustatius (82%), whereas this was the case for those from indigenous Dutch and other Western countries (22%) and Latin America and other non-Western countries (16%) on Saba – following their population composition [14]. In total, 102 women were vaccinated against HPV (*n* = 73, *n* = 27 and *n* = 2 in age groups 9–14, 15–19 and 20–29 years, respectively), with relatively most on St. Eustatius (*n* = 40 (8%)) and Saba (*n* = 17 (8%)), as routine HPV-vaccination was introduced two years earlier than on Bonaire.

Questions related to sexual behavior were completed from age 15 years (*n* = 1,209). Sixty percent reported to have a steady partner and 84% ever had sexual intercourse. Among the latter, median age of sexual debut was 17 years (interquartile range (IQR) 16–19). Men had an earlier sexual debut (17 (IQR: 15–18)) than women (18 (16–20)), being lowest for men on St. Eustatius (16 (IQR: 14–18) and Saba (16 (IQR: 15–18). Overall, 16% reported to have had ≥ 5 lifetime sexual partners. For Saba this percentage (26%) was higher than Bonaire (15%) and St. Eustatius (12%), however, nearly 50% of participants did not complete this question (mostly on St. Eustatius (62%)). Five percent had a self-reported history of a STD (chlamydia was most reported (*n* = 35), followed by gonorrhea (*n* = 15)), being highest on Saba (11%).

Table 1. Sociodemographic and sexual behaviour characteristics of participants with a blood sample for HPV IgG antibody determination in the Health Study Caribbean Netherlands, by island (*n* (%)).

Sociodemographic characteristic	Bonaire *n* = 1,124 (61.7%)	St. Eustatius *n* = 478 (26.2%)	Saba *n* = 221 (12.1%)	Total *n* = 1,823
Sex				
Men	503 (44.8%)	221 (46.2%)	96 (43.4%)	820 (45.0%)
Women	621 (55.2%)	257 (53.8%)	125 (56.6%)	1,003 (55.0%)
Age groups, years				
0–14	373 (33.2%)	183 (38.3%)	58 (26.2%)	614 (33.7%)
15–24	125 (11.1%)	53 (11.1%)	22 (10.0%)	200 (11.0%)
25–34	110 (9.8%)	62 (13.0%)	24 (10.9%)	196 (10.7%)
35–44	78 (7.0%)	34 (7.1%)	25 (11.3%)	137 (7.5%)
45–64	259 (23.0%)	90 (18.8%)	52 (23.5%)	401 (22.0%)
65–90	179 (15.9%)	56 (11.7%)	40 (18.1%)	275 (15.1%)
Ethnic background[a]				
Dutch overseas territories and Suriname	799 (71.2%)	384 (82.1%)	126 (57.5%)	1,309 (72.3%)
Indigenous Dutch and other Western countries	143 (12.7%)	30 (6.4%)	48 (21.9%)	221 (12.2%)
Latin America and other non-Western countries	181 (16.1%)	54 (11.5%)	45 (20.6%)	280 (15.5%)
(Maternal) educational level[b]				
High	170 (15.1%)	68 (14.2%)	85 (38.4%)	323 (17.7%)
Middle	297 (26.4%)	126 (26.4%)	45 (20.4%)	468 (25.7%)
Low	570 (50.7%)	232 (48.5%)	80 (36.2%)	882 (48.4%)
Unknown	87 (7.7%)	52 (10.9%)	11 (5.0%)	150 (8.2%)
HPV vaccination[c]				
Yes	45 (4.0%)	40 (8.4%)	17 (7.7%)	102 (5.6%)
No	1,079 (96.0%)	438 (91.6%)	204 (92.3%)	1,721 (94.4%)
Among participants from 15 years of age (n_{total}=1,209)				
Steady partner				
Yes	458 (61.0%)	178 (60.3%)	89 (54.6%)	725 (60.0%)
No	264 (35.1%)	91 (30.9%)	60 (36.8%)	415 (34.3%)
Unknown	29 (3.9%)	26 (8.8%)	14 (8.6%)	69 (5.7%)
Ever had sexual intercourse				
Yes	631 (84.0%)	249 (84.4%)	140 (85.9%)	1,020 (84.3%)
No	77 (10.3%)	12 (4.1%)	11 (6.7%)	100 (8.3%)
Unknown	43 (5.7%)	34 (11.5%)	12 (7.4%)	89 (7.4%)

Table 1. (Continued)

Sociodemographic characteristic	Bonaire *n* = 1,124 (61.7%)	St. Eustatius *n* = 478 (26.2%)	Saba *n* = 221 (12.1%)	Total *n* = 1,823
Among participants from 15 years of age who had sexual intercourse (i.e., excluding those without) (n_{total}=1,109)				
Median age at sexual debut	18 (16–20)	17 (15–18)	18 (16–19)	17 (16–19)
Age at sexual debut				
< 18	213 (31.6%)	94 (33.2%)	57 (37.5%)	364 (32.8%)
≥ 18	233 (34.6%)	67 (23.7%)	63 (41.5%)	363 (32.7%)
Does not want to answer	87 (12.9%)	45 (15.9%)	12 (7.9%)	144 (13.0%)
Unknown	141 (20.9%)	77 (27.2%)	20 (13.6%)	238 (21.5%)
Lifetime sexual partners				
1	110 (16.3%)	22 (7.8%)	26 (17.1%)	158 (14.3%)
2–4	150 (22.3%)	53 (18.7%)	27 (17.8%)	230 (20.7%)
≥ 5	102 (15.1%)	34 (12.0%)	40 (26.3%)	176 (15.9%)
Unknown	312 (46.3%)	174 (61.5%)	59 (38.8%)	545 (49.1%)
Ever had sexual transmitted disease				
Yes	30 (4.5%)	12 (4.2%)	16 (10.5%)	58 (5.2%)
No	644 (96.6%)	271 (95.8%)	136 (89.5%)	1,051 (94.8%)

[a] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten. Within ethnic group indigenous Dutch and other Western countries, *n* = 147 (66%) were indigenous Dutch. Within Latin America and other non-Western countries, *n* = 261 (93%) were born in Latin America.

[b] Maternal educational level was used for participants 0–11y, active education was used for participants 12–25y, and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1), lower general secondary education (MAVO/VMBO); Middle = intermediate/secondary vocational education (MBO-2-4), higher/senior vocational education (HAVO), pre-university education (VWO/Gymnasium); and High = higher professional education (HBO), University BSc., MSc., Doctorate.

[c] *n* = 71 women were vaccinated against HPV according to the vaccination registry and *n* = 31 without vaccination records were highly likely to be vaccinated based on IgG antibody concentration and age, see method section for detailed definition (in age groups 9–14y, 15–19y and 20–29y, *n* = 73, 27 and 2 women were vaccinated, respectively).

Missing: ethnic background *n* = 13.

Seroprevalence and GMC

Overall seroprevalence and GMC in CN

Seroprevalence for any of the seven hr-HPV types in CN (0–90 years, *n* = 1,823) was 31.3% (95% CI 28.6–34%) and amounted to 29.7% (95% CI 26.9–32.4) in those unvaccinated (*n* = 1,721). GMCs for all hr-types in vaccinated individuals were significantly higher than in unvaccinated individuals, especially for vaccine types HPV16 (GMCs of 246.8 vs. 0.56 IU/mL, respectively) and HPV18 (74.7 vs. 0.74 IU/mL, respectively) (all $p < 0.0001$) (*Figure 1*). Focusing on unvaccinated participants from age 15 years (*n* = 1,180), overall seroprevalence was 34% (95% CI 30.8–37.3), and antibodies against HPV16 and 52 were detected mostly (both 13.1%), followed by HPV58, HPV18, HPV31, HPV45 and HPV33 (8.9–12.7%) (*Table 2a*). Over half of those seropositive were positive for ≥ 2 hr-HPV types and a small proportion (2%) was positive for all hr-types.

Overall seroprevalence and GMC, by sex and island

Among those unvaccinated from age 15 years, seroprevalence for any hr-HPV type was significantly higher in women (51.4%) than in men (18.1%) (*Table 2a*). The same accounted for hr-type specific GMCs (all $p < 0.0001$) and hr-type specific seroprevalence, and this sex difference was observed on all islands. HPV16 and 52 were most common in women (20%), and HPV58 (8%), 16 and 52 (both 7%) in men. Women were over 3-fold more often seropositive against ≥ 2 hr-HPV types than men (28.8% vs. 8.8%), yet seropositivity against all hr-types did not differ between sexes.

St. Eustatius displayed a higher seropositivity against any hr-HPV types (38.4%) as compared to Bonaire (33.4%) and Saba (33.1%) (*Table 2b*), and this was due to a higher seropositivity in both men (23.0%) and (unvaccinated) women (55.7%) on this island. Overall GMCs for all hr-types were also highest on St. Eustatius, and significantly higher for HPV16, 18, 31, 33 and 58 as compared to Bonaire, and for HPV33 and 58 compared to Saba (all $p < 0.05$). Also, with exception of HPV52 – which was highest on Bonaire – seropositivity against all other six hr-types was highest on St. Eustatius (with HPV16, 31 and 58 being highest), attributable to higher seroprevalence in men as compared to Bonaire and Saba. Interestingly, seropositivity against all hr-types on St. Eustatius was higher for men (6.8%) than women (1.6%), whereas this was not the case on the other islands.

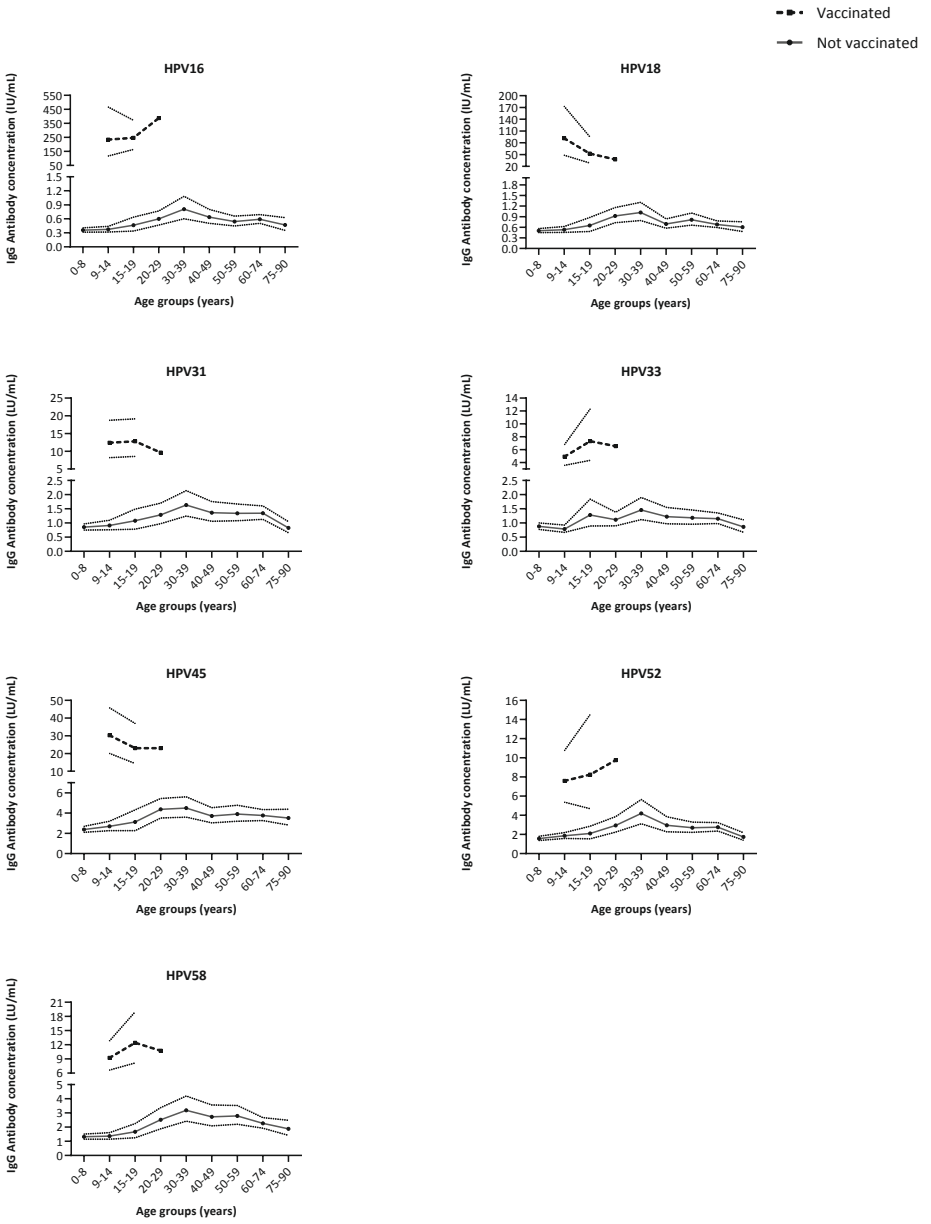

Figure 1. Age-specific geometric mean concentration (GMC) (with 95% confidence intervals (CI)) of seven high-risk types human papillomavirus (HPV) IgG antibodies in the general population of Caribbean Netherlands, 2017, by HPV vaccination. Note: 95% CI was not provided for vaccinated participants in age group 20–29 years due to the low number of participants in this group.

Age-specific seroprevalence and GMC in CN

In accordance with age of sexual debut, a sharp increase in seropositivity, i.e., a step-up, was observed from 6% in the 0–8 year-olds to 21.1% and 35.6% in the unvaccinated age groups 15–19 and 20–29 years respectively, with similar rise in GMC for all hr-types (*Figure 1* and *Supplement Figure S1*). Both GMC and seroprevalence peaked in age group 30–39 years (37.8%), remained stable up till age group 50–59 years and declined in persons of 60 years and above to levels comparable to that of 15–19 year-olds.

Age-specific seroprevalence and GMC, by sex and island

Unvaccinated women had a substantially higher HPV seroprevalence for any hr-type as compared to men between 15 and 74 years of age (*Figure 2*). Likewise, a sex difference in seroprevalence and GMC for all seven hr-types was observed for age groups 20–74 years. Although the step-up among adolescents was noticeable among both men and women, it was most pronounced in women in whom seroprevalence increased considerably from 18.8% (in 9–14 years) to 39.8% in 15–19 years, and almost reached 60% in those aged 20–29 years – with greatest step-up seen for HPV33 and HPV52. In women aged 20–39 years, seroprevalence was highest (all > 25%) for HPV16, 18, 31 and 52. Remarkably, seropositivity for HPV58 in women rose gradually with age, peaking at 50–59 years (23%), and being highest among all seven hr-types in that age group. In men, highest seroprevalence was observed in 15–19 year-olds for all seven hr-types, with rates being similar to women in that age group for HPV45, HPV52 and HPV58.

Seroprevalence was remarkably high for any hr-type in 65–90 year-olds on St. Eustatius (45%) as compared to Bonaire (24%; $p = 0.008$) and Saba (29%; $p = 0.13$). This was primarily due to HPV16, 18 and 58 which were all > 15% in this age group (*Supplement Figure S2*). Also, GMCs were significantly higher (data not shown). Specifically, besides women demonstrating a higher seroprevalence in this age group on St. Eustatius, men in particular had a higher seroprevalence as compared to those on the other islands (*Figure 3*) – with HPV16 and 58 being even higher in men than women on St. Eustatius (*Supplement Figure S3*). Interestingly, among the unvaccinated 9–17 year-olds on Saba no one was seropositive for any hr-type.

Table 2. Weighted seroprevalence (with 95% confidence interval (CI)) for seven high-risk HPV types and combinations in the total population of Caribbean Netherlands among those unvaccinated[a] and from 15 years of age, by sex (**A**) and island (**B**).

A	**Seroprevalence (95% CI)**						
	Overall ***n* = 1,180**		**Men** ***n* = 505 (42.8%)**		**Women** ***n* = 675 (57.2%)**		
	%	***(95% CI)***	***%***	***(95% CI)***	***%***	***(95% CI)***	***p* value[b]**
High-risk HPV types							
HPV16	13.1	(11.0–15.2)	6.8	(4.4–9.3)	19.9	(16.5–23.3)	**< 0.0001**
HPV18	11.8	(9.7–13.8)	5.6	(3.3–7.9)	18.5	(15.1–21.9)	**< 0.0001**
HPV31	10.9	(9.0–12.8)	5.6	(3.4–7.9)	16.3	(13.5–19.7)	**< 0.0001**
HPV33	8.9	(7.1–10.8)	6.0	(3.5–8.5)	12.2	(9.4–14.9)	**0.001**
HPV45	9.4	(7.6–11.3)	5.3	(3.1–7.5)	13.9	(11.0–16.9)	**< 0.0001**
HPV52	13.1	(10.8–15.4)	7.1	(4.3–9.9)	19.7	(16.2–23.2)	**< 0.0001**
HPV58	12.7	(10.5–14.9)	7.5	(4.7–10.3)	18.4	(14.9–21.8)	**< 0.0001**
HPV combinations							
HPV16 and 18	5.5	(4.1–7.0)	3.9	(2.1–5.7)	7.4	(5.2–9.5)	**0.02**
HPV16 or 18	19.3	(16.8–21.9)	8.5	(5.8–11.3)	31.1	(27.1–35.1)	**< 0.0001**
Positive for 1 or more high-risk HPV types	34.0	(30.8–37.3)	18.1	(14.0–22.2)	51.4	(47.1–55.7)	**< 0.0001**
Positive for 2 or more high-risk HPV types	18.1	(15.5–20.6)	8.8	(5.8–11.8)	28.1	(24.3–32.0)	**< 0.0001**
Positive for 7 high-risk HPV types	2.0	(1.1–3.0)	2.3	(0.8–3.8)	1.8	(0.6–2.9)	0.61

[a] *n* = 29 women were vaccinated (of which *n* = 12 according to the registry and *n* = 17 without vaccination records highly likely to be vaccinated (based on IgG antibody concentration and age, see method section for detailed definition)).

[b] Statistically significant different ($p < 0.05$) between men and women in bold type.

B	Seroprevalence (95% CI)					
	Bonaire *n* = 744 (63.0%)		St. Eustatius *n* = 278 (23.6%)		Saba *n* = 158 (13.4%)	
	%	*(95% CI)*	*%*	*(95% CI)*	*%*	*(95% CI)*
High-risk HPV types						
HPV16	**11.4**[st]	(9.0–13.8)	**20.7**[bo]	(15.0–26.4)	18.3	(11.5–25.2)
HPV18	11.3	(8.9–13.7)	15.2	(10.3–20.2)	11.0	(5.7–16.3)
HPV31	**9.6**[st]	(7.4–11.8)	**16.4**[bo]	(11.2–21.5)	14.9	(9.0–20.8)
HPV33	8.3	(6.2–10.5)	12.5	(7.8–17.2)	9.2	(4.5–14.0)
HPV45	8.9	(6.7–11.0)	12.6	(7.9–17.3)	9.9	(5.0–14.8)
HPV52	13.7	(11.0–16.4)	10.6	(6.3–15.0)	11.3	(6.1–16.4)
HPV58	12.3	(9.7–14.9)	16.6	(11.3–21.8)	10.0	(5.1–15.1)
HPV combinations						
HPV16 and 18	**4.9**[st]	(3.4–6.5)	**8.2**[bo]	(4.2–12.2)	7.4	(3.0–11.9)
HPV16 or 18	17.7	(14.8–20.6)	27.7	(21.5–33.9)	21.9	(14.6–29.2)
Positive for 1 or more high-risk HPV types	33.4	(29.6–37.3)	38.4	(31.7–45.1)	33.1	(24.8–41.3)
Positive for 2 or more high-risk HPV types	17.7	(14.7–20.7)	20.8	(15.4–26.3)	17.0	(10.7–23.3)
Positive for 7 high-risk HPV types	1.5	(0.5–2.6)	4.4	(0.8–7.9)	3.4	(0.3–6.6)

[a] *n* = 29 women were vaccinated (of which *n* = 12 according to the registry and *n* = 17 without vaccination records highly likely to be vaccinated (based on IgG antibody concentration and age, see method section for detailed definition)).

[bo] Statistically significant different from Bonaire ($p < 0.05$) in bold type.

[st] Statistically significant different from St. Eustatius ($p < 0.05$) in bold type.

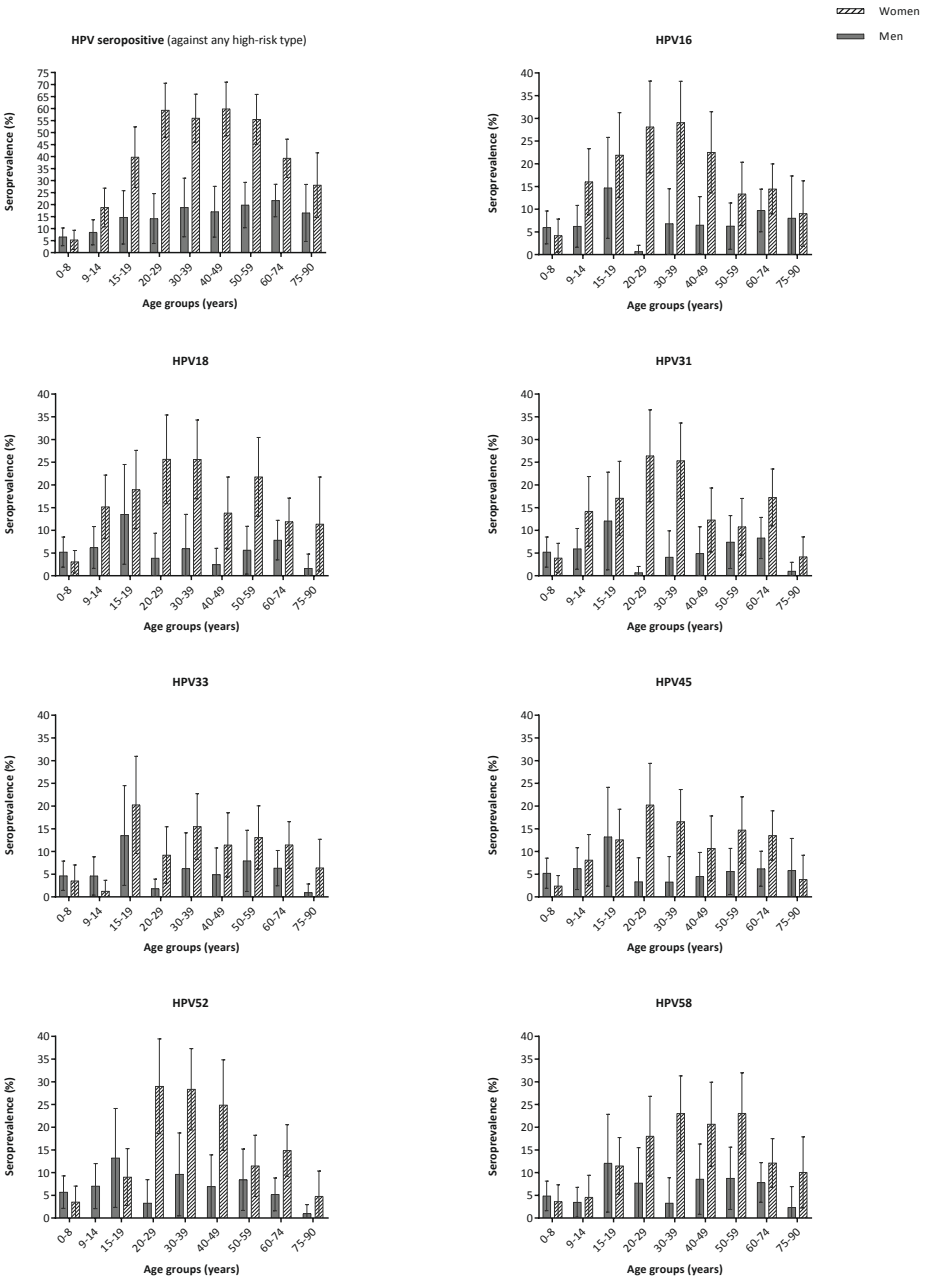

Figure 2. Age-specific seroprevalence (%) (with 95% confidence intervals) of any high-risk type and seven high-risk types human papillomavirus (HPV) IgG antibodies in the unvaccinated general population of Caribbean Netherlands, 2017, by sex.

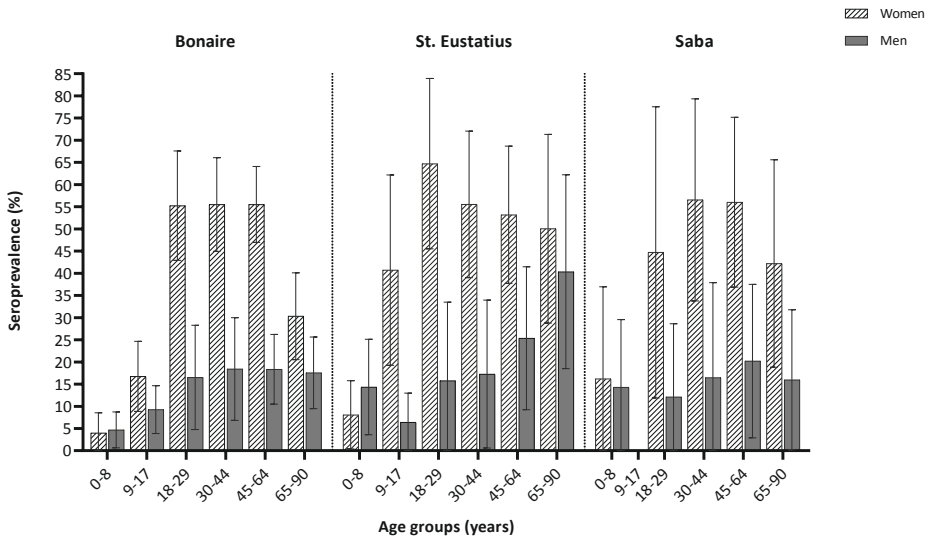

Figure 3. Age-specific seroprevalence (%) (with 95% confidence intervals) of any high-risk type human papillomavirus (HPV) IgG antibodies in the unvaccinated general population of Bonaire, St. Eustatius and Saba, 2017, by sex.

Risk factors for hr-HPV-seropositivity

Risk factors for hr-HPV-seropositivity were investigated in HPV-unvaccinated sexually active participants from age 15 years (*n* = 1,080) (*Table 3*). In univariate analyses the following variables were significantly associated with HPV-seropositivity: sex, age group, number of lifetime sexual partners and in the preceding year, and history of STD. In multivariate analysis, female sex was found to be the most pronounced determinant, followed by the number of lifetime sexual partners (2–4 and ≥ 5 vs. 1), being 25–34 years of age (vs. 15–24) and having a history of STD.

Table 3. Risk factor analysis for any high-risk type HPV IgG seropositivity among sexual active and unvaccinated participants from 15 years of age in the Health Study Caribbean Netherlands[a].

Potential risk factor for any high-risk type HPV seronegativity	*n* (%) *n* = 1,080	% HPV seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Island				0.61		
Bonaire	672 (62.2%)	37.5 (33.8–41.2)	Ref.			
St. Eustatius	261 (24.2%)	39.5 (33.5–45.4)	1.12 (0.85–1.47)			
Saba	147 (13.6%)	37.4 (29.6–45.2)	1.14 (0.81–1.61)			
Sex				**< 0.0001**		**< 0.0001**
Men	461 (42.7%)	19.5 (15.9–23.1)	Ref.		Ref.	
Women	619 (57.3%)	51.7 (47.8–55.6)	**2.94 (2.19–3.94)**		**3.34 (2.49–4.49)**	
Age group, years				**< 0.0001**		**0.0007**
15–24	102 (9.4%)	31.4 (22.4–40.4)	Ref.		Ref.	
25–34	189 (17.5%)	54.0 (46.9–61.1)	**1.74 (1.09–2.80)**		**1.68 (1.04–2.73)**	
35–44	134 (12.4%)	38.1 (29.8–46.3)	0.93 (0.55–1.57)		0.92 (0.54–1.59)	
45–64	383 (35.5%)	37.6 (32.7–42.5)	0.99 (0.62–1.57)		1.00 (0.62–1.61)	
65–90	272 (25.2%)	29.8 (24.3–35.2)	0.71 (0.43–1.17)		0.77 (0.46–1.28)	
Ethnic background				0.26		
Dutch overseas territories[d] and Suriname	675 (62.5%)	37.9 (34.3–41.6)	Ref.			
Indigenous Dutch and other Western countries	178 (16.5%)	32.0 (25.2–38.9)	0.85 (0.60–1.20)			
Latin America and other non-Western countries	227 (21.0%)	42.7 (36.3–49.2)	1.18 (0.89–1.58)			
Resident of Caribbean Netherlands since, years of age				0.37		
0–10	529 (49.0%)	38.4 (34.2–42.5)	Ref.			
11–17	25 (2.3%)	44.0 (24.5–63.5)	1.23 (0.64–2.34)			
18–39	286 (26.5%)	38.8 (33.2–44.5)	0.83 (0.62–1.11)			
≥ 40	189 (17.5%)	34.4 (27.6–41.2)	0.92 (0.66–1.29)			
Missing	51 (4.7%)	39.2 (25.8–52.6)	1.46 (0.79–2.69)			

Table 3. (Continued)

Potential risk factor for any high-risk type HPV seronegativity	*n* (%) *n* = 1,080	% HPV seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Educational level[e]				0.90		
High	225 (20.8%)	39.6 (33.2–46.0)	Ref.			
Middle	236 (21.9%)	39.4 (33.2–45.7)	0.98 (0.68–1.40)			
Low	520 (48.1%)	35.6 (31.5–39.7)	1.08 (0.81–1.45)			
Missing	99 (9.2%)	43.4 (33.7–53.2)	1.05 (0.69–1.60)			
Current smoking				0.93		
Yes	163 (15.1%)	33.1 (25.9–40.4)	Ref.			
No	888 (82.2%)	39.0 (35.8–42.2)	0.93 (0.65–1.33)			
Missing	29 (2.7%)	34.5 (17.2–51.8)	0.95 (0.40–2.26)			
Drunk alcohol in preceding year				0.33		
Yes	651 (60.3%)	39.3 (35.6–43.1)	Ref.			
No	381 (35.3%)	36.5 (31.6–41.3)	0.84 (0.66–1.07)			
Missing	48 (4.4%)	31.3 (18.1–44.4)	0.85 (0.45–1.64)			
Body Mass Index				0.27		
Underweight	15 (1.4%)	33.3 (9.4–57.2)	0.94 (0.41–2.13)			
Normal weight	280 (22.9%)	32.1 (26.7–37.6)	Ref.			
Overweight	352 (32.6%)	38.1 (33.0–43.1)	**1.39 (1.01–1.91)**			
Obesity	357 (33.1%)	43.1 (38.0–48.3)	1.30 (0.96–1.77)			
Missing	76 (7.0%)	35.5 (24.7–46.3)	1.12 (0.67–1.88)			
Steady partner				0.22		
Yes	689 (63.8%)	38.9 (35.3–42.5)	Ref.			
No	327 (30.3%)	35.8 (30.6–41.0)	1.03 (0.79–1.35)			
Missing	64 (5.9%)	39.1 (27.1–51.0)	**1.70 (1.02–2.83)**			

Table 3. (Continued)

Potential risk factor for any high-risk type HPV seronegativity	*n* (%) *n* = 1,080	% HPV seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Age at sexual debut, years				0.13		
< 18	356 (33.0%)	42.1 (37.0–47.3)	**1.39 (1.04–1.85)**			
≥ 18	359 (33.2%)	37.3 (32.3–42.3)	Ref.			
Does not want to answer	140 (13.0%)	37.1 (29.1–45.2)	1.34 (0.93–1.92)			
Missing	225 (20.8%)	32.9 (26.7–39.0)	1.25 (0.88–1.76)			
Lifetime sexual partners				**< 0.0001**		**< 0.0001**
1	155 (14.4%)	26.5 (19.5–33.4)	Ref.		Ref.	
2–4	225 (20.8%)	38.7 (32.3–45.0)	**1.89 (1.25–2.85)**		**1.85 (1.22–2.79)**	
≥ 5	171 (15.8%)	43.9 (36.4–51.3)	**2.42 (1.57–3.72)**		**2.24 (1.44–3.48)**	
Missing	529 (49.0%)	39.1 (35.0–43.3)	**2.91 (1.99–4.24)**		**2.88 (1.97–4.19)**	
Sexual preference				0.81		
Heterosexual	812 (75.2%)	37.3 (34.0–40.6)	Ref.			
Homosexual	14 (1.3%)	35.7 (10.6–60.9)	1.03 (0.43–2.45)			
Bisexual	32 (3.0%)	56.3 (39.0–73.5)	1.09 (0.65–1.84)			
Does not want to answer	74 (6.8%)	37.8 (26.8–48.9)	1.35 (0.84–2.16)			
Missing	148 (13.7%)	37.8 (30.0–45.7)	1.11 (0.77–1.61)			
Sexual partners preceding year				**0.043**		
0	244 (22.6%)	32.0 (26.1–37.8)	Ref.			
1	557 (51.6%)	41.7 (37.6–45.8)	**1.51 (1.09–2.10)**			
≥ 2	70 (6.5%)	40.0 (28.5–51.5)	1.33 (0.79–2.26)			
Does not want to answer	48 (4.4%)	29.2 (16.3–42.0)	1.51 (0.73–3.13)			
Missing	209 (14.9%)	34.4 (28.0–40.9)	**1.81 (1.19–2.75)**			

Table 3. (Continued)

Potential risk factor for any high-risk type HPV seronegativity	*n* (%) *n* = 1,080	% HPV seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Condom use last sexual intercourse				0.67		
Yes	166 (15.4%)	38.0 (30.6–45.3)	Ref.			
No	613 (56.7%)	37.7 (33.8–41.5)	0.81 (0.58–1.13)			
Does not want to answer	58 (5.4%)	29.3 (17.6–41.0)	0.88 (0.46–1.69)			
Missing	243 (22.5%)	40.7 (34.6–46.9)	0.87 (0.59–1.28)			
Oral contraceptive use last sexual intercourse				0.47		
Yes	117 (10.8%)	49.6 (40.5–58.6)	1.32 (0.95–1.85)			
No	626 (58.0%)	36.1 (32.3–39.9)	Ref.			
Does not want to answer	51 (4.7%)	29.4 (16.9–41.9)	1.02 (0.55–1.90)			
Missing	286 (26.5%)	38.8 (33.2–44.5)	1.09 (0.82–1.44)			
Ever had sexual transmitted disease				**0.01**		**0.02**
Yes	55 (5.1%)	65.5 (52.9–78.0)	**1.68 (1.18–2.39)**		**1.64 (1.12–2.40)**	
No	1,025 (94.9%)	36.5 (33.5–39.4)	Ref.		Ref.	

[a] *n* = 100 participants had not been sexual active, and *n* = 29 women were vaccinated (of which *n* = 12 according to the registry and *n* = 17 without vaccination records highly likely to be vaccinated (based on IgG antibody concentration and age, see method section for detailed definition)).

[b] Crude odds ratios were a priori adjusted for HPV high-risk type, sex and age group and significant (a)ORs are marked in bold type.

[c] *p* values were determined by means of Chi-Square tests for Generalized Estimating Equations (GEE) analysis, and significant *p* values (< 0.1 in univariate and < 0.05 in multivariate analysis) are marked in bold type.

[d] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten.

[e] Active education was used for participants 15–25y, and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1), lower general secondary education (MAVO/VMBO); Middle = intermediate/ secondary vocational education (MBO-2-4), higher/senior vocational education (HAVO), pre-university education (VWO/Gymnasium); and High = higher professional education (HBO), University BSc., University MSc., Doctorate.

Abbreviations: *aOR*, adjusted odds ratio; *CI*, confidence interval; *OR*, odds ratio; *Ref.*, reference category.

DISCUSSION

For the first time we describe the seroepidemiology of IgG-antibodies against the hr-HPV types 16, 18, 31, 33, 45, 52 and 58 in the population of Caribbean Netherlands, situated in a region with a high incidence of HPV-related cancers [2, 4, 5]. Seropositivity for multiple hr-HPV types was high in the unvaccinated population, with antibody responses against HPV16 and 52 being detected mostly. In general, women had a nearly 3-fold higher seroprevalence compared to men, predominantly peaking in women aged 20–59 years. Seropositivity for six hr-types was highest on St. Eustatius, which was particularly attributable to older men. Besides age and sex, risk factors related to sexual behavior were found to be associated with HPV-seropositivity in unvaccinated participants from age 15 years.

The incidence of cervical cancer is high in Caribbean countries. Recent estimations on Suriname and neighbouring island Curaçao revealed that incidence is 22.4 and 13.4 per 100,000, respectively [15, 16], whereas this is lower in the Netherlands (7.5 per 100,000) [17]. Despite this high incidence, only few studies have been conducted on HPV seroepidemiology in the Caribbean region; data which is key in developing preventive programs. In CN, over one third of the unvaccinated population from age 15 years was seropositive against any hr-HPV type measured, and over half of them had detectable antibodies against multiple hr-types. HPV16 and 52 were the most common hr-types (both 13%), followed by 58, 18 and 31. These observations are within a broad range found in (the few) other studies conducted in the Caribbean region [18-20], with exception of Jamaica where an even higher seroprevalence (50%) was found for HPV16 [21]. Conversely, seropositivity in CN was higher as compared to Western countries [22-25] for instance in the Netherlands [9, 26]; a country in which girls-only vaccination has been introduced since 2009 and population-based cervical screening has been in place since 1996. Still, prior to vaccination, seroprevalence in the Netherlands was lower than the present estimates in CN, with higher rates among people from Latin America and Caribbean descent [9], similar to the present study.

HPV-specific antibodies could already be detected in young children who are not likely to be sexually active which is in accordance with other population studies [24, 27]. This implies that the route of HPV-transmission is not only by sexual contact, but also for instance via vertical or horizontal transmission and autoinoculation [28]. Further, participants who had been vaccinated displayed an significant antibody response against hr-HPV vaccine types 16 and 18 as well as against the non-vaccine types. This cross-reactivity has been observed by others [29-32]. Interestingly, no one was HPV-seropositive among the unvaccinated 9–17 year-olds on Saba. Although HPV-vaccination was introduced for 9 year-old girls on Saba in 2013 and vaccine coverage has been high since a herd effect due to vaccination might seem too early. As ~25% of this total

age group on Saba responded in this study and the included numbers are low, future serosurveillance studies in CN should shed more light on this observation. Similar findings were not observed on the other islands; which might be explained by a lower vaccination coverage and very recent introduction of HPV-vaccination on Bonaire (two years prior to this study in 2015).

Female sex was the strongest predictor for being HPV-seropositive and women had a substantial higher overall seroprevalence than men in the total CN-population (51% vs. 18%). Seroprevalence (and GMC) rose quickly in adolescents and young adult women, corresponding to the age of sexual debut. This steep increase is in line with other studies [9, 19, 24, 33], and highlights the necessity to promote early education on HPV-vaccination and safe(r) sexual practices to prevent STDs in general. From age 60 years, seroprevalence decreased to rates comparable to 15–19 year-olds, possibly as a result of antibody waning or due to a cohort effect, i.e., decreasing sexual behavior over time, as earlier hypothesized [22]. Although the dissimilarity between sexes is in accordance with other studies, it was more pronounced than observed in other countries [9, 22]. After stratifying sexual risk behavior by sex, women were shown to have similar patterns as men (data not shown). It should be noted, however, that questions regarding sexual behavior were among the least well-completed, especially by men. Self-reporting of sexual behavior could lead to bias due to social desirability and this was also illustrated by our risk factor analysis for some variables (e.g., the missing category for lifetime sexual partners had the highest OR). It is known from literature, however, that Caribbean men more often report about multiple partnerships than women [34]. This could result in increased exposure to (multiple) HPV types in both sexes when compared to other populations. Subsequently, the fact that women display a substantial higher seroprevalence in this population might be explained by the different site of entry of the infection between sexes. As mucosal surfaces are infected in women predominantly, a detectable humoral immune response is more likely to be expected as compared to an infection at epithelial surfaces which mainly occur in men, as suggested by Desai and colleagues [22]. Hence, although increased sexual behavior in men will result in increased seropositivity, it will probably not be so pronounced as in women.

HPV-seropositivity for any hr-type was highest on St. Eustatius, followed by Saba and Bonaire, and seroprevalence (and GMC) for all measured hr-types, except HPV52, was highest on St. Eustatius too. Both women and men displayed higher seroprevalence rates on St. Eustatius as compared to the other islands, and particularly rates in men from 65 years of age were higher – predominantly due to HPV16 and 58. Increased sexual behavior on St. Eustatius most likely explains the difference between islands in general, and specifically among men. For instance, on this island, highest proportion for seropositivity against all seven hr-types was found in men as well as lowest age

of sexual debut (16 years of age). The questionnaire data could not confirm this for other sexual risk factors, probably due to the high number of missing values for these variables on St. Eustatius.

Various potential risk factors for HPV-seropositivity were investigated in this study. Beside female sex, and being a young adult (25–34 years), increased number of lifetime sexual partners and a history of STD are in line with other studies [19, 20, 35, 36]. Literature has been inconsistent on the influence of other factors, such as smoking, condom use, BMI and oral contraceptive use [18, 19, 36-40]. In this study, all these factors were not associated in our multivariate analysis, suggesting no relationship with HPV-seropositivity.

This study is subject to potential limitations. A direct comparison between HPV-serology studies is hindered by the use of different assays and methods [41]. Due to logistical reasons, we made use of the DBS-method in this study to collect our samples, and although we eluted these via a standardized and validated protocol, marginal difference with serum samples might not be inconceivable. Also, international standardization for all hr-HPV types, which has already been done for HPV16 and 18 and applied in this study, could help to overcome this difficulty in future studies. Direct comparison of data was possible with the population-based study performed in the Netherlands [9, 26], which was conducted, measured and analyzed in a similar way. Additionally, our cutoffs were determined via a statistically valid and widely used method in the field of immunoassays. Moreover, in particular men aged 18–34 years were relatively hard to include in our study; a common phenomenon in population-based studies [8, 42]. Hence, especially on the smaller islands St. Eustatius and Saba, this limits stratifying for multiple variables, and due to possible loss of power one should not exclude potential related bias. To minimize this, we have weighted our sample on a set of sociodemographic characteristics corresponding to the island's population at the time of enrollment. Further, we cannot draw firm conclusions on the rate of current HPV infections in this study as not all infected persons will develop a quantifiable antibody response, seroconversion might be delayed or HPV DNA has been cleared [43]. Likewise, risk factors for HPV-seropositivity do not necessarily reflect determinants for current HPV-infections.

