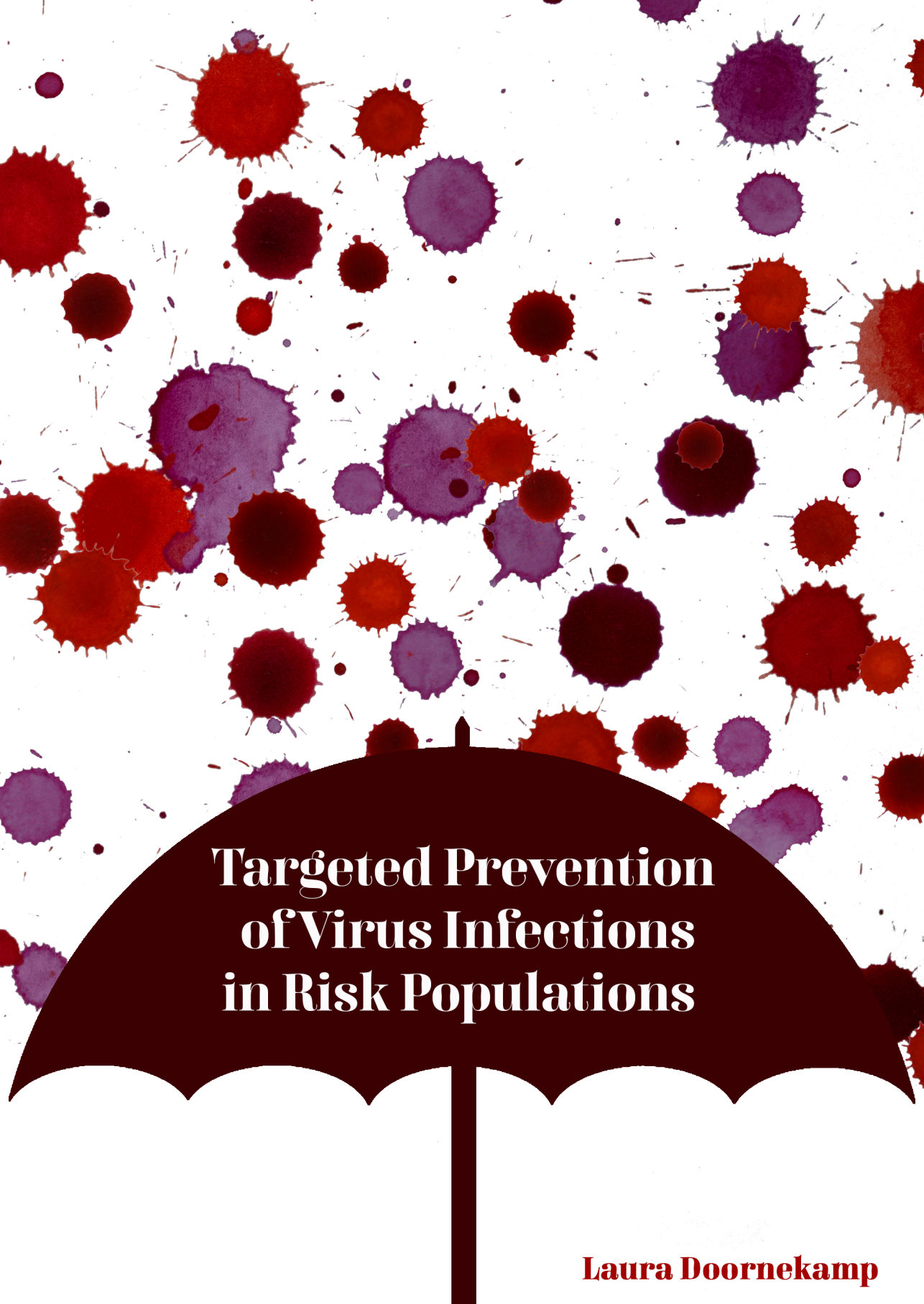

Targeted Prevention of Virus Infections in Risk Populations

Laura Doornekamp

TARGETED PREVENTION OF VIRUS INFECTIONS

IN RISK POPULATIONS

L. Doornekamp

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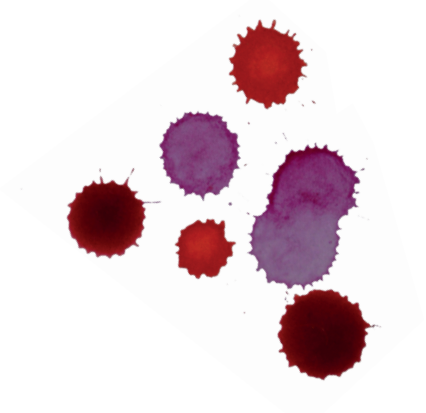
"Prevention is better than cure" (Hippocrates, 460-377 B.C.)

CONTENT

CHAPTER 1

Introduction



Chapter 1.1

General introduction and outline of the thesis

Prevention of virus infections

Although much progress has been made over the last century, infectious diseases still cause high morbidity and mortality worldwide. Distinct prevention and control strategies can be adopted to reduce the infectious disease burden. Prevention strategies are conventionally divided in primary, secondary and tertiary prevention. Primary prevention aims to reduce the incidence of a specific disease for example by prevention of an infection by vaccination. Secondary prevention intends to detect a disease in an early stage and prevent further harm like is being done by population screening programmes. Tertiary prevention measures are introduced when the disease has established and efforts include the prevention of long-term complications, for example the treatment of chronic HIV with antiretroviral therapy (1). In the field of infectious diseases – and particularly in virology – primary prevention is of most importance, as this does not only include the prevention of disease in individuals, but also prevention of spread of an infectious agent in a population at the earliest stage. Thereby, it is serving an important role in public health.

Primary prevention measures can be divided in psychosocial interventions (e.g. alter cognitive determinants and behaviour like condom use or use of mosquito repellents) and biomedical interventions (e.g. vaccinations or prophylactic medication like pre-exposure prophylaxis (PrEP) for HIV). Psychosocial interventions aim to influence human behaviour in such a way that individuals will not be exposed to disease or pathogens, or more specifically viruses. This can be achieved by individual initiation of behaviour, promoted behaviour or imposed behaviour. Many health behavioural models are available that discuss determinants that play a role in the establishment of behaviour, they will be discussed later in this chapter.

Biomedical interventions that prevent infections in human populations are recognized as immune- and chemoprophylaxis. Immune prophylaxis comprise immunizations i.e. vaccination, that provide protection by activation or support (passive immunization) of the immune system. Chemoprophylaxis consists of antibiotic, antiviral, antifungal, or antiparasitic medication. As compliance determines the uptake of these biomedical interventions, behaviour also plays an important role in the effectiveness of these interventions to prevent virus infections. Furthermore, the biological characteristics of a vaccine and the abilities of the immune system of the vaccinated individual determine vaccine efficacy. First, we will look into the different populations that might need vaccinations, thereafter we will discuss vaccine types.

Populations at risk for virus infections

As shown by the examples given before, many measures are in place to offer optimal protection at population level. National immunization programmes (NIP) aim to protect every child against childhood infections that are common in the country they are born in. In most European countries, at least the vaccinations against the following diseases are included in current NIP and freely provided to every child at a certain age: measles, mumps and rubella (MMR); diphtheria, tetanus, acellular pertussis (DTaP); poliomyelitis (IPV); H*aemophilus influenzae type B* (Hib); and pneumococcal disease (PCV-13/PPSV-23).

However, some subpopulations may need protection against a broader panel of pathogens as they are at risk for (severe) infections. This may be a consequence of their behaviour, health, or occupation. Three important groups that need attention here are travellers, immunocompromised patients (ICP), and healthcare workers (HCW). Their behaviour may put them at risk due to exposure to other viruses in other activities and places, as is the case when travelling. Individual's health status may make them vulnerable for severe infections due to diminished immunity. And finally, their occupation may induce increased exposure to (human or animal) individuals carrying an infection or even to viruses directly without intervention of a host (e.g. in the laboratory). As travellers, ICP and HCW are subject of the studies in this thesis, we will further elucidate them.

Travellers. Travellers are at additional risk for infections as at their destinations they may encounter pathogens they were not exposed to before. Travellers were not offered vaccinations from the NIP as the residents of that country may be (e.g. yellow fever), are not immune through natural infections which may be asymptomatic in childhood but severe in adulthood (e.g. hepatitis A), neither may they be aware of the prevalence of certain pathogens in that country and which behaviour to adopt to prevent getting infected. Therefore, travellers are advised to consult a travel clinic, to receive information on the epidemiology of infectious diseases at their destination and to receive immune and chemoprophylaxis if considered necessary. Recommended measures may be dependent on the destination (geographical region and altitude), the season during which they travel, the length of stay, their age and activities they have planned to undertake. Age, allergies and medical history may be contra-indication for certain vaccines.

Immunocompromised patients. ICP is a very heterogeneous group of patients that suffer from a diminished functioning of their immune system, making them more vulnerable for infectious diseases. This can be due to an underlying disease or treatment thereof. Distinction can be made between primary immunodeficiencies like agammaglobulinemia; acquired immunodefiencies, for example oncologic diseases and an infection with human

immunodeficiency virus (HIV); and iatrogenic, often induced by therapies for auto-immune diseases or after organ transplantations. Depending on the disorder or treatment, either the humoral or the cellular immunity or both can be compromised. Another category of ICP are asplenic patients. The spleen has an important role in the control of blood borne pathogens, especially the encapsulated bacteria. Their capsule consists of a polysaccharide membrane, around the cell membrane. Asplenic patients are at additional risk for an invasive infection when encountering these bacteria, as mononuclear phagocytic cells reside within the spleen and this organ produces components that directly activate complement pathways and normally clear these microbes from the blood (2).

Generally, live-attenuated vaccines are contra-indicated in ICP. Other types of vaccines can be given safely, however their efficacy may be compromised. Therefore, post-vaccination antibodies levels can be determined in the blood. If with active immunization desired effects are not reached, passive immunity can be applied for specific diseases, like hepatitis A.

Healthcare workers. At last but not least, HCW are a population with occupational risks for infections. They comprise all those who are involved in the care for patients. Nursing staff and doctors who are directly in contact with patients might be the first ones thought of, but also cleaning staff and laboratory workers are at risk by their contact with patient materials. Therefore, HCW should be protected against infectious diseases that are prevalent in the setting they work and measures are available for. For some diseases, like hepatitis B, measles and influenza vaccinations are available. For other diseases infection prevention measures can be taken in the hospital and personal protective equipment must be provided. In outbreak situations, HCW work in the frontline and also act as a barrier against the spread of infectious diseases in societies.

While travellers are vaccinated and considered protected, vaccine-induced protection is conventionally checked in HCW. For example for hepatitis B, it has to be sure that the HCW does not belong to the group of non-responders, requiring additional vaccinations.

Principles of immunization

Efforts to protect individuals against infectious diseases by immunization find their origin over two centuries ago, and many developments have been made since. Immunization is defined as "the deliberate induction of an immune response", while vaccination is more specifically described as "the induction of protective immune responses against common microbial pathogens in humans" (3). The purpose of vaccination is to generate long-lasting protection against a pathogen and the requirements for the generation of

this protective immunity depend on that pathogen and how it infects humans. Antibodies may provide protective immunity against many pathogens, but to resist some viruses or micro-organisms additional cellular immunity is required. The vaccines that are most similar to the virus normally elicit the best and broadest immune response. Therefore, the production and administration of vaccines is a continuous deliberation between efficacy and safety. The more hazardous the vaccine, the better the protection.

Vaccines may be administered oral, intranasal or parental (subcutaneous, intramuscular, intradermal or intravenous). The most commonly used vaccination routes nowadays are subcutaneous and intramuscular administration. Vaccinations elicit a fast, local innate immune responses and adaptive immune responses thereafter. Dendritic cells phagocytize antigens and present them to local lymph nodes and diffuse into the circulation. In the lymph nodes, the presented antigens activate lymphocytes and antibodies will be produced. When antigens enter the blood, they are delivered to the spleen and a systemic immune response is promoted. The exact mechanisms and quality of the immune response induced is determined by the type of antigen administered (3).

The different types of vaccinations that are available, each have their own advantages and disadvantages, as said often a reciprocity between efficacy and safety. In the following paragraphs, the most common vaccine types will be introduced. It is important to know the differences between these vaccine types, to understand the risks and gains each vaccine type may have. This elaboration will be restricted to well-established vaccine types that are widely used and evaluated in risk groups worldwide.

Live-attenuated vaccines are normally seen as the most effective type of vaccines (as they induce a strong antibody and cellular response), and are the oldest ones that are still being used. Attenuation is induced by mutations of the genetic material of the pathogen, in such a way that it can still replicate but does not cause disease. This attenuation used to be achieved through serial passage in animals or cell cultures, while nowadays genetic modification is more applied. Due to replication of the pathogen, an immune response closest to a natural infection occurs, including both humoral and cell-mediated immunity. As a consequence, one vaccination with a live-attenuated vaccine provokes a potent immune response and generally results in lifelong protection. On the other hand, due to the fact that the pathogen can still replicate, live-attenuated vaccines also bring a higher risk of causing disease, a potentially severe complication not seen by other types of vaccines. Consequently, live-attenuated vaccines are contra-indicated in certain risk groups, like immunocompromised patients. For example, a live-attenuated yellow fever vaccine can induce neurotropic or viscerotropic disease, which is very rare in healthy

individuals but constitutes a bigger risk to immunocompromised patients and elderly. This means that the vaccines provoking the most adequate immune response often are relatively or absolutely contraindicated in the persons who potentially benefit the most.

Inactivated vaccines. A vaccine type that does not involve the risk of causing a vaccine-induced infection, is an inactivated vaccine. This vaccine type contains a killed whole micro-organism or virus that is not capable of replication anymore due to inactivation by heat or chemicals. Inactivated vaccines mainly rely on humoral immunity. Cytotoxic T-cells are not activated, as inactivated viruses will not produce proteins in infected cells that can be presented by MHC class I molecules (3). The inactivation method differs per pathogen, to preserve optimal antigenicity and immunogenicity. An example of a widely used inactivated vaccine is a monovalent hepatitis A vaccine. For the production of this vaccine, the virus is inactivated with formaldehyde. This vaccine is safe and effective, as after two doses (with an interval of at least six months) it generates protection for at least two to three decades. Also for rabies successful inactivated vaccines are available. Rabies virus is cultured in different cell lines and thereafter inactivated with another chemical agent, namely beta-propiolactone (β-PL). Also this vaccine has a high immunogenicity and provides long-term protection.

Polysaccharide and conjugate vaccines. Another method of immunization is by vaccinating with fragments of a particular pathogen. This can be polysaccharides, protein subunits, or other purified components. Purified bacterial capsular polysaccharide vaccines (e.g. pneumococcal polysaccharide vaccine) induce a T-cell independent immune response, resulting in moderate immunogenicity and a lack of T-cell memory. Vaccines that contain proteins generate an immune response that is T-cell dependent. By linking polysaccharide vaccine components to a carrier protein, a process called conjugation, it becomes a T-cell dependent antigen, mounting an improved immune response. Examples of conjugated vaccines are vaccines against *Neissera meningitidis* (serogroup A, C, W, Y) and *Haemophilus influenzae type b.* This principle of an increased immune response due to conjugation is also being used for vaccination against pneumococcal disease (*Streptococcus pneumoniae*), where first a conjugated vaccine is administered mounting a vast immune response against few types of pneumococcal bacteria, followed by a booster with a polysaccharide vaccine containing more strains. Although the second vaccine contains more bacterial types, it is less immunogenic. The principle behind this order in which these vaccines are administered is to build upon the previously developed immune memory from the conjugated vaccine and expand the breath of protection.

Recombinant vaccines. One of the most used vaccines produced by recombinant DNA technology are hepatitis B vaccines. The technique consists of inserting a segment of the gene of the virus into the genome of a vector (for Engerix® e.g. the yeast *Saccharomyces cerevisiae*). This vector subsequently produces the hepatitis B surface antigen (HBsAg) that provokes an immune response when administered - mostly intramuscular - to humans. Another example of a recombinant vaccine is the meningococcal type B vaccine.

Toxoid vaccines. Some bacteria produce toxins that may - rapidly and with very little amount - damage host cells like phagocytes. Often the primary immune response is too slow to provide protection. However, if antibodies are present, they can immediately bind and neutralize these toxins. This principle is being used for vaccination by the production of detoxified exotoxins. After administration of these toxins, serum IgG is produced that can neutralize these toxins. However these vaccines normally provides strong immunity, it does not last long. This vaccine type therefore requires booster vaccinations after a certain period of time. Examples of toxoid vaccines are vaccines against diphtheria (*Corynebacterium diphtheria*), tetanus (*Clostridium tetani*) and pertussis (*Bordetella pertussis*).

Often for a specific pathogens different types of vaccines are available. For example, for influenza, live-attenuated vaccines, inactivated whole cell and subunit vaccines are currently approved by the Food and Drug Administration (FDA) (4). Although the live-attenuated vaccine induces a stronger immune response and provides long-term immunity compared to the inactivated ones, the vaccine still has to be administered yearly due to antigenic drift. Much research is done to develop a universal influenza vaccine to substitute the seasonal ones.

The previously mentioned vaccines types all make use of the principle of active immunization. Another option to provide immunity is passive immunization. Passive immunity is achieved by the administration of antibodies to an individual. This is mostly done via the intramuscular or intravenous route. However, vertical transmission of antibodies is also an example of passive immunity and also via breastmilk passive immunity is brought to newborns. Administered antibodies provide fast, or even direct, immunity. However, due to the decay of antibodies, the immunity is only short-lived and no immunological memory is developed. In principal protection after passive immunization will last for a few weeks, depending on the dose administered. This may last longer in case of maternal antibodies. Antibodies may derive from other human individuals or animals, but can also be produced artificially. Passive immunity is applied when the immune system of the recipient is not capable of producing a protective immune response fast enough. Post-exposure prophylaxis is for example applied after a needle stick injury with

hepatitis B positive materials or a bite of a potentially rabid animal in unvaccinated or immunocompromised individuals. Gammaglobulines provide an alternative to protect immunocompromised patients (ICP) against hepatitis A if active immunization will not provide the desired protection.

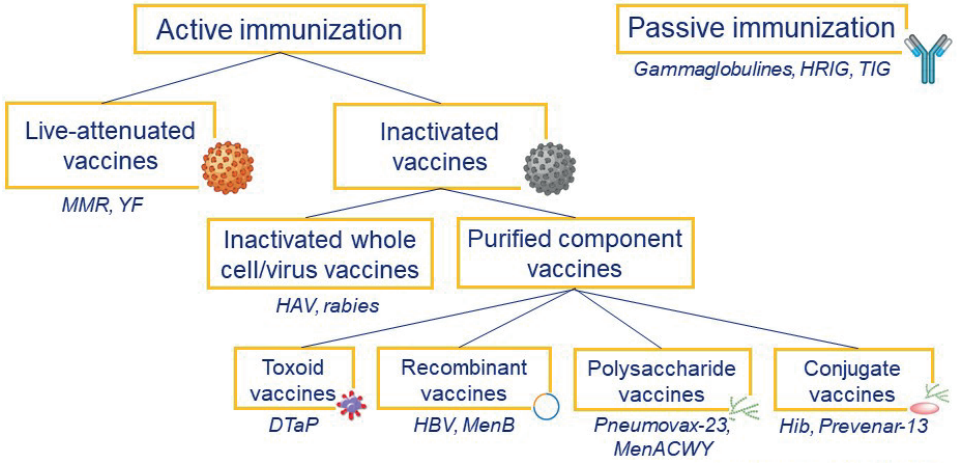


Figure 1. Different types of vaccines that are available
HRIG: Human rabies immunoglobulins; TIG: tetanus immunoglobulins; MMR: measles, mumps, rubella; YF: yellow fever, HAV: hepatitis A virus; DTaP: diphtheria, tetanus, acellular pertussis; HBV: hepatitis B virus; MenB: meningococcal B disease; Pneumovax-23: pneumococcal bacteria (23 serotypes); MenACWY: meningococcal bacteria type A, C, W, Y; Hib: Haemophilus influenzae type B; Prevenar-13: pneumococcal bacteria (13 serotypes).