Our findings are of great importance for policy implications. Firstly, girls in CN are currently vaccinated twice at age 9/10 years and this age is justifiable by the observed step-up in seroprevalence of multiple (vaccine-relevant) hr-types in those 15–19 years, which is indicative for the age of HPV-exposure in this population. Secondly, in June 2019 the Dutch Health Council advised to expand the National Immunization Program by offering the HPV vaccine also to boys [44]. As the burden of HPV-related cancers among men is substantial in the Caribbean region [2] and HPV-seropositivity among men was shown to be significant too, a sex-neutral vaccination program in CN will lead

to direct benefit of the male population. Thirdly, the high seroprevalence of multiple hr-types among adult women indicate towards a relative high-risk of (precursors of) HPV-related cancers and thereby underlines the need to consider routine cervical screening in CN and the potential value of a catch-up campaign.

Incidence of HPV-related cancers is high in the Caribbean region, and comprehensive and locally responsive cancer care is particularly challenging due to commonly under-resourced health-care systems, as highlighted by Spence and colleagues recently [45]. Besides the policy implications addressed, this study will be able to serve as a baseline for future investigations assessing the impact of a potential cervical cancer screening program and (catch-up) vaccination programs in CN by estimating vaccine uptake and monitoring epidemiological dynamics of HPV infection in the population (i.e., direct and indirect effects as well as impact on circulating HPV types) [7]. Few seroprevalence studies have been conducted in this region and we hereby would like to emphasize the need for serosurveillance data since that would be the first step in developing evidence-based public health policy and could eventually prevent HPV-infections and associated diseases as a whole.

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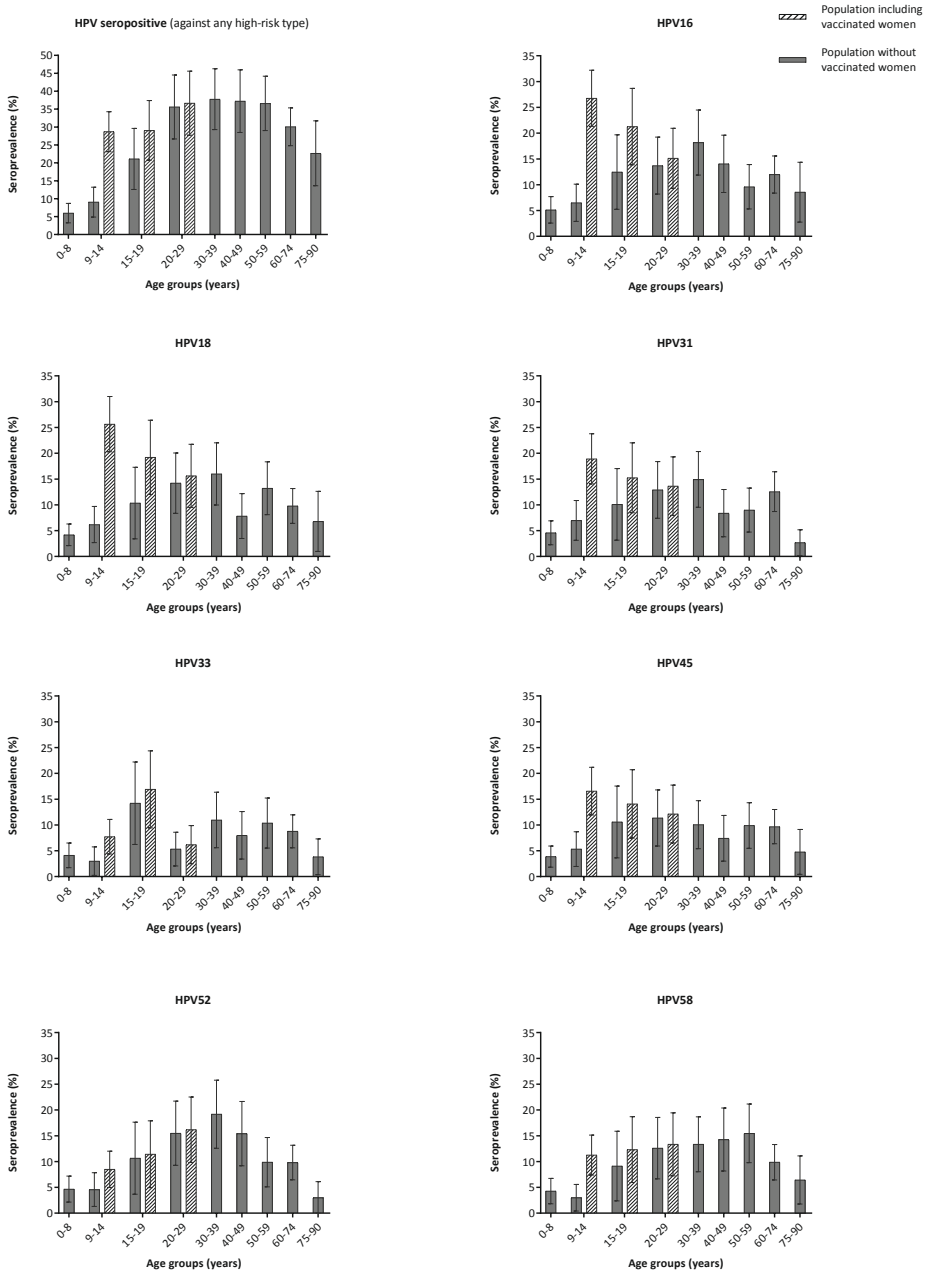
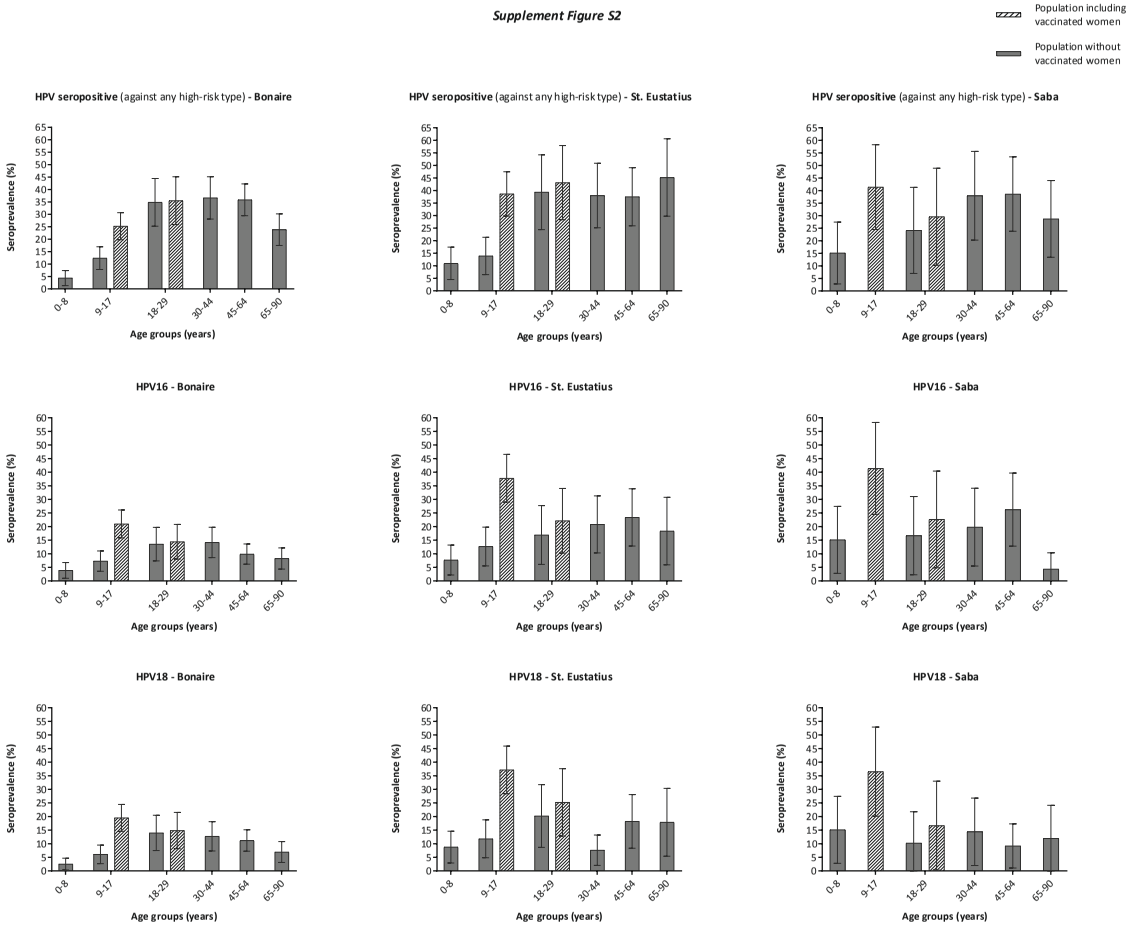
SUPPLEMENTARY MATERIALS



Supplement Figure S1. Age-specific seroprevalence (%) (with 95% confidence intervals) of any high-risk type and seven high-risk types human papillomavirus (HPV) IgG antibodies in the general population of Caribbean Netherlands, 2017, by HPV vaccination.

Supplement Figure S2



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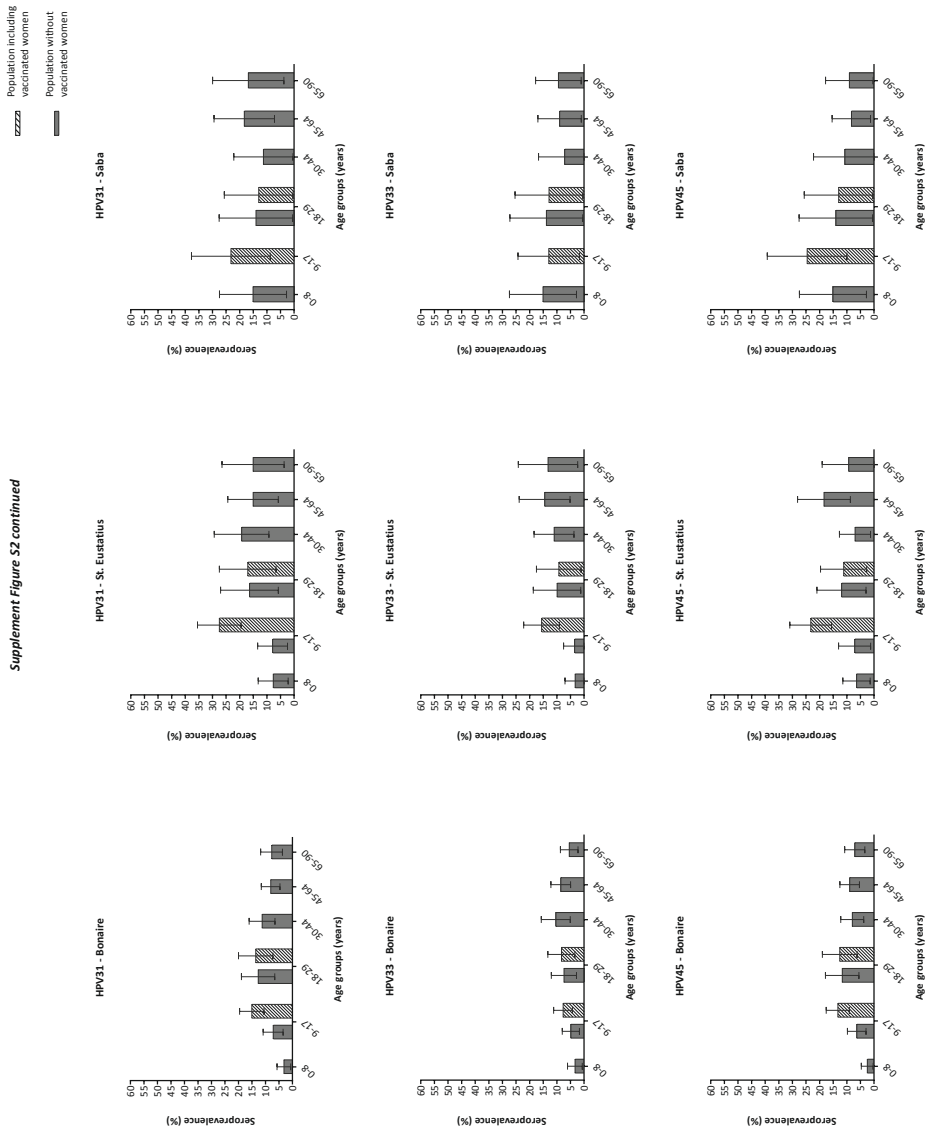

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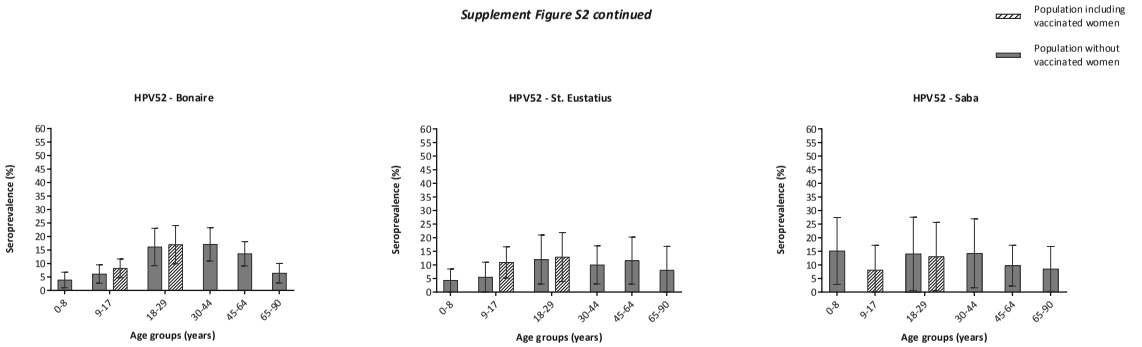
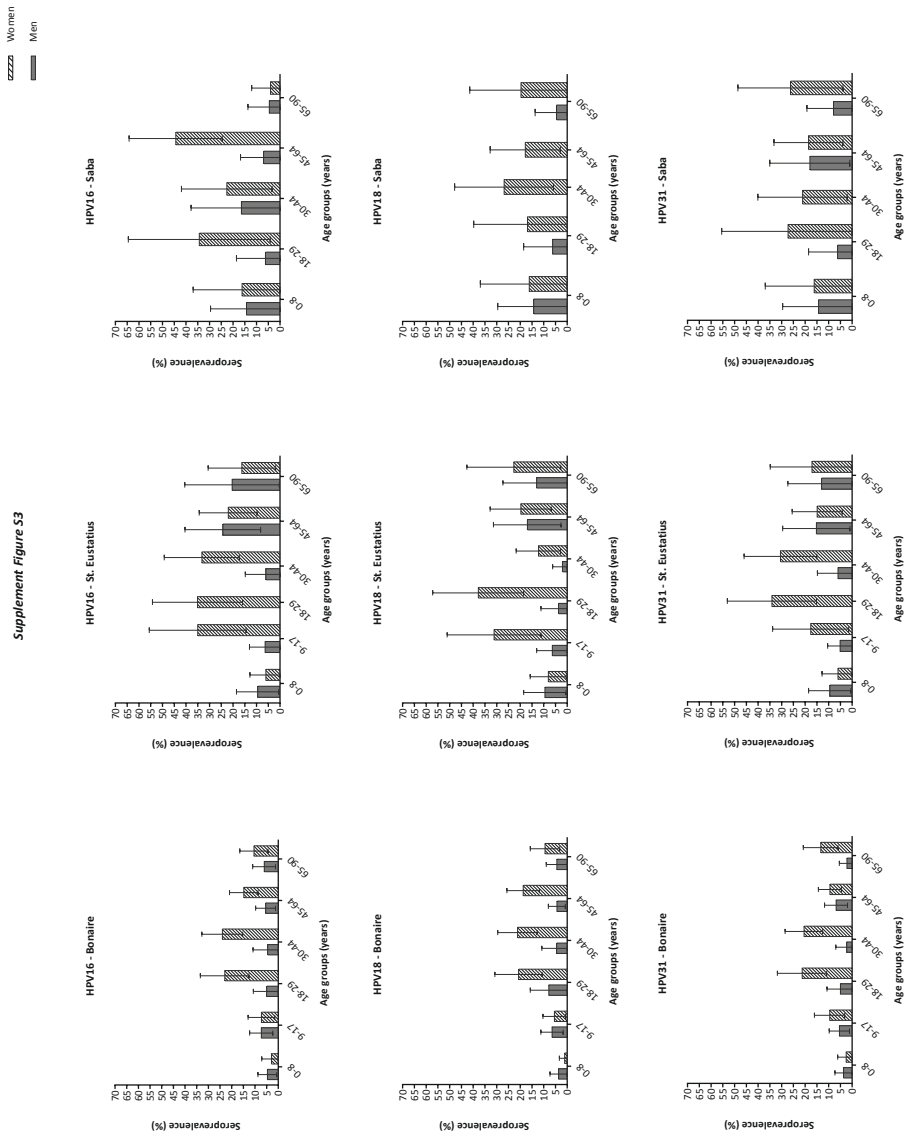


Note: overview of number of participants in each age group in this Figure S2, stratified by island and vaccination

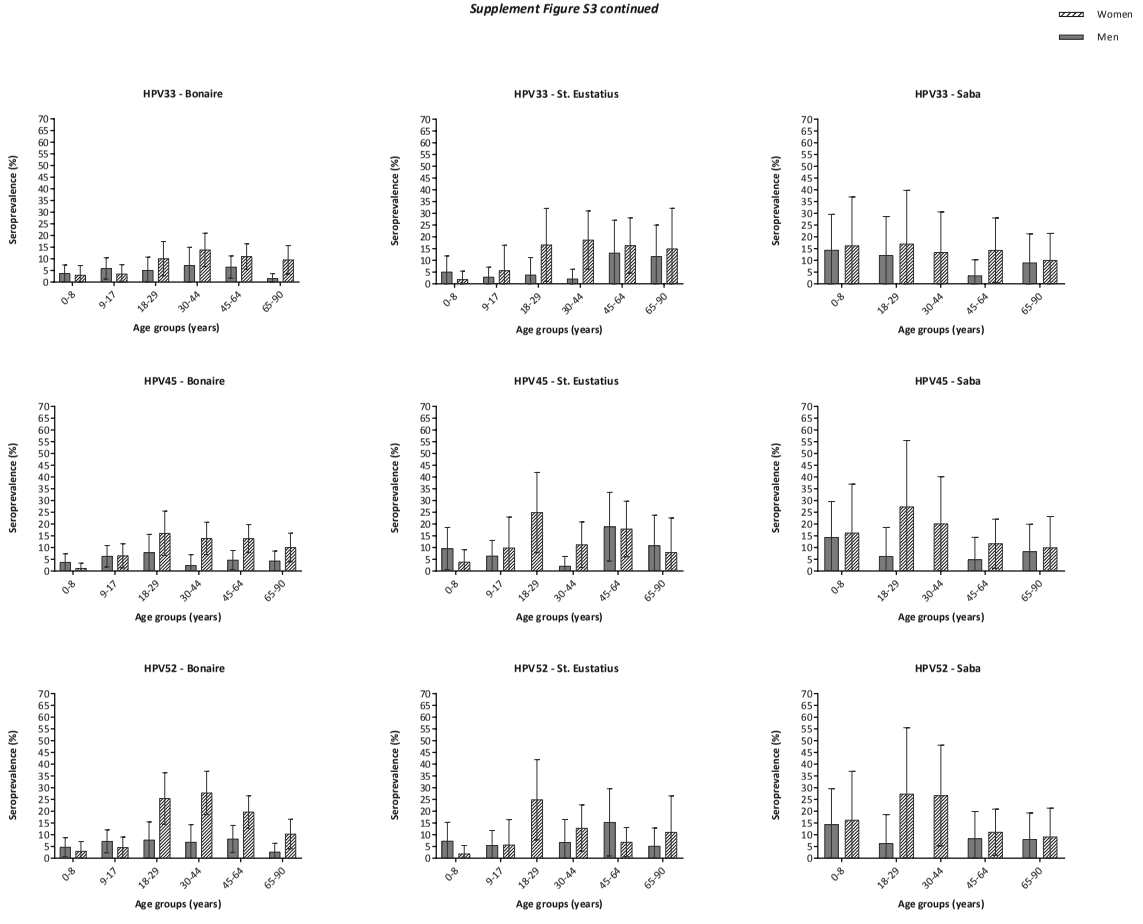
	Bonaire n (%) n= 1,124 (61.7)		St. Eustatius n (%) n=478 (26.2)		Saba n (%) n=221 (12.1)	
Age groups, years	*Population including vaccinated women*	*Population without vaccinated women*	*Population including vaccinated women*	*Population without vaccinated women*	*Population including vaccinated women*	*Population without vaccinated women*
0-8	193	0	88	0	35	0
9-17	211	44	90	37	21	16
25-34	102	1	46	3	22	1
35-44	135	0	68	0	34	0
45-64	259	0	90	0	52	0
65-90	179	0	56	0	40	0

Supplement Figure S2. Age-specific seroprevalence (%) (with 95% confidence intervals) of any high-risk type and seven high-risk types human papillomavirus (HPV) IgG antibodies in the general population of Bonaire, St. Eustatius and Saba, 2017.



Supplement Figure S3

Supplement Figure S3 continued



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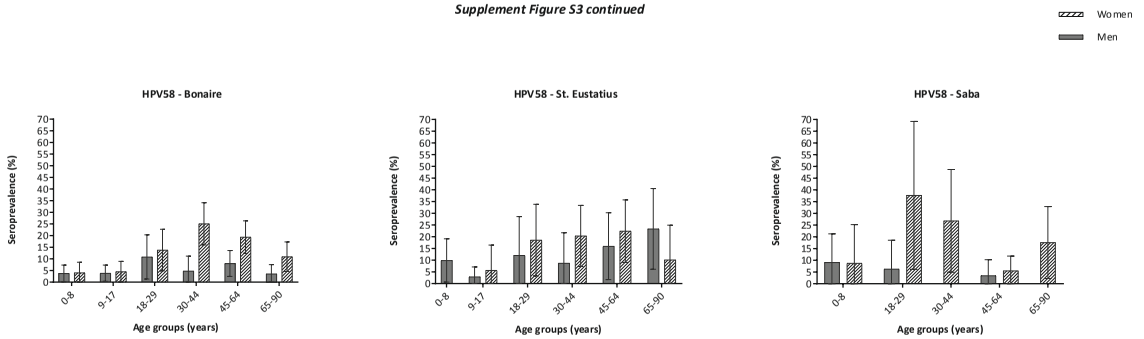

Note: overview of number of unvaccinated participants in each age group in this Figure S3, stratified by island and sex

	Bonaire *n* (%) *n*=1,079 (62.7)		St. Eustatius *n* (%) *n*=418 (24.4)		Saba *n* (%) *n*=204 (11.9)	
Age groups, years	*Men*	*Women*	*Men*	*Women*	*Men*	*Women*
0-8	102	91	40	48	21	14
9-17	122	89	69	21	16	5
25-34	36	66	19	27	12	10
35-44	43	92	24	44	11	23
45-64	130	149	44	46	19	33
65-90	90	89	25	31	17	23

Supplement Figure S3. Age-specific seroprevalence (%) (with 95% confidence intervals) of seven high-risk types human papillomavirus (HPV) IgG antibodies in the unvaccinated general population of Bonaire, St. Eustatius and Saba, 2017, by sex.

CHAPTER 6

High varicella zoster virus susceptibility in Caribbean island populations: implications for vaccination

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ABSTRACT

Objectives

Varicella zoster virus (VZV) infection is reported regularly among adolescents and adults in Caribbean island populations. The disease more often runs a severe course among these populations, causing a substantial burden. The aim of this seroepidemiological study was to obtain an insight into VZV susceptibility and its determinants in island populations of the Caribbean Netherlands (CN).

Methods

Participants from Bonaire, St. Eustatius, and Saba (*n* = 1,829, aged 0–90 years) donated a blood sample and completed a questionnaire. VZV-specific IgG antibodies were determined using a bead-based multiplex immunoassay. Risk factors were analysed using a logistic regression model.

Results

Overall seroprevalence in CN was 78%, being lowest on St. Eustatius (73%) and highest on Bonaire and Saba (79%). Seropositivity increased gradually with age, with 60% and 80% at ages 10 years and 30 years, respectively, and ranging between 80% and 90% thereafter. Higher odds for VZV seronegativity were seen among persons who were born in CN or had resided there since early childhood, and among single-person households.

Conclusions

VZV susceptibility is relatively high among adolescents and adults in CN. In order to reduce the burden of VZV-related disease in these populations, routine varicella vaccination is recommended. As data are scarce, the study findings can serve as a blueprint for the epidemiology in tropical regions.

INTRODUCTION

Varicella zoster virus (VZV) is a highly contagious herpes virus transmitted from person to person by direct contact or inhalation of aerosols. Primary infection with VZV causes varicella (chickenpox), a rash-like illness that confers immunity for life [1, 2]. Following primary infection, the virus remains dormant in sensory nerve ganglia. Viral reactivation, typically above age 50 years, leads to herpes zoster (shingles) [3]. Although varicella is usually a mild and self-limiting disease when acquired in childhood, the risk of severe complications – such as cerebellar ataxia, encephalitis, and pneumonia – increases with age and can lead to hospitalization and death [1].

Global estimated annual disease burden due to varicella is substantial, with 140 million cases, 4.2 million severe complications, and 4,200 related deaths [4]. VZV dynamics vary globally. In temperate climates, like most European countries, an apparent seasonality in varicella cases is noted (mostly during winter and spring) and 90% of people are infected before adolescence [4]. In tropical regions, this seasonality is less pronounced. Due to a higher proportion of susceptible adolescents and adults, acquisition of infection occurs at older ages, with varicella-related complications being reported more often [4-8]. Other groups with a particularly high risk of severe complications include immunocompromised persons as well as pregnant women and their offspring, in whom congenital varicella syndrome (during gestation) or neonatal varicella (in the newborn) may develop [1].

Immunization with vaccines using live-attenuated VZV is highly effective against all varicella disease (pooled vaccine effectiveness 81% (confidence interval (CI) 78–84%) for one dose and 92% (CI 88–95%) for two doses) and most likely long-lasting [9]. Hence, the World Health Organization (WHO) recommends routine varicella vaccination programmes in countries with a significant public health burden of varicella that are able to reach and maintain ≥ 80% vaccine coverage [4]. Since 2018, almost half of the countries in Latin America and the Caribbean have benefited substantially from lower disease burden due to the introduction of VZV vaccination, with coverage ranging between 74% and 91% [5, 10].

Varicella vaccination has not been included in the National Immunization Programme in the island populations of the Caribbean Netherlands (CN). Varicella is reported regularly among adolescents and adults in CN [11]. In 2017 (prior to the recent study), Saba was confronted with an outbreak of VZV that affected over 12.5% of its population (estimated varicella cases, minimum 250), causing substantial restlessness on the island [12]. Patients included infants, pregnant women, and the elderly, with some having to be admitted to the hospital on the nearby island of St. Maarten due to severe complications.

Seroepidemiological data enable the study of VZV dynamics in the population in terms of past infection and susceptibility among certain groups, and can be useful for vaccination policy. By means of our cross-sectional population-based seroepidemiological study conducted in 2017, we have been able to examine the age-specific VZV seroprevalence and determinants for seronegativity in CN for the first time. These findings will be of value for (island) populations with similar dynamics that consider varicella vaccination.

METHODS

Study population and design

A large representative biobank was established in CN in mid-2017 by means of the Health Study Caribbean Netherlands. A detailed description of the study design, data collection, and inclusion have been reported previously [13]. In brief, on the islands of Bonaire, St. Eustatius, and Saba, a random sample was drawn from the population registry (PIVA-V, January 1, 2017) and stratified by age, with strata 0–11, 12–17, 18–34, 35–59, and 60–89 years. In total, 7,768 persons were invited (Bonaire *n* = 4,667; St. Eustatius *n* = 2,062; and Saba *n* = 1,039) [13]. Participants were asked to donate a blood sample via a finger or heel prick, collected on air-dried filter paper (Whatman 903 protein saver cards) using the dried blood spot method (DBS). They were also asked to complete a questionnaire containing questions about (sociodemographic) characteristics possibly related to VZV infection, among others. Additionally, participants were requested to bring their vaccination certificate to check for possible VZV vaccination abroad. All procedures were performed in accordance with the 1964 Declaration of Helsinki and its later amendments. The study protocol was approved by the Medical Ethics Committee Noord-Holland (METC-number M015-022), and signed informed consent was obtained from all participants ≥ 12 years of age, as well as from the parents or legal guardians of minors (< 18 years of age) prior to participation.

Laboratory methods

DBS samples were air-shipped to the laboratory of the National Institute for Public Health and the Environment in the Netherlands after the fieldwork, and stored directly at −80 °C. Serological testing for VZV-specific IgG antibodies was performed with a fluorescent bead-based multiplex immunoassay using Luminex technology [14]. Using the standard protocol, 3.2 mm (1/8-inch) DBS were punched out of the filter paper and incubated in 300 µl phosphate-buffered saline containing 0.1% Tween-20 and 3%

bovine serum albumin (i.e., assay buffer) at 4 °C overnight on a shaker to release serum (resulting in a 1:200 dilution) [15, 16]. Sera were then further diluted to 1:4,000 in assay buffer. VZV strain VZ-10 (GenWay, San Diego, CA, USA) was coated onto the beads and R-phycoerythrin anti-human IgG was used as conjugate. All data were transformed into international units per milliliter (IU/mL) using the international standard for rubella (RUBI-1-94) that was calibrated against the international standard for VZV (as described in Smits *et al*. [14]). As there is no universal consensus on a cut-off for protection, an antibody concentration of ≥ 0.26 IU/mL was considered seropositive, following a previous analysis on a large representative cohort in the Netherlands, which had been performed in the same laboratory [17]. As a validation of the applied cut-off for the present data, mixture modelling was also performed and provided similar results (data not shown).

Data analysis

Seroprevalence and geometric mean concentrations (GMC)

Data were analysed in SAS v.9.4 (SAS Institute Inc., USA), R v.3.6, and Stan v.2.18.2. *P* values of < 0.05 were considered statistically significant. Overall seroprevalence and GMC for VZV-specific IgG antibodies were weighted, taking into account island, sex, age group, and country of birth (and for Bonaire neighbourhood too), to match the population distribution (as of January 1, 2017). Dissimilarities in overall seroprevalence between islands, sex, and ethnicity were identified by estimating the parameters of the beta distribution for these rates, using the methods of moments [18]. Thereafter, Monte Carlo simulations of these seroprevalence estimates were used to calculate risk ratios, 95% CI, and *p* values. Differences in overall GMC between islands, sex, and ethnicity were determined by calculating the difference in natural logarithmic (ln-) concentrations and tested by means of a t-test. Smoothed age-specific seroprevalence and GMC (with 95% CI) estimates were obtained for CN, and stratified by island, sex, and ethnicity, using Bayesian penalized splines [19]. Specifically, the logit-transformed prevalence and ln-transformed GMC were modelled with cubic splines taking 19 equally spaced knots on the age range (0–90 years). After preliminary testing of different alternatives (using Watanabe-Akaike Information Criterion (WAIC)), the Bayesian Lasso with inverse-gamma (1, 0.005) prior distribution for the variance parameter was used in all analyses.

Risk factors for VZV seronegativity

A logistic regression model was used to identify risk factors for VZV seronegativity among participants with a blood sample and questionnaire data for the studied variables, and excluding those vaccinated against varicella. Potential risk factors that were investigated included island, age group, sex, ethnicity, resident of CN since age, (maternal) educational level, household size, having a child in the household, attendance of day care/nursery

school of a child in the household, and number of social contacts (note: participants with a missing value for a specific variable were allocated to a missing category, hence a full case analysis could be applied). For all variables, unweighted VZV seroprevalence and 95% CI were estimated. Crude odds ratios (OR) in the univariate analyses were a priori adjusted for age and sex, thereby taking into account the survey design [17]. Besides age and sex, variables with $p < 0.10$ in the univariate analyses were included in the multivariate analysis. Backward selection (manually dropping variables one-by-one) was applied to detect risk factors that were associated with VZV seronegativity based on a $p < 0.05$. Adjusted ORs, corresponding 95% CIs, and an adjusted R^2 (as goodness-of-fit) were provided.

Validity of self-reported VZV history

The validity of self-reported history of VZV disease was assessed. Reports were compared to the serological results. Vaccinated participants and those with missing values for history of VZV disease were excluded from the analysis. Persons uncertain about their history were combined with those who did not have a history on VZV disease. Sensitivity, specificity, positive predictive value (PPV, proportion of people who were seropositive among those self-reporting to have a positive history of VZV disease), and negative predictive value (NPV, proportion of people who were seronegative among those self-reporting to have a negative or uncertain history of VZV disease) were determined.

RESULTS

Study characteristics

The study characteristics have been reported in depth previously [13]. In short, of the 1,900 persons included (response rate 24.5%), 1,829 participants aged 3 months to 90 years donated a blood sample and filled out the questionnaire. There were slightly more females (*n* = 1,005, 55%) than males (*n* = 824, 45%), and most participants resided on Bonaire (*n* = 1,129, 62%), followed by St. Eustatius (*n* = 477, 26%) and Saba (*n* = 223, 12%) – in accordance with their population size (*Table 1*). The vast majority originated from the Dutch overseas territories (comprising CN, Aruba, Curaçao, and St. Maarten) and Suriname (henceforth (former) overseas territories) (*n* = 1,312, 72%), followed by Latin America and other non-Western countries (henceforth Latin America) (*n* = 281, 15%), and indigenous Dutch and other Western countries (henceforth Western) (*n* = 223, 12%) (*n* = 13, 1% missing). Over half (*n* = 924, 51%) self-reported having had chickenpox (vs. not: *n* = 697, 38%, uncertain: *n* = 122, 7%, and missing: *n* = 86, 5%), and (obtained) from age 12 years, *n* = 77 (6%) self-reported having had shingles (vs. not: *n* = 1,133, 82%,

uncertain: *n* = 95, 7%, and missing: *n* = 75, 5%). Seven participants were vaccinated against varicella (*n* = 4 once and *n* = 3 twice).

Table 1. Sociodemographic characteristics of participants with a blood sample in the Health Study Caribbean Netherlands, by island, *n* (%).

Sociodemographic characteristics	Bonaire *n* = 1,129 (61.7%)	St. Eustatius *n* = 477 (26.1%)	Saba *n* = 223 (12.2%)	Total *n* = 1,829
Sex				
Men	506 (44.8%)	221 (46.3%)	97 (43.5%)	824 (45.1%)
Women	623 (55.2%)	256 (53.7%)	126 (56.5%)	1,005 (54.9%)
Age groups, years				
0–4	95 (8.4%)	45 (9.4%)	21 (9.4%)	161 (8.8%)
5–11	176 (15.6%)	83 (17.4%)	29 (13.0%)	288 (15.7%)
12–17	181 (16.0%)	86 (18.0%)	24 (10.8%)	291 (15.9%)
18–39	200 (17.7%)	102 (21.4%)	41 (18.4%)	343 (18.8%)
≥ 40	477 (42.3%)	161 (33.8%)	108 (48.4%)	746 (40.8%)
Ethnic background[a]				
Dutch overseas territories and Suriname	803 (71.2%)	383 (82.0%)	126 (57.0%)	1,312 (72.2%)
Indigenous Dutch and other Western countries	143 (12.7%)	30 (6.4%)	50 (22.6%)	223 (12.3%)
Latin America and other non-Western countries	182 (16.1%)	54 (11.6%)	45 (20.4%)	281 (15.5%)
Resident of the Caribbean Netherlands since, years of age				
0–4	679 (60.1%)	312 (65.4%)	115 (51.6%)	1,106 (60.5%)
5–11	69 (6.1%)	25 (5.2%)	6 (2.7%)	100 (5.5%)
12–17	30 (2.7%)	9 (1.9%)	4 (1.8%)	43 (2.3%)
≥ 18	321 (28.4%)	84 (17.6%)	84 (37.6%)	489 (26.7%)
Unknown	30 (2.7%)	47 (9.9%)	14 (6.3%)	91 (5.0%)
(Maternal) educational level[b]				
High	172 (15.2%)	68 (14.3%)	87 (39.0%)	327 (17.9%)
Middle	298 (26.4%)	125 (26.2%)	45 (20.2%)	468 (25.6%)
Low	571 (50.6%)	232 (48.6%)	80 (35.9%)	883 (48.3%)
Unknown	88 (7.8%)	52 (10.9%)	11 (4.9%)	151 (8.2%)
Household size, number of persons				
Single-person household	129 (12.3%)	51 (10.7%)	31 (13.9%)	221 (12.1%)
2–5	864 (76.5%)	350 (73.4%)	176 (78.9%)	1,390 (76.0%)
≥ 6	119 (10.6%)	72 (15.1%)	13 (5.8%)	204 (11.1%)
Unknown	7 (0.6%)	4 (0.8%)	3 (1.4%)	14 (0.8%)

Table 1. (Continued)

Sociodemographic characteristics	Bonaire *n* = 1,129 (61.7%)	St. Eustatius *n* = 477 (26.1%)	Saba *n* = 223 (12.2%)	Total *n* = 1,829
History of chickenpox (self-reported)				
Yes	603 (53.4%)	189 (39.6%)	132 (59.2%)	924 (50.5%)
No	418 (37.0%)	222 (46.5%)	57 (25.6%)	697 (38.1%)
Uncertain	63 (5.6%)	38 (8.0%)	21 (9.4%)	122 (6.7%)
Unknown	45 (4.0%)	28 (5.9%)	13 (5.8%)	86 (4.7%)
History of shingles (self-reported)				
Yes	62 (7.2%)	7 (2.0%)	8 (4.6%)	77 (5.6%)
No	693 (80.8%)	294 (84.2%)	146 (84.4%)	1,133 (82.1%)
Uncertain	61 (7.1%)	23 (6.6%)	11 (6.4%)	95 (6.9%)
Unknown	42 (4.9%)	25 (7.2%)	8 (4.6%)	75 (5.4%)

[a] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten. Within ethnic group indigenous Dutch and other Western countries, *n* = 147 (66%) were indigenous Dutch. Within Latin America and other non-Western countries, *n* = 261 (93%) were born in Latin America.

[b] Maternal educational level was used for participants 0–11y, active education was used for participants 12–25y, and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1), lower general secondary education (MAVO/VMBO); Middle = intermediate/ secondary vocational education (MBO-2-4), higher/senior vocational education (HAVO), pre-university education (VWO/Gymnasium); and High = higher professional education (HBO), University BSc., University MSc., Doctorate.
Missing: ethnic background *n* = 13.

Seroprevalence and GMC

The overall weighted seroprevalence of VZV-specific IgG antibodies in the general population of CN was 78.0% (95% CI 75.7–80.3%), with an overall GMC of 0.77 IU/mL (95% CI 0.72–0.83 IU/mL) (*Table 2*).

Seroprevalence and GMC were lowest on St. Eustatius (72.7% and 0.61 IU/mL, respectively), and differed considerably from Bonaire (seroprevalence: 78.8%, $p = 0.02$; GMC: 0.79 IU/mL, $p = 0.003$) and Saba (seroprevalence: 79.2%, $p = 0.11$; GMC: 0.89 IU/mL, $p = 0.002$). Seroprevalence and GMC did not differ significantly between the sexes in CN or on each island. Seroprevalence and GMC differed between participants from different ethnic backgrounds in CN, with the lowest seroprevalence among people from the (former) overseas territories (70.7%), followed by Latin America (87.7%), and the highest seroprevalence was in Western with 94.8% (all p values (also between GMCs) < 0.05).

Table 2. Weighted seroprevalence (%) and geometric mean concentration (GMC) (with 95% confidence intervals (CI) of varicella zoster virus (VZV)-specific IgG antibodies in the national population of Caribbean Netherlands.

	Seroprevalence[a] (≥ 0.26 IU/mL)		GMC[b]	
	%	*(95% CI)*	*IU/mL*	*(95% CI)*
Overall	78.0	(75.7–80.3)	0.77	(0.72–0.83)
Island				
Bonaire	78.8	(76.1–81.5)	0.79	(0.73–0.86)
St. Eustatius	72.7	(68.0–77.3)	0.61	(0.53–0.69)
Saba	79.2	(72.8–85.6)	0.89	(0.72–1.11)
Sex				
Men	79.0	(75.5–82.4)	0.79	(0.71–0.88)
Women	77.0	(74.1–79.9)	0.75	(0.68–0.82)
Ethnic background				
Dutch overseas territories[c] and Suriname	70.7	(67.6–73.8)	0.64	(0.58–0.70)
Indigenous Dutch and other Western countries	94.8	(91.2–98.4)	1.23	(1.08–1.41)
Latin America and other non-Western countries	87.7	(83.4–92.0)	0.97	(0.85–1.11)

[a] *P* values regarding differences in seroprevalence were derived using the methods of moments and Monte Carlo simulations (see Methods section). *P* value for Bonaire vs. St. Eustatius: **0.02**; Bonaire vs. Saba: 0.86; St. Eustatius vs. Saba: 0.11; Men vs. women: 0.39; Dutch overseas territories and Suriname vs. Indigenous Dutch and other Western countries: **< 0.0001**; Dutch overseas territories and Suriname vs. Latin America and other non-Western countries: **< 0.0001**; Indigenous Dutch and other Western countries vs. Latin America and other non-Western countries: **0.02**.

[b] *P* values regarding differences in GMC were derived using t-tests. *P* values: Bonaire vs. St. Eustatius: **0.003**; Bonaire vs. Saba: 0.33; St. Eustatius vs. Saba: **0.0002**; Men vs. women: 0.34; Dutch overseas territories and Suriname vs. Indigenous Dutch and other Western countries: **< 0.0001**; Dutch overseas territories and Suriname vs. Latin America and other non-Western countries: **< 0.0001**; Indigenous Dutch and other Western countries vs. Latin America and other non-Western countries: 0.07.

[c] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten.

Abbreviations: *IU/mL*, international units per mL.

Up until 12 months of age (n_{total} = 17), only one participant (aged 7 months) was VZV-seropositive in CN. After age 1 year, seroprevalence increased steadily to 40% at 5 years and to 60% at 10 years, with a corresponding rise in GMC to 0.60 IU/mL (*Figure 1*). Thereafter, seropositivity increased gradually with age, nearly reaching 70% at age 20 years, rising further to 80% and 90% at age 30 and 40 years, respectively. From there it remained range-bound between 80% and 90% until age 90 years. Likewise, GMC increased progressively with age, and was highest in the oldest age groups, approaching 1.70 IU/mL. No differences with age were observed between males and females in terms of seroprevalence and GMC.

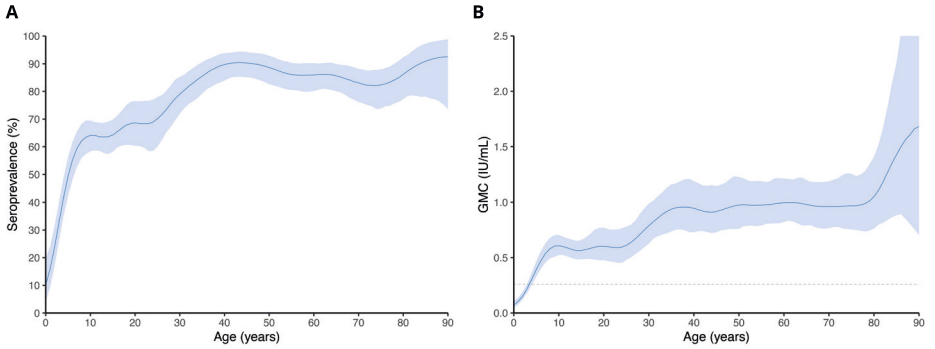

Figure 1. Age-specific seroprevalence (%) (**A**) and geometric mean concentration (GMC) (international units (IU)/mL) (**B**) (with 95% confidence intervals) of varicella zoster virus IgG antibodies in Caribbean Netherlands, 2017. Note: antibody concentrations ≥ 0.26 IU/mL were considered seropositive (dashed line in B).

Similar age patterns in seroprevalence and GMC were observed on Bonaire and Saba (*Figure 2*). A seroprevalence of 50% was reached before age 5 years on both islands and of 70% around age 10 years; after remaining constant for 15 years, it increased slowly from age 25 years, corresponding to the age pattern in CN. In contrast, on St. Eustatius the seroprevalence in children increased far slower as it only reached 50% in those aged 10 years, 60% in 20–year–olds, and 70% at age 25 years. From this age onwards, the pattern was similar to the other islands.

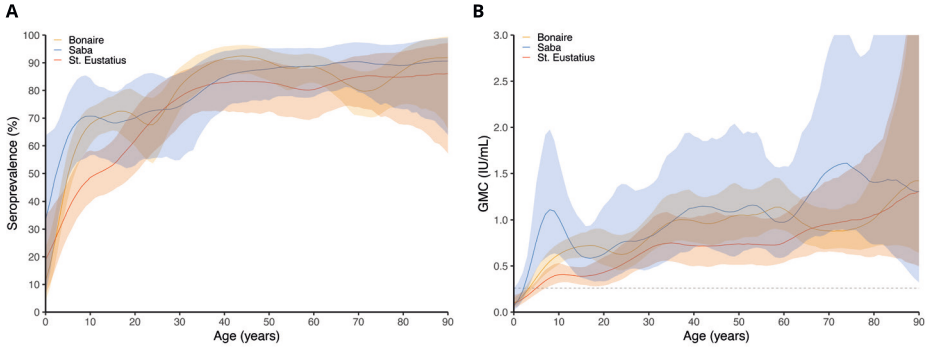

Figure 2. Age-specific seroprevalence (%) (**A**) and geometric mean concentration (GMC) (international units (IU)/mL) (**B**) (with 95% confidence intervals) of varicella zoster virus IgG antibodies in Caribbean Netherlands, 2017, by island. Note: antibody concentrations ≥ 0.26 international units (IU)/mL were considered seropositive (dashed line in B).

Age patterns in seroprevalence differed markedly between the three ethnic groups (*Figure 3*). In Western, seroprevalence rose quickly to almost 90% in 10–year–olds, and

from age 35 years it reached 95% and remained above that level. In Latin Americans and people from the (former) overseas territories, seropositivity levels were around 60% at 10 years of age. In Latin Americans this gradually increased to 80% at age 25 years and to 90% and above from 35 years of age, with the exception of those around 60 years being just below that level. In persons from the (former) overseas territories, a seroprevalence of 80% was only just reached at age 35 years, and from there it remained range-bound between 75% and 90% until 90 years of age.

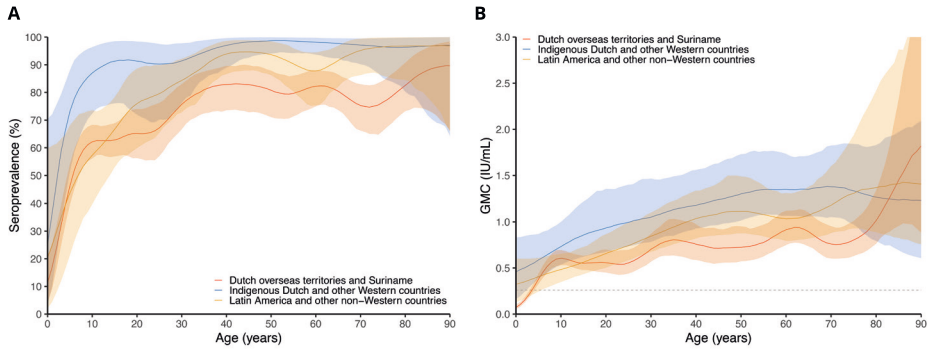

Figure 3. Age-specific seroprevalence (%) (**A**) and geometric mean concentration (GMC) (international units (IU)/mL) (**B**) (with 95% confidence intervals) of varicella zoster virus IgG antibodies in Caribbean Netherlands, 2017, by ethnic background. Note: antibody concentrations ≥ 0.26 international units (IU)/mL were considered seropositive (dashed line in B).

Risk factors for VZV seronegativity

Table 3 displays the risk factor analysis for VZV IgG seronegativity among the total unvaccinated (for VZV) study population (*n* = 1,809). The percentage of missing values ranged between 0.7% and 11.7%. In the multivariate analysis, the highest odds for VZV seronegativity were seen for the youngest age group 0–4 years (vs. ≥ 40 years), and the odds gradually declined with older age. People originating from the (former) overseas territories and Latin America had substantially higher odds as compared to Western. Those living on St. Eustatius (vs. Bonaire), as well as persons who had resided in CN since birth or up until 5 years of age, also displayed higher odds of being VZV-seronegative as compared to those who resided on the islands later. Finally, a single-person household (vs. ≥ 6-person household) was also found to be a risk factor for VZV seronegativity. The adjusted R^2 of the multivariate model amounted to 0.253.

Table 3. Risk factor analysis for varicella zoster virus (VZV) IgG seronegativity among the total VZV-unvaccinated study population of the Health Study Caribbean Netherlands.

Potential risk factor for VZV-seronegativity	*n* (%) *n* = 1,809[a]	% VZV-seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Island				**0.0003**		**0.003**
Bonaire	1,122 (62.0)	74.0 (71.4–76.5)	Ref.		Ref.	
St. Eustatius	466 (25.8)	63.3 (58.9–67.7)	**1.60 (1.25–2.05)**		**1.53 (1.18–1.98)**	
Saba	221 (12.2)	77.4 (71.9–82.9)	0.85 (0.58–1.23)		0.95 (0.65–1.40)	
Sex				0.22		0.14
Men	814 (45.0)	71.7 (68.6–74.8)	Ref.		Ref.	
Women	995 (55.0)	71.6 (68.8–74.4)	1.15 (0.92–1.44)		1.19 (0.94–1.50)	
Age group, years[d]				**< 0.0001**		**< 0.0001**
0–4	161 (8.9)	26.7 (19.9–33.5)	**17.16 (11.42–25.79)**		**13.84 (8.83–21.70)**	
5–11	283 (15.6)	61.1 (55.4–66.8)	**3.97 (2.89–5.45)**		**3.29 (2.29–4.73)**	
12–17	289 (16.0)	64.0 (58.5–69.6)	**3.50 (2.54–4.81)**		**3.20 (2.21–4.63)**	
18–39	337 (18.6)	76.9 (72.3–81.4)	**1.84 (1.32–2.55)**		**1.79 (1.27–2.52)**	
≥ 40	739 (40.9)	86.1 (83.6–88.6)	Ref.		Ref.	
Ethnic background				**< 0.0001**		**0.0001**
Dutch overseas territories[e] and Suriname	1,309 (72.3)	65.5 (63.0–68.1)	**5.29 (3.02–9.27)**		**3.79 (2.07–6.92)**	
Indigenous Dutch and other Western countries	220 (12.2)	93.2 (89.8–96.5)	Ref.		Ref.	
Latin America and other non-Western countries	280 (15.5)	83.2 (78.8–87.6)	**2.89 (1.53–5.47)**		**3.14 (1.63–6.04)**	

Table 3. (Continued)

Potential risk factor for VZV-seronegativity	*n* (%) *n* = 1,809[a]	% VZV-seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Resident of Caribbean Netherlands since, years of age				**< 0.0001**		**0.0004**
0–4	1,099 (60.7)	61.9 (59.0–64.7)	**2.71 (1.89–3.88)**		**1.87 (1.23–2.84)**	
5–11	100 (5.5)	77.0 (68.7–85.3)	1.34 (0.74–2.43)		1.05 (0.57–1.94)	
12–17	43 (2.4)	86.0 (75.7–96.4)	0.73 (0.28–1.89)		0.60 (0.23–1.58)	
≥ 18	485 (26.8)	89.9 (87.2–92.6)	Ref.		Ref.	
Unknown	82 (4.5)	80.5 (71.9–89.1)	1.19 (0.62–2.30)		0.77 (0.36–1.62)	
(Maternal) educational level[f]						
High	321 (17.8)	80.1 (75.7–84.4)	Ref.	**0.0006**		
Middle	465 (25.7)	65.8 (61.5–70.1)	**1.91 (1.32–2.78)**			
Low	876 (48.4)	70.4 (67.4–73.5)	**2.00 (1.40–2.85)**			
Unknown	147 (8.1)	78.9 (72.3–85.5)	1.29 (0.76–2.19)			
Household size, number of persons				**0.07**		**0.01**
Single-person household	218 (12.0)	78.9 (73.5–84.3)	**1.92 (1.17–3.15)**		**2.31 (1.39–3.86)**	
2–5	1,375 (76.0)	70.5 (68.1–72.9)	1.28 (0.90–1.82)		1.44 (0.99–2.07)	
≥ 6	204 (11.3)	72.1 (65.9–78.2)	Ref.		Ref.	
Unknown	12 (0.7)	66.7 (40.0–93.4)	0.83 (0.20–3.47)		2.06 (0.43–9.76)	
Child in the household				0.40		
Yes	1,026 (55.7)	66.4 (63.5–69.3)	Ref.			
No	662 (36.6)	79.8 (76.7–82.8)	1.21 (0.91–1.61)			
Unknown	121 (6.7)	71.9 (63.9–79.9)	1.16 (0.73–1.82)			

Table 3. (Continued)

Potential risk factor for VZV-seronegativity	*n* (%) *n* = 1,809[a]	% VZV-seropositive (95% CI)	Univariate Crude OR[b] (95% CI)	*p* value[c]	Multivariate aOR[b] (95% CI)	*p* value[c]
Child in the household attending day care/nursery school				0.52		
Yes	365 (20.2)	56.4 (51.3–61.5)	Ref.			
No	1,306 (72.2)	75.1 (72.8–77.5)	1.00 (0.74–1.36)			
Unknown	138 (7.6)	79.0 (72.2–85.8)	0.77 (0.46–1.28)			
Contact yesterday, number of persons[g]				**0.05**		
0–8	809 (44.7)	73.8 (70.8–76.8)	Ref.			
≥ 9	789 (43.6)	71.0 (67.8–74.1)	0.89 (0.70–1.14)			
Unknown	211 (11.7)	65.9 (59.5–72.3)	1.38 (0.97–1.96)			

[a] of *n* = 1,829 with a blood sample, *n* = 7 were vaccinated against VZV, and *n* = 13 had missing values for ethnic background.

[b] Crude odds ratios were adjusted for sex and age, and significant adjusted ORs are marked in bold type.

[c] *P* values were determined by means of Wald tests for logistic regression, and significant *p* values (< 0.1 in univariate and < 0.05 in multivariate analysis) are marked in bold type.

[d] The final age group consisted of participants ≥ 40 years of age as seroprevalence remained fairly stable after this age, thereby also separating those of reproductive age and above.

[e] Dutch overseas territories include the islands: Bonaire, Saba and St. Eustatius (i.e., Caribbean Netherlands), and Aruba, Curaçao and St. Maarten.

[f] Maternal educational level was used for participants 0–11y, active education was used for participants 12–25y, and highest accomplished educational level was used for participants > 25y. Low = no education, primary school, pre-vocational education (VMBO), lower vocational education (LBO/MBO-1), lower general secondary education (MAVO/VMBO); Middle = intermediate/ secondary vocational education (MBO-2–4), higher/senior vocational education (HAVO), pre-university education (VWO/Gymnasium); and High = higher professional education (HBO), University BSc., University MSc., Doctorate.

[g] Categories were based on median number of persons contacted.

Abbreviations: *aOR*, adjusted odds ratio; CI, confidence interval; *OR*, odds ratio; *Ref.*, reference category; *VZV*, varicella-zoster virus.

Validity of self-reported VZV history

A total of 1,739 VZV-unvaccinated participants had serological results and reported their history of VZV disease (*Table 4*). PPV was high in the total population (PPV = 91.5%, NPV = 51.8%, sensitivity = 69.0%, and specificity = 83.8%), as well as among all age groups, being highest in age group 18–39 years (95.7%) and lowest in age group 0–17 years (88.4%). This means that the majority of persons who self-reported having a history of VZV disease were indeed seropositive. NPV declined substantially with age from 74.0% in age group 0–17 years, to 48.2% in those aged 18–39 years and 23.0% in those ≥ 40 years. Sensitivity and specificity were similar for age groups 0–17 years and 18–39 years and lowest for age group ≥40 years.

Table 4. Varicella zoster virus (VZV) IgG antibody profile of VZV-unvaccinated participants in the Health Study Caribbean Netherlands with positive and negative/uncertain history of VZV disease, total population and by age groups.

		IgG antibodies against VZV		
	Self-reported history of VZV disease	**Positive**	**Negative**	**Total**
Total population[a]	Positive	858	80	938
	Negative/uncertain	386	415	801
	Total	1,244	495	1,739
Age groups, years of age				
0–17[b]	Positive	283	37	320
	Negative/uncertain	100	284	384
	Total	383	321	704
18–39[c]	Positive	180	8	188
	Negative/uncertain	72	67	139
	Total	252	75	327
≥ 40[d]	Positive	395	35	430
	Negative/uncertain	214	64	278
	Total	609	99	708

[a] **Total population** (*n* = 7 vaccinated; *n* = 83 missing; *n* = 127 uncertain about their history):
sensitivity (858/1,244) = 69.0%, specificity (415/495) = 83.8%, positive predictive value (PPV) (858/938) = 91.5%, negative predictive value (NPV) (415/801) = 51.8%.
[b] **Age group 0–17 years**:
sensitivity (283/383) = 73.9%, specificity (284/321) = 88.5%, PPV (283/320) = 88.4%, NPV (284/384) = 74.0%.
[c] **Age group 18–39 years**:
sensitivity (180/252) = 71.4%, specificity (67/75) = 89.3%, PPV (180/188) = 95.7%, NPV (67/139) = 48.2%.
[d] **Age group ≥ 40 years**:
sensitivity (395/609) = 64.9%, specificity (67/99) = 64.6%, PPV (395/430) = 91.9%, NPV (64/278) = 23.0%.

DISCUSSION

By means of a representative cross-sectional population-based serosurveillance study, we have estimated the VZV seroprevalence and determined possible risk factors for seronegativity in these unvaccinated Caribbean island populations for the first time. Overall seroprevalence was 78%, and seroprevalence was lower on St. Eustatius (73%) as compared to Bonaire and Saba (both 79%). Saba was confronted with a large varicella outbreak that ended shortly before the start of this study in 2017. Importantly, unlike populations in temperate climates, seroprevalence in CN increased gradually with age. Hence, relatively high susceptibility was observed among adolescents and adults (e.g., still 20% in 30-year-olds), which increases the risk of serious complications, and this was most pronounced in people originating from the (former) overseas territories and people who had resided on the islands since early childhood.

Seroprevalence for VZV differs substantially between countries. Studies conducted in the region, such as on the Caribbean islands of Puerto Rico and St. Lucia [20, 21] as well as Bolivia, Brazil, Mexico, Uruguay, and Argentina [22-27], have reported overall rates between 58% and 99%. In contrast, in most temperate countries, such as the Netherlands and Germany, varicella is typically a disease of childhood, as overall seroprevalence is > 95% and over 90% are seropositive before the age of 10 years [17, 28]. The age-specific profile of VZV seropositivity in this study is similar to that of most tropical countries and islands [20, 21, 29]: seroprevalence increased gradually with age, and with the mean age of seroconversion thus being higher, a larger proportion of adolescents and adults are susceptible to infection. Differences observed between temperate and tropical countries are not fully understood and several factors might influence this dissimilarity, including climate, viral, host, and social factors [29].

This study provides evidence of the role of host and social factors on VZV seropositivity. Firstly, a marked dissimilarity could be observed between ethnic groups, with specifically persons from the (former) overseas territories displaying higher rates of susceptibility throughout their course of life (e.g., still approximately 20% on average after age 40 years). Although host factors might play a role, as described previously [30], the most plausible explanation lies presumably in a lack of exposure to VZV. Since the CN islands can be compared with more rural/remote communities, especially in the pre-globalization era, the likelihood of exposure to VZV has been relatively low. This is also supported by the risk factors analysis, which revealed that people who were born in CN or who had been living there since early childhood had higher odds of being VZV-seronegative. We have observed the same phenomenon with other respiratory viruses of comparable infectivity, such as rubella, among elderly without vaccination in CN [31]. A study showing a discrepancy in VZV seroprevalence between rural and urban communities within the same tropical country supports this theory too [32]. Secondly,

living in a single-person household was shown to be a predictor of VZV seronegativity in this study, and the odds decreased with increasing household size. In other tropical settings this has also been revealed as a critical factor for transmission [33]. Close proximity of contacts in household settings is probably more crucial for becoming VZV-seropositive as compared to the number of contacts encountered (also supported by our analyses), as with highly infective respiratory pathogens such as measles. In our analyses, having a child in the household (who attends day care/nursery school) was not associated with seronegativity, suggesting that children may not be the main driver of transmission in this setting.

The lower seroprevalence and GMC on St. Eustatius as compared to the other islands appears to be due mostly to the current VZV dynamics. On Bonaire, small outbreaks of VZV are observed throughout the year, resulting in a gradual rise in age-specific seroprevalence. Saba, the smallest and most remote of the three islands, was confronted with a relatively large varicella outbreak, starting a few months prior to inclusion in the present study up until a week before the start; the high GMC in children as well as adults is illustrative of this event. Given the high susceptibility on the nearby island of St. Eustatius, one would also expect an outbreak in due time as a result of (indirect) human movement between these islands. In fact, shortly after completing the study, a small outbreak was reported, although not comparable to that on Saba in terms of the number of patients, probably due to the rapid preventive measures applied, such as recommending patients stay at home. Paramount is the higher age at infection on these islands compared to less-isolated populations, which increases the risk of more severe complications, including those in pregnant women and their (unborn) child, thus leading to higher morbidity, mortality, and economic burden of the disease [11]. Severely-ill patients need to be transported by helicopter to larger islands, such as St. Maarten, and admitted to hospitals there. This could additionally increase the risk of introduction and further dissemination on other islands. To prevent this, the exchange of patients between islands requires a good network, hospital hygiene, and standardized surveillance of data, principally in a time when the region is confronted with large numbers of migrants from unstable countries [34].

A possible limitation of this study might be its cross-sectional design. Some risk factors will not necessarily reflect an individual's situation at the time of infection per se, or will be subject to change, e.g., number of persons encountered yesterday. Nonetheless, in evaluating VZV vaccination policy, our findings are instrumental in describing the current VZV dynamics accurately. Further, there is no universal consensus on an IgG antibody level related to VZV protection; albeit we were able to accurately determine a cut-off for seropositivity, in line with our serosurveillance study in the Netherlands, using a highly valid method (of mixture modelling) discriminating the data strictly [17].

Currently, only 20% of countries in Central America and the Caribbean have introduced universal VZV vaccination [35]. Vaccination would be beneficial to prevent infection and severe complications in adolescents and adults on island populations like CN, given the considerable proportion of susceptible individuals. Hence, acceptance of vaccination will most likely be high, keeping in mind the suggested uptake of ≥ 80% recommended by the WHO [4] (World Health Organization, 2014). After the implementation of VZV vaccination, the burden of disease has declined considerably in many countries; for example, in Costa Rica, where a one-dose vaccination for all children aged 15 months was introduced in 2007 (with a vaccination coverage of 84% nowadays), there was a 97% reduction in cases in the target population and an indirect herd immunity effect in the general population, which reduced disease and hospitalization by 60% and 93%, respectively [5]. In designing a VZV vaccination programme for children, a catch-up campaign for susceptible persons might be considered on the basis of self-reported past disease, which was shown to be a highly valid method in this study population. An increasing incidence of herpes zoster in adults after vaccination, due to decreased exposure to circulating wild-type VZV, has been hypothesized (i.e., the exogenous boosting hypothesis), but has not been observed in countries that have implemented it so far, e.g., USA in 1996. Continued monitoring should still be anticipated though, because a presumable increase in herpes zoster is expected to occur several decades after the introduction of childhood varicella vaccination [36, 37].

In conclusion, by means of this representative population-based study, we have provided an insight into the epidemiology of VZV in CN [38]. This will be valuable in the decision-making process on the introduction of routine VZV vaccination (as this will be evaluated by the Dutch Health Council in 2020), and could serve as a baseline for future serosurveillance studies conceivably assessing the impact of vaccination. Furthermore, as the current epidemiology is most likely generalizable to other islands with corresponding population distribution, these results can guide them in consideration of VZV vaccination.

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PART II

Sero-monitoring the SARS-CoV-2 epidemic in the Netherlands

CHAPTER 7

Nationwide seroprevalence of SARS-CoV-2 and identification of risk factors in the general population of the Netherlands during the first epidemic wave

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ABSTRACT

Background

We aimed to detect SARS-CoV-2 serum antibodies in the general population of the Netherlands and identify risk factors for seropositivity amidst the first COVID-19 epidemic wave.

Methods

Participants (n = 3,207, aged 2–90 years), enrolled from a previously established nationwide serosurveillance study, provided a self-collected fingerstick blood sample and completed a questionnaire (median inclusion date 3 April 2020). IgG antibodies targeted against the spike S1-protein of SARS-CoV-2 were quantified using a validated multiplex-immunoassay. Seroprevalence was estimated controlling for survey design, individual pre-pandemic concentration, and test performance. Random-effects logistic regression identified risk factors for seropositivity.

Results

Overall seroprevalence in the Netherlands was 2.8% (95% confidence interval 2.1–3.7), with no differences between sexes or ethnic background, and regionally ranging between 1.3 and 4.0%. Estimates were highest among 18–39 year-olds (4.9%), and lowest in children 2–17 years (1.7%). Multivariable analysis revealed that persons taking immunosuppressants and those from the Orthodox-Reformed Protestant community had over four times higher odds of being seropositive compared to others. Anosmia/ageusia was the most discriminative symptom between seropositive (53%) and seronegative persons (4%, $p < 0.0001$). Antibody concentrations in seropositive persons were significantly higher in those with fever or dyspnea in contrast to those without ($p = 0.01$ and $p = 0.04$, respectively).

Conclusions

In the midst of the first epidemic wave, 2.8% of the Dutch population was estimated to be infected with SARS-CoV-2, that is, 30 times higher than reported. This study identified independent groups with increased odds for seropositivity that may require specific surveillance measures to guide future protective interventions internationally, including vaccination once available.

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), causative agent of coronavirus disease (COVID-19), emerged in Wuhan, China, in late 2019. On 11 March 2020, the World Health Organization (WHO) declared COVID-19 a pandemic, with over 10 million confirmed cases as of the beginning of July 2020 [1, 2]. The first patient in the Netherlands was confirmed on 27 February 2020 [3]. Cases primarily clustered in the southeastern part of the country, but were reported in other regions quickly hereafter. Multi-pronged interventions to suppress the spread of the virus, including social distancing, school and bar/restaurant closure, and stringent advice to home quarantine when feeling ill and work from home, were implemented on 16 March 2020 — and were relaxed gradually since 1 June 2020. By 1 July 2020, 50,273 cases, 11,877 hospitalisations, and 6,113 related deaths were reported in the Netherlands [3].

Reported COVID-19 cases worldwide are an underestimation of the true magnitude of the pandemic. The scope of undetected cases remains largely unknown due to difference in restrictive testing policy and registration across countries, and occurrence of asymptomatic infections [4, 5]. Large-scale nationwide serosurveillance studies measuring SARS-CoV-2-specific serum antibodies could help to better assess the number of infections, viral spread, and groups at risk of infection in the general population by incorporating extensive questionnaire data, for example, on lifestyle, behaviour and profession. This might yield different factors than those identified for (severely-ill) clinical cases investigated more frequently up until now [6, 7]. Unfortunately, such nationwide studies (e.g., in Spain [8] and Iceland [9]) also referred to as Unity Studies by the WHO [10], are scarce and mainly set up through convenience sampling.

Therefore, a nationwide serosurveillance study (PIENTER-Corona, PICO) was initiated quickly after the lockdown was in effect. This cohort is unique as it comprises data available from a previous serosurvey established in 2016/17 (PIENTER-3) of a randomised nationwide sample of Dutch citizens, across all ages and a separate sample enriched for Orthodox-Reformed Protestants, whom might have been exposed to SARS-CoV-2 more frequently due to their socio-geographical-clustered lifestyle [11, 12]. The presented serological framework and findings of our first round of inclusion can support public health policy in the Netherlands as well as internationally.

METHODS

Study design

In 2016/17, the National Institute for Public Health and the Environment of the Netherlands (RIVM) initiated a large-scale nationwide serosurveillance study (PIENTER-3) (*n* = 7,600; age-range 0–89 years). The primary aim was to obtain insights into the protection against vaccine-preventable diseases offered by the National Immunisation Programme in the Netherlands. A comprehensive description of PIENTER-3 has been published previously [13]. Briefly, participants were selected via a two-stage cluster design, comprising 40 municipalities in five regions nationwide (henceforth 'national sample', NS), and nine municipalities in the low vaccination coverage municipalities (LVC), inhabited by a relative large proportion of Orthodox-Reformed Protestants (*Figure 1*). Among other materials, sera and questionnaire data had been collected from all participants. Hence, the PIENTER-3 study acted as baseline sample of the Dutch population for the present cross-sectional PICO-study since 6,102 participants (80%) consented to be approached for follow-up (after updating addresses and screening of possible deaths). The study was powered to estimate an overall seroprevalence with a precision of at least 2.5% [13]. The PICO-study protocol was approved by the Medical Ethics Committee MEC-U, the Netherlands (Clinical Trial Registration NTR8473), and conformed to the principles embodied in the Declaration of Helsinki.

Study population and materials

On 25 March 2020, an invitation letter was sent. Invitees (age-range 2–92 years) willing to participate registered online. After enrolment, participants received an instruction letter on how to self-collect a fingerstick blood sample in a microtainer (maximum of 0.3 mL). Blood samples were returned to the RIVM-laboratory in safety envelopes. Serum samples were stored at −20°C awaiting analyses. Materials were collected between March 31 and May 11, with the majority (80%) in the first week of April 2020 (median collection date April 3). Simultaneous with the blood collection, participants were asked to complete an (online) questionnaire, including questions regarding sociodemographic characteristics, COVID-19-related symptoms, and potential other determinants for SARS-CoV-2 seropositivity, such as comorbidities, medication use and behavioural factors. All participants provided written informed consent.

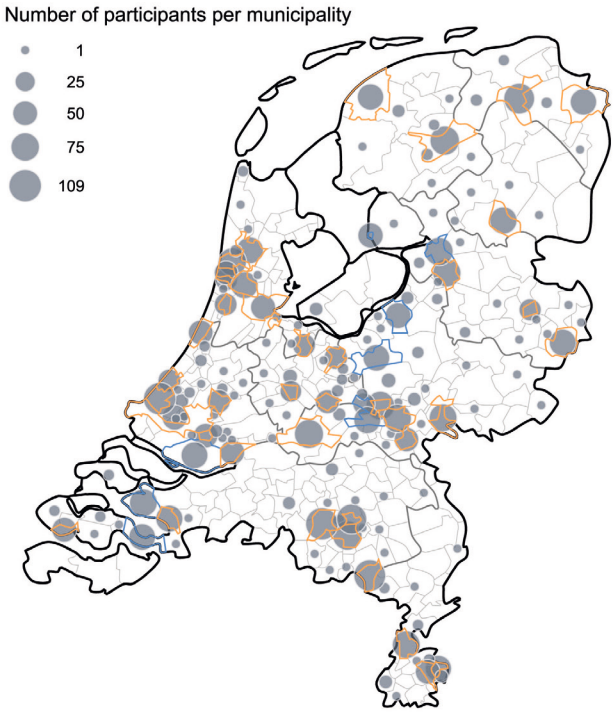

Figure 1. Geographical representation of number of participants in the PICO-study, the Netherlands, first round of inclusion, per municipality. The size of the dots reflect the absolute number of participants. Thicker grey and smaller light grey boundaries represent provinces and municipalities, respectively, and orange and blue boundaries characterize municipalities from the national and low vaccination coverage sample, respectively.

Laboratory methods

Serum samples (diluted 1:200) were tested for the presence of SARS-CoV-2 spike S1-specific IgG antibodies using a validated fluorescent bead-based multiplex-immunoassay as described [14]. A cut-off concentration for seropositivity (2.37 arbitrary units (AU)/mL; with specificity of 99% and sensitivity of 84.4%) was determined by ROC-analysis of 400 pre-pandemic control samples (including a nationwide random cross-sectional sample (*n* = 108)) as well as patients with confirmed influenza-like illnesses caused by coronaviruses and other viruses, and a selection of sera from 115 PCR-confirmed COVID-19 cases with mild, or severe disease symptoms. Seropositive PICO-samples and those with a concentration 25% below the cut-off were retested (*n* = 138), and the geometric mean concentration (GMC) was calculated. Paired pre-pandemic PIENTER-3-samples of these retested PICO-samples (available from *n* = 129/138) were tested correspondingly as described above to correct for false-positive results (*Supplement Figure S1A*).

Statistical analyses

Study population, COVID-19-related symptoms and antibody responses

Data management and analyses were conducted in SAS v.9.4 (SAS Institute Inc., USA) and R v.3.6. *P* values < 0.05 were considered statistically significant. Sociodemographic characteristics and COVID-19-related symptoms (general, respiratory, and gastrointestinal) developed since the start of the epidemic were stratified by sample (NS vs. LVC), or sex, respectively, and described for seropositive and seronegative participants. Differences were tested via Pearson's χ^2, or Fisher's exact test if appropriate. Differences in GMC between reported symptoms in seropositive participants were determined by calculating the difference in log-transformed concentrations of those who developed symptoms at least four weeks prior to the sampling — ensuring a plateaued response — and tested by means of a Mann-Whitney U-test.

Seroprevalence estimates

Seroprevalence estimates (with 95% Wilson CIs (CI)) for SARS-CoV-2-specific antibodies were calculated taking into account the survey design (i.e., controlling for region and municipality) and weighted by sex, age, ethnic background and degree of urbanisation to match the distribution of the general Dutch population in both the NS and LVC sample. Estimates were corrected for test performance via the Rogan & Gladen bias correction (with sensitivity of 84.4% and assuming a specificity of 100% after cross-validation with pre-sera) [15]. Smooth age-specific seroprevalence estimates were obtained with a logistic regression in a Generalised Additive Model using penalised splines [16].

Risk factors for SARS-CoV-2 seropositivity

A random-effects logistic regression model was used to identify risk factors for SARS-CoV-2 seropositivity, applying a full case analysis (n = 3,100; values were missing for < 5% of the participants). Potential risk factors included sociodemographic characteristics (sex, age group, region, ethnic background, Orthodox-Reformed Protestants, educational level, household size, (parent with a) contact profession, healthcare worker), and COVID-19-related factors (contact with a COVID-19 confirmed case, number of persons contacted yesterday, working from home (normally and in the last week), comorbidities (combining diabetes, history of malignancy, immunodeficiency, cardio-vascular, kidney and chronic lung disease (note: as a sensitivity analysis, comorbidities were also included separately)), and use of blood pressure medication, immunosuppressants, statins and antivirals/antibiotics in the last month). Models included a random intercept, potential clustering by municipality and region was accounted for, and odds ratios (OR) in univariable analyses were a priori adjusted for sex and age. Variables with $p < 0.10$ were entered in

the multivariable analysis, and backward selection was performed — manually dropping variables one-by-one based on $p \geq 0.05$ — to identify significant risk factors. Adjusted ORs and corresponding 95% CIs were provided.

RESULTS

Study population

Of 6,102 invitees, 3,207 (53%) donated a serum sample and filled-out the questionnaire, of which 2,637 persons from the NS and 570 from the LVC. Participants from across the country participated (*Figure 1*), with age ranging from 2 to 90 years (*Table 1*). In the NS, slightly more women (55%) participated, most (88%) were of Dutch descent, nearly half had a high educational level, and 45% was religious. 20 percent of persons between age 25–66 years were healthcare workers and 56% of the (parents of) participants reported to have had daily contact with patients, clients and/or children in their profession/volunteer work normally. Over half of the participants lived in a ≥ 2-person household, and 78% reported to have had physical contact with < 5 people outside their own household yesterday (during lockdown), of which more than half with nobody. Comorbidities most frequently reported included chronic lung and cardiovascular disease (both 13%), and a history of malignancy (5%). In line with the population distribution, the LVC sample was characterised by a relative high proportion of Orthodox-Reformed Protestants from Dutch descent (*Table 1*). Sociodemographic characteristics between responders and non-responders are provided in (*Supplement Table S1*).

Table 1. COVID-19-related symptoms since the start of the epidemic among all participants in the PICO-study reporting symptoms (*n* = 3,147), first round of inclusion.

	National sample			Low vaccination coverage sample		
	Total (*n* (%))	Weighted SARS-CoV-2 seroprevalence		Total (*n* (%))	Weighted SARS-CoV-2 seroprevalence	
		%	*(95% CI)*		*%*	*95% CI*
Overall	2,637 (100%)	2.8	(2.1–3.7)	570 (100%)	2.9	(1.4–6.3)
Sex						
Men	1,184 (44.9%)	2.9	(1.8–4.5)	233 (40.9%)	4.0	(1.5–10.6)
Women	1,453 (55.1%)	2.7	(1.7–4.1)	337 (59.1%)	1.9	(0.7–4.9)
Age categories (years)						
2–17	507 (19.2%)	1.7	(0.6–4.9)	93 (16.3%)	0.0	NA
18–39	735 (27.9%)	4.9	(3.2–7.5)	196 (34.4%)	6.8	(3.0–14.6)
40–64	919 (34.8%)	1.9	(1.2–3.2)	198 (34.7%)	2.4	(0.7–8.3)
65–90	476 (18.1%)	2.5	(1.2–5.1)	83 (14.6%)	1.0	(0.1–7.0)
Region						
North	566 (21.5%)	1.3	(0.4–3.2)	NA	NA	NA
Mid-West	427 (16.2%)	4.0	(1.8–8.0)	NA	NA	NA
Mid-East	508 (19.3%)	3.1	(1.3–6.2)	NA	NA	NA
South-West	468 (17.7%)	3.0	(1.5–5.3)	NA	NA	NA
South-East	668 (25.3%)	2.7	(1.4–4.7)	NA	NA	NA
LVC	NA	NA	NA	570 (100%)	2.9	(1.4–6.3)
Ethnic background						
Dutch	2,306 (87.5%)	2.8	(2.0–3.7)	555 (97.4%)	3.0	(1.4–6.5)
Non-Dutch Western	159 (6.0%)	2.0	(0.6–7.1)	12 (2.1%)	0.0	NA
Non-Western	172 (6.5%)	3.4	(1.4–8.4)	3 (0.5%)	0.0	NA
Educational level[a]						
High	1,257 (46.7%)	2.5	(1.6–3.9)	173 (30.7%)	2.3	(0.5–9.4)
Middle	883 (34.2%)	3.5	(2.0–6.2)	252 (44.8%)	4.4	(1.7–10.9)
Low	442 (17.1%)	2.2	(1.0–5.0)	138 (24.5%)	0.9	(0.1–6.3)
Religion						
No religion	1,329 (54.5%)	2.9	(1.9–4.4)	145 (28.0%)	0.3	(0.0–3.7)
Roman Catholic	613 (25.1%)	3.4	(1.7–6.6)	13 (2.5%)	0.0	NA
Other	119 (4.9%)	0.0	NA	14 (2.7%)	0.0	NA
Protestant	379 (15.5%)	3.0	(1.6–6.4)	346 (66.8%)	3.7	(1.5–8.8)
Orthodox-Reformed	28 (7.4%)	8.5	(2.4–26.9)	102 (29.5%)	7.4	(1.8–26.8)
other	351 (92.6%)	2.6	(1.0–6.5)	244 (70.5%)	2.2	(0.9–5.3)

[a] Maternal educational level was used for participants < 15 years of age.
Missing: in the national sample: (maternal) educational level *n* = 55, religion *n* = 197; in the low vaccination coverage sample: (maternal) educational level *n* = 7, religion *n* = 52.
Abbreviations: *LVC*, low vaccination coverage municipalities sample; *NA*, not applicable.

COVID-19-related symptoms and antibody responses

In total, 63% of participants reported to have had ≥ 1 COVID-19-related symptom(s) since the start of the epidemic, with runny nose (37%), headache (33%), and cough (30%) being most common (*Table 2*). All reported symptoms were significantly higher in seropositive compared to seronegative persons, except for stomach ache. The majority of those seropositive (93%) reported to have had symptoms (90% of men vs. 95% of women), of whom three already in mid-February, two weeks prior to the official first notification. Median duration of illness in the seropositive participants was 8.5 days (IQR: 4.0–12.5), 16% (n = 12) visited a general practitioner and one was admitted to the hospital. Among seropositive persons, most reported to have had ≥ 1 respiratory symptom(s) (86%), with runny nose and cough (both 61%) most regularly, and ≥ 1 general (84%) symptom(s), of which anosmia/ageusia (53%) was most discriminative as compared to the seronegative participants (4%, $p < 0.0001$) (*Table 2*). Symptoms were more common in women, except for anosmia/ageusia, cough and irritable/confusion. Almost 75% of the seropositive participants met the COVID-19 case definition of fever and/or cough and/or dyspnea, which improved to 80% when anosmia/ageusia was included — while remaining 36% in those seronegative. GMC was significantly higher among seropositive persons with fever vs. without (48.2 vs. 11.6 AU/mL, $p = 0.01$), and with dyspnea vs. without (78.6 vs. 13.5 AU/mL, $p = 0.04$).

Seroprevalence estimates

Overall weighted seroprevalence in the NS was 2.8% (95% CI 2.1–3.7), did not differ between sexes or ethnic backgrounds (*Table 1*), and was not higher among healthcare workers (2.7% vs. non-healthcare workers 2.5%). Seroprevalence was lowest in the northern region (1.3%) and highest in the mid-west (4.0%). Estimates were lowest in children — gradually increasing from below 1% at age 2 years to 3% at 17 years — was highest in age group 18–39 years (4.9%) and ranged between 2 and 4% up to 90 years of age (*Figure 2*). In both samples, seroprevalence was highest in Orthodox-Reformed Protestants (> 7%) (*Table 1*). *Supplement Figure S1B* displays the distribution of IgG concentrations for all participants by age, and *Supplement Figure S2* shows the seroprevalence smoothed by age in the LVC.

Table 2. COVID-19-related symptoms since the start of the epidemic among all participants in the PICO-study reporting symptoms (*n* = 3,147), first round of inclusion, by serostatus.