Besides the antigens that are responsible for the main goal of vaccination, vaccines contain additives to support and maintain its functions. Vaccines contain adjuvants, preservatives, stabilizers and suspending fluids. Adjuvants are immune modulators that generate aspecific activation of the immune system. Aluminium salt is an example of an adjuvant of which a very small amount can be added to a vaccine to stimulate the innate immune response. Preservatives, like antimicrobial agents, maintain the quality of vaccines, specifically important in case of multi-dose vials. Stabilizers are agents that aim to maintain the immunogenicity of vaccines in varying conditions, for example exposure to heat and light. Lastly, suspending fluids consist of sterile water or saline and may still contain components of the system in which vaccines are produced (e.g. egg products).

Health-behaviour

As shown, vaccines are available for many infectious diseases. However, if diseases becomes vaccine-preventable, it is not realistic to think that this is enough to decrease the

prevalence or even eradicate these diseases. Recent years, we even saw a rising incidence of some vaccine-preventable diseases (VPD) like measles, which was mainly caused by declining vaccination uptake (5). The decision to get vaccinated or not is part of human health-behaviour. This concept is defined as "behavioral patterns, actions and habits that relate to health maintenance, to health restoration and to health improvement" (6). Also, when vaccines are unavailable, health-behaviour plays a major role in prevention of infections, for example to comply hygiene measures. To better understand human health-behaviour, multiple behavioural models are designed. Here, the most commonly used models to explain preventive health behaviour like vaccination uptake will be discussed.

The Knowledge, Attitude, Behaviour (KAB) theory is long-existing concept, widely used to study the effects of public health education. This model underlines the importance of knowledge, as knowledge can lead to a positive attitude change. It is hypothesized that attitude would subsequently lead to a behavioural change on the long-term. However, this is under debate, with the main argument that solely attitude cannot predict or explain behaviour, psychological and social factors also have an important contribution to behaviour (7). However, the KAB model is still endorsed by the WHO to uniformly collect information about health related topics (8, 9).

A more comprehensive model to explain behaviour is the Health Belief Model (HBM), which was developed by Hochbaum, Kegels and Rosenstock (10). These three psychologists designed this social cognition model to predict health-related behaviours based on individuals' beliefs about health risks. The determinants of this model consist of the perceived severity and susceptibility of a disease and the perceived benefits and barriers of the health behaviour. Furthermore self-efficacy and call-to-action play a role (11). The concept self-efficacy was introduced by Bandura in his Social Cognitive Theory, and he defined this as "individuals belief in their capability to perform a behaviour" (12). Fishbein & Ajzen came up with their Theory of Reasoned Action (TRA), which was developed around ones intention to perform (or to not perform) certain behaviour (13). The concept self-efficacy was added to the TRA resulting in the Theory of Planned Behaviour (TPB). The TPB states that intention is determined by attitude to behaviour, subjective norms and perceived behavioural control. The TPB model is very similar to, and the foundation for, the ASE model in which intention to behaviour is explained by attitude, social norm and self-efficacy (ASE). Attitude is described as beliefs about the outcomes of the behaviour, and the social norm as beliefs about what other people think one should do and the willingness to conform to these opinions (14).

Recently, a Dutch psychologist named de Vries integrated several behavioural models, resulting in the integrated change model (I-Change model) (15). This model distinguishes three phases: a pre-motivational, a motivational and a post-motivational phase. In the pre-motivational phase knowledge, behavioural cognizance (awareness of own behaviour), risk perception and cues play a role. The motivational factors contain attitude, self-efficacy, social influence and intention and the post-motivational state ability factors and barriers (16, 17). Table 1 shows the evaluation of the models and the components that lay on the bases of each model.

Empowerment is another concept that contributes to health behaviour according to Kickbusch and Nutbeam and it includes people's abilities to gain control over and improve their health (24). The WHO states in their health promotion framework that empowerment needs to be an element of significant and sustainable behaviour change. The concept as a whole is not described in one of the health behavioural models mentioned before, but is an element of other components of the models.

Herd immunity

Although many of these health-behavioural models focus on individual behaviour, the decision to get vaccinated has implications for communities. This can best be explained by introducing the concept of herd immunity. Herd immunity is commonly defined as protection for a certain infectious disease in a population although not every individual is protected (25). As the proportion of protected individuals is large enough, they protect the susceptible individuals in that population by interrupting the chain of transmission. The necessary level of immunity in a population to prevent spread of a disease depends on the infectivity of the disease. A basic reproduction number (R_0) can be defined as "the number of cases generated by a typical infectious individual when the rest of the population is susceptible" and depends on the transmissibility of the disease and the proximity of individuals (25). For example, as measles is a very contagious infection with a R_0 of 11-18 the vaccination coverage must be at least 96 percent to reach herd immunity (26). Hereby, it is crucial that susceptible individuals are equally spread among protected populations. With this principle applied, ICP who cannot be vaccinated with the live-attenuated MMR vaccine, may be protected against measles by society.

Table 1. Health behaviour theories on the basis of the I-Change model

Health-behaviour model	Author, year	Components or Constructs or Stages (→)	Based on
Health Belief Model (HBM)	Hochbaum, Kegels & Rosenstock, 1952 (18)	• Perceived susceptibility, severity, benefits and barriers • Self-efficacy • Call-to-action	
Theory of Reasoned Action (TRA)	Fishbein, Ajzen, 1967 (19)	• Attitude toward behaviour • Subjective norms • Intention to engage in certain behavior	
Trans-Theoretical Model (TTM)	Prochaska, DiClemente, 1977 (20)	• Precontemplation → • Contemplation → • Preparation → • Action → • Maintenance → • Termination	
Theory of Planned Behaviour (TPB)	Ajzen, 1985 (21)	• Attitude toward behaviour • Subjective norm • Perceived behaviour control • Intention to behaviour • Behaviour	TRA
Social Cognitive Theory (SCT)	Bandura, 1986 (12)	• Reciprocal Determinism • Behavioural Capability • Observational Learning • Reinforcements • Expectations • Self-efficacy	Social Learning Theory (Bandura, 1960)
Precaution Adoption Process Model (PAPM)	Weinstein, 1988 (22)	• Unaware → • Unengaged → • Undecided → • Decided not to act OR • Decided to act → • Acting → • Maintenance	
Attitude - Social Influence - Efficacy model (ASE)	De Vries, Dijkstra, & Kuhlman, 1988 (23)	• Attitude • Social Influence • Self-Efficacy	
Integrated Change Model (I-Change)	De Vries, 2004(15)	• Predisposing determinants • Awareness determinants • Information determinants • Motivation determinants • Intention • Abilities • Barriers	ASE, TRA, SCT, TTM and PAPM

Evaluation of induced immunity; correlate(s) of protection

As becomes clear from the previous paragraphs, not every vaccination will by definition result in protection. To ascertain if a vaccination has generated protective immunity, concentrations of elicited antibodies in the peripheral bloodstream can be measured. Different assays are available to determine these levels, mostly known as enzyme immunoassays. However, the presence of antibodies in serum does not per definition conclude something about their functionality. Its match with the strain or subtype, isotype subclass, and affinity determine the ability to neutralize an invading pathogen. Isotype switching and affinity maturation requires an interaction of antigen specific B- and T-cell lymphocytes. Thus, the measurement of neutralizing antibodies does not only say something about humoral immunity, but also involves T-cell mediated immunity. Functional assays exist to measure the concentration of virus neutralizing antibodies (VNA) present in serum. However, as this requires live virus to test neutralization abilities of antibodies, this is not an assay that that can be performed easily. And still, if neutralizing antibodies are present, the questions is, which level provides protection against an encounter with the pathogen vaccinated for. In vaccinology the term 'correlate of protection' was introduced, to define an estimated measure for the effectiveness of vaccines.

With a correlate of protection set, vaccine efficacy can also be determined. Vaccine efficacy comprises the protective effect of a vaccine under ideal circumstances. With the development of new vaccines, the immune responses of recipients are extensively studied and if they meet the correlate of protection they are considered efficacious. This concept is not to be confused with effectiveness, which can be measured when a vaccine is implemented and the prevalence of disease is compared to vaccine receipt.

Dry blood spots

To determine the protection rate of populations, seroprevalence studies are performed. Generally, this is done in serum collected via venipuncture. However, as often only small volumes are needed to perform serology, microsampling is an alternative blood collection method that is less invasive. A frequently used microsampling method is the dry blood spot (DBS). DBS consist of whole blood, obtained with a finger stick, collected on filter paper cards. After the blood spots have dried, the cards may be saved in a foil bag. Subsequently, they can be stored at room temperature for two weeks, and may be refrigerated for longer lasting storage (27). All of these practical characteristics provide opportunities to collect materials from large populations and in remote settings. DBS were originally used for neonatal screenings for metabolic diseases (28), but its use has been extended for other purposes, including diagnostics of infectious diseases. Serological

analyses have been successfully performed on human DBS for example for hepatitis A, B and C, measles, mumps and rubella (27). With the ease of collection and storage of DBS samples, this technique provides opportunities for seroprevalence studies for a much broader range of viral infections, which we will subject of studies in this thesis.

Aim and outline of this thesis

In this thesis, the prevention of virus infections in three important risk populations - namely travellers, immunocompromised patients (ICP) and healthcare workers (HCW) - was investigated. These groups were chosen as they are at increased risk for (severe) infections due to their behaviour, health or occupation. The studies reported in the chapters give insight in how well these risk groups are protected, show methods to detect individuals in risk populations that are insufficiently protected and discover the causes of this suboptimal protection. Finally, the aim is to provide information regarding the current situation needed to intervene in the right places to optimize protection against virus infections in populations at risk.

Vaccines are one way to offer protection against virus infections in risk groups. However, they can only be of value if received by target populations. The decision to get vaccinated if influenced by many interacting cognitive determinants. In **Chapter 1.2** all different determinants that explain vaccination uptake in travellers, ICP and HCW were collected and compared. This resulted in a comprehensive overview presenting the most important promotors and barriers for uptake in these risk groups. Other, more biological factors that determine the state of protection were discussed in subsequent chapters. A separate chapter is devoted to each of the risk groups. In Chapter 2 we studied the vaccination statuses of travellers. In a national setting, questionnaires, vaccination records and seroprevalence data were collected from international travellers. The seroprevalence data was gathered with dry blood spots, a minimally invasive collection method generating reliable serological results which has therefore been gaining greater interest for population studies. This sample type has been validated for serology for some viral infections, among which also the viral infections studied in the following chapters. First, in **Chapter 2.1**, the vaccination rate for hepatitis A was studied. Hepatitis A is a widely recommended travel vaccine that is not included in the Dutch NIP and therefore most representative for all travel vaccines. Subsequently, in **Chapter 2.2**, the focus was on measles vaccination, a vaccine that is included in the Dutch NIP since 1975 but seem to be re-emerging with increasing numbers of measles outbreaks world-wide. Due to vaccine hesitancy the vaccination coverage lowers and the virus gets the chance of causing epidemics. Therefore, it is increasingly important to protect travellers who might visit regions where outbreaks happen. In Chapter 3, the vaccination status of the second risk population was assessed,

namely HCW. They are at risk for viral infections due to exposure to potentially infected patients or their materials. Some vaccines are indicated for almost all HCW, others only for a very specific group. In the next chapter the vaccination status and detection methods for one of each of these categories is studied. In **Chapter 3.1** the most important and widely recommended or even mandatory vaccine for HCW, namely hepatitis B, is studied. We evaluated seven years of hepatitis B vaccination policy in future HCW in a large tertiary centre and investigated if the number of booster vaccines given could be decreased. In **Chapter 3.2** the focus is on rabies vaccination. Although this vaccination is only recommended for a very specific subgroup of HCW, the efficacy is extremely important as exposure in insufficiently vaccinated individuals may lead to almost always fatal rabies disease. In this chapter, a protocol was developed and tested to use the - easy to collect and store - DBS for the determination of protective immunity against rabies virus. Implementation of this collection method, especially in remote regions where rabies is endemic, could ease the assessment of protective immunity in veterinary and laboratory workers. In Chapter 4, we shift to the last risk groups of this thesis, namely ICP, and brought to light causes for suboptimal protection in ICP. **Chapter 4.1** gives insight into the daily practice of HCW that are involved in the treatment of ICP and evaluated their suggestions for improvement. As this chapter revealed that seasonal influenza vaccine was considered one of the most important vaccines for ICP, it is important to know the efficacy of this vaccine in distinct ICP subpopulations. However, as new immunosuppressive therapies are continuously introduced to the market, the effect of new therapies on the vaccination response is often largely unknown. Therefore, the efficacy of influenza vaccination in Crohn's disease patients treated with a novel biological agent (ustekinumab) was studied in **Chapter 4.2.** Ustekinumab, is an interleukin-12 and interleukin-23 inhibitor which is an increasingly used immunosuppressive treatment for morbus Crohn. Finally, if vaccines are unavailable, education is most important to initiate sustained behaviour to prevent infections. Therefore, an international educational intervention that could change behaviour to prevent virus infections was studied in Chapter 5. In **Chapter 5.1** the impact of the module on knowledge, attitude and behaviour – assessed in three different countries – was reported. As these results made clear that knowledge and a positive attitude were not enough to change behaviour, we studied the presence of another element in the education module. In **Chapter 5.2** we reported the results of a qualitative study evaluating empowerment among participants. Empowerment includes people's abilities to gain control over and improve their health, being a concept that contributes to health behaviour. The results of all abovementioned studies are placed in perspective in the final chapter. **Chapter 6** gives a summary and general discussion on optimizing the protection of travellers, ICP and HCW against virus infections and gives future implications of this work.

Chapter 1.2

Determinants of vaccination uptake rates in risk populations -A comprehensive literature review

L. Doornekamp, L.P.M. van Leeuwen, E.C.M. van Gorp, H.A.C.M. Voeten, M. Goeijenbier

Vaccines, 2020

ABSTRACT

Vaccination uptake has decreased globally in recent years, with a subsequent rise of vaccine-preventable diseases. Travellers, immunocompromised patients (ICP), and healthcare workers (HCW) are groups at increased risk for (severe) infectious diseases due to their behaviour, health, or occupation, respectively. While targeted vaccination guidelines are available, vaccination uptake seems low. In this review, we give a comprehensive overview of determinants—based on the integrated change model—predicting vaccination uptake in these groups. In travellers, low perceived risk of infection and low awareness of vaccination recommendations contributed to low uptake. Additionally, ICP were often unaware of the recommended vaccinations. A physician's recommendation is strongly correlated with higher uptake. Furthermore, ICP appeared to be mainly concerned about the risks of vaccination and fear of deterioration of their underlying disease. For HCW, perceived risk of (the severity of) infection for themselves and for their patients together with perceived benefits of vaccination contribute most to their vaccination behaviour. As the determinants that affect uptake are numerous and diverse, we argue that future studies and interventions should be based on multifactorial health behaviour models, especially for travellers and ICP as only a limited number of such studies is available yet.

INTRODUCTION

Vaccinations have proven to play a major role in the prevention and control of many infectious diseases. However, in the twenty-first century, vaccination programs face multiple challenges (29). The first one is the need for fast development of effective and safe vaccines for new (re-)emerging pathogens. The recent SARS-CoV-2 pandemic is an example in which a vaccination is highly desired and may reduce the enormous impact of the current pandemic. The second challenge in the field of vaccinology is the upcoming trend of vaccine hesitancy and declining vaccination uptake.

Vaccine hesitancy is recognised by the World Health Organization (WHO) to be one of the ten threats to global health (30). Vaccination uptake is declining globally, resulting in a rise in outbreaks of vaccine-preventable diseases (VPD) (5). For instance, measles cases have increased—up to 300 percent—over the past years (31). Vaccine hesitancy has predominantly received attention in the light of parents rejecting the national immunization programs. However, low vaccination uptake among adult populations also raises concerns (32). Adults are progressively at risk for infectious diseases because life expectancy increases (33), the incidence of chronic diseases that require immunosuppressive treatment rises (34), and international travel expands (35). Other determinants will play a role in vaccination uptake in adult populations as compared to children.

Adults who are recommended to get vaccinated can be divided into several risk groups. Risk populations in this context are defined as groups of human individuals with an increased risk of acquiring a (severe) infection due to their behaviour, health, or occupation. To get a broad overview of determinants that play a role in the vaccination uptake among risk groups, this review will focus on three distinct risk groups which consult vaccination clinics frequently, namely: "travellers, immunocompromised patients (ICP) and healthcare workers (HCW)".

Travellers comprise a risk population, as at their destinations they can be exposed to infectious diseases they have not encountered before. Traveller vaccination guidelines are available to protect this population. These guidelines do not only differ per destination but are also dependent on the activities the travellers will undertake and the duration of their stay. Additionally, the country of origin is of importance, because of the endemicity of infectious diseases and therefore natural exposure, and national immunization programs. Moreover, travellers who are not properly vaccinated for their trip are not only at risk for getting sick themselves, they can also create a public health concern for communicable diseases, as they could carry an infection back home to a naïve population (36).

ICP have an increased risk for serious illnesses caused by infectious diseases due to a diminished function of their immune system. The compromised state of their immune system can be induced by either an underlying disease or the treatment of a disease. As a consequence of fast-developing immunosuppressive therapies for e.g., auto-immune diseases and malignancies, ICP are a constantly growing population (34). Therefore, optimal protection of this vulnerable group is of utmost importance.

HCW are another risk category for acquiring infectious diseases. Their occupation brings them in close contact with patients, that possibly carry an infectious disease. Furthermore, HCW are not only personally at risk, they may also put their—mostly vulnerable—patients at risk when they work while carrying an infection (37). On top of that, HCW play an important role in providing their (immunocompromised or travelling) patients with information or recommendations regarding vaccinations.

Vaccination uptake varies between risk populations and there may be differences in determinants that play a role in this behaviour. To find general patterns each risk group will be studied separately. However, as travellers, ICP, and HCW are interrelated, we aim to learn from similarities and differences between these groups. If we understand risk populations' motivations and concerns, we might be able to address these either separately or combined by effective interventions. To get a better overview of all determinants that have a possible impact on uptake, we classified these in a model of health behaviour change.