	SARS-CoV-2 seronegative *n* = 3,073		**SARS-CoV-2 seropositive *n* = 74**		**Total *n* = 3,147**		***p* value[a]**
	n	**%**	***n***	**%**	***n***	**%**	
Meets COVID-19 case definition							**< 0.0001**
Yes	1,096	35.7	55	74.3	1,151	36.6	
No	1,977	64.3	14	25.7	1,996	63.4	
Meets COVID-19 case definition, and self-reported to have had anosmia and/or ageusia							**< 0.0001**
Yes	1,113	36.2	59	79.7	1,172	37.2	
No	1,960	63.8	15	20.3	1,975	62.8	
Developed symptoms since the start of the epidemic							**< 0.0001**
Yes	1,903	61.9	69	93.2	1,972	62.7	
No	1,170	38.1	5	6.8	1,175	37.3	
General symptoms (one or more)	1,350	43.9	62	83.8	1,412	44.9	**< 0.0001**
Fever	361	11.8	32	43.2	393	12.5	**< 0.0001**
General malaise	332	10.8	34	46.0	366	11.6	**< 0.0001**
Headache	1,001	32.6	48	64.9	1,049	33.3	**< 0.0001**
Irritable/confused	232	7.6	17	23.0	249	7.9	**< 0.0001**
Muscle ache	312	10.5	22	29.7	334	10.6	**< 0.0001**
Arthralgia	497	16.2	42	56.8	539	17.1	**< 0.0001**
Anosmia and/or ageusia	111	3.6	39	52.7	150	4.8	**< 0.0001**
Respiratory symptoms (one or more)	1,622	52.8	64	86.5	1,686	53.6	**< 0.0001**
Cough	905	29.5	45	60.8	950	30.2	**< 0.0001**
Sore throat	798	26.0	33	44.6	831	26.4	**0.0003**
Runny nose	1,128	36.7	45	60.8	1,173	37.3	**< 0.0001**
Solely a runny nose & hay fever	22	0.7	1	1.4	23	0.7	0.42[b]
Dyspnea	251	8.2	13	17.6	264	8.4	**0.004**
Gastrointestinal symptoms (one or more)	668	21.7	32	43.2	700	22.2	**< 0.0001**
Diarrhea	388	12.6	18	24.3	406	12.9	**0.003**
Nausea/vomiting	207	6.7	13	17.6	220	7.0	**0.0003**
Stomach ache	364	11.9	13	17.6	377	12.0	0.13

[a] *p* values were calculated with Pearson's Chi-Square Test, unless depicted otherwise. Statistically significiant *p* values are shown in bold type.
[b] *p* value was calculated with Fisher's Exact Test.
Missing values for all symptoms *n* = 60.

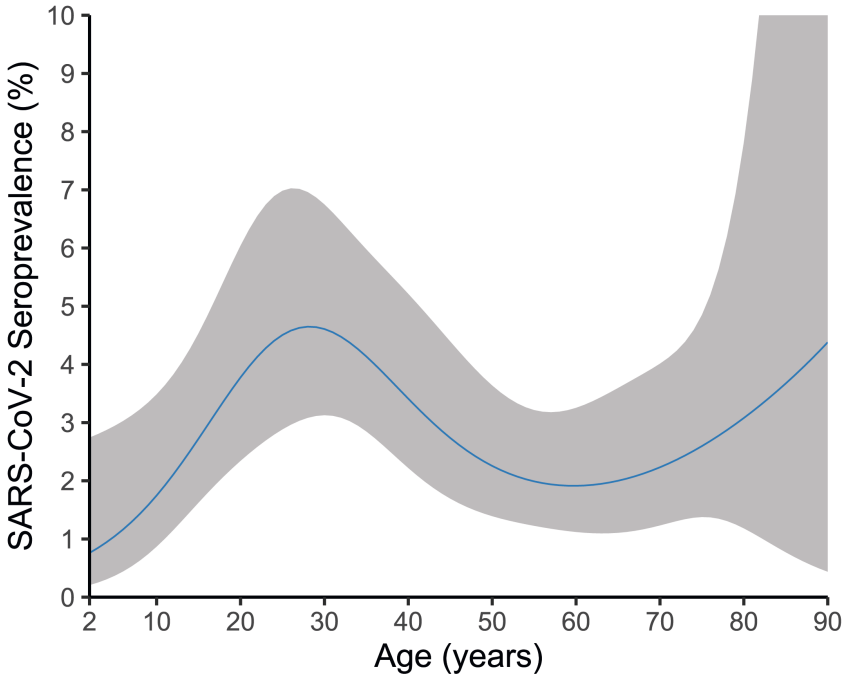

Figure 2. Smooth age-specific SARS-CoV-2 seroprevalence in the general population of the Netherlands, beginning of April 2020.

Risk factors for SARS-CoV-2 seropositivity

Variables that were associated with SARS-CoV-2 seropositivity in univariable analyses included age group, Orthodox-Reformed Protestant, had been in contact with a COVID-19 case, use of immunosuppressants, and antibiotic/antiviral medication in the last month (*Table 3*). In multivariable analysis, substantial higher odds were observed for those who took immunosuppressants the last month, were Orthodox-Reformed Protestant, had been in contact with a COVID-19 confirmed case, and from age groups 18–24 and 25–39 years (compared to 2–12 years).

Table 3. Risk factor analysis for SARS-CoV-2 seropositivity among all participants (*n* = 3,100; full case analysis) in the PICO-study, first round of inclusion.

		% SARS-CoV-2 seropositive	Univariable model[a]			Multivariable model		
	n_{total}	*n (%)*	*OR*	*(95% CI)*	*p value*	*aOR*	*(95% CI)*	*p value*
Age group					**0.016**			0.105
2–12	457	4 (0.9%)	Ref.			Ref.		
13–17	129	1 (0.8%)	0.88	(0.10–7.91)		0.87	(0.10–7.91)	
18–24	226	12 (5.3%)	6.47	(2.05–20.43)		4.52	(1.40–14.58)	
25–39	696	24 (3.5%)	4.17	(1.43–12.14)		3.10	(1.05–9.14)	
40–49	429	11 (2.6%)	3.05	(0.96–9.68)		2.48	(0.77–7.98)	
50–59	485	8 (1.7%)	1.94	(0.58–6.49)		1.49	(0.44–5.07)	
60–69	377	7 (1.9%)	2.16	(0.63–7.44)		1.71	(0.49–5.98)	
70–90	301	7 (2.3%)	2.64	(0.76–9.14)		2.46	(0.70–8.60)	
Sex					0.81			0.57
Men	1,368	32 (2.3%)	Ref.			Ref.		
Women	1,732	42 (2.4%)	1.06	(0.66–1.71)		1.15	(0.71–1.88)	
Region[b]					0.64			0.36
North	537	7 (1.3%)	Ref.			Ref.		
Mid-West	411	11 (2.7%)	2.14	(0.80–5.72)		2.27	(0.86–5.98)	
Mid-East	494	14 (2.8%)	2.27	(0.89–5.80)		2.00	(0.79–5.04)	
South-West	451	11 (2.4%)	1.83	(0.69–4.86)		1.80	(0.69–4.74)	
South-East	652	17 (2.6%)	2.04	(0.82–5.07)		2.08	(0.85–5.12	
LVC	555	14 (2.5%)	1.80	(0.71–4.61)		1.09	(0.40–2.94)	

Table 3. (Continued)

		% SARS-CoV-2 seropositive	Univariable model[a]			Multivariable model		
	n_{total}	*n (%)*	*OR*	*(95% CI)*	*p value*	*aOR*	*(95% CI)*	*p value*
Orthodox-Reformed Protestant					**0.001**			**0.0007**
No	2,972	65 (2.2%)	Ref.			Ref.		
Yes	128	9 (7.0%)	4.04	(1.72–9.48)		4.50	(1.89–10.74)	
Had been in contact with a COVID-19 confirmed case					**< 0.0001**			**< 0.0001**
No	2,074	33 (1.6%)	Ref.			Ref.		
Yes	192	16 (8.3%)	4.65	(2.44–8.87)		4.97	(2.58–9.56)	
Don't know	834	25 (3.0%)	1.75	(1.03–2.99)		1.88	(1.10–3.22)	
Took immunosuppressants, last month					**0.006**			**0.001**
No	3,039	69 (2.3%)	Ref.			Ref.		
Yes	61	5 (8.2%)	3.94	(1.50–10.39)		5.05	(1.89–13.48)	
Took antibiotics/antiviral medication, last month					**0.01**			
No	2,901	64 (2.2%)	Ref.					
Yes	199	10 (5.0%)	2.43	(1.21–4.89)				

[a] Variables that were not associated with SARS-CoV-2 seropositivity in univariable analyses (i.e., $p \geq 0.10$) – or that were not controlled for – included: ethnic background, (maternal) educational level, household size, (parent with a) contact profession, healthcare worker, number of persons contacted yesterday, working from home (normally and in the last week (during lockdown)), comorbidities (combining chronic lung disease, diabetes, history of malignancy, immunodeficiency, cardio-vascular disease, kidney disease), and use of blood pressure medication, immunosuppressants, statins and antivirals/antibiotics in the last month. *P* values that were statistically significant were depicted in bold type.

[b] Region North comprised provinces Groningen, Friesland, Drenthe and Overijssel, region Mid-West provinces Noord-Holland and Flevoland, region Mid-West provinces Utrecht and Gelderland, region South-West provinces Zuid-Holland and Zeeland, and region South-East provinces Noord-Brabant and Limburg.

Abbreviations: *aOR*, adjusted odds ratio; *CI*, confidence interval; *LVC*, low vaccination coverage municipalities; *OR*, odds ratio; *Ref.*, reference category.

DISCUSSION

Here, we have estimated the seroprevalence of SARS-CoV-2-specific antibodies and identified risk factors for seropositivity in the general population of the Netherlands during the first epidemic wave in April 2020. Although overall seroprevalence was still low at this phase, important risk factors for seropositivity could be identified, including adults aged 18–39 years, persons using immunosuppressants, and Orthodox-Reformed Protestants. These data can guide future interventions, including strategies for vaccination, believed to be a realistic solution to overcome this pandemic.

This PICO-study revealed that 2.8% (95% CI 2.1–3.7) of the Dutch population had detectable SARS-CoV-2-specific serum IgG antibodies, suggesting that almost half a million inhabitants (of in total 17,423,981 [17]) were infected (487,871 (95% CI 365,904–644,687)) in mid-March, 2020 (taking into account the median time to seroconvert [18]). Several seropositive participants reported to have had COVID-19-related symptoms back in mid-February, suggesting the virus circulated in our country at the beginning of February already. Our overall estimate is in line with preliminary results from another study conducted in the Netherlands in the beginning of April which found 2.7% to be seropositive, although this study was performed in healthy blood donors aged 18–79 years [19]. Worldwide, various seroprevalence studies are ongoing. A large nationwide study in Spain showed that around 5% (ranging between 3.7% and 6.2%) was seropositive, indicating that only a small proportion of the population had been infected in one of the hardest hit countries in Europe. Current studies in literature mostly cover COVID-19 hotspots or specific regions—with possibly bias in selection of participants and/or smaller age-ranges—with rates ranging between 1–7% in April (e.g., in Los Angeles County (CA, USA) [20] or ten other sites in the USA [21], Geneva (Switzerland) [22], and Luxembourg [23]). Estimates also very much depend on test performances. Particularly, when seroprevalence is relatively low, specificity of the assay should approach near 100% to diminish false-positive results and minimise overestimation. Although we cannot rule-out false-positive samples completely, our assay was validated using a broad range of positive and negative SARS-CoV-2 samples; PICO-samples were cross-linked to pre-pandemic concentration; and bias correction for test performance was applied to represent most accurate estimates. In addition, future studies should establish whether epidemiologically dominant genetic changes in the spike protein of SARS-CoV-2 influence binding to spike S1 used in our and other assays.

Seroprevalence was highest in adults aged 18–39 years, which is in line with the serosurvey among blood donors in the Netherlands, but contrary to the

low incidence rate as reported in Dutch surveillance, caused by restrictive testing of risk groups and healthcare workers at the beginning of the epidemic, primarily identifying severe cases [3, 19]. The elevation in these younger adults may be explained by increased social contacts typical for this age group, in addition to specific social activities in February, such as skiing holidays in the Alps (from where the virus disseminated quickly across Europe), or carnival festivities in the Netherlands (i.e., multiple superspreading events primarily in the mid and Southern part, explaining local elevation in seroprevalence). In correspondence with other nationwide studies [8, 9] and reports from the Dutch government [3, 24] seroprevalence was lowest in children. Although some rare events of paediatric inflammatory multisystem syndrome have been reported, this group seems to be at decreased risk for developing (severe) COVID-19 in general, which may be explained by less severe infection possibly resulting in a limited humoral response [25, 26]. Further, significantly higher odds for seropositivity were seen in Orthodox-Reformed Protestants. This community lives socio-geographically clustered in the Netherlands, that is, work, school, leisure and church are intertwined heavily. As observed in other countries, particularly frequent attendance of church with close distance to others, including singing activities, might have fueled the spread of SARS-CoV-2 within this community in the beginning of the epidemic [11, 12]. Whereas the comorbidities with possible increased risk of severe COVID-19 were not associated with seropositivity in this study, immunosuppressants use did display higher odds (note: we did not have information of specific drugs). Recent data indicate that immunosuppressive treatment is not associated with worse COVID-19 outcomes [27, 28], yet continued surveillance is warranted as these patients might be more prone to (future) infection, for instance due to a possible attenuated humoral immune response [29].

The majority of seropositive participants exhibited ≥ 1 symptom(s), mostly general and respiratory. A recent meta-analysis found a pooled asymptomatic proportion of 16% [5], hence the observed overall fraction in the present study (7%) might be a conservative estimate as the self-reported symptoms could have been due to other reasons or circulating pathogens along the recalled period (i.e., 62% of the seronegative participants reported symptoms too). The asymptomatic proportion might be different across ages [5] and should be explored further along with elucidating the overall contribution of asymptomatic transmission via well-designed contact-tracing studies. Interestingly, clinical studies have observed anosmia/ageusia to be associated with SARS-CoV-2 infection, and this notion is supported here at a population-based level [30]. In the pandemic context, sudden onset of anosmia/ageusia seems to be a useful surveillance tool, which can contribute to early disease recognition and minimise transmission by rapid self-isolation.

This study has some limitations. First, although half of the total municipalities in the Netherlands were included, some COVID-19 hotspots might be missed due to the study design. Second, our study population consisted of more Dutch (88%) than non-Dutch persons and relative more healthcare workers (20%) when compared to the general population (76% and 14%, respectively) [17]. Healthcare workers in the Netherlands do not seem to have had a higher likelihood of infection, and transmission seems to have taken place mostly in household settings [3, 31]. Although selectivity in response was minimised by weighting our study sample on a set of sociodemographic characters to match the Dutch population, seroprevalence might still be slightly influenced. Third, some potential determinants for seropositivity could have been missed as we might have been underpowered to detect small differences given the low prevalence in this phase, or because these questions had not been included in the questionnaire (as it was designed in the very beginning of the epidemic). Finally, at this stage the proportion of infected individuals that fail to show detectable seroconversion is unknown, potentially leading to underestimation of the percentage of infected persons.

To conclude, we estimated that 2.8% of the Dutch inhabitants, that is, nearly half a million, were infected with SARS-CoV-2 amidst the first epidemic wave in the beginning of April 2020. This is in striking contrast with the 30-fold lower number of reported cases (of approximately 15,000) [3], and underlines the importance of seroepidemiological studies to estimate the true pandemic size. The proportion of persons still susceptible to SARS-CoV-2 is high and the infection fatality rate (IFR) is substantial [4]. Globally, nationwide seroepidemiological studies are urgently needed for better understanding of related risk factors, viral spread, and measures applied to mitigate dissemination [7]. The prospective nature of our study will enable us to gain key insights on the duration and quality of antibody responses in infected persons, and hence possible protection of disease by antibodies [6]. Serosurveys will thus play a major role in guiding future interventions, such as strategies for vaccination (of risk groups), since even when vaccines become available, initial vaccine availability will be limited.

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SUPPLEMENTARY MATERIALS

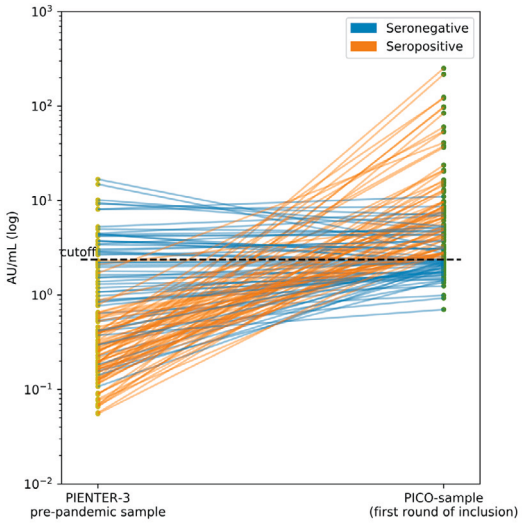
Supplement Table S1. Sociodemographic characteristics of responders and non-responders in the PICO-study, first round of inclusion (*n*, %).

	Non-responder		Responder		Total
	n	**%**	***n***	**%**	
Sex					
Men	1,360	47.0	1,417	44.2	2,777
Women	1,535	53.0	1,790	55.8	3,325
Age group, years					
2-12	691	23.9	468	14.6	1,159
13-17	238	8.2	132	4.1	370
18-24	269	9.3	232	7.2	501
25-39	580	20.0	699	21.8	1,279
40-49	292	10.1	440	13.7	732
50-59	248	8.6	492	15.4	740
60-69	224	7.7	401	12.5	625
70-90	353	12.2	343	10.7	696
Region					
North	447	15.4	566	17.7	1,013
Mid-West	395	13.6	427	13.3	822
Mid-East	414	14.3	508	15.8	922
South-West	483	16.7	468	14.6	951
South-East	598	20.7	668	20.8	1,266
Low vaccination coverage municipalities	558	19.3	570	17.8	1,128
Ethnic background					
Dutch	2,168	74.9	2,861	89.2	5,029
Non-Dutch Western	166	5.7	171	5.3	337
Non-Western	560	19.4	175	5.5	735
Educational level[a]					
High	787	29.2	1,262	41.8	2,049
Middle	984	36.4	1,122	37.1	2,106
Low	930	34.4	637	21.1	1,567
Religion					
No religion	1,070	40.6	1,474	49.8	2,544
Roman Catholic	501	19.0	626	21.2	1,127
Other (Islamic, Jewish, Buddhism, Hinduism, other)	379	14.4	133	4.5	512
Protestant	686	26.0	725	24.5	1,411
Orthodox-Reformed	182	26.5	130	17.9	312
Other	504	73.5	595	82.1	1,099

[a] Educational level during inclusion of the PIENTER-3 study (2016/17) was used for accurate comparison between responders and non-responders. Note: maternal educational level was used for participants < 15 years of age.

Missing: ethnic background *n* = 1; educational level *n* = 380; religion *n* = 508.

A



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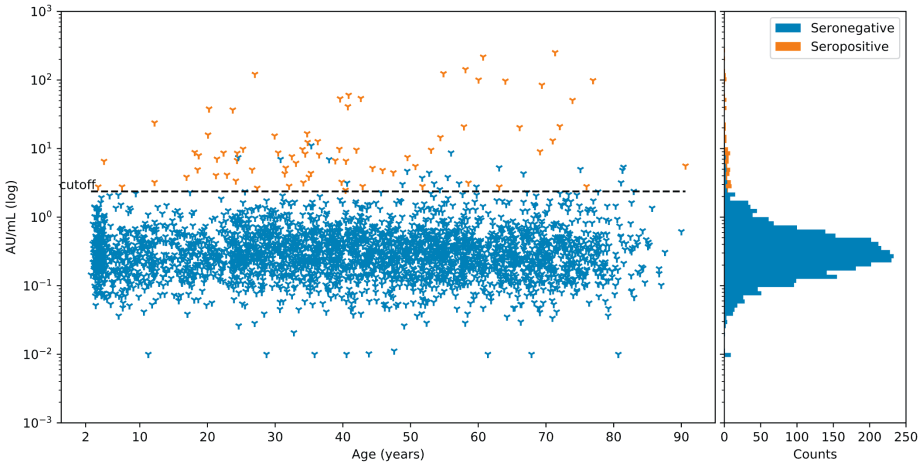
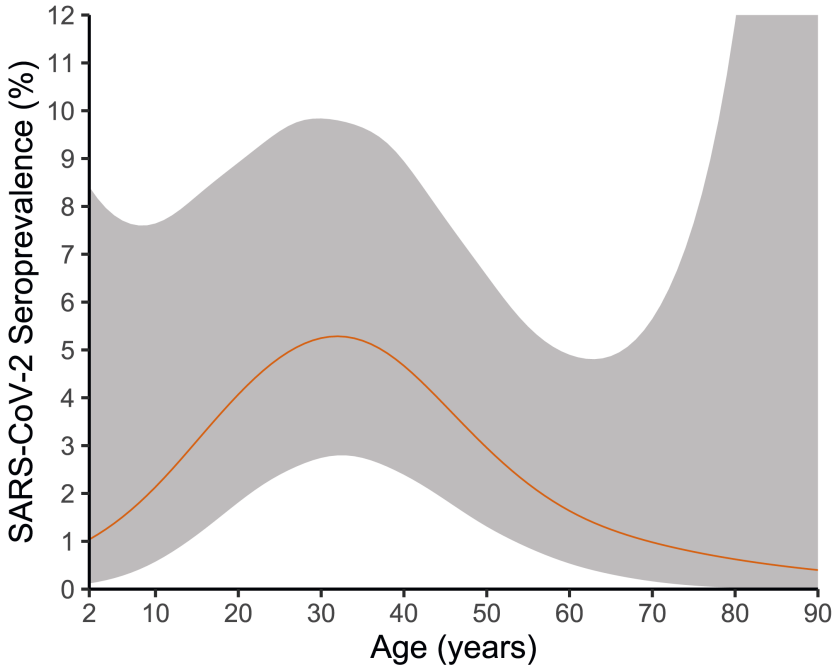


Supplement Figure S1. A. IgG antibody concentration (arbitrary units (AU)/mL) against SARS-CoV-2 of matched pre-pandemic PIENTER-3-samples (light green dots) and current PICO-samples (dark green dots) that were seropositive or 25% below the cutoff for seropositivity after the first measure *n* = 138). **B**. PICO-samples with seropositive pre-pandemic sera (based on the calculated cutoff by ROC-analysis) were considered seronegative (*n* = 26), thereby correcting for false-positivity. To note: a maximum concentration-fold increase of 1.5 was observed between a false-positive PIENTER-3-sample and its corresponding PICO-sample. Blue and orange lines represent seronegative and seropositive samples, respectively, and the dashed line depicted is considered the cutoff for seropositivity (2.37 AU/mL). IgG antibody concentration (AU/mL (log)) against SARS-CoV-2 of all individual PICO-samples, by age (years) (left side) and distributed by means of a histogram (right side). After correction for pre-pandemic cross-reactivity, samples were classified as seronegative (blue) and seropositive (orange). The dashed line depicted is considered the cutoff for seropositivity (2.37 AU/mL).



Supplement Figure S2. Smooth age-specific SARS-CoV-2 seroprevalence in the low vaccination coverage municipalities of the Netherlands, beginning of April 2020.

CHAPTER 8

Associations between measures of social distancing and severe acute respiratory syndrome coronavirus 2 seropositivity: a nationwide population-based study in the Netherlands

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ABSTRACT

This large, nationwide, population-based, seroepidemiological study provides evidence of the effectiveness of physical distancing (> 1.5 m) and indoor group size reductions in reducing severe acute respiratory syndrome coronavirus 2 infection. Additionally, young adults may play an important role in viral spread, contrary to children up until age 12 years with whom close contact is permitted.

INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic is an unprecedented global crisis. Stringent measures to suppress the spread of its causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), have been implemented to reduce incidence of disease and prevent health systems from becoming overwhelmed. Assessment of the impact of social-distancing measures is vital for informing public health decisions, particularly since the worldwide availability of vaccines is still very limited in this phase.

In the Netherlands, the first case of COVID-19 was reported on 27 February 2020. Key governmental interventions implemented since mid-March 2020 included keeping physical distance (≥ 1.5 m) from adults for those aged > 12 years, whereas close contact between children aged < 18 years was permitted; closing schools, restaurants/bars/cafés, cultural institutions, and sport facilities; working from home if possible; prohibiting contact professions; closing nursing homes to visitors; and reducing group sizes to a maximum of three visitors at home as well as three persons from different households outside and prohibiting all events and gatherings, except for weddings, funerals, religious gatherings (maximum of 30 persons), legally required meetings, and work-related meetings necessary for continuation of daily activities (maximum of 100 persons). In May, daycare and primary schools were reopened and contact professions were allowed to resume. Measures were further relaxed from June until the end of summer, while adhering to physical distancing measures and obligation of wearing a nonmedical mask in public transportation.

Seroprevalence of antibodies against SARS-CoV-2, acquired from validated laboratory assays and well-designed population-based studies, provides an important indicator of cumulative infection [1, 2]. In combining seroprevalence with questionnaire data, the current nationwide population-based study (PIENTER-Corona (PICO)) [3], performed after the first epidemic wave in the Netherlands in June 2020, enabled us to identify risk factors for infection to support assessment of the impact of globally applied social distancing measures.

METHODS

Randomly selected participants of all age groups from the first PICO serosurvey in April 2020 [3, 4], who were initially sampled from the PIENTER-3 serosurvey cohort established in 2016/2017 [4], were invited for the current study in June 2020, and 2,317 enrolled (of 4,926 invited). To enhance countrywide geographical coverage and given the low anticipated seroprevalence, the study sample from April 2020 was supplemented with an additional sample of 4,496 (of 26,854) randomly selected persons from the population registry, resulting in a total cohort of 6,813 participants in the current study (combined response rate, 21.4%; for further details on sampling, *see Supplementary Materials, Ch. 2*)). Participants were requested to collect a fingerstick blood sample in a microtainer (SARSTEDT) and return it by mail. A (online) questionnaire was completed on potential SARS-CoV-2 exposure (number and age group of non-household close contacts (< 1.5 m) the day before filling out the questionnaire, attendance of indoor meetings with > 20 persons, nursing home visits, working from home the previous week, profession, close contact (voluntary) work with patients/clients and children, and household size) and sociodemographic characteristics (sex, age, ethnic background, religion, educational level, and postal codes were used to determine geographical sites).

Quantitative measures of serum immunoglobulin G (IgG) antibodies against SARS-CoV-2 spike S1 antigen were derived via a validated immunoassay [5] (see *Supplementary Materials, Ch. 4* for further details on the assay). Based on low anticipated seroprevalence [3], we aimed for a specificity of 99.9% to keep false-positive rates to a minimum. Mixture model analyses (using a validation panel as prior distribution) showed that such specificity could be obtained (at a cutoff for seropositivity of 0.04 log (arbitrary units (AU))/mL) with associated sensitivity of 94.3% (95% confidence interval (CI), 90.6–96.7) (*Supplementary Materials, Ch. 4*–7). Applying this cutoff, all seroprevalence estimates (and 95% CIs) for the general Dutch population took into account the survey design, included weighting factors to match the distribution of the general Dutch population (based on sex, age, ethnic background, and degree of urbanization; *Supplementary Materials, Ch.* 3), and were controlled for test characteristics subsequently [6, 7]. Smooth age-specific seroprevalence was modeled with B-splines (second degree, 3 percentile-placed internal knots, following lowest Akaike's Information Criteria (AIC)).

Risk factors for seropositivity were identified using random-effects logistic regression, taking into account municipality as a unit of clustering. In the main analysis, all participants without missing data for the tested determinants were included (n = 6,331). Odds ratios (ORs) and 95% CIs were derived from univariable analyses, and 2-way interactions with age were tested for significance. Variables with an overall $p < 0.15$ were tested in multivariable analysis in which stepwise-backward selection was applied, yielding a final model that included only independent risk factors (based on lowest AIC). Sensitivity analyses were

performed applying forward selection and by testing models without the variable 'being religious' (*n* = 6,487), as it comprised the most missing values, without 'educational level' (*n* = 6,339) and without non-household contact data (*n* = 6,338), the latter two to test potential associations with profession.

Analyses were performed using Stan v.2.21 (mixture modeling) and SAS v.9.4 (SAS Institute Inc). The Medical Research Ethics Committees United (MEC-U) approved the study, and all participants provided written informed consent.

RESULTS

Median inclusion date was 14 June 2020 (range, 9 June–24 Augustus; 90% were enrolled by 22 June) (note: sociodemographic characteristics available from non-responders were compared with those of responders and are shown in *Supplementary Materials, Ch. 2 & 3*). The cohort comprised 55% women, and regions were equally represented following population size (*Supplementary Materials, Ch. 3 & 4*). Half of the participants reported to have had ≥ 2 non-household close contacts the day before filling out the questionnaire. Since the start of the epidemic, one quarter had attended an indoor meeting with > 20 persons, and 8% had visited a nursing home. Among those aged 18–66 years, 36% (voluntarily) worked in close contact with clients/patients, 18% were healthcare workers, and 40% had been (partly) working from home the previous week.

After the first epidemic wave, overall seroprevalence in the Dutch population was 4.5% (95% CI 3.8–5.2). No statistically significant differences were observed between sexes or ethnic backgrounds. Estimates were low (0–2%) in children aged 1–12 years, high (9%) in young adults in their early twenties, and 4–7% in individuals aged ≥ 35 years (*Figure 1A*). Low urbanized areas were hit hardest, predominantly in the southeast (up to 16%) (*Supplementary Materials, Ch. 8*).

All potential risk factors for seropositivity tested in univariable analyses are shown in *Figure 1B* (see *Supplementary Materials, Ch. 8* for additional details). Close contact (voluntary) work with children and work with clients/patients was not associated with seropositivity. Social distancing–related risk factors in the multivariable model (*Figure 1B & C*, and *Supplementary Materials, Ch. 8*) included non-household close contacts with ≥ 50% persons aged ≥ 10 years, but not close contact with ≥ 50% children aged < 10 years compared with no contacts (see also *Figure 1D*); attending indoor meetings with > 20 persons; working in a nursing home (rather than visiting); increased household size; and age, with low adjusted odds in children aged ≤ 12 years, with greater than 2.5 times higher odds in adults aged 18–30 and ≥ 50 years compared with those aged 12 years (*Figure 1C*). Notably, total number of non-household close contacts did not remain in the final model after including the variable nature of close contact. Sensitivity analyses yielded similar results (*Supplementary Materials, Ch. 8*).

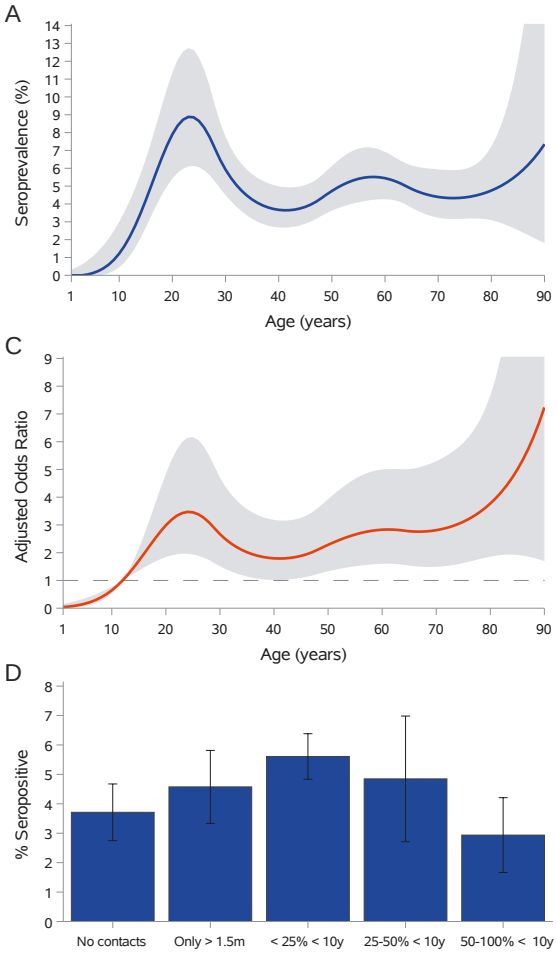
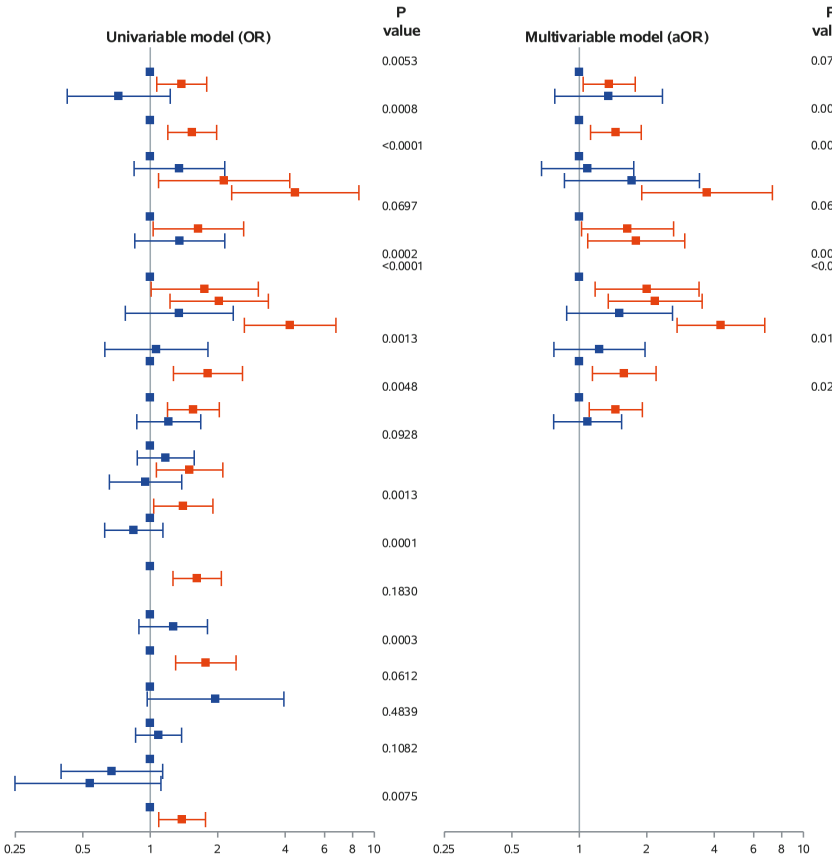


Figure 1. A. Weighted smooth age-specific SARS-CoV-2 seroprevalence (with 95% confidence envelope) in the general Dutch population after the first epidemic wave. **B**. Risk factor analyses for SARS-CoV-2 seropositivity. Nr. and % of total as well as seropositive participants per potential risk factor category are provided. Forest plots are shown with *p* values, and crude odds ratios (ORs) for univariable analyses and adjusted odd ratios (aORs) the multivariable analysis, both including 95% confidence intervals (95% CIs). Red squares are significantly associated with seropositivity, and those in blue are not. Time window of attending indoor meetings with > 20 persons and visiting a nursing home started from the beginning of the epidemic in the Netherlands (27 February 2020) until the day of completing a questionnaire or until closure (for visitors) of nursing homes (20 March 2020), respectively. Nature and number of non-household close contacts yesterday and working from home last week were related to the day or week before the questionnaire was completed, respectively. ROC analysis of the multivariable model yielded an AUC (as a measure of goodness of fit) of 0.72. **C**. The aOR with 95% confidence envelope for age (which was included with a flexible (spline) function) derived from the multivariable model, with 12 years as reference category. **D**. The % of SARS-CoV-2 seropositive participants (and 95% CIs) by number and nature of non-household close contact the day before the questionnaire was completed. Nature of non-household close contact was defined as the proportion of non-household close contacts with children aged < 10 years of the total number of non-household close contacts.

B

Risk factor	No. total	% total	No. pos.	% pos.	P value
Nature of close contact, yesterday					0.0053
No close contact	2328	36.8	94	4.0	
< 50% with persons < 10y	3411	53.9	186	5.5	
50-100% with persons < 10y	592	9.3	17	2.9	
Attended indoor meeting(s) with > 20 pers.					0.0008
No	4728	74.7	197	4.2	
Yes	1603	25.3	100	6.2	
Visited a nursing home					<0.0001
No	5801	91.6	254	4.4	
Yes, 1-5 times	348	5.5	21	6.0	
Yes, >= 6 times	111	1.8	10	9.0	
Nursing home worker	71	1.1	12	16.9	
Household size					0.0697
Single-person	670	10.6	22	3.3	
Two-person	2462	38.9	132	5.4	
Three or more persons	3199	50.5	143	4.5	
Age (spline)					0.0002
Region					<0.0001
North	1257	19.9	29	2.3	
Mid-West	1116	17.6	44	3.9	
Mid-East	1374	21.7	64	4.7	
South-West	1171	18.5	37	3.2	
South-East	1413	22.3	123	8.7	
Urbanization degree					0.0013
High (large cities)	1223	19.3	46	3.8	
Middle (moderate cities)	1946	30.8	65	3.3	
Low (village to countryside)	3162	49.9	186	5.9	
Educational level					0.0048
High	3089	48.8	119	3.9	
Middle	2042	32.3	120	5.9	
Low	1200	18.9	58	4.8	
Nr. of close contacts, yesterday					0.0928
0-1	3125	49.3	135	4.3	
2-4	1476	23.3	75	5.1	
5-9	789	12.5	49	6.2	
>= 10	941	14.9	38	4.0	
Had been working from home, last week					0.0013
No	1712	27.0	107	6.3	
Yes/partly	1738	27.5	79	4.6	
No job (including < 15y)	2881	45.5	111	3.9	
Contact profession/voluntary work with clients/patients					0.0001
No	4725	74.6	192	4.1	
Yes	1606	25.4	105	6.5	
Contact profession/voluntary work with children					0.1830
No	5640	89.1	258	4.6	
Yes	691	10.9	39	5.6	
Healthcare worker					0.0003
No	5609	88.6	243	4.3	
Yes	722	11.4	54	7.5	
Works in a pub/restaurant/café					0.0612
No	6222	98.3	288	4.6	
Yes	109	1.7	9	8.3	
Sex					0.4839
Men	2812	44.4	127	4.5	
Women	3519	55.6	170	4.8	
Ethnic background					0.1082
Dutch	5593	88.3	274	4.9	
non-Dutch Western	473	7.5	16	3.4	
non-Western	265	4.2	7	2.6	
Religious					0.0075
No	3562	56.3	140	3.9	
Yes	2769	43.7	157	5.7	



DISCUSSION

Here, we provide evidence from a large population-based study on the effectiveness of physical distancing (> 1.5 m) as well as indoor group size reductions on SARS-CoV-2 infection. These data suggest that close contact with children up until age 12 years may pose little risk for infection.

Our results on physical distancing are in line with the few previous reports mostly derived from healthcare settings and households [8]. Seroprevalence rates were low in children aged ≤ 12 years despite close contact and similar to observations from other European countries with comparable nationwide estimates [1, 9]. Interestingly, the likelihood of infection among persons in close contact with children was not statistically significantly increased, most likely indicating a low contribution in transmission, as suggested previously [10-13]. In contrast, particularly young adults who engage in relatively more social interaction as opposed to older age groups [14] and often living in larger (student) households in the Netherlands, most probably played an increased role. Applying physical distancing measures within households may not always be feasible; however, stressing its relevance in outbreak management could help to reduce (ongoing) transmission. Further, as observed in other countries [15], these data underline the increased risk of infection among nursing home workers. Hence, while working with the most vulnerable, this requires specific attention.

Our study has strengths and limitations. A strength is that our study provides a large population sample that covers a full age range from young to old, combining a sound indicator of prior infection, that is, seropositivity, with extensive questionnaire data. Also, samples could be classified accurately since antibodies were measured with a highly specific and sensitive immunoassay. A limitation includes the relatively low response rate, which may have introduced potential selection bias, for example, participation of relatively more health-conscious individuals who possibly adhere to social distancing measures, healthcare workers, and persons from Dutch descent; however, we expect little effect on our main outcome. Further, some variables might be proxies of risk of viral exposure, for example, on contacts, thus associations should be interpreted with care as they may not reflect causal effects.

In conclusion, these results underscore the effectiveness of social distancing–related measures to reduce SARS-CoV-2 transmission in an era of limited availability of vaccines. Additionally, our data suggest a diminished role of young children in viral spread that, combined with a proactive testing policy, might justify keeping primary schools open, while young adults may seem to play a more considerable role.

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We gratefully acknowledge the participants of the study. This study would not have been possible without the contribution of colleagues from the National Institute of Public Health and Environment (RIVM), more specifically the Department of Immunology of Infectious Diseases and Vaccines (related to logistics and laboratory analyses) and the Department of Epidemiology and Surveillance (EPI; related to logistics and methodological and statistical insights). We also thank Maarten Schipper for performing the sampling and Susan van den Hof (EPI Department head) for reviewing the manuscript. The study protocol was approved by the Medical Research Ethics Committees United (MEC-U), the Netherlands, and conformed to the principles embodied in the Declaration of Helsinki. Our data are accessible to researchers upon reasonable request for data sharing to the corresponding author.

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SUPPLEMENTARY MATERIALS

1. Introduction

In this supplement we detail our sampling strategy, provide information on non-response rates, explain how we have included post-stratification weighting in the analyses, and provide additional information on the laboratory assay [1]. The risk factor analyses in the main text use random-effects logistic regression based on binary classification of the data (seronegative versus seropositive). Here, we also provide an underpinning of this classification using a two-component mixture model. In this model, samples are not rigidly classified as either seronegative or seropositive, but belong to either the negative or positive component with certain probability [2, 3]. As the probability of seropositivity may depend on age, we model the mixing parameter (i.e. the probability of seropositivity, or seroprevalence) with an age-dependent penalized spline [4]. We fit the model to antibody concentration measurements from the population sample described in the main text while incorporating information from a test panel of proven negative and positive samples [1]. Subsequently, we derive test characteristics (sensitivity, specificity) for various cut-offs, showing that the binary classification used in the main text performs well. Finally, we present additional weighted seroprevalence estimates by Municipal Health Services (GGD) region, and we show detailed results of our main analyses, i.e. risk factors for seropositivity, as well as of the sensitivity analyses.

2. Sampling

The PIENTER-3 serosurvey cohort was established in the Netherlands in 2016/17 (for details see [5]). Primary aim of this seroepidemiological study was to evaluate the National Immunization Program and to monitor (re-)emerging infectious diseases. In respect of the current study, prior randomly-selected participants (from the Dutch population registry) previously enrolled in PIENTER-3 and who had provided consent to be approached for potential follow-up, were invited for the first PIENTER-Corona (PICO-)study in April, 2020. In this first PICO-serosurvey 2,634 participants (of initially 4,926 invited) had been included (for details see [6]). Subsequently, these participants were invited to the second PICO-serosurvey in June, 2020, i.e., the current study, in which 2,317 enrolled.