An abundance of behaviour change models are available that describe determinants affecting preventive health behaviour (38). In 2003, the integrated change (I-Change) model was developed (15, 39). This model is derived from the attitude-social norm-self-efficacy (ASE) model and integrates several other models, among which are the often-used health belief model (HBM) and the theory of planned behaviour (TPB) (Supplementary Table S1). According to the I-Change model, vaccination behaviour is shaped by the intention to get vaccinated which is subject to barriers and facilitators. Intention is established by motivation, awareness, information, and predisposing determinants. As this I-Change model comprises a wide variety of determinants that are used by other studies, for example those based on the HBM and ASE model, we use this model as a conceptual framework.

With this comprehensive review, we aim to better understand determinants that play a role in the uptake of vaccinations in travellers, ICP, and HCW and explore similarities and differences in these three groups. Hereby, we aim to create a solid ground for the development of evidence-based interventions to increase vaccination uptake in the

populations that need optimal prevention strategies for infectious diseases.

METHODS

Search Strategy

We performed a systematic database search on 19 February 2020. We performed one search for all three risk groups (Supplementary File S1). For each risk groups we combined search terms for vaccination uptake and health behavioural models. We searched the following databases: Embase, Medline, Cinahl, Web of Science Core Collection, ERIC, PsychINFO, and SocINDEX. As determinants of vaccination uptake may vary over time, we limited our search to studies published during the last ten years (between 1 January 2010 and 1 January 2020). We excluded research papers written in another language than English. All records were retrieved into an EndNote database. Duplicates were removed and titles and abstracts were screened (by L.D.). Thereafter, papers were sorted in the three different groups and full texts articles were reviewed for suitability using inclusion and exclusion criteria (by L.D. and L.v.L.) using EndNote X9.

Study Selection

Studies were included if they met all of the following criteria: (1) at least 75% of the included respondents are either ICP (patients with autoimmune diseases, malignancies, HIV, asplenia and solid organ or stem cell transplantations) or travellers (including travellers visiting friends and relatives (VFR), short- and long-term business travellers) or HCW (including general practitioners (GPs), physicians and nurses working in a hospital); (2) addressing self-reported cognitive determinants that may explain vaccination uptake; and (3) being performed in Western countries (defined as Europe, North America, Australia, and New Zealand).

We excluded studies that focussed on: (1) children; (2) HCW who care for populations other than the ICP defined in our study (e.g., paediatricians, elderly home physicians) or who are not directly involved in the care for this group (e.g., pharmacists, dentists); (3) future healthcare workers (e.g., medicine or nursing students); (4) uptake of the national immunization programme (e.g., HPV vaccination); (5) hypothetical vaccinations (e.g., a HIV vaccine); (6) vaccinations administered in outbreak situations (e.g., H1N1 vaccine, Ebola vaccine); (7) other very specific target groups (e.g., Roma travellers, migrants, pregnant women; and (8) predisposing factors exclusively. We also excluded qualitative studies and non-peer reviewed articles such as conference abstracts.

In case any doubt or disagreement between the two researchers who performed the study selection (by L.D. and L.v.L.) arose, the specific papers were discussed in a plenary session with all co-authors.

Data Extraction

The following background characteristics from included studies were extracted: first author and year of publication; study design; enrolment period; enrolment site; sample size; study population; theoretical framework; and targeted outcome variables. Extracted data was collected in Microsoft Excel 2016 and the presence and impact of determinants were rated in separate sheets per study group (by L.D. and L.v.L.). Random samples were taken to check the data extraction and disagreements were discussed plenary with all co-authors. Furthermore, the quality of studies was assessed using the the AXIS tool (40), which is a screening tool specifically designed for cross-sectional studies, as those in our review, and includes 20 items relevant to this design. Scores 1–9 are rates as low, 10–14 as medium and 15–20 as high.

Labelling of Determinants

The I-Change model was used to organize all determinants that could explain vaccination uptake. A simplified version of this model is shown in Figure 1. The following concepts are used: (1) predisposing factors, including baseline characteristics of studied populations; (2) information factors, including information retrieved via media, social contacts and HCW; (3) awareness, of the infectious agent being present or a vaccine being available; (4) knowledge (either examined or self-evaluated), about the consequences of the infection, or about the efficacy and duration of protection of vaccination; (5a) perceived risk of the infection, which is divided into perceived severity of the disease and perceived susceptibility to get infected; (5b) perceived risk of vaccination, including vaccine-specific considerations such as fear of side-effects and trust in the effectiveness of the vaccine; (6) attitude, defined as a person's disposition to respond favourably or unfavourably to vaccinations (21), often reflected by a person's general believes about vaccinations; (7) social influence, which can be social norms imposed by family, friends or religion, but also recommendations from a healthcare professional or tour guide; (8) self-efficacy, defined as beliefs in one's own capacity to perform certain behaviour (12); (9) intention to behaviour, expressed by people before they perform the behaviour; (10) barriers and facilitators, that withhold individuals from or enable them to certain behaviour, such as time, costs, or accessibility.

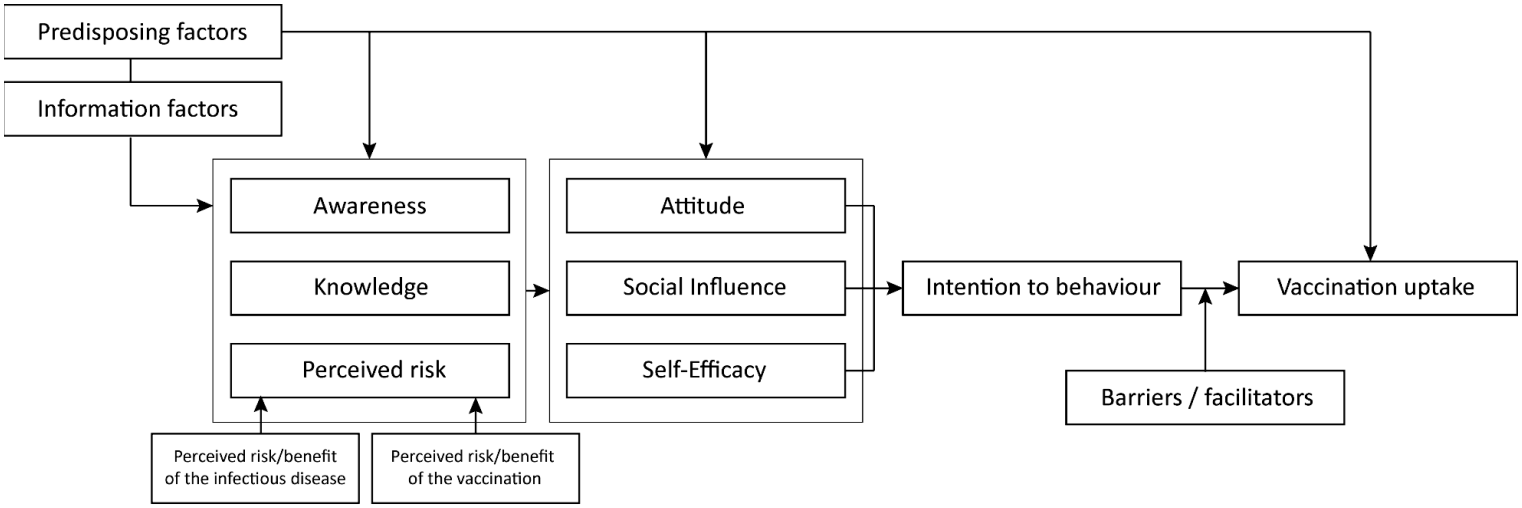


Figure 1. Simplified I-Change model summarizing the studied determinants that could predict vaccination uptake. We used a simplified version of the I-Change model applied to vaccination uptake. Uptake is shaped by the intention to get vaccinated which is subject to barriers and facilitators. Intention is established by motivation (attitude, social influence, and self-efficacy), awareness (awareness, knowledge, and perceived risk) and information and predisposing determinants. Predisposing factors include baseline characteristics of studied populations and influence awareness, motivation and uptake. Information factors include information retrieved via media, social contacts and healthcare workers.

RESULTS

The literature search generated 2227 hits (Figure 2). After removing duplicates and excluding articles published before 2010, 1260 articles were available on the topic. These were screened based on title and abstract, resulting in 242 articles that were eligible for full-text assessment. These were divided into the three subgroups (some were included in more than one category): 30 for travellers, 95 for ICP, and 122 for HCW. Finally, 17, 29, and 44 articles were included in the data analysis for the three groups, respectively. The most common reason for exclusion was that no determinants (other than predisposing factors) were reported. Table 1 describes the characteristics and quality of included studies for travellers, ICP, and HCW. Determinants that play a role in vaccination uptake were retrieved from the articles and summarized in Tables 2–4 for travellers, ICP, and HCW respectively. The results of the quality assessment are presented in Supplementary Table S2.

Table 1. Study characteristics of included studies for travellers, ICP and HCW.

Study	Study Design	Enrolment Period	Enrolment Site	Sample Size	Study Population	Theoretical Framework	Outcome Measures *	Vaccination Coverage	Quality Score **
Balaban, 2013 (38)	Pre- and post-travel surveys	2009	USA	186	American Hajj pilgrims	None	Seasonal influenza	-	Low
Barasheed, 2014 (39)	Cross-sectional survey	2011–2012	Mina (Mecca)	966	Australian Hajj pilgrims	None	Influenza	62%	High
Duffy, 2013 (40)	Cross-sectional survey	2007 (Aug.–Sept.)	United States	1691	American travellers to Asia	None	JE	11%	Medium
Frew, 2017 (41)	Cross-sectional survey	2015 (Feb.–March)	Ferry ports of 2 popular islands, Thailand	1680	Backpackers from Europe, Canada, Australia and New-Zealand (94%)	None (KAP)	HBV	31% completed series	High
Goodman, 2014 (42)	Online cross-sectional survey	2010 (Feb.)	UK	302	Travellers to the meningitis belt of Africa in last 3 years or planned to do so next 6 months	None	MenAWCY	30%	Medium
Herbinger, 2011 (43)	Online cross-sectional survey	2009 (Dec.)	Netherlands, Czech Republic, Spain, Sweden	4203	Travellers to countries of moderate or high prevalence for HBV in the last 5 years	None	HBV	39% in the previous 5 years	Medium
Heywood, 2016 (44)	Online cross-sectional survey	2014 (Aug.–Oct.)	Australia, Finland, Germany, Norway, Sweden, UK, Canada	27,386	Travellers (18–65 years) who travelled to HAV endemic countries in Africa, Asia, South/Central America in the last 3 years	None	HAV/HBV	27% for 3-dose combined HAV and HBV and 37% for 2-dose monovalent HAV schedules	Medium
Igreja, 2019 (45)	Cross-sectional survey	2019 (May–June)	Travel Clinic, Lisbon, Portugal	55	Portuguese travellers	None	Attitudes to vaccinations in general	-	Low
Lammert, 2016 (46)	Retrospective study	2012–2014	clinics from Global TravEpiNet, USA	24,478	International travellers who sought pre-travel health advice	None	Refusal rates of recommended vaccines and reasons	25% refused one or more recommended vaccine(s)	High
Paudel, 2017 (47)	Prospective enhanced surveillance study	2013 (Feb.)–2014 (Jan.)	Australia	180	confirmed cases of typhoid, paratyphoid, measles, HAV, HEV, chikungunya, malaria	None	Seeking pre-travel advice and uptake	25% sought pre-travel advice and 16% got vaccinated	Medium
Pavli, 2019 (48)	Cross-sectional survey by email	2015 (Nov.)–2016 (Mar.)	Greece	231	Greek (non-healthcare) students from 36 universities, planning to study abroad	None	Men, intention to vaccinate	23% vaccinated, 15% intention	Medium
Pfeil, 2010 (49)	2 cross-sectional surveys	2009 (Jan.–Feb.), 2010 (Jan.)	Centre for Travel Health, Zurich, Switzerland	623	Travellers to a resource-limited destination	None (KAP)	Seasonal (and pandemic) influenza	14% seasonal influenza	High
Selcuk, 2016 (50)	Cross-sectional survey	2013 (July)	Istanbul Ataturk Airport, Istanbul, Turkey	124	Turkish travellers to Africa	None	Recommended for destination	53% vaccinated pre-travel	Medium
Tan, 2017 (51)	Retrospective cohort study	2012 (Jan.)–2013 (Dec.)	Mayo Clinic Travel and Tropical Medicine Clinic, Minnesota, USA	2073	Children and adults who sought pre-travel advice (19% VFR)	None	Documented receipt or positive serology or completion of series	94% men in VFR, 12% rabies in VFR	High

Tashani, 2016 (52)	Cross-sectional survey	2014–2015	Immunization clinic, Sydney, Australia	300	Travellers (>18 year) planning to attend Hajj	None	Pneu and DTP when recommended	17% pneu, 14% DTP	Medium
Wiemken, 2015 (53)	Cross-sectional study	2013 (Nov.)–2014 (July)	University of Louisville, Travel Clinic, USA	183	American travellers before their consultation	TPB	Intention to get vaccinated	Not given	High
Yanni, 2010 (54)	Pre- and post-travel surveys	2008 (June–Sept.)	Departure lounges at airports in New York, Chicago, Los Angeles, and San Francisco	1301 (pre) (337 post)	American travellers who will travel to Asia	KAP	Influenza	41%	High
Akin, 2016 (55)	Cross-sectional survey	2015 (July–Sept.)	Daycare chemotherapy unit of Hacettepe University Cancer Institute, Ankara, Turkey	229	Adult patients with cancer receiving chemotherapy	None	Adult vaccination coverage (influenza, tetanus, hepatitis, pneu)	54% were vaccinated at least once, only 9% after cancer diagnosis	Medium
Althoff, 2010 (56)	Nested influenza study (interview administered surveys)	2006–2007 and 2007–2008	5 cities in the USA	1462	HIV+ women	HBM	Influenza	55–57% of women reported vaccination (about 44% not vaccinated)	Medium
Battistella, 2019 (57)	Cross-sectional observational study	2017 (Jan.–July)	7 large dialysis services, Italy	703	Dialysis patients	None	Influenza	58% adherence	High
Chehab, 2018 (58)	Cross-sectional study (in longitudinal cohort)	2012 (Nov.)–2013 (Oct.)	Germany	579	SLE patients (48% on IS)	None	Influenza, tetanus, pneu, men and previous refusal	45% influenza (last year); 65% tetanus; 32% pneu; 6% men	High
Chin-Yee, 2011 (59)	Cohort study (one time follow-up)	2009 (Oct.)–2010 (Mar.)	Tertiary care cancer center, Canada	129	Patients with hematologic malignancies (92% chemotherapy, 76% in past 3 mo)	None	Seasonal influenza (and pandemic)	57% seasonal influenza	Medium
Gagneux-Brunon, 2019 (60)	Cross-sectional survey	Unknown	France	468	HIV+ patients	None	Pneu, HAV, HBV, seasonal influenza	30% IPD; 24% HAV; 64% HBV; 40% influenza	Low
Haroon, 2011 (61)	Cross-sectional survey (audit)	2009 (Sept.)	Outpatient clinics, tertiary university hospital, Ireland	110	Rheumatology patients on IS	None	Seasonal influenza and pneu	34% influenza; 11% pneu; 11% both	Medium
Harrison, 2017 (62)	Cross-sectional survey	2015 (Aug.–June)	HIV out-patient department of the University Hospital of Vienna, Austria	455	HIV patients	None	Seasonal influenza	12% influenza	Medium
Harrison, 2018 (63)	Cross-sectional survey	2017 (July–Oct.)	Outpatient clinic, Medical University of Vienna, Austria	490	Inflammatory rheumatic disease patients on IS	None	Seasonal influenza	25% influenza	Medium
Lachenal, 2010 (64)	Cross-sectional survey (standardized questionnaire)	2008 (Jan.)	Centre Léon-Bérard, Lyon, France	200	Patients with haematological malignancies (hospitalized or at outpatient clinic)	None	Influenza	26%	Medium
Loubet, 2015 (65)	Self-reported cross-sectional survey	2013 (Summer)	AVNIR, a group of associations whose goal is to support ICP, France	3653	79% autoimmune, 13% SOT, 8% treated for hematological malignancies. 85% on IS.	KAP	Influenza and pneu	59% seasonal influenza and 49% pneu	Medium

Loubet, 2018 (66)	Self-reported cross-sectional survey	2015 (Dec.)–2016 (March)	AFA, national association of patients with IBD, France	199	IBD patients (62% receiving IS)	KAP	Influenza and pneu	34% influenza, 38% pneu	Medium
Malhi, 2015 (67)	Cross-sectional survey (self-reported, paper-based)	2013 (Sept.)–2014 (Jan.)	IBD Clinic or Endoscopy Suite at Mount Sinai Hospital, Toronto, Canada	305	IBD patients (53% using biologicals/steroids)	None	Influenza, pneu, HAV, HBV, VZV, men, HVZ, HPV	61% influenza, 10% pneu, 61% HBV, 52% HAV, 26% VZV, 21% men, 5% HZV, 11% HPV	High
Miller, 2018 (68)	Cross-sectional survey	2016 (June–Sept.)	3 tertiary autologous and allogeneic HSCT centres, UK	93	HSCT patients (79% autologous)	adjusted HBM	Intention to receive seasonal influenza	76% expressed high intent	High
Mouthon, 2010 (69)	Cross-sectional survey (standardized questionnaires)	2006 and 2007	Dept. Of Internal Medicine, Cochin Hospital, France	177	Patients with systemic sclerosis	None	Influenza	39% (last year)	Medium
Narula, 2012 (70)	Cross-sectional survey	2010 (May–Aug)	McMaster University Medical Centre Digestive Disease Clinic, Canada	250	IBD patients (63% on IS)	None	Seasonal (and H1N1) influenza	25% seasonal influenza	High
Nguyen, 2017 (71)	Cross-sectional survey with invitation RCT for new pneu vaccine)	2014 (Oct.–Nov.)	Outpatients clinic of rheumatology at 2 hospitals in Graasten, Denmark	192	RA patients	None	Influenza and pneu	59% seasonal influenza ever, 49% last year, 6% pneu	High
Poeppl, 2015 (72)	Cross-sectional survey	2013 (July)–2013 (Oct.)	Outpatient departments of the General Hospital Vienna, Austria	444	Patients with malignancies (55% solid tumours, 22% haematological malignancy, and 17% had no diagnosed malignancy)	None	Influenza	18% influenza last year	Medium
Price, 2019 (73)	Cross-sectional survey	2014 (June–July)	Cancer center providing ambulatory care, USA	703	Patients (83%) (and caregivers and family (17%) of patients) treated for malignancies	None	Influenza	Patients 72%, caregivers 71% (last year)	Medium
Restivo, 2017 (74)	Prospective observational study	2014 (Oct.)–2015 (April)	SOT Reference Center in Palermo, Sicilia, Italy	82	SOT recipients during hospital admission for transplantation	None	Influenza	38%	Medium
Ruiz-Cuesta, 2016 (75)	Prospective observational study	2012 (Jan.–March)	Reina Sofía University Hospital, Córdoba, Spain	153	IBD (50% UC, 50% CD) patients (>14 years old), 34% on biologicals/corticosteroids	None	HAV, HBV, VZV, MMR assessed by registry	84%	Medium
Sadlier, 2015 (76)	Retrospective study, with provider-delivered survey	2014 (Jan.–Feb.)	Tertiary university hospital in Ireland	170	Dermatology patients prescribed systemic IS	None	Influenza and pneu	38% seasonal influenza last year, 21% pneu last 5 years, 18% both.	Medium
Sandler, 2016 (77)	Cross-sectional, telephone survey	2013 (July–Sept.)	Memorial Medical Center in Chicago, USA	102	RA patients (85%–91% taking IS)	None	Self-reported and EHR influenza, pneu, and HZV	79% influenza last season, 54% pneu and 8% HZV	High
Savage, 2011 (78)	Retrospective audit	2010 (Aug.–Oct.)	Outpatient dermatology clinics in Aberdeen Royal Infirmary, Scotland	87	Immunocompromised dermatology patients	None	Influenza and pneu	70% influenza (last year), 22% pneu	Medium