Correspondingly, anticipating a 10% drop-out rate from the first PICO-serosurvey in April, 2020, and given the low estimated seroprevalence (2.8%), we aimed to increase the overall power of the current study as well as enhance countrywide geographical coverage. Hence, the cohort was supplemented with an additional sample of randomly-selected persons from the Dutch population registry (as of May, 2020). These persons were randomly drawn from five regions with roughly similar population size (North:

provinces of Groningen, Friesland, Drenthe and Overijssel; Mid-West: provinces of Flevoland and Noord-Holland; Mid-East: provinces of Gelderland and Utrecht; South-West: provinces of Zuid-Holland and Zeeland; South-East: provinces of Noord-Brabant and Limburg), and from 17 pre-defined age groups (1–4, 5–9, 10–14, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–79, 80–89 years). A total sample size of 6,400 participants, i.e. with an average of 380 participants per age group, would enable us to estimate an overall and age-specific seroprevalence with a precision of 1.25% and 5%, respectively. Following previous experience, we anticipated a response rate of at least 15%. Hence, for this additional sample, we randomly selected 27,200 persons from the population registry, of which 26,854 remained eligible for participation after an initial screening and these were invited. Of these, 4,496 participated.

Taken together, the current PICO-survey in June, 2020, consisted of 6,813 participants (combined response rate 21.4%).

3. Non-response and weighting

Supplement Table S1 shows the number of participants and response rates, stratified by sex, age group, region, and ethnic background.

Post-stratification weights were assigned to each participant to standardize seroprevalence estimates, using census data from the Statistics Netherlands of January 1, 2020. Since our cohort consists of two samples, weights were calculated for each sample separately. Per study sample, weights were assigned to each participant based on their membership to specific census strata (in total 112): for Dutch ethnic background, strata are designed for age group (1–4, 5–9, 10–14, 15–19, 20–24, 25–29, 30–34, 35–39, 40–44, 45–49, 50–54, 55–59, 60–64, 65–69, 70–74, 75–90 years), urbanization level (high, middle, low), and sex; and for other ethnicity groups strata were based on age group (1–9, 10–34, 35–59, 60–90 years) and sex.

Subsequently, post-stratification weights were defined as the proportion of each stratum represented in the Dutch population divided by the analogous proportion in the study sample. Specifically, weights W_{ij} for participants in stratum i and study j were calculated as:

$$W_{ij} = \frac{\frac{X_i}{N}}{\frac{x_{ij}}{n_j}},$$

where X_i is the total number of persons in stratum i, N is the total population size (i.e., the Netherlands), x_{ij} is the number of participants in stratum i in study sample j and n_j is number of participants in sample j.

Supplement Table S1. Overview of responders vs. non-responders.

	Non-responder		Responder		Total
	n	%	*n*	%	
Total	24,967	78.6	6,813	21.4	31,780
Sex					
Men	12,609	50.5	3,042	44.7	15,561
Women	12,358	49.5	3,771	55.4	16,129
Age groups, years					
1–4	1,740	7.0	220	3.2	1,960
5–9	1,637	6.6	285	4.2	1,922
10–14	1,567	6.3	319	4.7	1,886
15–19	1,591	3.4	304	4.5	1,895
20–24	1,542	6.2	300	4.4	1,842
25–29	1,779	7.1	398	5.8	2,177
30–34	1,293	5.2	369	5.4	1,662
35–39	1,519	6.1	408	6.0	1,927
40–44	1,439	5.8	448	6.6	1,887
45–49	1,423	5.7	457	6.7	1,880
50–54	1,365	5.5	548	8.0	1,913
55–59	1,323	5.3	544	8.0	1,867
60–64	1,226	4.9	591	8.7	1,817
65–69	1,250	5.0	626	9.2	1,876
70–74	1,326	5.3	501	7.4	1,827
75–79	1,410	5.7	134	2.0	1,752
80–90	1,537	6.2	153	2.3	1,690
Region					
North	5,029	20.1	1,357	19.9	6,386
Mid-West	4,957	19.9	1,211	17.8	6,168
Mid-East	4,825	19.3	1,469	21.6	6,294
South-West	5,060	20.3	1,248	18.3	6,308
South-East	5,096	20.4	1,528	22.4	6,624
Urbanization degree					
High (large cities)	6,038	24.2	1,319	19.4	7,357
Middle (moderate cities)	7,670	30.7	2,101	30.8	9,771
Low (village to countryside)	11,259	45.1	3,393	49.8	14,652
Ethnic background					
Dutch	18,598	74.5	5,996	88.0	24,594
Non-Dutch Western	2,389	9.6	512	7.5	2,901
Non-Western	3,964	15.9	305	4.5	4,269

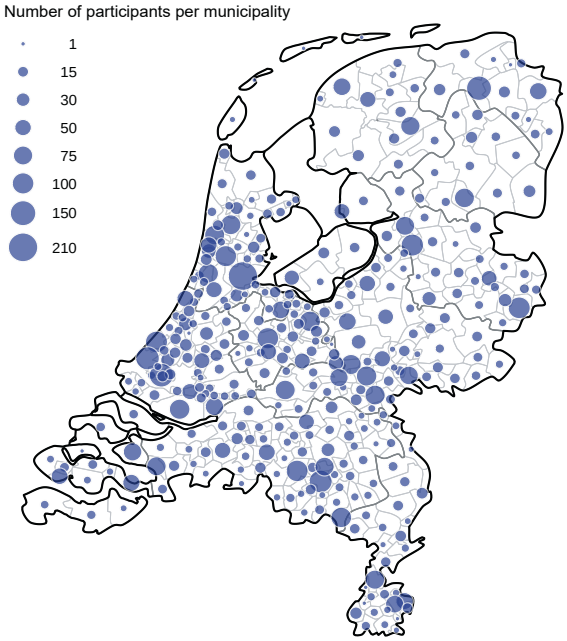
Missing: ethnic background *n* = 16.

4. Data and immunoassay

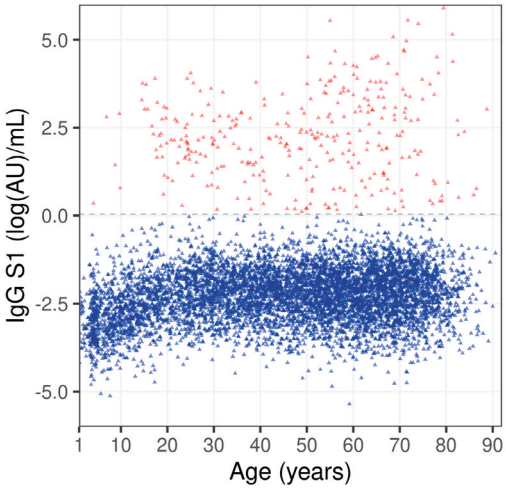
Supplement Figure S1 shows the regional distribution of participants in the Netherlands, and *Supplement Figure S2* depicts the individual antibody concentration by age. Participants' fingerstick blood samples were centrifuged at the RIVM laboratory and serum was stored at –20 degrees Celsius awaiting analyses. Using a validated fluorescent bead-based immune assay [1], which was improved recently [7]), concentrations of IgG antibodies to the SARS-CoV-2 spike S1 protein (Wuhan isolate, GenBank YP-009724390.1) were measured. More specifically, serum samples were diluted 1:200 and 1:8,000 and incubated with spike S1-coupled beads in SM01 buffer (Surmodics, USA) supplemented with 2% FCS while shaking (600 rpm) at room temperature for 45 minutes. Hereafter, plates were washed three times (with PBS), incubated with PE-conjugated anti-human IgG (Jackson ImmunoResearch Laboratories) and incubated for an additional 30 minutes. After final washing steps, samples were acquired on a LX200 or FlexMap3D (using Luminex technology). Concentrations were interpolated from an in-house reference consisting of pooled sera using a 5-parameter logistic fit.
For the mixture modelling analyses below, we included a validation panel that has been used for validation of the assay [1]. Specifically, a set of 384 pre-pandemic samples comprising participants from the PIENTER-2 (2006/2007) and PIENTER-3 (2016/2017) cohorts (representative of the Dutch population) as well as a panel of cases with influenza-like illness, and a set of 115 proven SARS-CoV-2 infections covering asymptomatic and mild to severe cases [1]. Mean and standard deviation of the (log-transformed) measurements were $\mu_{\text{uninfected}} = -2.3$ (arbitrary units (AU)/mL) and $\sigma_{\text{uninfected}} = 1.0$ for the uninfected group, and $\mu_{\text{infected}} = 3.0$ and $\sigma_{\text{infected}} = 2.1$ for the infected group.

5. Mixture model

Survey participants are assumed to be either seropositive or seronegative. These two classes were characterized by distributions for antibody measurements, denoted by f_{neg} and f_{pos} and specified by parameters $\boldsymbol{\theta}_{\text{neg}}$ and $\boldsymbol{\theta}_{\text{pos}}$. The mixing parameter (probability of seropositivity) depends on age and is denoted by $p(a)$. For $n = 6{,}813$ participants, the set of participant ages and observed measurements were given by $\mathbf{a} = (a_k)$ $\mathbf{x} = (x_k)$ $(k = 1, \ldots, n)$, respectively. Throughout we used normal distributions for the components of the mixture of the log-transformed data, so that $\boldsymbol{\theta}_{\text{neg}} = (\mu_{\text{neg}}, \sigma_{\text{neg}})$ and $\boldsymbol{\theta}_{\text{pos}} = (\mu_{\text{pos}}, \sigma_{\text{pos}})$, while the mixing parameter was modelled with a Bayesian penalized-spline using cubic basis functions and first order penalization [8, 9]. Throughout, we considered the age range [0, 100] years, placing knots at 10-year intervals (11 knots in total), so that the total number of basis functions was 13 [8, 9].



Supplement Figure S1. Regional distribution of participants. Notice that the western part of the Netherlands is the most densely populated area and also has large number of samples, thus attaining good population coverage.



Supplement Figure S2. Overview of the data. Shown are (log-transformed) antibody concentrations of all 6,813 samples in the national sample as function of age. Here, samples are classified as seronegative below the cut-off of 0.04 (log(arbitrary units (AU))/mL) (blue) and as seropositive above the cut-off (red).

6. Estimation

Parameters were estimated in a Bayesian framework using Hamiltonian Monte Carlo, implemented in Stan [10]. To improve performance at low prevalence, we employed a logistic transformation for the age-specific prevalence.

Prior distributions for the means and standard deviations of the seronegative and seropositive components were based on the uninfected and infected samples from the validation set as described before. As the uninfected set is obtained from random samples from the Dutch population in 2006/2007 and 2016/2017 as well as a panel comprising cases with influenza-like illness, and the seropositive set contained mostly cases with symptoms and may be less representative of cases in the population, we took informative prior distributions for the parameters of the seronegative component, a weakly informative prior distribution for the mean of the seropositive component, and provided no explicit prior distribution for the standard deviation of the seropositive component. Specifically, we took

$$\mu_{\text{neg}} \sim N\,(\mu_{\text{uninfected}}, 0.01)$$
$$\sigma_{\text{neg}} \sim N\,(\sigma_{\text{uninfected}}, 0.1)$$
$$\mu_{\text{pos}} \sim N\,(\mu_{\text{infected}}, 0.5)$$

For the spline smoothing parameter (RW_{var}) we took an inverse gamma distribution [9],

$$\text{RW}_{\text{var}} \sim \text{inverse gamma}\,(1, 0.0005),$$

and for the weights of the spline base functions w_i ($i = 1 \ldots 13$), we took

$$w_i \sim N\,(0, 4),$$

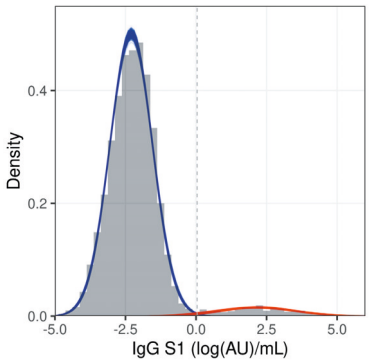
where it should be noted that the prior weights were defined on the logistic scale.

Supplement Table S2. Parameter estimates (selected posterior quantiles) with selected convergence diagnostics.

Parameter	$\widehat{R}$	n_{eff}	2.5%	50%	97.5%
μ_{neg}	0.997	1071	-2.311	-2.297	-2.284
σ_{neg}	0.997	964	0.742	0.756	0.770
μ_{pos}	0.996	1066	1.967	2.168	2.336
σ_{pos}	1.003	1126	1.1216	1.339	1.501
RW_{var}	1.000	1030	0.008	0.042	0.169

Estimates for the parameters defining the mixing distribution and the spline smoothing parameter are given in *Supplement Table S2*, together with convergence diagnostics $\hat{R}$ and n_{eff} [10]. In a sensitivity analysis we have re-run the fitting procedure with uninformative prior distributions (only assuming that $\mu_{pos} > \mu_{neg}$). These analyses yield virtually identical results (not shown).

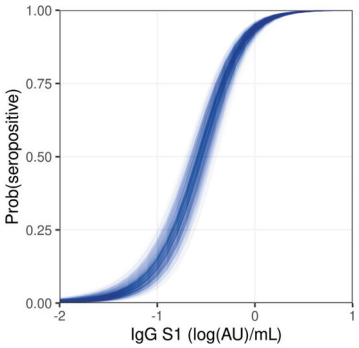
Supplement Figure S3 gives a visualization of the data (gray histograms) and model fit (colored lines), suggesting good agreement between the two. Notice also that over-lap between the negative and positive component is small which bodes well for efforts to distinguish seronegative from seropositive samples. To further investigate the implications of the analyses, *Supplement Figure S4* shows the estimated probability of infection as function of antibody concentration. Here, the probability of infection calculated as the estimated positive density (at a certain concentration) divided by the sum of the positive and negative densities (at that concentration) [2]. The figure shows that, in the absence of information on age-specific prevalence, the estimated probability of infection is close to 0 for concentrations of −1 (log(AU)/mL) and lower, and close to 1 at concentrations of 0 (log(AU)/mL) and higher.



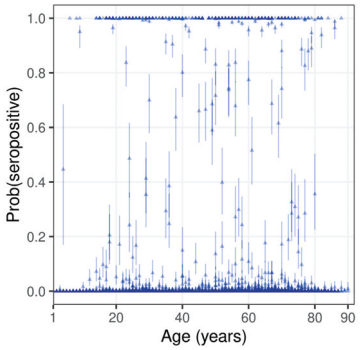
Supplement Figure S3. Data and model fit. Shown are the data (gray histograms) and fit of the mixture model (blue: seronegative component; red: seropositive component). The age-specific prevalence was modelled with a penalized spline, and the mixing distributions were weighted with the overall posterior probability of infection. Shown are 1,000 samples from the posterior distribution.

In a next step we estimated the probability of seropositivity for each of the *n* = 6,813 samples. Here we weighted the posterior seropositive density by the posterior prevalence, and the posterior seronegative density by 1 minus the posterior prevalence, and applied the same procedure as in *Supplement Figure S4*. The figure shows that for the majority of samples (6,722), the posterior median for the probability of infection is either low (< 0.05, 6,437 samples) or high 0.95, 285 samples), indicating that only for a small minority of samples (< 100) classification would not be straightforward. This is

a robust result that also holds when using less informative priors or when including a random effect at the municipality level (not shown). It is due to the clear separation of the negative and positive components in the analyses (*Supplement Figure S5*).



Supplement Figure S4. Estimated probability of seropositivity. Shown are estimated probabilities of seropositivity as function of the (log-transformed) antibody concentration. No weighting for prevalence was applied. Shown are 1; 000 samples from the posterior distribution.

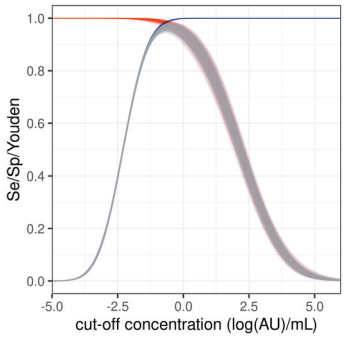


Supplement Figure S5. Estimated probability of seropositivity. Shown are estimated prob-abilities of seropositivity for each of the 6, 813 samples as function of age. Esti-mates were weighted with age-specific prevalence. Dots and whiskers represent posterior medians and 95% credible intervals, respectively. Notice that the posterior probability of seropositivity (i.e. posterior median) is either very low (< 0.05) or very high (> 0.95) for the majority of samples (> 98%).

7. Binary classification

The above results show that for the majority of samples there is limited uncertainty as to whether they should be classified as seronegative or seropositive. Therefore, we feel confident that reliable binary classification of the samples is feasible. Here, we investigated the optimal cut-off value for such binary classification, and associated test characteristics (sensitivity and specificity).

For a given cut-off, the proportion of the negative distribution with concentrations higher than the cut-off defines specificity of the test (high proportion implies low specificity), while the proportion of the positive distribution with concentrations lower than the cut-off defines sensitivity of the test. Technically, both sensitivity and specificity are calculated using cumulative density functions of the negative (specificity) and positive distributions (sensitivity) [2]. *Supplement Figure S6* shows the test characteristics and the Youden index (*Se* + *Sp* − 1) as function of the cut-off. For low values of the cut-off, sensitivity of the test is high, at the price of a low specificity. Conversely, at high values of the cut-off, specificity of the test is high, at the price of low sensitivity. At intermediate values both sensitivity and specificity are reasonably high, and the Youden index is maximal.



Supplement Figure S6. Sensitivity, specificity, and Youden index. Shown are the estimated sensitivity (red), specificity (blue), and Youden index (gray, superposed on top of sensitivity and specificity) as function of the cut-off concentration for seropositivity. Shown are 1,000 samples from the posterior distribution.

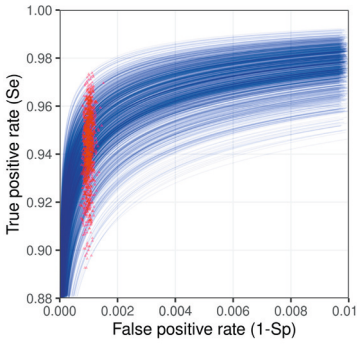
In *Supplement Table S3* we show test characteristics for two specific scenarios. The first takes cut-offs that maximize the Youden index. Here, the estimated optimal cut-off was -0.56 (95% CrI -0.67–-0.44) and the estimated maximal Youden index was 0.97 (94% CrI 0.95–0.98). This cut-off, however, is not useful in practice as expected seroprevalence is low (< 10%), and control of the false positive rate is more important than control of the false negative rate. Therefore, in a second scenario we aimed at a specificity of 0.999. Such specificity can be reached with the test, at a cut-off of 0.04 and a sensitivity of 0.943. In the following and in the main text we have opted for a cut-off of 0.04.

Supplement Table S3. Test characteristics for cut-off that maximizes the Youden index or that selects for high test specificity (*Sp* = 0.999). Shown are posterior medians with 95% credible intervals (CrI).

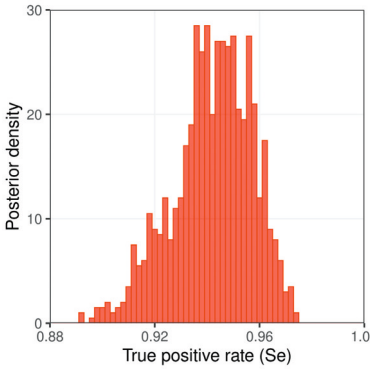
Scenario	cut-off (95% CrI)	Se (95% CrI)	Sp (95% CrI)	Youden (95% CrI)
Youden	0.56 (-0.67–-0.44)	0.979 (0.965–0.987)	0.989 (0.985– 0.993)	0.97 (0.95–0.98)
Sp	0.04 (0.0–0.08)	0.943 (0.910– 0.966)	0.999	0.94 (0.91–0.97)

Supplement Figure S7 presents the results of a Receiver Operating Characteristic (ROC) diagram (blue lines), together with true and false positive rates at the cut-off of 0.04 (red dots). Variation in the false positive rate was minimal (*Sp* = 0.9990 (95% CrI 0.9987–0.9992)), while estimated sensitivity was still high (*Se* = 0.944 (95% CrI: 0.910–0.967)). Estimated Youden index is 0.94 (95% CrI: 0.91–0.97).

Finally, *Supplement Figure S8* shows the posterior distribution of test sensitivity at a cut-off of 0.04 (log(AU)/mL). Mean and standard deviation of the distribution are 0.942 and 0.0151, respectively. These values can be incorporated in Rogan-Gladen-type corrections for estimating true prevalence from observed apparent prevalence in binary classification [11, 12].



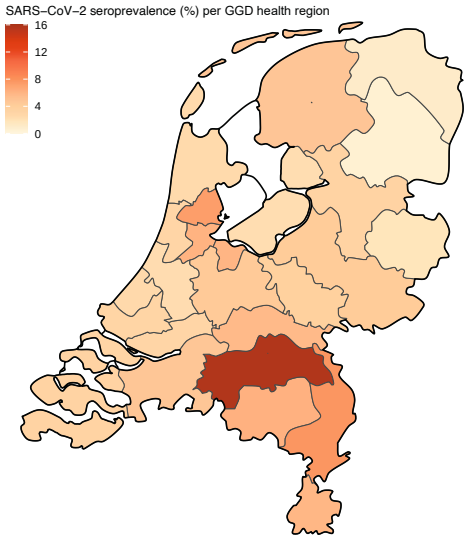
Supplement Figure S7. Receiver Operator Characteristic (ROC) diagram. Shown are the false positive rates (1 − *Sp*) and true positive rates (*Se*) for 1, 000 samples from the posterior distribution (blue). Also shown are the false and true positive rates for cut-off of 0.04 log(AU)/mL, i.e., 1.04 AU/mL (red).



Supplement Figure S8. Posterior distribution of the true positive rate (sensitivity) when the cut-off is set at 0.04 log(AU)/mL, i.e., 1.04 AU/mL. Shown is a histogram of 1, 000 samples from the posterior distribution. Mean and standard deviation of the distribution are 0.942 and 0.0151, respectively.

8. Regional seroprevalence and risk factor analysis

Supplement Figure S9 shows the regional weighted seroprevalence estimates, i.e., by Municipal Health Service (GGD) region. Further, the manuscript provides main results and interpretation of the analyses with random-effects logistic regression using the binary classification described in the above. *Supplement Table S4* provides detailed results of the main risk factor analysis (*n* = 6,331, these results were similar after applying both backward and forward selection), including age-specific estimates of the unadjusted odds ratios for seropositivity derived from the univariable model (*Supplement Figure S10*). Finally, *Supplement Table S5* shows the results of the multivariable models derived from the sensitivity analyses as described in the manuscript: Model 1 - without the variable 'being religious', *n* = 6,487; Model 2 - without the variable 'educational level', *n* = 6,339; and Model 3 - without contact data (i.e., nature of close contacts as well as total number), *n* = 6,338.



Supplement Figure S9. Weighted seroprevalence by Municipal Health Service (GGD) region.

Supplement Table S4. Main risk factor analysis (*n* = 6,331).

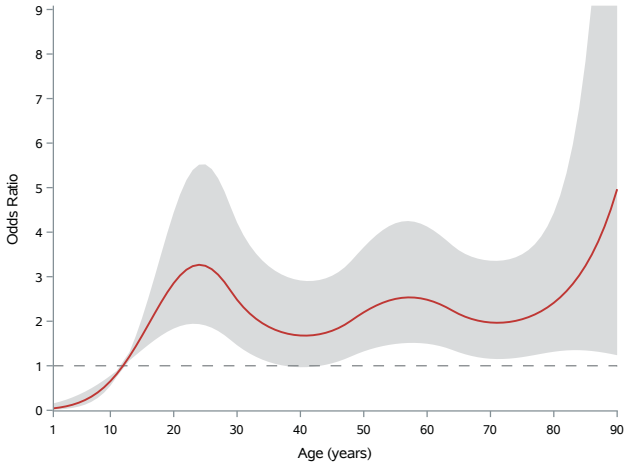
Risk factor	Univariable Model			Multivariable Model		
	OR	***(95% CI)***	***p value***	***aOR***	***95% CI***	***p value***
Nature of close contact, yesterday			**0.0053**			**0.0752**
No close contact	Ref.			Ref.		
< 50% with persons < 10y	**1.38**	**(1.07–1.79)**		**1.36**	**(1.04–1.78)**	
50–100% with persons < 10y	0.72	(0.43–1.23)		1.35	(0.78–2.35)	
Attended indoor meeting(s) with > 20 persons			**0.0008**			**0.0047**
No	Ref.			Ref.		
Yes	**1.54**	**(1.20–1.98)**		**1.46**	**(1.12–1.89)**	
Visited a nursing home			**< 0.0001**			**0.0009**
No	Ref.			Ref.		
Yes, 1-5 times	1.35	(0.85–2.15)		1.09	(0.68–1.75)	
Yes, ≥ 6 times	**2.14**	**(1.09–4.19)**		1.72	(0.86–3.44)	
Nursing home worker	**4.44**	**(2.31–8.53)**		**3.72**	**(1.90–7.27)**	
Household size			**0.0697**			**0.0667**
Single-person	Ref.			Ref.		
Two-person	**1.64**	**(1.03–2.61)**		**1.64**	**(1.02–2.63)**	
Three or more persons	1.35	(0.85–2.15)		**1.79**	**(1.09–2.95)**	
Age (spline)			**0.0002**			**0.0002**
Region			**< 0.0001**			**< 0.0001**
North	Ref.			Ref.		
Mid-West	**1.75**	**(1.01–3.04)**		**2.01**	**(1.18–3.42)**	
Mid-East	**2.03**	**(1.23–3.37)**		**2.18**	**(1.35–3.53)**	
South-West	1.35	(0.77–2.35)		1.51	(0.88–2.61)	
South-East	**4.21**	**(2.63–6.74)**		**4.28**	**(2.73–6.72)**	

Supplement Table S4. (Continued)

Risk factor	Univariable Model			Multivariable Model		
	OR	*(95% CI)*	*p value*	*aOR*	*95% CI*	*p value*
Urbanization degree			**0.0013**			**0.0197**
High (large cities)	1.07	(0.63–1.81)		1.23	(0.77–1.96)	
Middle (moderate cities)	Ref.			Ref.		
Low (village to countryside)	**1.81**	**(1.27–2.58)**		**1.59**	**(1.14–2.20)**	
Educational level			**0.0048**			**0.0216**
High	Ref.			Ref.		
Middle	**1.56**	**(1.19–2.03)**		**1.45**	**(1.11–1.91)**	
Low	1.21	(0.87–1.68)		1.09	(0.77–1.54)	
Nr. of close contacts, yesterday			**0.0928**			
0–1	Ref.					
2–4	1.17	(0.88–1.57)				
5–9	**1.50**	**(1.07–2.11)**				
≥ 10	0.95	(0.66–1.38)				
Had been working from home, last week			**0.0013**			
No	**1.40**	**(1.04–1.90)**				
Yes/partly	Ref.					
No job (including < 15y)	0.84	(0.63–1.14)				
Contact profession/voluntary work with clients/patients			**0.0001**			
No	Ref.					
Yes	**1.62**	**(1.26–2.08)**				

Supplement Table S4. (Continued)

Risk factor	Univariable Model			Multivariable Model		
	OR	***(95% CI)***	***p value***	***aOR***	***95% CI***	***p value***
Contact profession/voluntary work with children			0.1830			
No	Ref.					
Yes	1.27	(0.89–1.80)				
Healthcare worker			**0.0003**			
No	Ref.					
Yes	**1.77**	**(1.30–2.42)**				
Works in a pub/restaurant/café			**0.0612**			
No	Ref.					
Yes	1.96	(0.97–3.95)				
Sex			0.4839			
Men	Ref.					
Women	1.09	(0.86–1.38)				
Ethnic background			**0.1082**			
Dutch	Ref.					
non-Dutch Western	0.67	(0.40–1.14)				
non-Western	0.54	(0.25–1.12)				
Religious			**0.0075**			
No	Ref.					
Yes	**1.39**	**(1.09–1.76)**				



Supplement Figure S10. Estimates of the unadjusted odd ratios for seropositivity as function of age (see main text for adjusted odds ratios). The estimate is based on random-effects univariable logistic regression. Also shown is the 95% confidence envelope. Reference age is 12 years (odds ratio = 1).

Supplement Table S5. Sensitivity analyses.

Risk factor	Model 1 *n* = 6,487			Model 2 *n* = 6,339			Model 3 *n* = 6,338		
	OR	***(95% CI)***	***p value***	***OR***	***(95% CI)***	***p value***	***OR***	***(95% CI)***	***p value***
Nature of close contact, yesterday			**0.0849**			**0.068**			NA
No close contact	Ref.			Ref.			NA		
< 50% with persons < 10y	**1.35**	**(1.03–1.76)**		**1.37**	**(1.05–1.79)**		NA	NA	
50–100% with persons < 10y	1.30	(0.75–2.26)		1.35	(0.77–2.34)		NA	NA	
Attended indoor meeting(s) with > 20 persons			**0.005**			**0.0047**			**0.0028**
No	Ref.			Ref.			Ref.		
Yes	**1.45**	**(1.12–1.88)**		**1.45**	**(1.12–1.89)**		**1.49**	**1.15–1.93)**	
Visited a nursing home			**0.0012**			**0.0004**			**0.0006**
No	Ref.			Ref.			Ref.		
Yes, 1–5 times	1.14	(0.71–1.81)		1.10	(0.69–1.77)		1.11	(0.69–1.78)	
Yes, ≥ 6 times	1.70	(0.85–3.40)		1.80	(0.90–3.59)		1.75	(0.87–3.49)	
Nursing home worker	**3.61**	**(1.85–7.03)**		**3.94**	**(2.02–7.69)**		**3.83**	**(1.96–7.49)**	
Household size			**0.0657**			**0.069**			**0.0777**
Single-person	Ref.			Ref.			Ref.		
Two-person	**1.67**	**(1.04–2.68)**		**1.63**	**(1.02–2.62)**		**1.62**	**(1.01–2.60)**	
Three or more persons	**1.78**	**(1.08–2.92)**		**1.79**	**(1.09–2.94)**		**1.76**	**(1.07–2.90)**	
Age (spline)			**0.0003**			**0.0003**			**0.0004**
Region			**< 0.0001**			**< 0.0001**			**< 0.0001**
North	Ref.			Ref.			Ref.		
Mid-West	**1.98**	**(1.16–3.37)**		**1.99**	**(1.17–3.39)**		**2.01**	**(1.18–3.42)**	
Mid-East	**2.14**	**(1.32–3.46)**		**2.13**	**(1.32–3.43)**		**2.16**	**(1.34–3.50)**	
South-West	1.52	(0.88–2.60)		1.50	(0.88–2.58)		1.50	(0.87–2.58)	
South-East	**4.18**	**(2.67–6.55)**		**4.20**	**(2.68–6.57)**		**4.25**	**(2.71–6.65)**	

Supplement Table S5. (Continued)

Risk factor	Model 1 *n* = 6,487			Model 2 *n* = 6,339			Model 3 *n* = 6,338		
	OR	***(95% CI)***	***p value***	***OR***	***(95% CI)***	***p value***	***OR***	***(95% CI)***	***p value***
Urbanization degree			**0.0181**			**0.0115**			**0.0156**
High (large cities)	1.22	(0.76–1.95)		1.20	(0.75–1.90)		1.23	(0.77–1.95)	
Middle (moderate cities)	Ref.			Ref.			Ref.		
Low (village to countryside)	**1.59**	**(1.15–2.21)**		**1.62**	**(1.17–2.24)**		**1.60**	**(1.16–2.22)**	
Educational level			**0.0275**			NA			**0.0199**
High	Ref.			NA			Ref.		
Middle	**1.43**	**(1.09–1.88)**		NA	NA		**1.46**	**(1.11–1.92)**	
Low	1.07	(0.76–1.52)		NA	NA		1.09	(0.77–1.55)	

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CHAPTER 9

Persistence of antibodies to severe acute respiratory syndrome coronavirus 2 in relation to symptoms in a nationwide prospective study

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ABSTRACT

Background

Assessing the duration of immunity following infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a first priority to gauge the degree of protection following infection. Such knowledge is lacking, especially in the general population. Here, we studied changes in immunoglobulin isotype seropositivity and immunoglobulin G (IgG) binding strength of SARS-CoV-2-specific serum antibodies up to seven months following onset of symptoms in a nationwide sample.

Methods

Participants from a prospective representative serological study in the Netherlands were included based on IgG seroconversion to the spike S1 protein of SARS-CoV-2 (n = 353), with up to three consecutive serum samples per seroconverted participant (n = 738). Immunoglobulin M (IgM), immunoglobulin A (IgA), and IgG antibody concentrations to S1, and increase in IgG avidity in relation to time since onset of disease symptoms, were determined.

Results

While SARS-CoV-2-specific IgM and IgA antibodies declined rapidly after the first month after disease onset, specific IgG was still present in 92% (95% confidence interval (CI) 89–95) of the participants after seven months. The estimated 2-fold decrease of IgG antibodies was 158 days (95% CI 136–189). Concentrations were sustained better in persons reporting significant symptoms compared to asymptomatic persons or those with mild upper respiratory complaints only. Similarly, avidity of IgG antibodies for symptomatic persons showed a steeper increase over time compared with persons with mild or no symptoms (p = 0.022).

Conclusions

SARS-CoV-2-specific IgG antibodies persist and show increasing avidity over time, indicative of underlying immune maturation. These data support development of immune memory against SARS-CoV-2, providing insight into protection of the general unvaccinated part of the population.

INTRODUCTION

The persistence of specific antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), is as of yet not fully understood, partly because the follow-up time of studies investigating antibody kinetics is short owing to the novelty of the disease. Multiple studies show seroconversion to specific proteins following recent infection with SARS-CoV-2 [1-12]. Concurrently, studies report on the decay of antibodies over time, which raises the concern to what degree infected persons may remain protected to reinfection [4, 6, 8, 9, 11]. In addition, rapid decay of these antibodies would make seroprevalence estimates more difficult to interpret later after infection.

Specific antibodies are produced in different isotypes. Following most infections, immunoglobulin M (IgM) production is rapidly upregulated after infection and subsequently declines quickly [13-15]. Specific immunoglobulin A (IgA) and immunoglobulin G (IgG) antibodies typically are initiated later than IgM production. In blood, IgG is the dominant circulating antibody isotype, whereas at mucosal surfaces, including the respiratory tract, IgA antibodies are more dominant [16]. The reported decay of SARS-CoV-2 antibodies will likely differ per isotype, necessitating detailed analyses of the distribution of different antibody isotypes over longer periods of time. The presence of antibodies longer after infection, and rapid upregulation of antibody secretion following reinfection, depends on the presence of B-cell memory. Memory B cells are responsible for the induction of high-quality antibodies that are produced after class switching from IgM to IgG and require editing of the specificity of the antibody to provide an increased fit and binding strength of antibodies, collectively referred to as avidity maturation [17]. Hence, stronger avidity of antibodies is expected to be associated with an underlying cellular response, immune memory, and better ability to confer protection against future infection [18]. In addition to memory B cells, long-lived plasma cells contribute to the secretion of antibodies that can be detected multiple months and even years after an infection [19].

Specifically, spike S1-specific antibodies may neutralize the virus [1-3, 7, 20], for which reason many vaccines aim to induce immunity to this part of the virus [21]. Understanding of anti-spike antibody kinetics over prolonged periods of time is therefore of crucial importance [1, 5, 22, 23]. Very recent reports describe the presence of antibodies for ≥ 6 months after infection in specific populations such as healthcare workers or hospitalized patients [24, 25]. The duration of the antibody responses in the general population with generally mild symptoms however, has received little attention thus far.

Using samples of seroconverted individuals (n = 353) from the nationwide prospective PIENTER-Corona (PICO) serosurveillance study covering all ages, we studied the decay in SARS-CoV-2 spike S1-specific IgM, IgA, and IgG antibodies over a period of seven months after infection, and investigated the effect of COVID-19-related symptoms on antibody

concentrations. In addition, we studied the development of avidity of anti-SARS-CoV-2 spike S1 IgG antibodies as a marker of underlying cellular immunity and functionality of detected antibodies.

METHODS

Study participants

Participants from the PICO-serosurvey (design and inclusion are described in [7, 26, 27]) were requested to return a self-collected finger-prick blood sample in a microtainer (Sarstedt) by mail [7]. Participants were invited for a first round (PICO1) in April 2020 and for consecutive donations in June 2020 (PICO2) and October 2020 (PICO3). In the PICO2 round, the study was extended with an additional nationwide random sample [28]. Three hundred sixty-five participants seropositive for IgG to SARS-CoV-2 spike S1 were available; symptom data were missing for 12 (3.3%) participants, so 353 were included in the present study. Since we aimed to study antibodies in the general population, no other exclusion criteria were applied. Every study round, participants were asked to complete a questionnaire to collect type and date of onset of COVID-19–related symptoms data. The study was ethically approved by the Medical Research Ethics Committees United MEC-U and registered under trial number NL8473 (https://www.trialregister.nl/trial/8473). The study was performed in accordance with the Declaration of Helsinki (2008), and all participants provided written informed consent.

Laboratory analyses

Finger-prick blood samples were centrifuged and serum stored at –20°C until analyses. The concentrations of IgG antibodies to SARS-CoV-2 spike S1 (Wuhan isolate, GenBank accession number YP_009724390.1) were determined using a fluorescent bead-based immune assay as published previously [12], which was further improved recently (*Supplementary Figure 1*). The assay selectively discriminates between antibodies to SARS-CoV-2 and the four known coronaviruses OC43, HKU-1, NL63, and 229E [12]. The specificity (99.7%) and sensitivity (91.6%) of the assay were determined using a heterogeneous sample including asymptomatic and mild to severe COVID-19 cases as representative of COVID-19 cases in the general population. Since previous publication, the assay was extended to detect IgM and IgA antibodies to spike S1 (*Supplementary Figure 2*). Thresholds for seropositivity were determined based on receiver operating characteristic curve (ROC) analysis maximizing specificity and set at 1.20 arbitrary units (AU)/mL for IgM, 0.50 AU/mL for IgA, and 1.04 AU/mL for IgG.

Serum samples were diluted 1:200 and 1:8,000 and incubated with spike S1-coupled beads in SM01 buffer (Surmodics, Eden Prairie, Minnesota) supplemented with 2% fetal calf serum while shaking (600 rpm) at room temperature for 45 minutes. Next, plates were washed three times in phosphate-buffered saline, incubated with phycoerythrin-conjugated anti-human IgG (Jackson ImmunoResearch Laboratories), IgA (Southern Biotech), or IgM (Jackson ImmunoResearch Laboratories) and incubated for an additional 30 minutes. Samples were washed and acquired on a LX200 or FlexMap3D (Luminex). Concentrations were interpolated from an in-house reference consisting of pooled sera using a 5-parameter logistic fit. The coefficient of variation between independent assay runs ranges from 13.3 to 17.6.

Avidity of anti-spike S1 IgG was performed on 73 samples of randomly selected participants with varying concentrations of IgG by testing samples within the linear range of detection in the absence or presence of 1.1 M of the chaotropic agent ammonium-thiocyanate [29, 30]. This concentration was confirmed to provide an optimal balance in discriminating antibodies of low and high avidity. Avidity is expressed as percentage of binding remaining when ammonium-thiocyanate is added.

Statistical analyses

All statistical analyses were performed in R version 4.0.2 [31]. Participants with fever, dyspnea, muscle ache, extreme tiredness, general malaise, painful respiration, joint pain, diarrhea, and/or stomach ache were considered symptomatic for COVID-19. Asymptomatic participants and participants with mild upper respiratory tract complaints only (runny nose, sore throat, anosmia/ageusia, headache) were grouped together since these symptoms suggest contained, nonprogressive infection. Sera of 365 participants were available, of which 12 were excluded because symptom data were missing.

Days since onset of symptoms for symptomatic and mildly symptomatic participants was defined as the number of days between symptom onset and the blood collection date. For asymptomatic participants, the mean number of days since onset of symptoms of symptomatic persons was used as a surrogate measure to calculate their days since infection. To show seropositivity over time, time since onset of symptoms was categorized into month 1 (0–30 days) — the period of induction of antibody production — and subsequently in months 2–3 (31–92 days), months 4–5 (93–152 days), and ≥ 6 months (> 152 days).

To study the change in the antibody concentrations and IgG avidity over time, antibody concentrations (AU/mL) were natural log-transformed and modeled separately. For each isotype, participants were included based on evidence of seroconversion to exclude persons who did not convert for IgM or IgA to influence decay rates (*Supplementary Table 1*). For IgG avidity, all available data were used. Generalized

Estimating Equations with an exchangeable correlation structure was used to take into account correlation due to repeated sampling (using *geepack version 1.3.1* [32-34]). We selected the model with exponential decay over time if it resulted in a decrease in QIC (quasi-likelihood under independence model criterion) of at least 2 compared to a model with a linear change over time [35]. Hereafter, age, sex, days since onset of symptoms, presence and duration of symptoms, and an interaction term between days since onset of symptoms and symptoms were included in the model as potential predictor variables. Age and duration of symptoms were dichotomized at their median (i.e., ≥ 50 vs. ≤ 49 years of age and ≥ 11 vs. ≤ 10 days, respectively). Variables with $p < 0.10$ in univariable analyses were included in the multivariable model. Backwards selection was performed manually, excluding variables one-by-one with $p > 0.05$. Reported p values are from model coefficients. The 2-fold decrease of IgG antibodies was calculated using the slope estimate and its 95% confidence interval (CI) (i.e., -log 2/slope) [29].

RESULTS

Description of the study population

Sera of 353 participants with specific IgG antibodies to spike S1 were available for analysis (*Figure 1A*). In total, 738 samples of these participants were analyzed, which are shown relative to date of onset of symptoms in *Figure 1B*.

The majority of participants reported a date of onset of symptoms that was close to the peak of the first wave of COVID-19 infections in the Netherlands [36]. Of the 353 participants, 214 reported symptoms and 139 reported no (n = 77) or only very mild (n = 62) upper respiratory tract symptoms (*Table 1*). The median age was 48 years (interquartile range (IQR), 30–61 years) and 51 years (IQR, 32–66 years) for symptomatic and asymptomatic/mildly symptomatic persons, respectively. Of the symptomatic and asymptomatic/mildly symptomatic participants, 60% and 53%, respectively, were female. The most frequently reported symptoms were headache (67%), coughing (63%), fever (57%), muscle ache (52%), and general malaise (49%), while 35% reported dyspnea. Forty percent of those from the symptomatic participant group visited the general practitioner and 2% were admitted to the hospital.