Struijk, 2015 (79)	Cross-sectional survey	Unknown	Renal Transplant Unit, Academic Medical Center, Amsterdam, NL	526	77% renal transplant recipients (and their nephrologists)	KAB	Influenza, tetanus, pneumococci, HAV, HBV	56% influenza, 15–30% tetanus, 0–5% pneu, 5–30% HAV, 10–20% HBV	High
Teich, 2011 (80)	Cross-sectional survey	2009 (April–Sept.)	Germany	203	IBD patients who had not received vaccination counseling ≥1 year (54% on IS)	None	Vaccinations in general	67% tetanus (<10 years), 21% pertussis, 28% seasonal influenza, 9% pneu	High
Urun, 2013 (81)	Cross-sectional survey (with face-to-face interviews)	2012 (Jan.–March)	Medical Oncology Department of Ankara University Faculty of Medicine, Turkey	359	Patients with malignancies	None	Influenza and pneu	17% influenza 4% pneumococcal	Medium
Waszczuk, 2018 (82)	Cross-sectional survey (self-completed)	Unknown	Wrowclaw, Poland	195	IBD patients (70% on IS)	None	Influenza, HBV and pneu	HBV 55%; Tdap 12%; HAV 7%; annual influenza 6%; VZV/HZV 3%, and pneu 2%	High
Wilckens, 2011 (83)	Cross-sectional survey	2009 (April–Oct.)	IBD outpatients' clinic, a tertiary referral center, Lueneburg, Germany	102	IBD patients (57% CD, 91% on IS)	None	Vaccinations in general	19% influenza, 3% pneumoccous, 22% HBV, 5% VZV, 55% MMR, and 63% tetanus. Of those who had traveled, 9% HAV and 1% YF	High
Akan, 2016 (84)	Cross-sectional study	2014 (June–Sept.)	family health care centres in Turkey	596	GPs	used, name not mentioned	Seasonal influenza	27%	High
Asma, 2016 (85)	Cross-sectional study	2015 (Jan.)	6 university hospitals in Turkey	642	177 (28%) physicians and 448 (71%) nurses	None	Seasonal influenza	9%	Medium
Boey, 2018 (86)	Cross-sectional study	2015 (Nov.–Dec.)	13 hospitals and 14 nursing homes in Belgium	5141	4506 hospital staff, 635 HCW nursing home staff.	HBM, HIM and ASE	Seasonal influenza	2014: 62% (hospital) 2015: 65% (hospital)	High
Bonaccorsi, 2015 (87)	Cross-sectional study	2010 (Oct.–Nov.)	Careggi University Teaching Hospital, Florance, Italy	2576	10% physicians, 39% nurses, 23% students, 4% health care assistant, 15% other	None	Seasonal influenza	18%	Medium
Castilla, 2013 (88)	Cross-sectional study	2012 (Mar.–May)	PHC workers, Spain	1956	47% GP, 10% paediatricians, 43% nurses	None	Seasonal influenza	52–61% (2008–2011)	High
Ciftci, 2018 (89)	Cross-sectional study	2015 (Sept.–Dec.)	University Hospital, Ankara, Turkey	470	Tertiary healthcare setting (18% physicians, 29% nurses, 11% assistants, 23% auxillary, 9% paramedics, 10% secretaries)	None	Seasonal influenza	27%	High
Costantino, 2019 (90)	Cross-sectional study	Influenza seasons 2016–2019	University Hospital of Palermo, Italy	1237	Hospital HCW that had not received influenza vaccination	None	Seasonal influenza	0%	High

Dedoukou, 2010 (91)	Cross-sectional study	2018 (Oct.–Nov.)	76 PHCs in Greece	1617	PHC: 35% physicians, 32% nurses, 23% paramedical/technical, 8% administrative	None	Seasonal influenza	41%	Medium
deSante, 2010 (92)	Cross-sectional study	2009 (Apr.)	2 tertiary care hospitals in Pennsylvania, USA	227	House officers and attending physicians in emergency/internal medicine depts.	None	Seasonal influenza	94%	Medium
Dominguez, 2013 (93)	Cross-sectional study	2012 (Mar.–May)	PHC workers in 7 Spanish regions	1749	Familiy physician (47%), paediatrician (10%), nurses (43%).	None	Seasonal influenza	51%	High
Durando, 2016 (94)	Cross-sectional study	2013 (Oct.)–2014 (Apr.)	San Martino Teaching Hospital/Scientific Research Institute, Italy	830	HCW	None	Seasonal influenza	26%	High
Ehrenstein, 2010 (95)	Cross-sectional study	2006 (Feb.)	Tertiary care university hospital in Germany	652	HCW (physicians 36%, nurses 42%, administrators 22%)	None	Seasonal influenza	34%	Medium
Giese, 2016 (96)	Cross-sectional study	2013	Ireland	164	HCW in a study group of Irish residents	None	Seasonal influenza	28%	Medium
Gramegna, 2018 (97)	Cross-sectional study	2016	Italy	144	Italian Respiratory Society members		Seasonal influenza	55%	Medium
Gutknecht, 2016 (98)	Cross-sectional study	2016 (Feb.–Mar.)	Poland	77	Physicians	None	Seasonal influenza	-	Low
Hagemeister, 2018 (99)	Cross-sectional study	2015 (June-July)	University Hospital Würzburg, Germany	677	Physicians and nursing staff	None	Seasonal influenza	55%	Medium
Harrison, 2016 (100)	Cross-sectional study	-	Vienna General Hospital, Austria	116	Nursing staff	None	HAV/HBV, DTP/Tdap, MMR, influenza, VZV, men, pneu	Seasonal influenza: 42%; Measles: 60%	Medium
Hopman, 2011 (101)	Cross-sectional study	2008 (Nov.–Dec.)	All 8 University Medical Centers in NL	1238	HCW at medium and high risk for influenza	HBM, BIM, ASE	Seasonal influenza	38%	Medium
Hulo, 2017 (102)	Cross-sectional study	2014	University Hospital Lille, France	344	HCW in the emergency departments and the IC units	None	Seasonal influenza	18%	Medium
Johansen, 2012 (103)	Cross-sectional study	2007 (May)	North and South Dakota	155	Randomly selected nurses (52% hospital, 13% clinic, 12% long term)	Triandis	Seasonal influenza	-	Medium
Kalemaki, 2020 (104)	Cross-sectional study	-	Crete, Greece	260	GPs	None	Seasonal influenza, measles HBV, Tdap	Seasonal influenza 57%; Measles 26% HBV 68%; Tdap 47%	High
Karlsson, 2019 (105)	Cross-sectional study	-	Public hospitals in Finland	2962	Hospital personnel who may work with vaccinations (14% physicians)	None	Seasonal influenza	-	High
Kisic-Tepavcevic, 2017 (106)	Cross-sectional study	2015 (Dec.)	Clinical Centre of Serbia, Belgrade, Serbia	352	HCW	None	HBV	66%	High
Lehmann, 2015 (107)	Cross-sectional study	2013 (Feb.–Apr.)	20 hospitals in Belgium, Germany and NL	1022	56% nurse, 15% physicians, 14% paramedics	None	Seasonal influenza	Total: 37%; Netherlands: 28%; Belgium: 53%; Germany: 36%	High
Maridor, 2017 (108)	Cross-sectional study	2013	3 medium-sized, non-teaching hospitals, Switzerland	252	Nursing staff	None	Seasonal influenza	58%	Medium