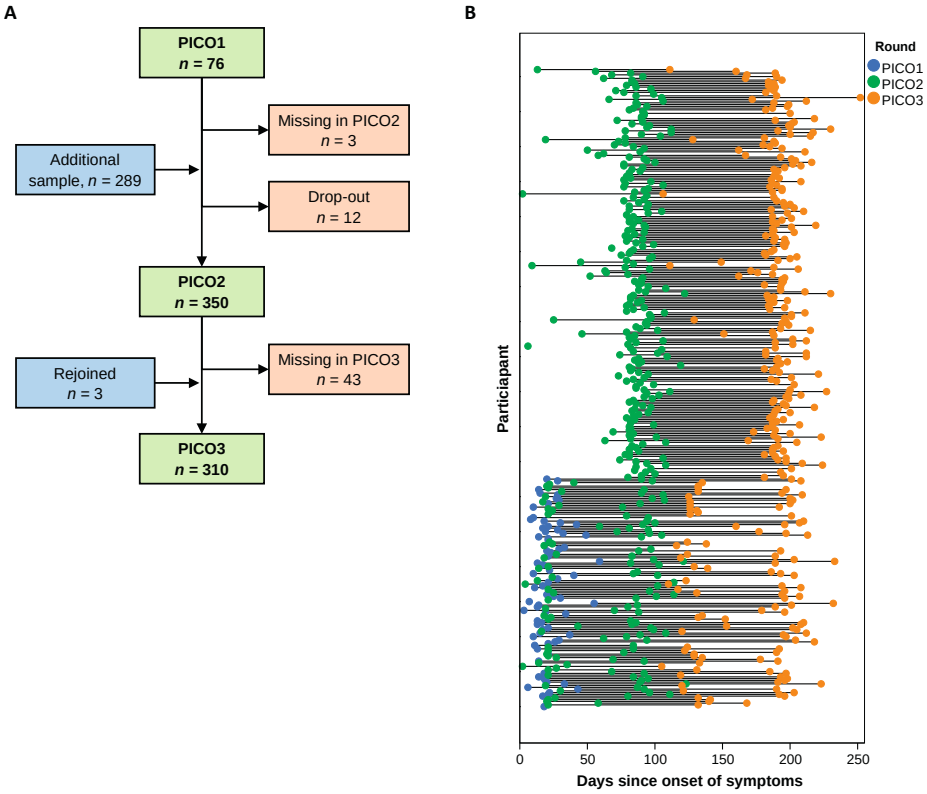

Figure 1. A. Flow diagram of number of participants throughout the study. **B.** The availability of consecutive samples from the three PIENTER-Corona (PICO) rounds relative time since onset of disease to days since onset of symptoms (x-axis). Each line represents a participant, with the dot indicating the days since onset of disease and the lines the availability of consecutive samples.

Seropositivity to IgM, IgA, and IgG anti-spike S1

The majority of individuals had anti-spike S1 IgM (64%) and IgA (62%) antibodies in the first month after SARS-CoV-2 IgG seroconversion (*Figure 2A*). The proportion of IgM- and IgA-positive participants decreased after the first month to approximately 50% at 2–3 months after onset of symptoms. After six months since onset of symptoms, 33% (95% CI 28–39) and 37% (95% CI 31–43) remained positive for IgM and IgA, respectively. In the first month, 99% of the participants were IgG positive, which increased to 100% in months 2–3. After six months, 92% (95% CI 89–95) were still positive for IgG.

Table 1. Characteristics of seroconverted individuals.

	Symptomatic *n* **= 214**	**Asymptomatic / only mild symptoms** *n* **= 139**
Symptoms, *n* (%)		
Runny nose	103 (48%)	23 (17%)
Sore throat	79 (37%)	15 (11%)
Cough	135 (63%)	27 (19%)
Ageusia/anosmia	98 (46%)	18 (13%)
Headache	144 (67%)	20 (14%)
Fever	133 (57%)	NA[a]
Dyspnea	74 (35%)	NA[a]
Muscle ache	112 (52%)	NA[a]
Extreme Fatigue	73 (34%)	NA[a]
Painful respiration	34 (16%)	NA[a]
Diarrhea	61 (29%)	NA[a]
Joint pain	52 (24%)	NA[a]
Stomach ache	44 (21%)	NA[a]
General malaise	104 (49%)	NA[a]
No symptoms	NA	77 (56%)
Age, years, median (IQR)	48 (30–61)	51 (32–66)
Male sex, *n* (%)	85 (40%)	65 (47%)
Median duration of symptoms[b], days (IQR)	11 (6–18)	6 (2–9)

[a] Participants with these symptoms are included in the 'symptomatic' group and therefore shown as 'NA' in the 'asymptomatic/only mild respiratory symptoms' group.
[b] Data on the duration of symptoms were available for 153 participants in the symptomatic group and 26 participants in the asymptomatic/only mild upper respiratory symptoms group.
Abbreviations: *IQR*, interquartile range; *NA*, not applicable.

Seropositivity in relation to symptoms

Symptomatic individuals were more frequently positive for IgM or IgA in the first month after SARS-CoV-2 IgG seroconversion (*Figure 2B* and *2C*; *Supplementary Table 2A*) compared with asymptomatic/mildly symptomatic persons. This difference gradually decreased over time, though it was still present after six months with 10% and 14% more symptomatic participants being positive for IgM and IgA, respectively, compared with asymptomatic/mildly symptomatic persons. IgG anti-spike S1 seropositivity was observed regardless of COVID-19 symptoms. However, after 6 months, the individuals who had turned negative for IgG were mostly asymptomatic/mildly symptomatic: 87% positive for asymptomatic/mildly symptomatic persons vs. 95% positive for symptomatic persons (*Figure 2D*; *Supplementary Table 2A*).

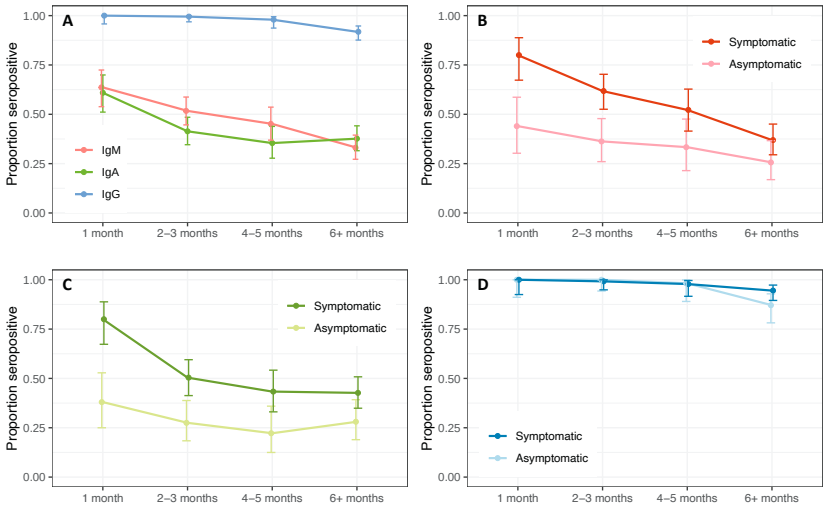

Figure 2. A. The proportion of immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (IgG) and 95% confidence intervals (CIs) of positive samples in relation to months since onset of symptoms. The proportion of individuals positive for IgM (**B**), IgA (**C**), and IgG (**D**) with symptoms, or with mild or no symptoms.

Concentrations of anti-spike S1 antibodies over time in relation to symptoms

Among persons who seroconverted to spike S1 IgM (*n* = 86), IgM concentrations showed a linear decline over time and initially were higher in symptomatic persons than asymptomatic/mildly symptomatic persons, but were similar from two months post onset of symptoms onward (*Figure 3A*; *Supplementary Table 2B*). The average concentration of IgM decreased to the threshold for seropositivity after around 150 days. Among persons who seroconverted to spike S1 IgA (*n* = 82), IgA concentrations showed an exponential decrease over time (*Figure 3B*). The presence of symptoms resulted in higher IgA concentrations (*Supplementary Tables 2B*, *2C*, and *3*). Average IgA concentration reached the threshold concentration after around 140 days. IgG concentrations showed a linear decrease over time, and symptomatic persons had significantly higher concentrations (*Figure 3C*; *Supplementary Table 2B*). The average concentrations of IgG did not intersect the threshold value for seropositivity within the studied time frame of seven months after onset of symptoms. IgM and IgA antibody concentrations over time for the entire study population — including those who did not seroconvert to IgM and IgA in the first 60 days following symptom onset — are shown in *Supplementary Table 2C*. IgG and IgA, but not IgM, levels were higher in males and persons older than 50 years (*Supplementary Table 3*). In addition, duration of symptoms for longer than ten days resulted in increased IgG levels.

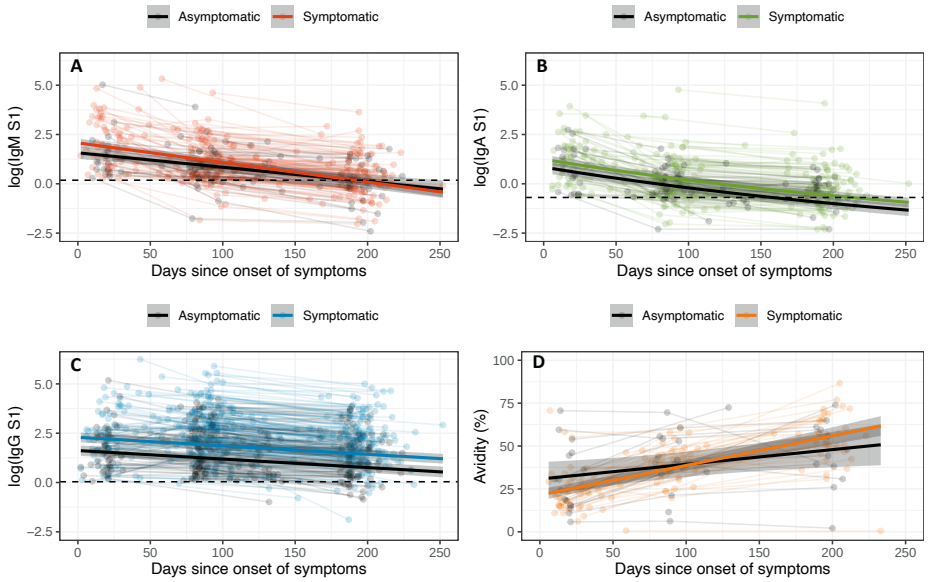

Figure 3. The concentrations of immunoglobulin M (IgM, **A**), immunoglobulin A (IgA, **B**), and immunoglobulin G (IgG, **C**) are shown relative to days since onset of symptoms for individuals having symptoms (colored lines) or those without or only mild symptoms (black lines). **D**. Development of IgG avidity for persons with or without symptoms. Data were fitted using generalized estimating equations and show 95% confidence intervals (CIs) around the fit, with an exponential decay over time for IgA and a linear relationship for IgM, IgG, and avidity. The fit was adjusted for age, sex, symptoms, and the duration of symptoms where appropriate (see *Supplementary Table 3).* For IgM, univariable regression analysis did not show an association between symptoms and IgM levels (i.e., $p > 0.10$; see Methods) but results by group are shown here for consistency. Transparent dots and connected lines represent (repeated) measures per individual.

Decrease in concentration and avidity maturation of IgG anti-spike S1

Since IgG antibodies persist, we calculated the 2-fold decrease and measured avidity for IgG. The 2-fold decrease of IgG concentrations, corrected for age, sex symptoms, and duration of symptoms, was estimated to be 158 days (95% CI 136–189 days). In addition to the duration of IgG in serum, we assessed the maturation of IgG to spike S1 by assessing the avidity. The avidity index of spike S1-specific IgG antibodies increased > 2-fold during the seven months after onset of symptoms ($p < 0.015$; *Figure 3D*). Symptomatic individuals showed a stronger increase over time than asymptomatic/mildly symptomatic individuals ($p = 0.022$; *Supplementary Table 3*).

DISCUSSION

In light of the urgent question of the duration of immunity to SARS-CoV-2 following infection in the general population, we systematically studied the dynamics in seropositivity and concentrations of IgM, IgA, and IgG antibodies to the SARS-CoV-2 spike S1 protein among cases with different symptom profiles and investigated IgG maturation over time. Our data confirm that antibodies decline rapidly in the case of IgM and IgA isotypes. In contrast, 87% of the asymptomatic/mildly symptomatic and 95% of the symptomatic participants remained positive for IgG seven months after onset of COVID-19 symptoms. Moreover, the estimated 2-fold decrease in concentration of 158 days and the increasing avidity of anti-spike IgG antibodies indicate the presence of memory B cells and/or long-lived plasma cells.

We showed that IgM and IgA antibodies start to decay within a few months after onset of symptoms, which may help explain the decline in seropositivity in some studies [6, 11, 13-15]. Since IgG antibodies persist much longer than IgM and IgA antibodies, the detection of IgG provides better sensitivity longer after infection, and therefore, IgG should be the isotype of choice in studies aiming to assess seroprevalence > 2 months after the infection and in longitudinal studies. IgG may also be the most informative for identifying memory induction, since specific IgG antibody development requires multiple cell divisions and class-switch recombination, processes that are a hallmark of memory formation. The hallmarks of memory formation — IgG antibodies with high avidity and persistence of antibodies — are presented in this study. The 2-fold decrease of IgG estimated in this study was 5- to 6-fold longer than the decay of passively transferred maternal antibodies [29, 37, 38]. This decrease rate may still be underestimated since the decay of antibodies is the most pronounced in the first months after the induction of the antibodies. Therefore, longer follow-up studies should reassess the persistence of antibodies to spike S1 of SARS-CoV-2 and compare these to persistence as observed for other viruses [39, 40].

The formation of B-cell memory implies that antibodies can be rapidly upregulated in response to reinfection in order to effectively control the virus [18, 41]. It is still unknown which antibody levels confer protection against reinfection or COVID-19 disease. While the antibodies detected in this study are restricted to spike S1, we cannot exclude the detection of antibodies not necessarily contributing to virus neutralization. In light of newly emerging strains with mutations that may escape neutralization by antibodies, the cross-protection by preexisting immunity, either through infection or vaccination, needs to be closely monitored. Interestingly, having had COVID-19-like symptoms resulted in higher antibody concentrations for IgG and IgM and faster development of IgG avidity, compared with persons who remained asymptomatic/mildly symptomatic after SARS-CoV-2 infection. The reason for this may be a stronger

inflammatory response, a higher or longer viral replication period, or both, that may result in better and longer-lasting immunity.

This study is unique in analyzing samples collected in the general population including all ages and COVID-19 disease severities. While the findings reflect SARS-CoV-2 antibody dynamics of the general public, the study has several limitations. Participants were included based on IgG anti-spike S1 seropositivity, and therefore we may have missed a few persons who seroconverted for IgM or IgA, but not, or insufficiently, for IgG. The time since onset of COVID-19 was based on self-reported symptoms on a presumed SARS-CoV-2 infection, and therefore may be less accurate since symptoms could be caused by other infections still prevailing during the peak of the epidemic. However, the reported date of onset of symptoms of the participants matched the national epidemiological data of COVID-19 cases in the Netherlands [36]. In addition, the paired samples of seroconverted individuals collected six months apart confirm that IgG antibodies persist for > 6 months in 92% of seroconverted individuals [42]. Despite the persistence of IgG antibodies, the decay cannot be neglected and will eventually result in an underestimation of the proportion of infected persons in the population once this proportion has crossed the cutoff levels of specific antibody detection.

In conclusion, our analyses included 353 individuals participating in a nationwide population study with seven months' follow-up for most participants, which is a substantially longer follow-up period than most other population studies [3, 10]. We show that anti-SARS-CoV-2 spike S1 IgG antibodies persist for an extended time (i.e., > 6 months). Therefore, we propose that analysis of IgG anti-spike S1 of SARS-CoV-2 will generate the most consistent seroprevalence estimates and provide understanding of the duration of protective immunity. In view of an IgG decay rate 5- to 6-fold slower than reported for passively transferred maternal IgG and the improving IgG avidity over time, B-cell memory is likely established in most individuals. In addition, our data suggest that the duration of the IgG response is likely longer for symptomatic COVID-19 cases due to higher initial concentrations. Our results aid the interpretation of the duration of immunity in unvaccinated persons and provide a framework for the evaluation of immunity induced by vaccines for SARS-CoV-2.

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SUPPLEMENTARY MATERIALS

Supplement Table S1. Number of included participants and repeated samples for estimates of antibody decay per immunoglobulin isotype.

Symptoms	Isotype	Total	One sample	Two samples	Three samples
Asymptomatic or only upper respiratory					
	IgM	58	5	44	9
	IgA	44	6	30	8
	IgG	139	13	113	13
	Avidity	18	2	5	11
Symptomatic					
	IgM	135	17	84	34
	IgA	119	15	71	33
	IgG	214	27	149	38
	Avidity	55	11	11	33

Supplement Table S2A. Seropositivity over time and in relation to having symptoms (*n*, %).

Months		**1**			**2–3**			**4–5**			**≥ 6**		
Symptoms		***No***	***Yes***	***p value***	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***
IgM	Negative	28 (54.9%)	12 (20.0%)		51 (63.7%)	47 (38.2%)		36 (66.7%)	43 (47.8%)		61 (74.4%)	100 (63.7%)	
	Positive	23 (45.1%)	48 (80.0%)	< 0.001	29 (36.3%)	76 (61.8%)	< 0.001	18 (33.3%)	47 (52.2%)	0.027	21 (25.6%)	57 (36.3%)	0.094
IgA	Negative	31 (60.8%)	12 (20.0%)		58 (72.5%)	62 (50.4%)		42 (77.8%)	53 (58.9%)		59 (72.0%)	90 (57.3%)	
	Positive	20 (39.2%)	48 (80.0%)	< 0.001	22 (27.5%)	61 (49.6%)	0.002	12 (22.2%)	37 (41.1%)	0.021	23 (28.0%)	67 (42.7%)	0.027
IgG	Negative	1 (2.0%)				1 (0.8%)		1 (1.8%)	2 (2.2%)		12 (12.9%)	9 (5.5%)	
	Positive	50 (98.0%)	60 (100%)	*	80 (100%)	122 (99.2%)	*	54 (98.2%)	90 (97.8%)	0.883	81 (87.1%)	155 (94.5%)	0.037

p values were calculated using two-sided Chi-square tests.
* No Chi-square test results could be obtained.

Supplement Table S2B. Geometric mean concentrations (AU/mL) and avidity (%) (with 95% confidence intervals) over time and in relation to symptoms of participants seropositive for IgM and IgA.

Months	**1**			**2–3**			**4–5**			**≥ 6**		
Symptoms	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***
IgM	4.10 (2.54–6.61) *n* = 22	7.87 (5.59–11.08) *n* = 48	0.020	2.45 (1.73–3.47) *n* = 32	4.36 (3.38–5.61) *n* = 81	0.007	2.27 (1.61–3.19) *n* = 25	2.39 (1.85–3.08) *n* = 55	0.805	1.16 (0.84–1.58) *n* = 38	1.49 (1.22–1.82) *n* = 103	0.1
IgA	2.22 (1.32–3.74) *n* = 19	3.50 (2.52–4.84) *n* = 48	0.117	1.00 (0.71–1.41) *n* = 25	1.40 (1.07–1.83) *n* = 69	0.076	0.82 (0.54–1.22) *n* = 18	1.15 (0.82–1.62) *n* = 47	0.165	0.68 (0.51–0.89) *n* = 28	0.79 (0.64–0.98) *n* = 92	0.349
IgG	5.54 (4.06–7.55) *n* = 50	9.28 (6.8–12.66) *n* = 60	0.017	5.40 (4.22–6.93) *n* = 80	14.19 (11.32–17.78) *n* = 123	< 0.001	5.20 (3.82–7.07) *n* = 55	11.03 (8.35–14.57) *n* = 92	< 0.001	3.87 (3.05–4.89) *n* = 93	7.47 (6.19–9.00) *n* = 164	< 0.001
Avidity, %	25 (18–37) *n* = 16	22 (19–26) *n* = 41	0.207	30 (16–56) *n* = 9	28 (21–37) *n* = 32	0.507	46 (37–57) *n* = 8	41 (37–46) *n* = 22	0.272	36 (20–66) *n* = 12	48 (37–63) *n* = 37	0.131

p values, from generalized estimating equations (GEE), testing the effect of symptoms from within timepoint using log-transformed data, are shown. Participants for IgG concentration and avidity data are identical between *Supplement Table S2B* and *S2C* due to the selection criterium to include participants that are positive for IgG.

Supplement Table S2C. Geometric mean concentrations (AU/mL) and avidity (%) (with 95% confidence intervals) over time and in relation to symptoms of all participants.

Months	**1**			**2–3**			**4–5**			**≥ 6**		
Symptoms	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***	***No***	***Yes***	***p value***
IgM	1.16 (0.77–1.75) *n* = 50	4.13 (2.65–6.46) *n* = 60	< 0.001	0.83 (0.63–1.08) *n* = 80	2.15 (1.68–2.77) *n* = 123	< 0.001	0.82 (0.59–1.14) *n* = 54	1.25 (0.97–1.60) *n* = 90	0.042	0.59 (0.47–0.74) *n* = 82	0.92 (0.77–1.10) *n* = 157	0.002
IgA	0.41 (0.25–0.65) *n* = 50	1.88 (1.24–2.86) *n* = 60	< 0.001	0.28 (0.21–0.36) *n* = 80	0.60 (0.47–0.76) *n* = 123	< 0.001	0.26 (0.19–0.36) *n* = 54	0.47 (0.35–0.62) *n* = 90	0.007	0.24 (0.18–0.31) *n* = 82	0.42 (0.35–0.51) *n* = 157	< 0.001
IgG	5.54 (4.06–7.55) *n* = 50	9.28 (6.8–12.66) *n* = 60	0.017	5.40 (4.22–6.93) *n* = 80	14.19 (11.32–17.78) *n* = 123	< 0.001	5.20 (3.82–7.07) *n* = 55	11.03 (8.35–14.57) *n* = 92	< 0.001	3.87 (3.05–4.89) *n* = 93	7.47 (6.19–9.00) *n* = 164	< 0.001
Avidity, %	25 (18–37) *n* = 16	22 (19–26) *n* = 41	0.207	30 (16–56) *n* = 9	28 (21–37) *n* = 32	0.507	46 (37–57) *n* = 8	41 (37–46) *n* = 22	0.272	36 (20–66) *n* = 12	48 (37–63) *n* = 37	0.131

p values from generalized estimating equations (GEE), testing the effect of symptoms from within timepoint using log-transformed data, are shown. Participants for IgG concentration and avidity data are identical between *Supplement Table S2B* and *S2C* due to the selection criterium to include participants that are positive for IgG.

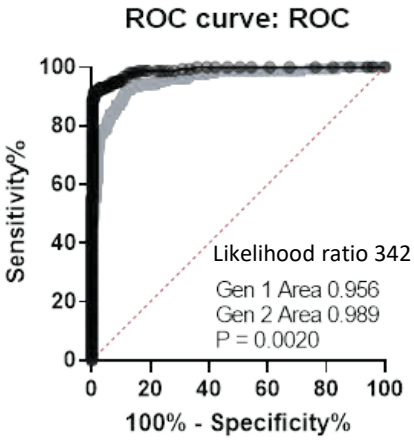
Supplement Table S3. Univariable and multivariable Generalized Estimating Equations (GEE) regression models for antibody concentration to SARS-CoV-2 spike S1 per isotype.

		Univariable model		Multivariable model	
	n/total	***Coefficient***	***p value***	***Coefficient***	***p value***
IgG					
Time[a]	NA	-0.004	< 0.001	-0.004	< 0.001
Sex: Male	150/353	0.227	0.079	0.230	0.012
Age: 50+ years	174/353	0.390	0.002	0.440	< 0.001
Symptoms: Yes	214/353	0.736	< 0.001	0.671	< 0.001
Duration: ≤ 10 days	169/353	Ref.		Ref.	
11+ days	122/353	0.621	< 0.001	0.395	0.004
Unknown[b]	62/353	0.029	0.870	-0.003	0.986
Time * symptoms	NA	-0.001	0.360		
Time	NA	-0.004	< 0.001		
Symptoms	214/353	0.827	< 0.001		
IgM					
Time[a]	NA	-0.009	< 0.001		
Sex: Male	80/193	-0.009	0.950		
Age: 50+ years	96/193	0.042	0.760		
Symptoms: Yes	135/193	0.306	0.032		
Duration: ≤10 days	88/193	Ref.		Ref.	
11+ days	78/193	0.219	0.140	0.256	0.120
Unknown[b]	27/193	-0.390	0.036	-0.477	0.012
Time * symptoms	NA	-0.002	0.049	-0.003	0.042
Time	NA	-0.007	< 0.001	-0.007	< 0.001
Symptoms	135/193	0.586	0.001	0.503	0.009
IgA					
Exp(-k*time)[a,c]	NA	4.240	< 0.001	4.366	< 0.001
Sex: Male	74/163	0.332	0.019	0.354	0.010
Age: 50+ years	88/163	0.308	0.028	0.570	< 0.001
Symptoms: Yes	119/163	0.280	0.049	0.397	0.005
Duration: ≤ 10 days	74/163	Ref.			
11+ days	67/163	0.009	0.951		
Unknown[b]	22/163	-0.439	0.038		
Exp(-k*time)[b]*** symptoms**	NA	0.818	0.160		
Exp(-k*time)[b]	NA	3.628	< 0.001		
Symptoms	119/163	-0.301	0.510		

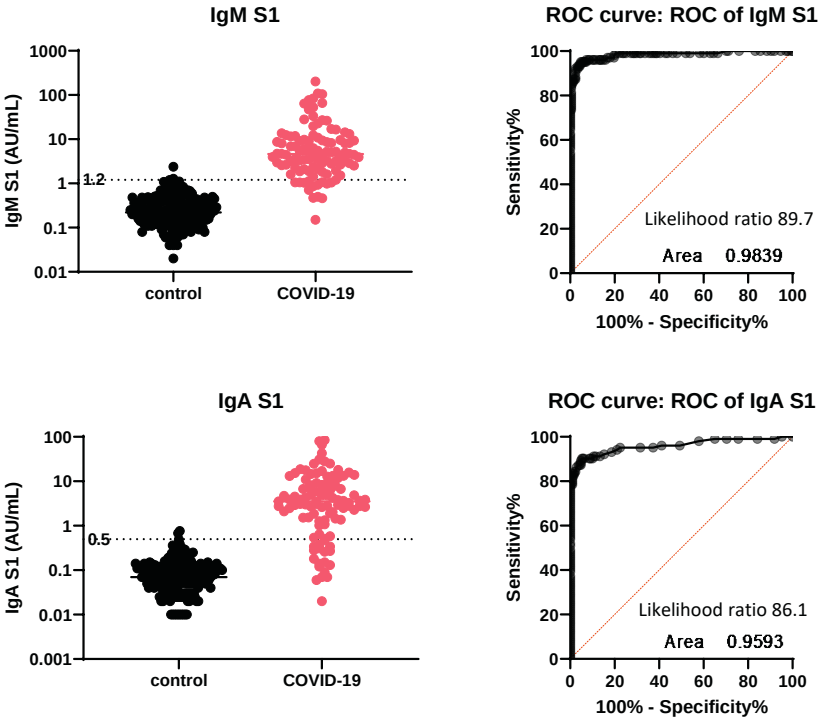
Supplement Table S3. (Continued)

		Univariable model		Multivariable model	
	n/total	***Coefficient***	***p value***	***Coefficient***	***p value***
IgG avidity					
Time[a]	NA	0.150	< 0.001		
Sex: Male	32/73	2.610	0.380		
Age: 50+ years	27/73	-0.508	0.880		
Symptoms: Yes	55/73	-1.350	0.740		
Duration: ≤ 10 days	33/73	Ref.			
11+ days	28/73	-3.650	0.210		
Unknown[b]	12/73	-5.650	0.280		
Time * symptoms	NA	0.087	0.022	0.087	0.022
Time	NA	0.086	0.015	0.086	0.015
Symptoms	55/73	-9.321	0.079	-9.321	0.079

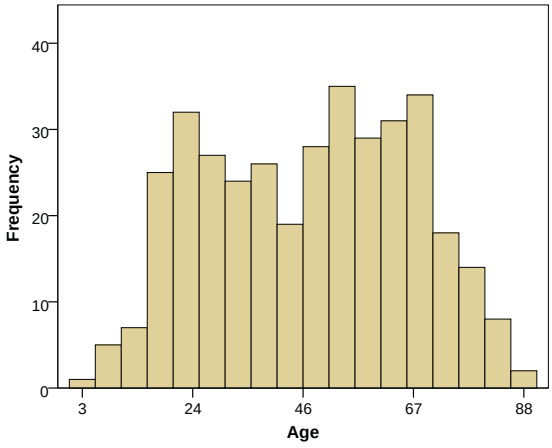
[a] Time is defined as days since onset of symptoms (see Methods).
[b] Data on the duration of symptoms was available for 153 participants in the symptomatic group and 26 participants in the asymptomatic/only mild upper respiratory symptoms group. The duration of symptoms was dichotomized at 11 days or longer or 10 days or shorter. Participants without any symptoms were included in the 10 days or shorter group. Participants with ongoing symptoms at the sampling date were only included if their symptoms persisted for 11 days or longer at the time of sampling: 24/27 with ongoing symptoms in the symptomatic group and 13/15 with ongoing symptoms in the asymptomatic/only mild upper respiratory symptoms group.
[c] For IgA the model for exponential decay over time showed improved fit compared to a model with linear decay over time (see Methods). In the exponential model, the decay constant (*k*) was determined using log(2)/t; where *t* is the time period studied (i.e., 252 days).
Abbreviations: *Ref.*, reference category; *NA*, not applicable.



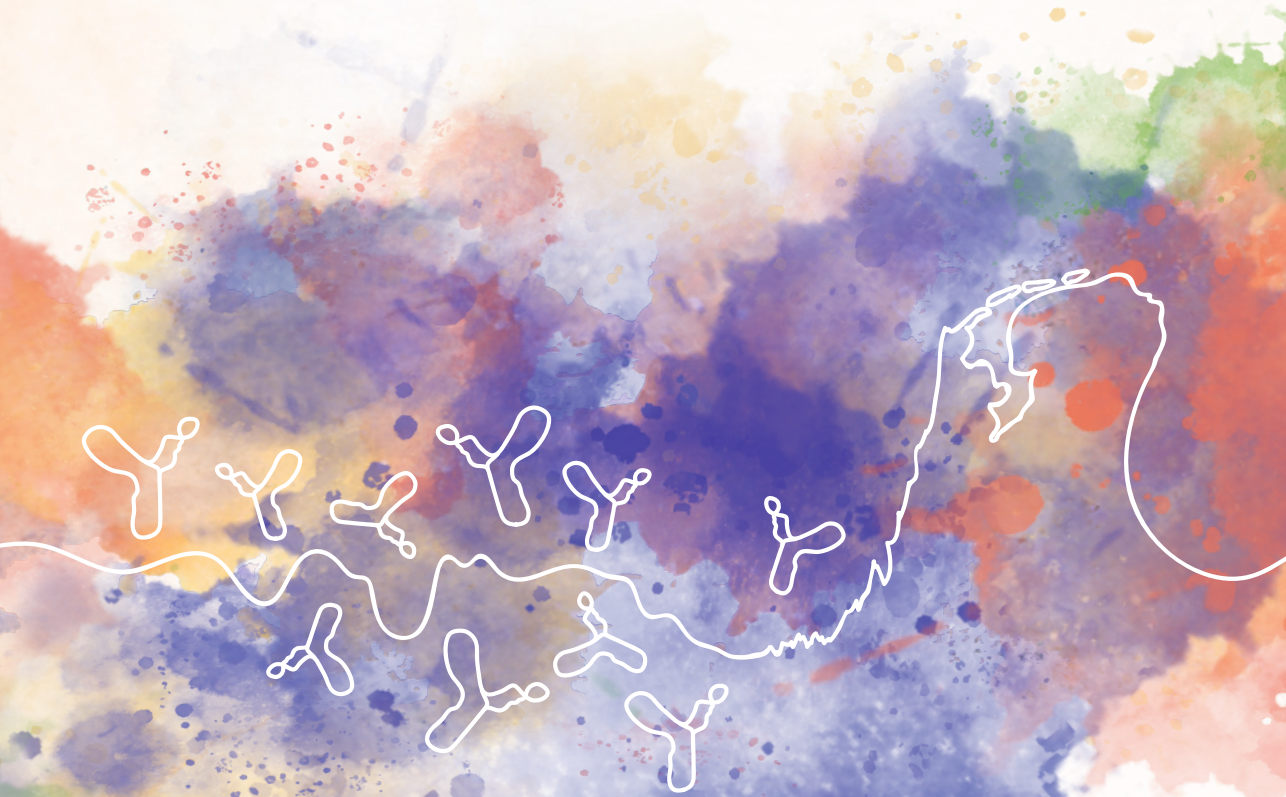
Supplement Figure S1. Improvement of the assay.



Supplement Figure S2. Validation of the detection of IgM and IgA.



Supplement Figure S3. Age distribution of the participants in the study.



CHAPTER 10

Summary

Seroepidemiology is essential in the prevention and control of infectious diseases. This surveillance tool complements other tools by assessing the presence of serum antibodies, which provides information on previous exposure to an antigen in a cohort that represents the target population most optimally. Firstly, seroepidemiology is a key pillar in guiding vaccination policy. Consecutive sero-monitoring of the National Immunization Program (NIP) provides insight into immunity against diseases over time that have already been included in the past. Examples of such insights are increased understanding of susceptible pockets, waning immunity, shifts in circulating serotypes, and changes in vaccination uptake to supplement vaccination registries. The ultimate aim is to optimize the NIP for the benefit of the whole population. Secondly, seroepidemiology is valuable in monitoring candidate vaccine-preventable diseases that might require specific attention (in the future), as well as novel pathogens, e.g., during a pandemic. Conventional surveillance tools are usually not able to provide a complete picture of cases due to test restrictions or (differential) -behavior, or because these cases remain unnoticed as a fraction is (generally) asymptomatic. Vaccination may not be available for emerging pathogens (yet), and short-term monitoring is required to enhance our knowledge on the extent of an outbreak, risk factors or longevity of humoral responses (after infection).

Outcomes of seroepidemiological assessments thus provide important input for shaping public health policy in the short, acute phase, as well as the long, more controlled term. In this regard, large population-based serosurveillance studies have been set up in the kingdom of the Netherlands. This thesis provides a unique overview of the multi-applicability of the serosurveillance tool in different phases and settings of infectious disease prevention and control. Below we will summarize the key findings as presented in this thesis, starting with the evaluating of vaccine-preventable diseases in the Caribbean Netherlands (CN) (**part 1**), and thereafter, monitoring the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) epidemic in the Netherlands during the first year of the coronavirus disease 2019 (COVID-19) pandemic (**part 2**).

Part 1: Evaluation of population immunity of vaccine-preventable diseases in Caribbean Netherlands

Since 10 October 2010, Bonaire, St. Eustatius and Saba (together referred to as CN) have become special municipalities within the (European) Netherlands. Public health became the direct responsibility of the Dutch government as a result, including supply, execution and monitoring of the NIP. Although recent data show that vaccine coverage is generally moderate to good in these island populations, it has only been monitored routinely since a few years. Population immunity has never been assessed which complicates insights on potential susceptibility and risk factors. Surveillance mostly

relies on syndromic surveillance, yet potential cases could remain undetected due to a lack of facilities. The Health Study CN was the first serosurveillance study to fill these knowledge gaps in order to support public health policy.

Chapter 2 described the methodology, set up and data collection of the third nationwide population-based serosurvey in the Netherlands (PIENTER-3) – which plays a role in the second part of this thesis – including the Health Study CN in 2017. Similar designs were applied for both studies. Over 10,000 persons (randomly-drawn from the population registries), of which 1,900 in CN, participated at on-site consultations, provided multiple biological samples and completed extensive questionnaires. The study samples were generally a good reflection of the total population, covering a complete age range (0–90 years) and were a fair representation regarding other relevant sociodemographic characteristics. The majority provided consent for a potential follow-up study, and suggestions for improvements in future studies with regards to increasing response rates and overcoming logistical hurdles were addressed.

An ongoing unstable (political) situation in Venezuela since mid-2010s has led to a prolonged humanitarian crisis. Disruption of the health system as a whole has caused large outbreaks of vaccine-preventable diseases. A significant number of Venezuelans have fled to surrounding countries which led to outbreaks of measles and diphtheria and fatalities elsewhere in the Region of the Americas. Our first priority was to assess the population immunity against measles and diphtheria on Bonaire given its location near the coast of Venezuela. **Chapter 3** showed that the population-based sero-protection for measles was suboptimal, especially for those aged < 5 years originating from the Dutch Caribbean islands (and Suriname), and for adolescents from other Latin American countries. Likewise, the proportion of persons with a minimum protective level against diphtheria was rather low (< 80%), especially among women from 30 years and older, and those originating from the Dutch Caribbean islands and Latin America. Health authorities on Aruba, Bonaire and Curaçao, as well as surrounding islands, were advised to be on alert to detect cases early and prevent potential transmission, whereby rapid supply of antitoxins against diphtheria should be facilitated. Vaccination status of refugees (at arrival) should also be verified as soon as possible and vaccination offered if applicable. Risk groups, including those in close contact with refugees, were recommended to update their vaccinations to reduce susceptibility.

Other viral pathogens, such as rubella and mumps, most likely also circulate more frequently in the region given the unprecedented situation in Venezuela. **Chapter 4** provided an in-depth evaluation of measles, mumps and rubella (MMR) on all CN-islands, with focus on identification of susceptible pockets, waning immunity, and exposure (recently and in the pre-vaccination era). First of all, robust antibody responses were seen after MMR-vaccination, and two doses of MMR (vs. one) showed prolonged humoral immunity, underlining the purpose of booster vaccination congruent with other

studies. Overall seroprevalence in CN for measles (94%) was higher than rubella and mumps (both 85%), but notably, all hovering around the levels for herd immunity. In NIP-eligible persons, particularly among those who became CN-residents at an adolescent age, mainly originating from Latin America, seropositivity for all diseases was below 90%. This is especially concerning for a highly transmissible pathogen as measles that necessitates high levels of population immunity to protect the herd. Likewise, although rubella is somewhat less transmissible than measles, the observed susceptibility also requires close (direct) monitoring of women of childbearing age to prevent Congenital Rubella Syndrome (CRS). This should preferably be prevented by vaccination at an earlier stage, hence an additional catch-up vaccination moment at adolescent age (for all missed NIP-vaccinations) should be considered. Our data further suggested circulation of mumps among young adults on Bonaire (but not on St. Eustatius or Saba at that time) which has not been detected via other means of surveillance. Sensitive disease surveillance is warranted in light of the recent outbreaks of viral pathogens in the region, as well as a sustained high MMR-vaccination coverage, with particular focus on offering vaccination to migrants at arrival if applicable. High uptake is ultimately required to meet the World Health Organization (WHO) elimination goals for measles and rubella. We further observed a relative low seroprevalence for rubella and mumps in elderly who were born on one of the Dutch Caribbean islands or resided there since childhood in a period when routine vaccination had not been introduced. This is indicative of an interesting and typical island epidemiology with reduced circulation of these pathogens in the pre-vaccination era, and contrasts greatly for instance with the Netherlands where seroprevalence in older individuals nears 100%.

In **chapter 5** we covered another viral pathogen that causes a large burden in Caribbean countries: human papillomavirus (HPV). Incidence and mortality due to HPV-related cancer is high in the region, but vaccination has only recently been added to the NIP in CN for young adolescents. Our data showed robust antibody responses against the vaccine types in those vaccinated as well as some cross-reactivity against high-risk non-vaccine types of the virus. Seropositivity among the unvaccinated persons – a marker for cumulative exposure (against one of seven high-risk types investigated here) – was relatively high (34%), and over half them were seropositive for at least two types (HPV16 and -52 primarily). Highest seropositivity rates were observed on St. Eustatius. Strikingly, substantial proportions of previous exposure with hr-HPV types were observed among adults below 60 years of age for both sexes, more specifically over half of the women and nearly one in five men. Other risk factors for seropositivity besides sex and age were predominantly related to increased sexual behavior. These data underline the relevance for a sex-neutral HPV-vaccination program in CN such as recently introduced in the Netherlands, as well as routine cervical screening in adult women.

Varicella-zoster virus (VZV), causative agent of chickenpox and herpes zoster (shingles), has also been of specific interest in CN. VZV has a typical epidemiology in tropical (and more remote) regions: less pronounced seasonality/endemicity, usually resulting in larger fractions of susceptible adolescents and adults, whilst more intense epidemics such as experienced on Saba in 2017. Infection at older age of infection increases the likelihood of varicella-related complications and thus a higher burden of disease (individually, including unborn children, as well as nationwide) relative to temperate climates. In **chapter 6** we showed that the seroprevalence is indeed relatively low in CN (78%), particularly when compared to the Netherlands (95%), especially on St. Eustatius (73%). Most importantly, relative high susceptibility, i.e., the proportion being seronegative, was observed among adolescents (40%) and adults (10–30%), and most noticeable in those who were born in CN or resided there since early childhood. These results have been essential in the decision-making process of introduction of a highly effective childhood VZV-vaccination in the NIP in CN, including a catch-up campaign for those without a history of infection. These data can also be of guidance for other countries in Central America and the Caribbean in their consideration of vaccination as only 20% has implemented this vaccination in their NIP as of yet.

Part 2: Sero-monitoring the SARS-CoV-2 epidemic in the Netherlands

A novel coronavirus (most likely) of zoonotic origin, SARS-CoV-2, emerged in China in late 2019 and disseminated swiftly across the globe due to a completely immune-naive population. Lockdowns and stringent control measures were installed worldwide, including the Netherlands as of mid-March 2020, to curb transmission, reduce cases, and prevent the health system from being overstretched due to an enormous inflow of severely-ill patients. The true extent of the epidemic was unknown as well as the groups foremost infected as testing was restricted to at-risk groups and information on symptomatology (frequency and type) in the general population was uncertain in the beginning. Durability of the humoral immunity after infection, and thus likelihood of re-infection, was also questioned, and necessitated investigation. Hence, to provide (seroepidemiological) insights in the Netherlands as well as globally, and guide policymakers during the course of the pandemic, the longitudinal population-based serosurveillance PIENTER-Corona (PICO) study was set up quickly in this first phase. Information was gathered by means of home/self-collected (fingerprick) blood samples, using seropositivity against the spike S1 antigen as a marker of past infection, and serology was linked to extensive risk factor questionnaires.

Chapter 7 described the primary insights at the peak of the first SARS-CoV-2 wave in the Netherlands in the beginning of April 2020. The overall seroprevalence in the Dutch population was very low (~3%) despite the enormous pressure on the health

system. Equivalent to half a million persons infected, this was 30 times higher than the number of cases officially reported, but demonstrated that severity of disease was substantial given the relative large number of severe cases and deaths. Seroprevalence estimates were highest among 18–39 year-old adults and lowest in children < 18 years. Orthodox-Reformed Protestants, who generally have a low uptake of NIP-vaccinations and reside socio-geographically clustered from the Southwest to Northeast part of the country (a region referred to as the 'Bible belt'), had among the highest odds of being infected (four times higher as compared to the rest). The standard COVID-19 case definition by the European Centre for Disease Prevention and Control (ECDC) was met by 75% of the seropositive participants. Interestingly, we showed that anosmia/ageusia was the most discriminative symptom between cases and controls on a population level, and adding this symptom to the case description could therefore contribute to improved disease recognition. We also observed that immunoglobulin (Ig)G antibody concentrations in seropositive persons were significantly higher in those with systemic symptoms like fever and dyspnea, which suggested that severity of disease had impact on the strength of the humoral response and potentially its longevity.

The first lockdown in the Netherlands lasted until May 2020 and included stringent social distancing measures similar to most countries worldwide. In **chapter 8** we investigated the effects of some of these measures on infection by using data from the (2nd round of the) PICO-study aiming to inform and support global decisionmakers for potential waves of SARS-CoV-2 infections to come. Firstly, still only 4.5% of the Dutch population had been infected after the first wave, with low urbanized areas and the Southeastern part of the country hit hardest – potentially due to Carnaval festivities that may have functioned as super spread events. Nursing home workers had also been infected relatively frequently, which requires specific attention with regards to (personal) protection to prevent infection as well as transmission whilst working with the most vulnerable persons. Most outstandingly, we provided evidence for the effectiveness of physical distancing (> 1.5m) and indoor group size reductions on risk of infection as those with less adherence to these measures had higher odds of infection. Young adults were shown to have the highest rates of infection and could have played a considerable role in viral dissemination, especially since they are among the age groups with the highest frequency of contacts. Contrarily, these data suggested a diminished role for young children < 12 years of age during circulation of wild-type SARS-CoV-2. Seroprevalence rates were very low in these children despite the fact that close contact with adults had not been restricted during the lockdown, and persons reporting to have had close contact with young children foremost (e.g., at work, schools, etc.) also did not have higher odds of infection as compared to those without contacts.

Knowledge on the persistence and functionality (avidity) of antibodies after a SARS-CoV-2 infection among persons in the general population (who generally experience

mild to moderate symptoms) was lacking in the beginning of the pandemic. This is important information as it might provide relevant information for the degree of protection against future infection and/or (severe) disease, and may offer insights on a response after vaccination – which was investigated concurrently in clinical trials already in the beginning of the first pandemic year. **Chapter 9** provided results of this analysis using consecutive samples obtained in the first three rounds of the PICO-study (April, June, September 2020). Serum IgM and IgA antibodies against the spike S1 antigen declined relatively rapid the first month post infection with only 50% seropositive at 2–3 months, whereas IgG was present in most participants (> 90%) up to seven months. Hence, the longevity of seropositivity over time (i.e., sensitivity) in combination with being highly specific made spike S1 IgG the preferred marker for seroprevalence studies, in relative contrast to antibodies targeting Nucleoprotein – a more preserved antigen in the core of the virus. Participants that reported substantial (and longer duration of) symptoms – particularly men and those aged > 50 years – displayed higher initial and more sustained antibody concentrations, and also showed a stronger increase of avidity of IgG antibodies than mild or asymptomatic persons over time. All in all, this strongly indicated development of immune memory and thus maturation of the response, which probably contributes to conferring protection against disease (with a similar antigen) in the future – especially in symptomatic individuals – as spike S1-specific antibodies are vital in neutralizing SARS-CoV-2.

Conclusions and future perspective

Following the presentation of the main results in this chapter (**chapter 10**), the public health implications of our seroepidemiological findings in the kingdom of the Netherlands during various epidemiological stages are discussed in **chapter 11**. In short, seroepidemiology can be of utmost value in identifying group at-risk (in terms of susceptibility or past infection) and timely adapting or expanding the NIP. The tool can be applied during phases of alert, e.g., in case of (regional) pathogenic threats, but also in the longer run, e.g., in reacting to a changing climate, global migration or in relation to vaccine uptake, hesitancy and -equity. Alongside enhancement of its surveillance system, CN will benefit from a regional-driven approach when it comes to introduction, adaptation and implementation of preventive public health measures, and can lead the path for other countries in the Caribbean region. Serological studies will play an important role in evaluating future interventions specifically targeted at the island populations. Seroepidemiology has further demonstrated its practicality and multi-applicability in an acute, pandemic phase during emergence of a novel pathogen, by swiftly providing data and information for decisionmakers on several topics related to (population) immunity. In that perspective, we subsequently reflect on the

importance of accurate and valid population estimates, including serological markers, methodological/statistical correction, survey designs and representation of groups. Recommendations for future areas of research and the role of seroepidemiology is further discussed, such as concerning mucosal immunity, post-COVID syndrome and changing epidemiological patterns of other pathogens during the pandemic. In the post-pandemic/endemic phase it will be paramount to apply and further develop the population-based serological frameworks that have been set up globally during the pandemic in order to remain dynamic and flexible in a fast- and ever-changing world.

CHAPTER 11

General discussion

Surveillance efforts have been deployed for centuries to generate information for action in order to prevent and control infectious diseases and optimize the health of the public [1-3]. In this thesis we had the unique opportunity to apply and investigate the multifaceted utility of one of the main surveillance pillars: seroepidemiology [4-8]. Large nationwide population-based seroepidemiological studies were set up in the kingdom of the Netherlands, including the overseas Caribbean islands Bonaire, St. Eustatius and Saba (Caribbean Netherlands, CN) (**part 1**) as well as the (European) Netherlands (**part 2**), to enhance surveillance during various epidemiological phases [9-11]. This chapter will reflect on the public health implications of our findings during control-, alert- and pandemic phases, incorporate recommendations for areas of future research, and discuss opportunities and challenges of future seroepidemiology.

Seroepidemiology in the control and alert phase

A regular evaluation of population immunity against vaccine-preventable diseases is recommended by the World Health Organization (WHO) [12]. Such information, including identification of risk groups, is valuable for decisionmakers and can lead to timely adaptions of the National Immunization Program (NIP) to prevent the population from potential pathogenic threats and/or swiftly halt transmission upon introduction [8, 13, 14]. Below, we will contemplate on the implications of our seroepidemiological findings in CN during phases of control and alert, and how a regional-driven approach can be beneficial.

Timely adaptations

Elimination of measles requires ongoing efforts to increase vaccination coverage globally [15, 16]. The number of outbreaks are increasing worldwide, and endemic transmission has returned to some regions, e.g., the Americas [17-19]. Overall seropositivity for measles was rather high and similar between the CN-islands, nearing the herd immunity threshold of 95% (**chapter 3** and **4**). However, seropositivity in NIP-eligible inhabitants of Bonaire was < 90% and in children aged < 5 years even below the target level (for elimination) of 85% [20]. For optimal long-term immunity and to prevent interference with maternal antibodies, measles-mumps-rubella (MMR)-1 vaccination is preferred from age 12 months and only suggested between 6–12 months in outbreak situations (MMR-0) [21-23]. The exact timing is an interplay with the epidemiological situation: high-risk countries should offer it as close to 12 months as possible, and those with lower anticipated risk can extend it (e.g., in Iceland MMR-1 is administered at 18 months [24]). Lowering the age of MMR-1 on Bonaire from 14 months to 12 months is thus recommended to reduce susceptibility optimally in this context. This is already in place on the other CN-islands and most Latin American countries, and will ensure no changes in uptake or interference with other vaccines [25, 26]. Ultimately, two-dose measles

vaccination regimen is needed for successful elimination [16, 18]. MMR-2 enhances a persistent immune response (**chapter 4**), besides offering a second opportunity for those who missed the first dose or suffered from primary/secondary vaccine failure. The minimum interval between the first and second dose is four weeks (and for MMR-varicella (MMRV) three months) [25]. Quickly after our seroepidemiological assessment, the public health department of Bonaire decided to lower MMR-2 from nine years to 18 months in 2019 to reduce susceptibility and increase overall population immunity given the regional outbreaks. A swift catch-up campaign for everyone < 9 years to rule-out any potential risk was not initiated, primarily due to limited workforce, yet solely the < 5 year-olds were offered a catch-up at their four-year vaccination moment. Capacity building initiatives and close collaboration within the kingdom remains essential. This applies to the short term, e.g., in controlling outbreaks when epidemiological situations change rapidly, such as during the coronavirus disease 2019 (COVID-19) pandemic, as well as the longer run, particularly considering the potential increased impact of infectious diseases due to climate change that is likely to affect CN first [27-29].

MMR-vaccination coverage data could already shed some light on the impact of the NIP-adaptation in 2019. Recent updates on Bonaire show an uptake of 91% for MMR-1 and 56% for MMR-2, which is lower than the preceding years [26]. This vindicates strict monitoring as the novel stand-alone vaccination moment might be perceived unfavorable and thus be counter-effective. Importantly, coverage of most infant vaccinations tend to have decreased on Bonaire and St. Eustatius lately (not on Saba) [26]. The latest MMR coverage data from Latin America display a similar decreasing trend when compared to pre-pandemic years, with an average uptake of 85% for MMR-1, whilst large difference between countries (from < 60% in Paraguay and Grenada to 100% in Cuba), and of 68% for MMR-2 (e.g., 58% in Suriname and 97% in Mexico) [30]. Drops in uptake are observed in many parts of the world [31, 32]. Reduction could be due to delays in reporting or missed vaccination opportunities resulting from the COVID-19 pandemic, which was also apparent in the Netherlands initially [33]. Several outbreaks and signs of circulation of vaccine-preventable diseases in previously non-endemic areas have been reported worldwide in the aftermath of the pandemic, such as diphtheria and measles in Western Europe and poliovirus in sewer samples in New York and London [19, 34-37]. An increasing universal trend of vaccine hesitancy already started pre-pandemic, but seems to have accelerated, e.g., due to less trust in the government and increased dissemination of misinformation (predominantly via social media) [38-40]. Focus groups and questionnaires are held in the Netherlands to unravel factors associated with potential changes in willingness, knowledge and behavior [41]. These are also warranted for CN as factors might be dissimilar. Enhanced understanding and a multidisciplinary approach, including implementation science, are needed as willingness does not always reflect actual behavior and effectiveness of

interventions can be context-specific. Evaluation of interventions and behavioral change is ultimately required for the success of the program [42-44].

Identification of risk groups

Our seroepidemiological data suggest circulation of mumps in young adults on Bonaire which had not been detected by other surveillance tools (**chapter 4**), and a similar observation for pertussis was reported by Immink and colleagues recently [45]. Vaccine effectiveness against mumps infection tends to wane over time, and persons can be susceptible to infection (although frequently asymptomatic) approximately ten years after vaccination [46-48]. This is particularly the case in populations where persons cluster together, foremost adolescents and young adults, nevertheless effectiveness against (long-term) sequelae remains high, especially among twice vaccinated [49-55]. No clear correlate of protection has been defined for mumps, so identifying person at-risk is complicated [46, 56]. Whilst lowering the age of MMR-2 on Bonaire, it will be important to have sensitive surveillance in place to monitor potential changes in the mumps epidemiology, e.g., a lower age of infection, changes in transmission or disease severity. Countries that offer MMR-2 already in early childhood or have lowered the age do not show consistent epidemiological shifts over time, with the majority of outbreaks among young adults [57]. St. Eustatius and Saba have lowered MMR-2 to the age of four years the past decade and have not reported an increase in mumps cases nor was circulation detected by our serosurveillance data. However, these are smaller and more isolated populations than Bonaire, limiting comparison. Part of the sensitive surveillance should include raising awareness on this topic to general practitioners as the success of the current symptom-based (early-warning) surveillance greatly relies on their indication and whether suspected cases undergo laboratory surveillance.

The WHO has called for greater focus on vaccination of vulnerable groups in their Immunization Agenda 2030 to ensure equitable access [58]. We observed particularly low levels of immunity against measles (70%) among persons who became residents of CN at an adolescent age, i.e., after the regular NIP, who predominantly migrated from (other) Latin American countries, and the same was true for rubella (**chapter 4**). Similarly, minimum protective levels against diphtheria were < 70% in those > 30 years – which is substantially lower than in the Netherlands [59] – and, again, particularly low (~60%) in non-Dutch (women) (**chapter 3**). International migration, in terms of absolute numbers of refugees and labor-related migrants (the majority), has increased the last decade, also exemplified by inflows on the Caribbean islands [60, 61]. Many countries have health assessment programs for refugees and migrants in place as they are generally at increased risk of infection as well as worse disease outcomes, and decreasing traveling time nowadays has elevated the likelihood of introduction of pathogens in the country of arrival [62-64]. (Cost-)effectiveness and feasibility of

active infectious disease screening among these minority populations was evidenced in a recent systematic review by Seedat *et al*., indicating better patients outcomes compared to routine detection, especially when high-risk groups are targeted [63]. Recent research from the United Kingdom (UK) also delineated that large proportions of refugees arrive underimmunised due to vaccine unavailability in their country of origin or increased vaccine hesitancy, which is further complicated by a lack of vaccination administration [65]. Particularly adolescents and adults are more likely to have unrecorded vaccinations. Likewise, a Dutch serosurvey conducted in 2016 among adult refugees predominantly from the Middle-East, showed insufficient protection against several vaccine-preventable diseases, particularly measles, although higher than we observed in CN [66]. Following Dutch guidelines, a youth/consultation physician constructs a vaccination plan for children in the first months after arrival in addition to an overall medical examination [67]. However, our seroepidemiological results clearly underline that there are challenges to reach various groups of migrants with regards to updating their vaccination status. This also corresponds to the low completion rates of treatment and follow-up screening as observed in other countries [63], and justifies enhanced awareness among professionals. We advocate for further exploration with local partners on targeted approaches and potential bottlenecks to effectively screen and timely deliver free-of-charge vaccination (that may have been missed) to protect individuals, sustain herd immunity – including sufficient high population antibody levels – and prevent clustering of susceptible pockets.

Multiple countries, e.g., the United States of America (USA) and Australia, offer catch-up campaigns among adolescents for missed vaccinations [68, 69]. A final catch-up vaccination moment could also be considered in CN to further close the observed immunity gap. This can protect them before leaving the island for study or work (abroad) around adulthood as well as before childbearing age (with respect to MMRV) – especially as the rate of teenage pregnancies is relatively high on the Dutch Caribbean islands [70]. Delivery of vaccination at schools (class-based), in combination with health education, is shown to be the most effective and efficient approach to reach adolescents, hence the current vaccination moment at age 14 years might be appropriate [71-73]. Furthermore, regarding our diphtheria findings, a recent study found that countries with routine adult vaccination did not have a significant decline in diphtheria cases compared to those without, yet only highly vaccinated countries with zero to low circulation had been included, i.e., different from Latin America [74]. Introduction of the maternal Tdap vaccine for pregnant women [26] – to reduce pertussis incidence in infants – can enhance population immunity for diphtheria to some extent, yet updating the vaccination status of adult risk groups may also be considered (**chapter 3**).

During the data collection in CN we noticed that registration of vaccinations differed per island, e.g., via paper vaccination certificates, stand-alone digitized overviews, or

patient files (mostly elderly) at the hospital or general practitioners. Data could also be absent or incomplete, which necessitated using questionnaire data as proxies in analyses. Such registration issues apply to all Dutch Caribbean islands, and since inhabitants move frequently between islands as well as the Netherlands it would be beneficial to have an online secured vaccination database, e.g., as part of the national register (such as 'Praeventis'). This will enable quicker and more accurate analyses on uptake to keep track of risk groups, and could also aid in standardizing invitations and reminders for missed vaccinations [75].

A regional-driven approach

The WHO aims to eliminate cervical cancer worldwide and achieving this requires a high (90%) vaccination uptake – besides setting high targets for screening and early treatment [76]. Recent data from the UK and Sweden have revealed that HPV-vaccination is highly protective against HPV-related cancer (instead of using persistent infections or cervical abnormality (CIN) grades as proxies) [77-79]. Girls-only HPV-vaccination for 9-year-olds has recently been introduced in CN, but consistent with other Latin American countries coverage remains low, and future uptake might also be influenced by the COVID-19 pandemic/vaccination [30, 75, 80]. The burden of HPV-related disease is relatively high in the Caribbean region, similar to other sexual-transmitted infections [81-83]. This was underlined by the high rates of HPV seropositivity, i.e., cumulative incidence, in both CN-men and -women, specifically in those aged < 60 years (**chapter 5**). Adolescents on the Dutch Caribbean islands have a younger age at sexual debut, higher number of sexual partners and unsafe sexual activity as compared to those in the Netherlands [70]. Gender-neutral HPV-vaccination, such as introduced in the Netherlands, has the potential to induce herd protection in the longer run if coverage is high, and is strongly advised for all islands [84]. Strong communication efforts (via multiple means of media and face-to-face) and involvement of key figures providing information unambiguously and tackling misconceptions would be required [71-73]. This can be an effective approach – also applied to inform the public about the Health Study CN (**chapter 2**) – especially since implementation of girls-only HPV-vaccination has already been challenging in CN and adolescents are a difficult group to reach [71, 75]. Interestingly, following Australia in the beginning of 2023 [85] and supported by recommendation of the WHO [86], the UK has recently adjusted their HPV-vaccination schedule to one-dose (for immunocompetent persons) [87] following emerging evidence showing that single vaccination is non-inferior compared to two doses in terms of immunogenicity, and incidence of persistent infections and pre-cancer lesions [88-92]. The Dutch Health Council has nonetheless advised in 2022 to offer two doses, awaiting results from clinical trials [93]. A one-dose schedule could facilitate initial introduction (for boys) and increase uptake [94], particularly in low- and middle-income countries with limited access to preventive measures, such as

in the Caribbean. This could result in overall sustainability of the HPV-program, next to simplification and reduction of costs of vaccine purchase and delivery. In anticipation of their re-evaluation and future update, the Health Council should take into account the potential beneficial effects of a one-dose approach for CN given the dissimilar context with the Netherlands. A regional-driven approach for CN is required to achieve successful implementation of public health interventions. Differences in epidemiology, vaccination coverage, political landscape and cultural factors should be taken into account when developing the regional strategy. Our serological HPV-data (**chapter 5**) further underscored the importance for routine cervical cancer screening in adult CN-women to detect hr-HPV DNA early; a preventive measure that has shown to be (cost-)effective consistently across numerous studies [95-97]. In collaboration with the National Institute for Public Health and the Environment (RIVM), all CN-women aged 30–60 years will be invited to participate in a population-based pilot up till 2025, and the program is planned to run routinely thereafter [98].

Seroepidemiology can also be a valuable tool in the decision-making process on introduction of a novel vaccine [5]. The WHO recommends routine vaccination against varicella-zoster virus (VZV) for countries with a significant public health burden where ≥ 80% coverage can be achieved [99]. Opposed to temperate regions [100-103], high VZV-susceptibility was observed among adolescents and adults in CN (40% at 25 years and still 20% > 40 years), with highest rates among those born in CN, followed by (other) Latin American countries (**chapter 6**); a typical island epidemiology with lower circulation and absence of endemicity [104-107], potentially similar to mumps and rubella pre-vaccination (**chapter 4**). Such high susceptibility may cause relatively large outbreaks, as exemplified on Saba in 2017, resulting in a high overall burden (including costs) for society as VZV-complications rise with age [108]. Several live-attenuated vaccines are available (either monovalent or multivalent (MMRV)), and are licensed from age 11 or 12 months [99, 102, 109]. Modelling studies from Latin America show unanimously that routine vaccination (one/two doses) reduces societal- and healthcare costs significantly [110]. VZV-vaccines are safe and highly effective against infection, transmission and severe disease with long-lasting immunity (especially two-doses) [109]. Hospitalization in Costa Rica reduced with > 90% in the years following one-dose introduction [111], and in the USA a disease reduction of > 97% among all ages was seen once the two-dose schedule was introduced in 2007, indicative of indirect effects [112, 113]. Decreasing circulation due to VZV-vaccination has been hypothesized to reduce exogenous boosting of the immune system (following Hope-Simpson's theory in the 1960s), which in turn may cause a higher incidence of (and burden to) shingles in elderly in the long-term [114]. Recent data from the USA have not displayed an increase in shingles among adults attributable to the vaccination program 25 years after introduction though, which could suggest a more important role of endogenous boosting through

subclinical reactivation [115]. Although resurgence of the live-attenuated VZV-strain is still possible and might potentially cause shingles among vaccinated, there is strong epidemiological evidence of a ~80% lower risk in vaccinated American children, and a continued decline in cases over time is expected [116]. Together, supported by our data and given the high burden and expected high uptake, the Dutch Health Council advised the minister in 2020 to introduce routine childhood VZV-vaccination (two-dose MMRV) and a catch-up campaign (monovalent) for uninfected persons [117]. The Dutch minister approved the advice and implementation is planned in due time [118]. Half of the countries in Latin America, yet solely 20% of the Caribbean, have implemented childhood VZV-vaccination recently, and report high coverage [30, 119]. We strongly warrant consideration of routine VZV-vaccination on all (Dutch) Caribbean islands given the expected similar susceptibility in the adult population [120]. Future studies in CN should assess its impact and effectiveness and use our current results as a baseline measure pre-vaccination. Long-term surveillance of varicella and shingles is desired, and in case of outbreaks it will be valuable to conduct a case-control study to have more in-depth information on vaccine-effectiveness and severity of cases.

Moreover, in the context of a regional-driven approach for CN and with regards to considerations of new vaccines/adaptations to its NIP, it is noteworthy that a novel dengue-vaccine has recently become available (for travelers) in (European) Netherlands [121]. Dengue inflicts a significant economic-, health- and social burden in endemic regions, including the Caribbean region, and is one of the most important mosquito-borne emerging global threats [122]. Hence, the CN population may potentially benefit from regular introduction into the NIP. The Health Study CN samples can be of importance for this consideration as implementation requires understanding of previous exposure in the population – which can be evaluated following presence of antibodies – and can thus provide knowledge on susceptibility and specific at-risk groups that might benefit from vaccination [29, 123].

Seroepidemiology in the pandemic phase

Besides evaluation of population-based immunity against vaccine-preventable diseases during phases of control and alert, seroepidemiology can be a powerful tool after the emergence of a novel pathogen [4, 124]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in late 2019 and disseminated swiftly across the globe, causing respiratory illness (COVID-19) and initiated a pandemic that required stringent control measures [125-129]. Early studies primarily focused on patient populations and case-based testing was restricted to symptomatic at-risk groups, limiting the use of conventional surveillance methods [126, 130, 131]. Hence, the prospective population-based PIENTER-

Corona (PICO) serosurvey was set up rapidly in the Netherlands (**chapter 7**) to provide increased understanding. Below we will discuss the key insights and lessons learned.

Outbreak investigation and valid markers

The PICO-study provided key information on the extent of the outbreak, groups infected (and their symptoms), and development of (humoral) immunity early in the pandemic. Using seropositivity as a marker of previous exposure, we revealed that nationwide ~3% had been infected at the peak of the first wave (**chapter 7**), and 4.5% after the first wave. Highest proportions were seen in young adults, the Southeastern part of the country and 'Bible belt' region (**chapter 8**). Despite the heavy burden on society, including 10,000 deaths [132], our estimates reflected that only ~800.000 inhabitants had been infected. Although this was multifold higher than the cases reported [133], it clearly showed that the largest part of the population remained susceptible, and future waves with excessive pressure on the health system would be imminent without control measures. A highly specific and sensitive marker is needed to distinguish individuals with past infection from those without to be able to accurately approximate prevalence/cumulative incidence over a long period [134, 135]. This requires a high-throughput immunoassay that is validated using panels of positive (polymerase chain reaction (PCR)-confirmed cases ranging from asymptomatic to severe/hospitalized) and negative controls (preferably pre-pandemic samples from cases with respiratory symptoms) [136]. Spike S1 – being an important target for neutralization, together with its receptor-binding domain (RBD) – immunoglobulin (Ig)G was observed to be most promising in terms of test specifics (over time) in our data (**chapter 9**) as well as multiple other early studies [137-140]. Alarming reports of swift antibody waning appeared, suggesting susceptibility to re-infection and potentially disease rather quickly. However, most of these studies measured total Ig and/or predominantly targeted Nucleoprotein (N). The latter is a more preserved antigen in the core of the virus, which is less essential as an immunological target, and somewhat less specific and sensitive in most assays; an important feature to consider from a seroepidemiological stance [141-143]. Van den Hoogen *et al*., also confirmed that anti-N waned quicker than anti-S1 (e.g., from onset of infection up to six months: 85% to 59% vs. 90% to 80%, respectively), especially in milder cases [144]; and this was in line with our results for anti-S1 (**chapter 7** and **9**). The above underlines the importance of correcting for test specifics while calculating population estimates, and this is even further substantiated by the fact that one-to-one comparisons between assays often show differences in their abilities against several targets [141, 145-147]. High specificity to rule out false-positivity is particularly required when prevalence of infection is low, which was (in)famously illustrated in a Californian serosurvey that largely overestimated prevalence when extrapolated to the population without (initial) adjustment [148]. When vaccination was introduced in

the beginning of 2021, anti-N became the preferred marker to detect breakthrough infections as most vaccines are targeted against spike S1 [144], despite some data showing (marginally) reduced sensitivity in vaccinees [149]. The power of longitudinal measures and quantitative antibody assessment became evident with increasing numbers of re-infections over time as the difference in height of the concentrations between timepoints enabled such identification.

Informing public health policy

Findings from the PICO-study were shared constantly with the Outbreak Management Team, Ministry of Health and Dutch Health Council to inform decision-making. Age-specific population-based estimates of infection – and contact data – have been used as input for modelling purposes to forecast future waves, and assess the impact/severity of disease to comprehend dynamics of different waves (with different variants of concern (VOC), and the impact of vaccination later) [150-154]. Effects of control measures were also evaluated, and although causality cannot be derived from these cross-sectional seroepidemiological analyses, they do represent risk profiles that can support policy (**chapter 7** and **8**). For instance on contacts: despite external-, pathogen-, and host-specific factors, transmission is affected by the number, duration and nature (i.e., protected/distance/age) of contacts [155, 156]. PICO-participants were requested to list the frequency and age of contacts they had yesterday (a previously validated method [157, 158]) and whether this was close or distant. Most individuals had limited contacts during the lockdown, although we cannot completely exclude potential information bias. Higher odds for infection were seen among those with more close contacts – consistent with reviews ranking physical distancing as one of the most effective measures [159, 160] – albeit this did not apply to contact with young children. Children are generally suggested to play an important role in transmission of respiratory infections, such as influenza [157, 161]. Infectiousness and susceptibility seemed however reduced with younger age early in the pandemic, which also corresponded with our lowest observed seroprevalence as well as estimates worldwide [162, 163]. While the latter may pertain to a diminished adaptive immune response relative to their reduced severity, or higher asymptomatic proportion and generally quick recovery [164] – which may be a reason for being less infectious [165] – they did seroconvert with higher proportion than adults later into the pandemic [166-168]. A recent systematic review by Zhu *et al.*, indeed underlined enhanced infectiousness and susceptibility of younger children with emergence of more transmissible VOCs [169].]. Collectively, numerous studies supported a lower risk of close contact with young children (vs. adolescents and adults) in the first phase, justifying policy to keep daycare and primary schools open as much as possible provided that testing was carried out proactively [170-175]. We observed that young adults, on the contrary, were infected foremost. This is an unsurprising

observation as this age group generally has the most close contacts in the community [151, 157, 170], and later rounds pointed to a similar direction [166]. Closure of higher education and remote-working during periods of high circulation are defendable by these data from a purely medical/epidemiological stance. It is however essential for policymakers to evaluate the optimal trade-off between reducing viral circulation and the burden of disease for the population as a whole given today's increased knowledge on mental health issues and cognitive disadvantages due to the measures [176].

Survey design and sampling approaches

For accurate and valid population estimates a robust survey design is required in which the study population represents the general population optimally and has enough power to detect differences [5, 177]. Survey designs can improve precision and/or efficiency of the sampling process by using clustered designs in which e.g., municipalities are randomly drawn within regions, or households within neighborhoods, and apply pre-defined stratification, e.g., age groups. Invitees are randomly-selected within this framework using existing registries like a population registry. The PIENTER-3 study used such a two-stage cluster design which enabled comparison with the previous two serosurveys, and the Health Study CN followed a similar approach using age strata within each island (**chapter 2**). This design greatly relies on an up-to-date registry, especially important with large demographic mutations, e.g., population in- or outflux, and hampers inclusion of relocated individuals. These issues were relatively more often noticed in CN, which stresses a regular update of the registry more frequently as well as consideration of oversampling in future research. The PIENTER-3 design enables assessment of the population immunity against vaccine-preventable diseases robustly, and arranged on-site visits (in selected municipalities) to collect various material. The design is however less effective with emergence of a novel pathogen that may cluster throughout the country, and hence may have affected our estimates in the first PICO round marginally (**chapter 7**), yet the direction is unclear. Moreover, as on-site visits were impossible due to the constrains, PICO-participants were asked to self-collect a fingerstick blood sample (which is more efficient in terms of data processing than e.g., dried-blood spot samples that we used in CN, or elsewhere [178]). This enabled us to improve the initial study design after the first round by supplementing the cohort with participants who could be randomly-selected proportional to the size of each municipality (and age-stratified) to better represent the country geographically (**chapter 8**). A downside of self-sampling is that it might be experienced as a burden, despite taking it at one's own pace at home. This especially concerns (young) children for whom parents conduct the sampling, alongside the fact that this group is already more reluctant to partake in blood sampling and usually drops-out more often [166, 179]. Other sampling approaches, such as using residual sera, e.g., from laboratories or

blood donors [180, 181] can be efficient (in acute settings), are less time-consuming and less costly. Completion of in-depth questionnaires, e.g., on risk factors or vaccination data, is usually not possible though, and selective response is generally worse due to inclusion of persons with enhanced health seeking behavior, those with comorbidities or persons who are overly healthy, which hampers generalization, as was for instance illustrated greatly by an early SARS-CoV-2 serosurvey among donors in Brazil [182].

A downward trend in response rates has been observed across population-based studies the last decades, mainly due to a combination of factors including less trust in (the) government (institutions), greater time pressure, survey fatigue and privacy concerns [179, 183]. Reduced inclusion is especially concerning for already hard-to-reach groups, such as young adults, men, those of other ethnic background and lower education, and requires additional attention [184, 185]. Our cohorts indeed display an overrepresentation of female Dutch adults of higher educational level from non-urbanized areas [10, 11, 186], and are therefore weighted on a set of characteristics – that are available and important for the outcome – to enhance representativeness; yet, may not overcome selective response completely. Consequently, SARS-CoV-2 infection estimates can be slightly underestimated, especially among (middle-aged) adults, as our participants are likely to adhere better to control measures, and conversely, the seroprevalence induced by infection and vaccination combined – as studied in later rounds – is likely an overestimation due to a relative higher vaccination uptake. Oversampling of underrepresented groups can increase power and enhance representativeness as shown by simulation studies [187]. Some other approaches have shown to effectively improve response rates, including incentives (e.g., test results), reminders and improving study material [184, 188, 189]. Reminders were also highly effective in CN [9], however incentives can raise ethical considerations and in fact increase selective response. Improving study material can include easy-to-read invitation letters, especially among illiterate groups, in multiple languages. Due to time constraints we were not able to apply the latter in PICO, which potentially had an effect on response rates in non-Dutch migrants. The impact on the length of questionnaires is less clear, but could influence follow-up participation [190]. QR codes to access online questionnaires had a significant impact on response rate in a study from the UK [184]. Recent studies have started to use mobile app(lication)s, which provide notifications on tasks and enable easy access to results and questionnaires, which is potentially interesting for young adults especially. Targeted social media campaigns to trigger participation are more often applied too (also at the RIVM). It should be helpful to have enhanced understanding of the effects of participation rates across sociodemographics with these types of communication. All in all, the most appropriate design and approach should be balanced against the desired outcome, i.e., questions to be answered and samples needed, timing and timeliness of the survey and continued sources of funding.

Challenges and opportunities in the post-pandemic phase

After multiple SARS-CoV-2 waves causing large burden on societies, the WHO ended the public health emergency of international concern in May 2023; more than three years after its initiation [191, 192]. Vaccination has been very effective in reducing the risk of COVID-19 mortality [193]. The vast majority of persons have acquired immunity, either via vaccination, infection (with different strains) or both (hybrid immunity) [166, 167]. Hence, although the picture of population immunity has become increasingly complex, seropositivity can best be considered as a marker of protection against severe disease (at least in the moderate term), and we have recently transitioned from a pandemic to an endemic/post-pandemic state. This final section will discuss the future challenges and opportunities, and role of seroepidemiology.

Monitoring immunity

Our insights on the persistence and functionality/avidity of antibodies after infection (**chapter 9**) have shed light on the degree of protection and contributed to prioritizing and speeding-up the COVID-19-vaccination campaign as those with a previous infection could be offered one instead of two doses [194]. The epidemiological landscape changed and re- and breakthrough infections became more prevalent with waning antibodies and emergence of VOCs – that escaped previously induced antibodies more easily and show improved binding affinity [195]. Recent systematic reviews highlight that hybrid immunity is optimal in terms of durable effectiveness against infection (~50% at 12 months) and severe disease (over 95% in primary series- and booster-vaccinated individuals) [196, 197]. These epidemiological findings are substantiated by immunological data demonstrating high levels of neutralizing antibodies with boosting of pre-existing variants and reactivity against new variants, illustrative of a broadened immune response [198-200]. This reinforces the importance of vaccination with primary series, also in those previously infected. Some challenges and uncertainties exist that could initiate novel waves of infections, for instance differential imprinted (hybrid) immunity or robustness of effectiveness of novel Omicron-containing boosters with new variants emerging [199, 201-205]. Monitoring and further understanding of the durability of protection is of great importance for vaccination policies and control of COVID-19, especially concerning elderly and immunocompromised. Despite observations of waning humoral immunity, the more pre-served cellular immune response is expected to have a pivotal role in maintaining protection against severe disease, and hence should remain an important topic of research [206, 207]. Longitudinal serosurveillance studies using quantitative antibody measurements to identify infection following boosting of antibodies become even more important for estimation of (vaccine-)effectiveness with limited PCR/home-testing and increasing

re-infections. Our existing serological frameworks can also be of value in monitoring circulating variants via self-sampling of participants with respiratory symptoms [208].

A long-term strategy to effectively combat SARS-CoV-2 infection and disease could include novel vaccine platforms, e.g., intranasal-, oral- or inhaled vaccines, which have lagged behind development of injected vaccines [209, 210]. These vaccines are suggested to be less invasive and provide a durable local protection due to induction of mucosal antibodies that may be less affected by immune escape (due to relative higher avidity) and potentially block transmission more effectively in the nasal mucosa [211-213]. Some recent clinical trials show promising results, but comparative research with existing booster regimens is needed [214-216]. Correlates of protection, which have been established for some vaccine-preventable pathogens, are a valuable tool for assessing and comparing (population) immunity, yet are often a serum antibody concentration as these are easily measured and standardized [46]. Some initial efforts using serum IgG have been successful, however the occurrence of VOCs have hampered their implementation as neutralizing- and binding antibodies correlated worse over time, hindering determination of protective levels [217-219]. Mucosal vaccines usually induce low serum antibody responses which makes it harder to compare and establish a protective correlate, and mucosal immune responses are harder to measure and standardize than serum responses [211]. In recent PICO rounds we revealed that those who acquired (nasal) mucosal IgA were significantly protected against future infection, predominantly observed in those with hybrid immunity [220]. Future study rounds should investigate the persistence of these responses, also in relation to future vaccine boosters and other characteristics. Additional insights on SASR-CoV-2 mucosal immunity and potential indirect effects on the population, as well as further development and standardization of sampling and immune assays, could boost the development of other mucosal vaccines, e.g., for influenza and respiratory syncytial virus (RSV), and prepare for emerging pathogens.

Future focus

Now that the acute pandemic phase has passed, conditions like post-COVID should gain focus as a relative large proportion of the population experiences sustained symptoms after infection [221, 222]. Prospective seroepidemiological studies collecting large amount of data have the ability, and duty, to contribute to this piece of the 'COVID-19-puzzle' by investigating risk factors and immunological markers [223]. In addition, relative few SARS-CoV-2 serosurveys have been performed in children, yet they are an interesting group to further study as they consist of a immunologically heterologous group [168]. Examining their humoral responses with respect to the probability of future infection and linking these to risk factors and symptoms will be insightful. Serosurveys should also incorporate a multi-pathogen approach [224]. The epidemiology of numerous infectious diseases

has changed during the pandemic, with incidence of most (other) respiratory pathogens nearing zero due to the stringent control measures [225]. Incidence peaked substantially for some after easing, e.g., chickenpox, group A streptococcus, or RSV during summer, completely outside the normal seasonal pattern [226-228]. It will be important to monitor whether and how these trajectories have changed to explain atypical outbreaks and be prepared for future ones. Furthermore, some large seroepidemiological studies have ongoing data collection, like NHANES in the USA, but most (cross-sectionally) collect (residual) sera every 5–10 years, e.g., Australia, depending on funding and public-health relevance [181, 229]. Many countries initiated seroepidemiological initiatives during the acute pandemic phase [167, 230]. Due to a lack of funding most have halted these, however they should take advantage of the established framework, and flexibility and knowledge gained by the participants. Future sampling in PICO or (nested within) other large established cohorts at the RIVM can provide a wealth of information for the NIP, and beyond. More frequent and quicker updates on population immunity can be valuable given the recent epidemiological context and potential increased blurry vision on vaccination coverage due to privacy constrains [75, 231].

Concluding remarks

Seroepidemiology is an indispensable and key element in the toolbox of surveillance for prevention and control of infectious diseases. Its multi-applicability alongside conventional methods during different epidemiological phases has been demonstrated here and will remain essential besides novel approaches, such as sewage surveillance and community participatory surveillance (e.g., RIVM's Infectieradar) [232, 233]. We have arrived at an era of increased vaccine hesitancy and historic backslide in immunization globally, which may require optimization of the NIP and improved communication. Effects of the pandemic, wars and climate change are apparent, and cause large migrant flows and cross-border pathogenic threats that presumably will be worse in the foreseeable future. Outbreaks of vaccine-preventable diseases, (re-)emergence of pathogens and (permanently) changing epidemiology, as well as uncertainties regarding SARS-CoV-2, ask for robust ongoing seroepidemiological assessment. Established serological frameworks should be further optimized to answer the questions of tomorrow, and be prepared for the next pandemic. New approaches should be explored to enhance inclusion of hard-to-reach groups. And finally, vaccine equity should be high on the global agenda as the unequal vaccine distribution to low- and middle-income countries and disproportional consequences thereof have been shown ingloriously during the pandemic [234]. The pandemic has taught us one thing undeniably: we have to work collectively and across (overseas) borders to be adequately prepared for the challenges ahead.

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APPENDICES

NEDERLANDSE SAMENVATTING

Seroepidemiologie is essentieel in de preventie en bestrijding van infectieziekten. Deze vorm van surveillance is een belangrijke en onmisbare aanvulling op de andere instrumenten uit de 'gereedschapskist', zoals notificaties van ziektegevallen, ziekenhuisopnames of sterfte. Het meten van antilichamen in het serum van deelnemers in een studie geeft informatie over eerdere bloostelling aan een antigeen (stukje eiwit van een ziekteverwerker), bijvoorbeeld door infectie en/of vaccinatie. De deelnemers uit zo een (cohort)studie moeten de doelpopulatie zo goed mogelijk representeren, bijvoorbeeld de algemene bevolking. Seroepidemiologie is allereest een belangrijke pijler ten aanzien van het bijsturen en optimaliseren van het vaccinatiebeleid. Opeenvolgende studies om het Rijksvaccinatieprogramma (RVP) te '(sero)-monitoren', bijvoorbeeld iedere 5–10 jaar, geeft belangrijke inzichten in (mogelijke wijzigingen in) de populatie-immuniteit over de tijd tegen ziekten die reeds zijn opgenomen in het programma. Denk hierbij aan inzicht over vatbare groepen, afnemende immuniteit, verschuivingen in circulatie van sero-typen, of veranderingen in de vaccinatiegraad die een aanvulling kunnen zijn op gegevens uit vaccinatieregisters. Het uiteindelijke doel is om het RVP te optimaliseren ten gunste van de gehele bevolking. Daarnaast is seroepidemiologie waardevol bij het monitoren van mogelijk toekomstige vaccinatie-voorkombare ziekten ('kandidaten') die specifieke aandacht vereisen, evenals nieuwe (opkomende) pathogenen, bijvoorbeeld tijdens een pandemie zoals we hebben gezien bij het *severe acute respiratory syndrome coronavirus 2* (SARS-CoV-2). Conventionele surveillance-instrumenten zijn doorgaans niet in staat een volledig beeld te geven van het totale aantal ziektegevallen als gevolg van testbeperking of (fluctuerend) testgedrag, of omdat ze onopgemerkt blijven doordat een fractie van hen asymptomatisch blijft. Veelal is vaccinatie in het geval van opkomende infectieziekten (nog) niet beschikbaar, maar in dat geval is monitoring op korte termijn wel vereist om onze kennis te vergroten over bijvoorbeeld de omvang van een uitbraak, risicofactoren voor infectie of vatbaarheid, of de duur van de antistofrespons (na infectie).

Bevindingen uit seroepidemiologisch onderzoek leveren dus belangrijke input voor het vormgeven van het volksgezondheidsbeleid zowel op de korte termijn (mogelijk meer acuut), als ook op de lange termijn (wanneer er sprake kan zijn van meer controle). Om die reden hebben we in het koninkrijk der Nederlanden grote serologische populatiestudies opgezet. Dit proefschrift biedt een uniek overzicht van de veelzijdige toepasbaarheid van het serosurveillance-instrument dat we hebben ingezet in verschillende epidemiologische fasen en (geografische) settings ten behoeve van de preventie en bestrijding van infectieziekten. In dit hoofdstuk vatten we de belangrijkste bevindingen samen uit dit proefschrift, te beginnen met de evaluatie van de populatie-immuniteit tegen de door vaccinatie te voorkomen ziekten in Caribisch Nederland (CN) (**deel 1**), en daarna

aangaande de monitoring van de SARS-CoV-2 epidemie in Nederland tijdens het eerste jaar van de *coronavirus disease 2019* (COVID-19) pandemie (**deel 2**).

Deel 1: Evaluatie van de populatie-immuniteit tegen de door vaccinatie te voorkomen ziekten in Caribisch Nederland

Sinds 10 oktober 2010 zijn de Caribisch Nederlandse eilanden Bonaire, St. Eustatius en Saba (samen CN) bijzondere gemeenten binnen (Europees) Nederland. De Nederlandse overheid is hierdoor verantwoordelijkheid geworden voor de volksgezondheid, en dat behelst ook de levering, uitvoering en monitoring van het RVP. Hoewel uit recente gegevens blijkt dat de vaccinatiegraad over het algemeen redelijk tot goed is in deze eilandpopulaties, wordt dit pas sinds een paar jaar routinematig en systematisch bijgehouden en gerapporteerd. De populatie-immuniteit van de door vaccinatie te voorkomen ziekten is hier echter nooit beoordeeld/geëvalueerd, waardoor er weinig inzicht is over de potentiële vatbaarheid en risicofactoren (van groepen) in de bevolking. Momenteel is de surveillance van infectieziekten grotendeels gebaseerd op de zogenaamde syndroom-surveillance. Een (representatief) deel van de huisartspraktijken houdt wekelijks een lijst met syndromen/symptomen bij en geeft de frequentie daarvan door aan het publieke gezondheidskantoor van Curaçao welke het geheel van alle eilanden van de voormalige Nederlandse Antillen bundelt en monitort. Afhankelijk van de relevantie en/of urgentie zal van een deel van de gevallen die bij de huisarts komen een sample worden afgenomen voor een verdere bepaling, maar veel potentiële ziektegevallen blijven door een gebrek aan faciliteiten op de eilanden vaak onopgemerkt. De Health Study CN is het eerste serosurveillance-onderzoek om de genoemde kennislacunes op te vullen ter ondersteuning van het volksgezondheidsbeleid in CN.

Hoofdstuk 2 beschrijft de methodologie, opzet en dataverzameling van de derde landelijke populatie serosurveillance studie in Nederland (PIENTER-3) – welke in deel 2 van dit proefschrift aan bod komt – inclusief de Health Study CN in 2017. Voor beide onderzoeken werd een vergelijkbare opzet toegepast. Ruim 10.000 personen (die gerandomiseerd zijn getrokken uit het bevolkingsregister), waarvan 1.900 in CN, namen deel aan consultaties ter plaatse, verstrekten meerdere biologische monsters, en vulden uitgebreide vragenlijsten in. De studiepopulaties waren over het algemeen een goede weergave van de totale bevolking, bestreken een grote leeftijdsreeks (0–90 jaar), en waren een redelijk tot goede afspiegeling voor wat betreft andere relevante socio-demografische kenmerken. De meerderheid van de deelnemers gaf toestemming voor het meedoen aan een eventueel vervolgonderzoek. In dit hoofdstuk bespraken we verder suggesties voor het verbeteren van de responspercentages in toekomstige onderzoeken, alsmede de logistieke hindernissen.

Een aanhoudende instabiele (politieke) situatie in Venezuela sinds medio jaren 2010 heeft geleid tot een voortslepende humanitaire crisis in het land. De ontwrichting van het gezondheidszorgsysteem als geheel heeft grote uitbraken van door vaccinatie te voorkomen ziekten tot gevolg gehad. Een aanzienlijk aantal Venezolanen is naar omliggende landen gevlucht, resulterend in uitbraken van mazelen en difterie, inclusief dodelijke slachtoffers elders in *the Americas*. Gezien de nabije ligging van Bonaire ten opzichte van de kust van Venezuela was onze eerste prioriteit het evalueren van de immuniteit van de bevolking tegen mazelen en difterie op dit eiland. **Hoofdstuk 3** liet zien dat de bescherming tegen mazelen suboptimaal was, vooral voor mensen jonger dan 5 jaar afkomstig van de Nederlands Caribische eilanden (en Suriname), evenals voor adolescenten uit andere Latijns-Amerikaanse landen. Ook het aandeel personen met een minimaal beschermingsniveau tegen difterie was vrij laag (< 80%), vooral onder vrouwen van 30 jaar en ouder, en degenen afkomstig van de Nederlands Caribische eilanden en Latijns-Amerika. De gezondheidsautoriteiten op Aruba, Bonaire en Curaçao, evenals de omliggende eilanden, kregen het advies alert te zijn om gevallen vroegtijdig op te sporen en zo mogelijke transmissie te voorkomen, waarbij snelle aanvoer van antitoxinen tegen difterie sterk aanbevolen is. Daarnaast is geadviseerd om de vaccinatiestatus van vluchtelingen zo snel mogelijk te verifiëren (bij binnenkomst) en, indien van toepassing, vaccinatie aan te bieden. Risicogroepen, waaronder degenen die in nauw contact staan met vluchtelingen, is aangeraden hun vaccinatiestatus te updaten als nodig om zodoende de kans op besmetting (en transmissie) te reduceren.

Mede door de ongekende humanitaire situatie in Venezuela is het mogelijk dat andere virale ziekteverwekkers, zoals rubella en de bof, waarschijnlijk ook vaker circuleren in de regio. **Hoofdstuk 4** beschrijft een diepgaande analyse van mazelen, bof en rubella (BMR) op alle CN-eilanden, waarbij de nadruk lag op de identificatie van vatbare groepen in de samenleving, mate van afnemende humorale immuniteit (antistoffen), en mogelijk bewijs van blootstelling (recentelijk en in het pre-vaccinatietijdperk). Allereerst zagen we robuuste antilichaamresponsen na de BMR-vaccinatie, waarbij twee vaccin doses (vs. één) een persistente/langdurige immuniteit liet zien tot jaren later. In lijn met andere studies onderstreept dit wederom het doel van boostervaccinatie. De algehele seroprevalentie (dat wil zeggen, de proportie mensen waarbij we antistoffen detecteerden in de bevolking) in CN voor mazelen (94%) was hoger dan voor rubella en bof (beide 85%). Belangrijk om te vermelden is dat deze schattingen allemaal rond het niveau van kudde-immuniteit (voor zover van toepassing) schommelden. Bij personen die zijn geboren in het vaccinatietijdperk (van deze ziektes) lag de seropositiviteit voor al deze ziekten onder de 90%, en dit was met name laag onder groepen die op adolescente leeftijd CN-inwoner zijn geworden; het gaat dan vooral om migranten afkomstig uit Latijns-Amerika. Dit is zorgwekkend voor een zeer besmettelijke ziekteverwekker als mazelen, waarvoor een hoge mate van populatie-immuniteit nodig is om kudde-immuniteit te waarborgen en de

gehele bevolking te kunnen beschermen. Hoewel rubella een lager reproductiegetal (dat wil zeggen het aantal secundaire gevallen dat een primaire case kan besmetten) heeft dan mazelen, en dus minder besmettelijk is, vereist de waargenomen lagere seroprevalentie (en dus verhoogde vatbaarheid) eveneens nauwgezette monitoring. Dit is vooral van belang voor vrouwen in de vruchtbare leeftijd ter voorkoming van het congenitaal rubellasyndroom. Bij voorkeur moet deze vatbaarheid al in een vroeg stadium worden voorkomen door vaccinatie, liefst tijdens het reguliere programma, maar een nieuw in te plannen inhaalmoment voor alle RVP-vaccinaties op adolescente leeftijd zou overwogen moeten worden. Onze data suggereerden verder dat er waarschijnlijk sprake is van circulatie van bof onder jongvolwassenen op Bonaire (maar destijds niet op St. Eustatius en Saba). Dit is door andere surveillance-middelen nooit opgemerkt. Gevoelige (ziekte) surveillance door (huis)artsen is nodig in het licht van de recente uitbraken van virale ziekteverwekkers in de regio. Daarnaast is een hoge BMR-vaccinatiegraad essentieel, en in het bijzonder het aanbieden van vaccinatie aan migranten (indien van toepassing) bij aankomst. Alleen op die manier kan er worden voldaan aan de eliminatiedoelstellingen van de Wereldgezondheidsorganisatie (WHO) voor mazelen en rubella. Een opmerkelijk bevinding was daarnaast dat we een relatief lage seroprevalentie van rubella en bof hebben waargenomen bij ouderen die op één van de Nederlands Caribische eilanden zijn geboren, of daar sinds hun kindertijd zijn komen wonen, toen routinematige vaccinatie nog niet was ingevoerd. Dit is indicatief voor een typische eiland-epidemiologie, gekenmerkt door verminderde circulatie van deze ziekteverwekkers in het pre-vaccinatietijdperk, en staat in schril contrast met bijvoorbeeld Nederland, waar de seroprevalentie bij oudere personen de 100% nadert.

In **hoofdstuk 5** hebben we een andere virale ziekteverwekker besproken die in Caribische landen een grote ziektelast veroorzaakt: het humaan papillomavirus (HPV). De incidentie en mortaliteit als gevolg van HPV-gerelateerde kankers is hoog in de regio, maar vaccinatie is pas onlangs toegevoegd aan het RVP in CN voor jonge adolescenten. Robuuste antilichaamresponsen tegen de vaccin-typen waren te zien in de gevaccineerde deelnemers, evenals enige kruisreactiviteit tegen hoog-risico serotypen (die kanker kunnen veroorzaken) die niet in het vaccin zitten. De seropositiviteit onder de niet-gevaccineerde personen – in dat geval een marker voor cumulatieve blootstelling gedurende het leven (tegen één van de zeven hier onderzochte hoog-risico typen) – was relatief hoog (34%). Ruim de helft van de seropositieven was positief voor ten minste twee typen (voornamelijk HPV16 en -52). Op St. Eustatius werd de hoogste seropositiviteit waargenomen. Opvallend was dat voor beide geslachten gold dat een aanzienlijk deel van de (ongevaccineerde) volwassenen onder de 60 jaar seropositief was, namelijk meer dan de helft van de vrouwen en bijna één op de vijf mannen. Naast geslacht en leeftijd hielden andere risicofactoren voor seropositiviteit vooral verband met verhoogd seksueel risicogedrag. Deze gegevens onderstrepen de relevantie voor

routinematige screening van baarmoederhalskanker bij volwassen vrouwen en een sekseneutraal HPV-vaccinatieprogramma, waarbij net als in Europees Nederland de HPV-vaccinatie aan jongens wordt aangeboden.

Het laatste virale pathogeen uit dit deel van het proefschrift waarbij seroepidemiologie van specifiek belang is geweest in CN is het varicella-zostervirus (VZV), de veroorzaker van waterpokken en herpes zoster (gordelroos). VZV heeft een typische epidemiologie in tropische (en meer geïsoleerde) gebieden: een minder uitgesproken seizoensgebondenheid en endemisch karakter die doorgaans resulteren in grotere fracties van vatbare (niet eerder geïnfecteerde/seronegatief) adolescenten en volwassenen. Dit kan leiden tot relatief grote uitbraken, zoals geïllustreerd op Saba in 2017. Infectie op oudere leeftijd verhoogt de kans op varicella-gerelateerde complicaties en dus een hogere ziektelast (individueel, inclusief dat van ongeboren kinderen, maar ook op populatieniveau) in vergelijking met gematigde klimaten. In **hoofdstuk 6** lieten we zien dat de seroprevalentie inderdaad relatief laag is in CN (78%), met name in vergelijking met Nederland (95%), vooral op St. Eustatius (73%). Een belangrijk inzicht is tevens dat er een relatief hoge vatbaarheid bij adolescenten (40%) en volwassenen (10–30%) werd gezien, en dat was het meest opvallend bij degenen die in CN geboren zijn of daar sinds hun vroege kinderjaren wonen. Deze resultaten zijn essentieel geweest in het besluitvormingsproces omtrent de introductie van (zeer effectieve) VZV-vaccinatie bij kinderen in het RVP in CN, inclusief een inhaalcampagne voor mensen zonder een voorgeschiedenis van infectie. Deze gegevens kunnen ook leidraad zijn voor andere landen in Midden-Amerika en het Caribisch gebied bij hun overwegingen over VZV-vaccinatie, aangezien slechts 20% van hen deze vaccinatie tot op heden in haar RVP heeft geïmplementeerd.

Deel 2: Sero-monitoring van de SARS-CoV-2-epidemie in Nederland

Een nieuw coronavirus (zeer waarschijnlijk van) zoönotische oorsprong, SARS-CoV-2, dook eind 2019 op in China, en verspreidde zich zeer snel over de hele wereld als gevolg van een volledig immuun-naïeve bevolking. Nagenoeg wereldwijd, inclusief in Nederland, werden vanaf medio maart 2020 lockdowns en zeer strenge maatregelen ingevoerd om te transmissie te onderdrukken en het aantal gevallen terug te dringen. Hiermee moest voorkomen worden dat het gezondheidszorgsysteem overbelast raakte als gevolg van een enorme toestroom van ernstig zieke patiënten (met COVID-19). Inzicht in de omvang van de epidemie en meest geïnfecteerde groepen ontbrak op dat moment, aangezien het testen beperkt was tot risicogroepen en ook informatie over de symptomen (frequentie en type) in de algemene bevolking niet geheel bekend was. Het aanhouden van langdurige (humorale) immuniteit na infectie werd in de eerste maanden daaropvolgend in twijfel getrokken, en daarmee samenhangende de kans op her-infectie, waardoor onderzoek hiernaar noodzakelijk was. Om (sero-

epidemiologische) inzichten te verschaffen ten gunste van onderzoekers wereldwijd en beleidsmakers te begeleiden tijdens het verloop van de pandemie werd daarom in deze eerste fase de populatie serosurveillance PIENTER-Corona (PICO) studie opgezet. In deze studie werden deelnemers op meerdere momenten tijdens de pandemie (longitudinaal, iedere 3–6 maanden) gevolgd. Informatie werd verzameld door middel van thuis/zelf-afname van (vingerprik)bloedmonsters, waarbij seropositiviteit tegen het spike S1-antigeen werd gebruikt als marker voor eerdere infectie, en de serologische analyses werden gekoppeld aan uitgebreide vragenlijsten over risicofactoren.

Hoofdstuk 7 omvat de belangrijkste inzichten op het hoogtepunt van de eerste SARS-CoV-2 infectiegolf in begin april 2020 in Nederland. De algehele seroprevalentie onder de Nederlandse bevolking was op dat moment zeer laag (~3%), ondanks de enorme druk op het gezondheidszorgsysteem. Dit kwam overeen met ongeveer een half miljoen infecties, en hoewel dit 30 keer hoger was dan het aantal officieel gerapporteerde gevallen, toonde het aan dat de ernst van de ziekte aanzienlijk was gezien het relatief grote aantal ernstige gevallen en doden. De seroprevalentie schattingen voor infectie waren het hoogst onder volwassenen van 18 tot 39 jaar en het laagst bij kinderen < 18 jaar. Orthodox-gereformeerde protestanten, die over het algemeen een lage vaccinatiegraad hebben tegen RVP-ziekten en sociaalgeografisch geclusterd wonen in de zogenoemde 'Bijbelgordel' welke zich uitstrekt van het zuidwesten tot het noordoosten van het land, hadden een van de hoogste kansen om besmet te zijn geweest (vier keer hoger in vergelijking met de rest). Aan de standaard COVID-19-casusdefinitie van het European Centre for Disease Prevention and Control (ECDC) werd voldaan door 75% van de seropositieve deelnemers. Interessant genoeg zagen we dat anosmie/ageusie (geur/smaakverlies) het meest onderscheidende symptoom was tussen ziektegevallen (seropositieven) en controles (seronegatieven) op populatieniveau. Het toevoegen van dit symptoom aan de casusdefinitie zou daarom kunnen bijdragen aan het bevorderen van de ziekteherkenning. Daarnaast namen we waar dat de concentraties immunoglobuline (Ig)G antilichamen bij seropositieve personen significant hoger waren bij mensen met systemische symptomen, zoals koorts en kortademigheid, wat suggereerde dat de ernst van de ziekte invloed had op de sterkte van de humorale respons en mogelijk op de duur ervan.

De eerste lockdown in Nederland duurde tot mei 2020 en omvatte strenge en beperkende *social distancing* maatregelen, vergelijkbaar met de meeste landen in de wereld. In **hoofdstuk 8** hebben we de effecten van een aantal van deze maatregelen op infectie onderzocht door gebruik te maken van gegevens uit de (tweede ronde van de) PICO studie, die tot doel had om besluitvormers (mondiaal) te informeren gegeven de mogelijke golven van infectie in het verschiet. Ten eerste, na de eerste golf was nog steeds maar slechts 4,5% van de Nederlandse bevolking besmet, waarbij de laag verstedelijkte gebieden en het zuidoostelijke deel van het land het zwaarst

waren getroffen – mogelijk als gevolg van carnavalsfestiviteiten die waarschijnlijk als *superspread* evenementen hebben gefunctioneerd. Ook verpleeghuismedewerkers zijn relatief vaak besmet geraakt in deze periode, wat specifieke aandacht behoeft op het gebied van (persoonlijke) bescherming om zowel infectie als overdracht te voorkomen aangezien ze werken met de meest kwetsbare personen. Eén van de meest belangrijke resultaten uit deze analyse was dat we bewijs leverden voor de effectiviteit van het houden van fysieke afstand (> 1,5 m) en het verkleinen van de groepsgrootte in binnenruimten; degenen die zich minder aan deze maatregelen hielden bleken vaker besmet. Jongvolwassenen lieten de hoogste besmettingspercentages zien en spelen waarschijnlijk een aanzienlijke rol bij de verspreiding van het virus, vooral omdat zij tot de leeftijdsgroep behoren met de hoogste frequentie van contacten zoals we weten uit contact-studies. Daarentegen suggereerden deze data juist een verminderde rol voor jonge kinderen onder de 12 jaar ten tijde van circulatie van het wildtype SARS-CoV-2 (de eerste variant uit de eerste golf). De seroprevalentie bij deze kinderen was erg laag, ondanks het feit dat nauw contact met volwassenen voor hen tijdens de lockdown niet beperkt was. Ook personen die meldden nauw contact met jonge kinderen te hebben gehad (bijvoorbeeld werk/school), hadden geen hogere kans op infectie vergeleken met degenen zonder contacten.

Kennis over de persistentie en functionaliteit (in dit geval aviditeit, oftewel bindingssterkte) van antilichamen na een SARS-CoV-2 infectie in personen uit de algemene bevolking (die over het algemeen milde tot middelmatige klachten hebben) ontbrak veelvuldig in het begin van de pandemie. Dit is echter belangrijke informatie omdat het kan dienen als maatstaf voor de mate van bescherming tegen toekomstige infecties en/of (ernstige) ziekte. Ook kan het eerste inzichten opleveren ten aanzien van een respons na vaccinatie – welke tegelijkertijd in klinische onderzoeken werd onderzocht al vroeg in het begin van dit eerste pandemische jaar. **Hoofdstuk 9** bescrhijft de resultaten van deze analyse door gebruik te maken van opeenvolgende monsters uit de eerste drie PICO studie rondes (april, juni en september 2020). Serum IgM en IgA antilichamen tegen het spike S1 antigeen namen relatief snel af in de eerste maand na infectie, met slechts 50% seropositief na 2–3 maanden, terwijl IgG bij de meeste deelnemers (> 90%) tot zeven maanden aanwezig was. Deze relatief langdurige seropositiviteit (dat wil zeggen de sensitiviteit/gevoeligheid over de tijd) in combinatie met de hoge specificiteit (dat wil zeggen het met grote betrouwbaarheid aan kunnen tonen van antistoffen die specifiek gericht zijn tegen SARS-CoV-2) maakte spike S1 IgG tot de meest optimale marker voor seroepidemiologische studies. Dit overigens enigszins in tegenstelling tot antilichamen die zich richten op Nucleoproteïne – een meer geconserveerd antigeen in de kern van het virus. Deelnemers die ernstigere/systemische (en langere duur van) symptomen rapporteerden – dat bleken vooral mannen en personen ouder dan 50 jaar – hadden hogere initiële antilichaamconcentraties, evenals na verloop van tijd. Ook vertoonden

zij een sterkere toename van de aviditeit van IgG antilichamen in de loop van de tijd dan personen met milde of asymptomatische klachten. Samengenomen duidde deze analyses op een sterke ontwikkeling van immuungeheugen en een duurzame respons, wat waarschijnlijk zal bijdragen aan bescherming tegen ziekte (met een vergelijkbaar antigeen) in de toekomst – vooral bij symptomatische individuen – aangezien spike S1-specifieke antilichamen van groot belang zijn bij het neutraliseren van SARS-CoV-2.

Conclusies en toekomstperspectief

Na de presentatie van de belangrijkste resultaten in dit hoofdstuk (**hoofdstuk 10**), worden de implicaties voor de volksgezondheid van onze seroepidemiologische bevindingen in het koninkrijk der Nederlanden tijdens verschillende epidemiologische stadia besproken in **hoofdstuk 11**. Kortgezegd, seroepidemiologie kan van groot belang zijn bij het identificeren van risicogroepen (ten aanzien van vatbaarheid of, andersom, juist het hebben van infectie(geschiedenis)) en het tijdig aanpassen of uitbreiden van het RVP. Het instrument kan worden ingezet tijdens verschillende epidemiologische fases. Bijvoorbeeld in tijden van waakzaamheid en alertheid, zoals in het geval van (regionale) pathogene dreigingen. maar ook op de langere termijn, zoals bij het reageren op een veranderend klimaat, mondiale migratie of het monitoren van vaccinatiegraad, -bereidheid en -gelijkheid. CN zal kunnen profiteren van een regionaal-gestuurde aanpak als het gaat om de introductie, aanpassing en implementatie van preventieve volksgezondheidsmaatregelen, en kan een voorbeeldfunctie zijn voor andere landen in het Caribisch gebied. Serologische studies zullen een belangrijke rol spelen bij het evalueren van toekomstige interventies die specifiek gericht zijn op de eilandpopulaties. Seroepidemiologie heeft haar bruikbaarheid en veelzijdige toepasbaarheid ook aangetoond in een acute, pandemische fase tijdens de opkomst van een nieuwe ziekteverwekker, door op korte termijn data en informatie te verstrekken aan besluitvormers over verschillende onderwerpen gerelateerd aan (populatie)immuniteit. In dat perspectief reflecteren we vervolgens ook over het belang van nauwkeurige en valide bevolkingsschattingen, inclusief serologische markers, methodische/statistische correcties, studie designs, en representatie van groepen. Aanbevelingen voor toekomstige onderzoeksgebieden en de rol van seroepidemiologie worden verder besproken, zoals die in relatie tot mucosale immuniteit, het post-COVID syndroom, en veranderende epidemiologische patronen van andere pathogenen tijdens de pandemie. In de post-pandemische/endemische fase zal het belangrijk zijn om de wereldwijd opgezette 'serologische raamwerken' (dat wil zeggen de populatiestudies en biobanken) verder te ontwikkelen en in te zetten, om op die manier dynamisch en flexibel te blijven in een steeds veranderende wereld.

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DANKWOORD

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Shout-out ook naar alle **mede (oud-)promovendi** tijdens mijn PhD-traject (die ik nog niet genoemd heb): **Abigail**, **Anke**, **Daan**, **Daantje**, **Elise**, **Elsbeth**, **Esther**, **Joske**, **Leon**, **Liz**, **Maarten**, **Mariette**, **Marit**, **Marta**, **Michiel**, **Nora**, **Pauline**, **Sara**, en **Saskia**. De meeste van jullie hebben de titel inmiddels binnen, of gaan nog/niet meer verdedigen. Hoe dan ook, we hebben allemaal in hetzelfde schuitje gezeten, wees vooral trots op jezelf. Dank voor alle gezellige etentjes, borrels, en dagelijkse praatjes; het heeft mijn tijd er leuker van gemaakt!

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heb ik me altijd thuis gevoeld. En daardoor ook plezier gehad in die paar maandjes pipeteerwerk op het MIA-lab. Ik denk dat het een pré is om als epidemioloog te zien hoe de samples in het lab verwerkt en gemeten worden, inclusief alle nuances die daarbij horen. Veel dank ook voor het uitpakken van het studiemateriaal dat terugkwam van het veldwerk – toen ik zelf nog met een cocktail op het Caribische strand lag. Hopelijk denken jullie niet meer al te vaak terug aan de geur die er uit één van de boxen kwam, haha. Dank ook voor jullie tomeloze inzet voor de PICO studie tijdens de pandemie; wat is er wat verwerkt, afgedraaid, ingezet en geanalyseerd door jullie. Tegelijkertijd wil ik de medewerkers van de **PICO-onderzoekskamer** ook bedanken voor het maken van pakketjes, het verwerken van toestemmingsverklaringen, vragenlijsten, het nabellen, en alle andere hands-on taken.

Uiteraard vergeet ik mijn **huidige collega's van EPI** niet, en in het bijzonder de **RVP-groep**! We zijn tijdens de pandemie als afdeling behoorlijk gegroeid, of zal ik zeggen, naar elkaar toegegroeid? Het voelt altijd als een warm bad. Dat blijkt ook wel uit het aantal collega's dat na een uitstapje elders vaak weer terugkomt. Iedereen is behulpzaam en staat altijd klaar voor een ander; dat werd niet alleen duidelijk tijdens de pandemie, maar ook daarna. Dank voor jullie interesse en motiverende woorden in de laatste fase van mijn boekje. Dat heeft me goed gedaan! Ik ben blij dat ik onderdeel mag zijn van deze groep en mijn steentje kan bijdragen aan de inhoudelijke uitdagingen die er voor ons liggen.

En dan zijn er nog mijn **oud collega's van VU** (G&L) waar ik even kort bij stil wil staan. Na twee jaar werkzaam te zijn geweest bij jullie, ging ik **Brechje** achterna en vertrok ik voor mijn PhD-avontuur naar het RIVM. Al mijn opgedane kennis en ervaring als docent is zeer waardevol geweest tijdens de coördinatie van de studies en daarna. Leuk om met een aantal van jullie nog contact te hebben; het voelt altijd direct weer als vanouds. **Esther**, leuk om ieder jaar (als het lukt) een gastcollege te verzorgen bij jouw cursus. En een bijkomend voordeel dat we dan ook direct even gezellig kunnen bijpraten. **Jelske**, vrienden en ook nog eens oud-collega's. Bewonderingswaardig hoe jij je PhD naast het lesgeven hebt gedaan. Inmiddels een nieuwe baan in het VUmc; grote klasse hoe je altijd maar doorgaat. Dank voor je interesse (in mijn onderzoek), tips en vooral gezelligheid. Hopelijk zien we elkaar weer wat vaker als mijn verdediging straks is geweest.

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Naast iedereen die direct betrokken was bij mijn PhD-traject, waren er vrienden en familie die voor veel gezelligheid, plezier, afleiding en ondersteuning hebben gezorgd de afgelopen jaren. Hoewel jullie inhoudelijk niet altijd van de hoed en de rand wisten, heeft ieder op zijn/haar eigen manier wel degelijk bijgedragen.

Daniel & Maya, Lieblings-Schweizer. Dennie, de tank, geen bankier zo humoristisch, stoïcijns en behulpzaam als jij. Jouw doorzettingsvermogen is een voorbeeld voor mij en vele anderen. Inmiddels hebben jij een Maya helemaal jullie leven in Zürich opgebouwd, respect, maar we missen jullie nog steeds. Thanks voor alle oneindig mooie momenten samen, hopelijk volgen er nog vele! **Stef & Joyce**, "salid y disfrutad", ga eropuit en geniet, is wat een wijs man ooit zei. Precies jullie levensmotto en inmiddels zelfs (letterlijk en figuurlijk) onlosmakelijk verbonden met kleine Liv. Jullie top-adresje in Barcelona (hoe kan het ook anders) was meermaals genieten. Dank voor jullie gastvrijheid, oprechte belangstelling in mijn werk, luisterende oor en de vele mooie feestjes; jullie zijn schatten. Naast Dennie en Stefke, zijn er natuurlijk de overige Ajax-boys: **Robert**, **Robbie** en **Bert**. Hoge bergen en diepe dalen lijkt me de beste samenvatting van de situatie in de JC-ArenA de afgelopen jaren. Fan ben je voor het leven. En eerlijk is eerlijk, wie doet ons wat; gezellig is het sowieso. De Europese 'away days' zijn altijd dolle pret met jullie. Nu mijn boekje af is, sluit ik komend seizoen hopelijk weer wat vaker aan. Zeker nu de lange zijde lonkt! "Wij zijn Ajax, wij zijn de beste." **Floris & Lindy**, al zien we elkaar niet vaak, het voelt altijd vertrouwd. Flo, we kennen elkaar al vanaf de middelbare school en zijn elkaar daarna eigenlijk nooit meer uit het oog verloren. We delen de passie voor muziek en draaien en, niet te vergeten, een goed gesprek (oeverloos aan de telefoon). Je bent een gouden gozer en ik waardeer onze vriendschap. En Lindy, hopelijk doe ik het straks net zo goed als jij deed in het Academiegebouw! **Guus & Sanne**, "Words are very unnecessary, they can only do harm." Need I say more? Eigenlijk niet, maar ik doe het toch. Guus, broertje, ouwe sommelier, al ruim 15 jaar onafscheidelijk. Was het nou assay of essay? Of allebei? Man, man, man, wat een tijden hebben we al meegemaakt; we kunnen al heel wat afvinken. Ik waardeer je oprechtheid, adviezen (hoewel ik aardig volhardend kan zijn) en onze onvoorwaardelijke vriendschap. Voor ons is dat vanzelfsprekend; en daar gaat het om. Sanne, the star that keeps on rising, jij bent de perfecte aanvulling voor Guus, een super team. Ik geniet van je oprechte interesse in mensen en je saamhorigheidsgevoel; het goed willen doen voor anderen. Dank voor de mooie reisjes samen (waarop Guus tóch weer stiekem z'n gezicht liet zien), ik hoop ik dat er nog vele volgen. Heerlijk toch, 'als je later groot bent', met een wijntje bij een ondergaand Siciliaans zonnetje. **Jense & Kelly**, Kel en Jens, mijn Mokumers. Op iedere afspraak te laat, maar altijd als eerste als er hulp nodig is. Jens, draaimaat, andere helft van Calippo collective, harde werker, luisterend oor, en vooral lieve vriend. Mooi dat je altijd met je persoonlijke ontwikkeling bezig bent maar anderen nooit uit het oog verliest. Dank dat je er altijd voor me bent en zal zijn. Kel, ongeëvenaarde projectmanager met een goed gevoel voor humor en een snufje luchtigheid. Moet je iets geregeld hebben, bel deze meid; moet je iets niet geregeld hebben, heeft ze het al gedaan. "Wat wordt er straks van ons verwacht bij die verdediging, Vossie?" Niets hoor, deze keer mag je gewoon rustig aanschouwen. Dank voor je hart van

goud en fijne vriendschap. **Munay & Kiki**, de 020-Brabo's. Mun, met deze lopende encyclopedie en parate-kennis-absurdist wil je in een pub-quiz team. De etentjes met de 'knappe mensen' zijn altijd een welkome afleiding, vooral omdat we het eerste half uur altijd even gezellig met zijn vieren kunnen bijpraten. Op nog veel feestjes en gezelligheid met jullie samen! **Nick**, **Esther** en, ik kan wel zeggen, 'petekindjes' **Bo & Jari**. Waar moet ik beginnen, het voelt als familie. Gelukkig heb ik onlangs tijdens jullie geregistreerd partnerschap al in wat langere bewoording kunnen vertellen wat jullie voor mij betekenen. Dat is namelijk wat jarenlang samenwonen doet. Bittersweet is het dat jullie nu op een meer-jaarlijks avontuur naar Mozambique zijn gegaan, maar het is jullie zo gegund. Akka Vos en Dibbie komen snel 'even buurten', hoor. En tsja, hoe vet is het dat jullie gewoon bij deze mijlpaal aanwezig kunnen zijn...obrigado por tudo! **Niiwino & Anne**, kon minder, toch? We hebben elkaar de afgelopen jaren helaas niet zo regelmatig gezien, maar ondanks dat gaan we way back. Dank voor jullie belangstelling in mijn onderzoek en de voortgang ervan. Kunnen we straks eindelijk écht op niveau converseren, dr. Buunk! **Robin & Kim**, Aussiesss! Ik heb weer wat leesvoer voor je geprepareerd, Rob. Dit keer gebundeld en met een mooie kaft. Ook al zijn jullie er tegenwoordig maar ongeveer één keer per jaar, het voelt nooit alsof het lang geleden is. We kunnen niet wachten om jullie straks op te zoeken! **Ronald & Chrissie**, en sinds kort kleine Otis. Ronnie, als er iemand is met karakter ben jij het. Iedere maand een marathon, wie dut mie wat? Ik vergeet alleen nooit meer die blik in je ogen toen ik je op de finishstreep van de halve marathon voorbij had gelopen, haha. Eens en nooit meer overigens. Thanks voor alle leuke momenten samen met jullie, en de vele die er nog volgen. **Simon**, mate. Ik doe het in het Nederlands, want je bent inmiddels een Dutch pro! Dank voor je uitzinnige enthousiasme, dat werkt aanstekelijk voor iedereen. Ik kijk uit naar nog vele feestjes en gezelligheid samen, ouwe stuiterbal! **Vincent**, ouwe dj, vastgoedbaron, amigo en inmiddels ook daddy Vinnie cool. Altijd vol nieuwe ideeën en projecten, in binnen- en buitenland. Dank voor je belangstelling in mijn werk en gezelligheid; santé!

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gekregen; wat een gezelligheid en wat fijn dat het klikt. Het voelt altijd als thuiskomen bij jullie, het enige jammere is toch dat je voor die club uit 010 bent. Bob, hoe leuk dat we nu een soort van huisgenoten zijn! Wat mij betreft gaat die nieuwbouw niet door, hoor. Het doet me goed dat we de afgelopen jaren steeds closer zijn geworden en ik waardeer onze gesprekken over alle zaken in het leven. Evenals het urenlang discussiëren over Ajax, Oranje, opstellingen, spelers, trainers, en alles wat met voetbal te maken heeft natuurlijk. Keep the spirit high, vamos!

Rianne & Johan, helaas ver weg in het hoge Noorden en allemaal onze eigen levens, maar het is altijd fijn als we elkaar weer zien. Lekker een weekendje ontspannen bij jullie met een BBQ in de tuin of juist even crossen door de bossen. Bedankt voor jullie interesse en support al die jaren. **Maarten**, **Tygo** en **Myrle**, ik ben stuk voor stuk trots op jullie. Ondanks dat jullie nog te jong zijn om mijn onderzoek écht te begrijpen, stellen jullie altijd hele pientere vragen tussen al het stoeien, voetballen en ravotten door. Dit oompje is straks écht dr. En als ik jullie één gouden tip mag geven: als het zover is, ga dan studeren in Amsterdam. Wel zo gezellig!

Papa en **mama**, **Bert & Lia**, dit proefschrift heb ik niet voor niets aan jullie opgedragen. Jullie hebben mij van jongs af aan alle kansen geboden om te komen waar ik nu ben. Niets was te veel, ook niet als we weer eens het halve land door moesten voor voetbal. Al die uren wachten en aanmoedigen op trainings- en wedstrijddagen. En toen ik besloot een tweede studie in Amsterdam te gaan doen, stonden jullie volledig achter me. Zonder jullie ondersteuning was dat nooit gelukt en daar ben ik jullie eeuwig dankbaar voor. Helaas is het niet mogelijk om even snel op de koffie te gaan en mis je daarom wel eens de kleine dingen van elkaar. Maar als het even kan, is het altijd fijn om weer op 't plattelaaand te zijn en ontvangen jullie ons met open armen. Hopelijk kunnen we nog vele jaren van elkaar genieten!

Lieve **Debbie**, mijn Deb. Het is moeilijk om in woorden uit te drukken wat jij voor me hebt betekend al die jaren. Jij gunde het mij om een paar maanden naar de Caribbean te gaan. Elkaar los laten is denk ik onze grootste kracht. En jouw nuchterheid en schaterlach niet te vergeten, haha. Het is waar wat ze zeggen: achter al dat harde werk staat ook een krachtige partner. Er leek soms geen einde aan te komen; een pandemie en daarna moest dat boekje ook nog af. Jij zal ongetwijfeld ook blij zijn dat we deze bladzijde nu om kunnen slaan. Hoe cool dat je sinds kort 'even tussendoor' personal trainer bent geworden! "Want ja, waarom niet? Ik wil weer ff wat nieuws leren." Met jouw positivisme probeer je van iedere dag wat moois te maken. En daarbij sta je het liefst ook altijd klaar voor anderen. Weet dat ik onwijs trots op je ben. Dank voor je onuitputtelijke steun, knuffels, kookkunsten, lieve woorden, onze mooie reizen samen, maar bovenal je onvoorwaardelijke liefde. Nu dit avontuur is afgerond, kijk ik uit naar onze toekomstige avonturen. Love joeeeee!

CURRICULUM VITAE

Eric Vos was born on the 31st of March 1987 in Kloosterburen (GN), the Netherlands. After finishing secondary school (VWO) at Het Hogeland College in Warffum in 2005, he obtained a bachelor's degree in Business Administration at the Hanze University of Applied Sciences in Groningen in 2009. After a year of working and traveling he moved to Amsterdam, and started his bachelor studies Health and Life Sciences at the VU University after having developed great interest in health. Eric acquired a double major (Health Sciences as well as Biomedical Sciences) and discovered a passion for infectious disease epidemiology and control. Finalizing his bachelors, he did an internship at the Onze Lieve Vrouwe Gasthuis (OLVG) hospital in Amsterdam, where he studied the progression of HIV diagnoses over the past decade – using data from the clinic and the HIV Monitoring Foundation – under supervision of prof. dr. Kees Brinkman.

After obtaining his bachelor's degree in 2013, Eric continued his master studies in Health Sciences, with a specialization in infectious diseases and public health at the VU University. During that period, he was also involved in a research project at the OLVG hospital (conducting lab- and statistical analyses) investigating the effects of statins on immune parameters in HIV patients. He conducted his master internship at the Municipality Health Service (GGD) Amsterdam, where he studied the epidemiology of high-risk human papillomavirus (HPV) infections among different ethnic groups in Amsterdam – using data from the HELIUS study – under the supervision of dr. Nienke Alberts and prof. dr. Maarten Schim van der Loeff. Both projects at the OLVG and GGD were later published.

In 2014, Eric received his master's degree cum laude. He then started working as a lecturer for the bachelor Health and Life Sciences at the VU University, alongside involvement in a research project on water, sanitation and hygiene (WASH) in Cambodia using data from the Demographic and Health Survey (DHS). He acquired his university teaching degree (BKO) at the VU University in 2016, and developed and coordinated several courses. His teaching predominantly concentrated on infectious diseases, epidemiology and statistics, academic skills and career guidance. Hereafter, he started as a PhD candidate at the Centre for Infectious Disease Control (CIb) of the National Institute for Public Health and the Environment (RIVM), under supervision of dr. Fiona van der Klis, dr. Hester de Melker, and prof. dr. Ymkje Stienstra (UMC Groningen). His PhD research focused on seroepidemiology of vaccine-preventable diseases in Caribbean Netherlands and SARS-CoV-2 during the first year of the COVID-19 pandemic.

In 2020, Eric started working as an epidemiologist at the department of Epidemiology and Surveillance (EPI) at the RIVM. He was involved in several research projects and (sero)surveillance efforts in support of the control of SARS-CoV-2 in the Netherlands. Since 2023 he holds a position as an epidemiologist with emphasis on seroepidemiology of infectious diseases at the National Immunization Program department within EPI at the RIVM.

ABOUT THE COVER

It all comes down to interconnectedness.

Interconnectedness comes in various ways and on multiple levels. From the antibodies circulating through our bloodstream ready to neutralize pathogens, to the tight bonds we share within the kingdom of the Netherlands, and the solidarity we felt to overcome the COVID-19 pandemic together. In this thesis we had the unique opportunity to combine all of these aspects. The cover attempts to capture these mutual connections.

The background colors are a mixture of all the colors from the flags of Bonaire, St. Eustatius, Saba (BES-islands) and the (European) Netherlands to reflect the connectivity within our kingdom. The splashy and cloudy way of presenting these colors refers to the blood samples that have been collected in this seroepidemiological research to assess the presence of antibodies; one of the main data sources that are linked to other relevant characteristics.

The concept of interconnectedness is further specified in the design of the antibodies as the shapes of all three BES-islands are combined into a single-line antibody. Ten antibodies are displayed, referring to the 10th of October 2010 ('10-10-10'); the date at which the BES-islands became special municipalities within the Netherlands and public health fell under direct responsibility of the Dutch government.

The single-line that runs through these antibodies connects the islands to the Netherlands. Yet when looking more closely, the west coast of the Netherlands shapes up to become an ascending epi-curve too, referring to the second part of this thesis. Fortunately, waves of infection tend to fade away, whether or not following adequate interventions. Population immunity is build up and epidemic peaks reduce over time, as depicted on the back side.

Seroepidemiology is a key surveillance tool for the prevention and control of infectious diseases. *Let's remain interconnected.*