Napolitano, 2019 (109)	Cross-sectional study	2018 (Sept.–Nov.)	8 hospitals in Italy	531	Random sample of HCWs (29% physicians, 59% nurses)	None	HBV, influenza, MMR, VZV, pertussis	HBV: 98%; DTP: 91%; MMR: 64%; VZV: 59%; TBC: 50%; Influenza: 30%; Men C: 41%	High
Nowrouzi, 2014 (110)	Cross-sectional study	2011 (Sept.–Nov.)	University of Toronto	963	Medical trainee's (post graduate)	HBM	Seasonal (and pandemic) influenza	Seasonal influenza 69%–76% (2008–2010)	High
Pielak, 2010 (111)	Cross-sectional study	2005 (Apr.)	British Columbia, Canada	719	Immunization nurses of all health units and all physicians that administer vaccinations	TPB	Seasonal influenza	-	High
Prematunge, 2014 (112)	Cross-sectional study	2010 (June)	Tertiary care hospital Ontario, Canada	3275	35% nurse, 5% physician, 11% allied HCW's, 22% administrative/clerical	None	Seasonal (and pandemic) influenza	Seasonal influenza: 74%	Medium
Quan, 2012 (113)	Retrospective cohort study	2006–2011	University of California Irvine Healthcare	32,808	all HCWs	None	Seasonal influenza	44–92% (2007–2011)	Medium
Rabensteiner, 2018 (114)	Cross-sectional study	2016 (Oct.–Dec.)	South Tyearolean Health Service, Italy	4091	13% physicians, 20% administrative, 67% sanitary or executive non-medical staff	None	Seasonal influenza	10%	High
Real, 2013 (115)	Cross-sectional study	-	Academic medical center in Lexington, USA	318	80% clinical, 20% non-clinical	RPA	Seasonal influenza	66% already received the vaccination or planned to get one soon	Medium
Rebmann, 2012 (116)	Cross-sectional study	2011 (Apr.–June)	Saint Louis region, USA	3188	54% non-hospital HCW, 46 % hospital HCW	None	Seasonal (and pandemic) influenza	2010/11: 79%	High
Scatigna, 2017 (117)	Cross-sectional study	2015 (Apr.–May)	San Salvatore Hospital, L'Aquila, Italy	334	Nurses 53%, physicians 23%, other 24%	None	HBV, influenza, MMR, VZV	-	Medium
Surtees, 2018 (118)	Cross-sectional study	2016	Tertiary referral hospital in Victoria, Australia	1835	HCW	None	Seasonal influenza	97%	High
Taddei, 2014 (119)	Cross-sectional study	2011 (June–Oct.)	6 public hospitals in Florence, Italy	436	59% nurses, 21% physicians, 13% nursing assistants, and 7% were midwives	None	MMR, VZV, Pertussis	11% measles, 7% mumps, 17% rubella, 2% VZV, 7% pertussis	Medium
Tanguy, 2011 (120)	Cross-sectional study	2009 (Nov.)–2010 (Feb.)	Tertiary care centre in Pays de la Loire Region, France	532	24% medical staff, 65% nursing staff, 11% ancillary staff	None	Seasonal (and pandemic) influenza	22%	Medium
Vallée-Tourangeau, 2018 (121)	Cross-sectional study	2014 (June–July)	A single metropolitan hospital group, UK	784	11% physicians, 36% nurses, 30% allied health professionals, 17% assistants	CME	Seasonal influenza	-	Medium
Verger, 2016 (122)	Cross-sectional study	2014 (Apr.–July)	France	1582	GPs	None	Seasonal influenza, DTP, HBV	72% influenza, 84% DTP, 86% HBV	High
Virseda, 2010 (123)	Cross-sectional study	2009 (Dec.)–2010 (Jan.)	University Hospital 12 de Octubre, Madrid, Spain	527	HCW (23% physician, 29% nurse, 19% nursing assistant, 29% ancillary staff)	None	Seasonal (and pandemic) influenza	50%	Medium

Wicker, 2010 (124)	Cross-sectional study	2010 (Jan.–May)	Frankfurt University Hospital, Germany	1504	Physicians 26%, nurses 35%, other HCW 23%, students 16%	None	Pertussis	22% in last 10 years	Medium
Wilson, 2019 (125)	Cross-sectional study	Influenza seasons 2015–2017	Southeast France	1539	74% hospital nurses, 26% community nurses	None	Seasonal influenza	Both seasons: 24% at least one season: 34%	Medium
Wilson, 2020 (126)	Cross-sectional study	2017–2018	Southeast France	1539	74% hospital nurses, 26% community nurses	None	Mandatory and recommended vaccines in France	96% BCG, 73% DTP (<10 years), 61% HBV, 58% pertussis, 64% measles, 39% VZV, 27% seasonal influenza (last year)	Medium
Zhang, 2011 (127) and 2012 (128)	Cross-sectional study	2010 (May-Oct.)	University Hospital London, UK	522	Qualified nurses (79% working in hospital)	None	Seasonal influenza	36%	Medium

* concerns vaccination uptake unless otherwise specified. ** Quality is assessed with the AXIS tool. A low score represents fulfillment of 1–9 out of 20 items, medium 10–14 and high 15–20 items (Exact scores are given in Supplementary Table S2). The following abbreviations are used (organized per column, in alphabetical order): Enrolment sites: USA = United States of America; UK = United Kingdom; NL = the Netherlands. Study populations: CD = Crohn's Disease; GP = general practitioner; HCW = healthcare workers; HIV = human immunodefiency virus; HSCT = hematological stem cell transplantation; IBD = inflammatory bowel disease; ICP = immunocompromised patients; IS = immunosuppressive treatment; PHC = primary healthcare; RA = rheumatoid arthritis; SOT = solid organ transplantation; UC = colitis ulcerosa; VFR = travellers visiting friends and relatives. Theoretical frameworks: ASE = attitude, social influence and self-efficacy model; HBM = health belief model; KAP = knowledge, attitude, practice; HIM = the Health Intention Model; BIM = behavioral intention model; CME = Cognitive model of empowerment; RPA = risk perception attitude framework; Triandis = Triandis model of interpersonal behavior. Vaccinations: BCG = Bacillus Calmette-Guerin (vaccine for tuberculosis); DTP = diphtheria, tetanus, poliomyelitis; HAV = hepatitis A virus; HBV = hepatitis B virus; HZV = herpes zoster virus; JE = Japanese encephalitis; Men = meningococcal disease; menACWY = meningococcal serotype A, C, W and Y; MMR = measles, mumps, rubella; Pneu = pneumococcal disease; TBC = tuberculosis; Tdap = tetanus, diphtheria, acellular pertussis; VZV = varicella zoster virus, YF = yellow fever.

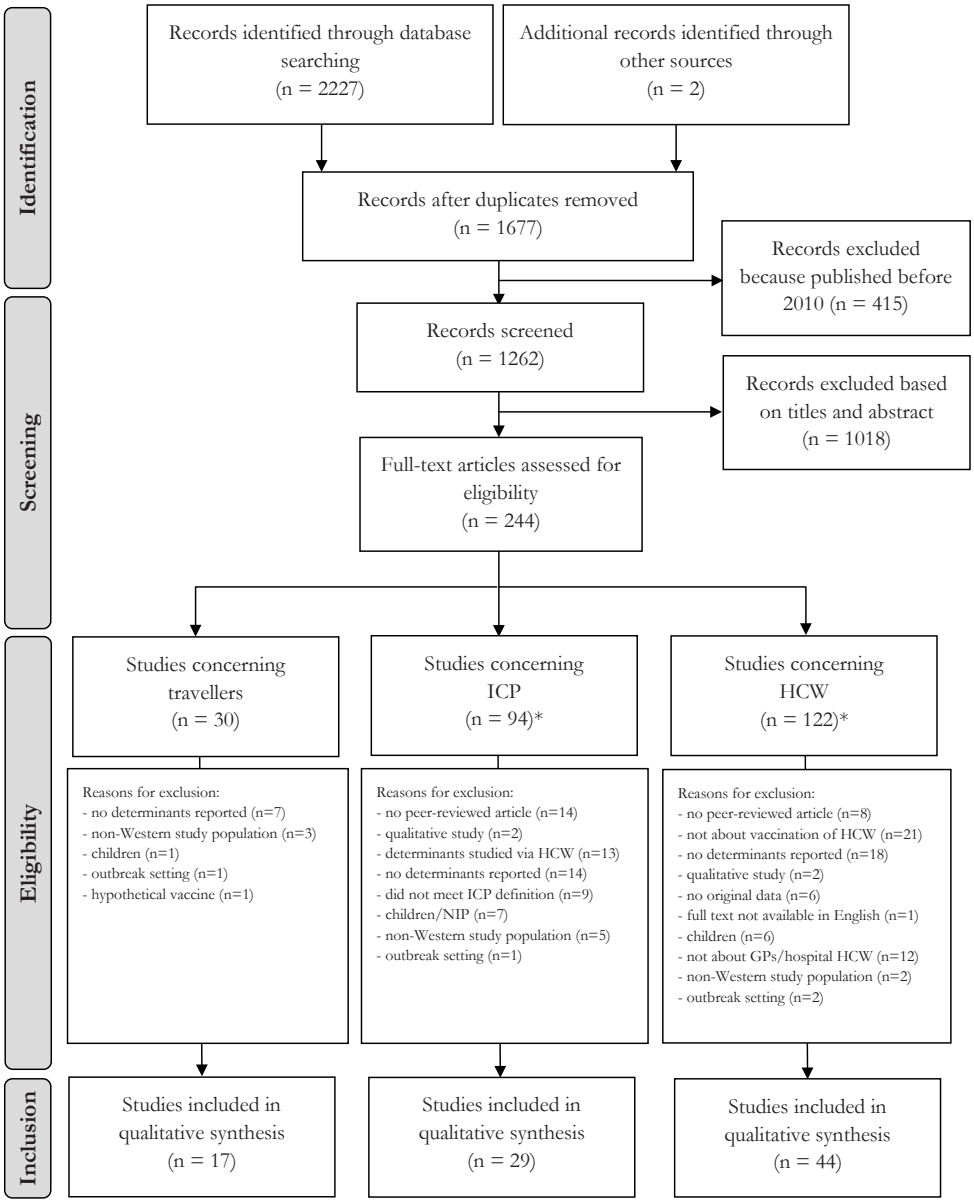


Figure 2. Flow diagram of study selection procedure.

* *n* = 2 articles were included in both ICP and HCW.

Vaccination Uptake Among Travellers

The 17 articles that studied determinants of vaccination uptake among travellers comprised 12 cross-sectional surveys, two pre- and post-travel surveys, and three retrospective studies of which one was based on confirmed cases of VPD (Table 1). Travellers that were studied originated from the USA (6 studies), Australia (4 studies), Europe (5 studies), or mixed continents (2 studies). Sample sizes ranged from 55 to 27,386 and comprised Hajj pilgrims in three studies, travellers to Africa in two studies and to Asia in two studies. Other studies had broader inclusion criteria. Three studies used KAP (knowledge-attitude-practices) surveys and one study mentioned a health behavioural model (theory of planned behaviour) as theoretical background for their study.

Predisposing Factors

Ten articles studied baseline characteristics of travellers that could be associated with vaccination uptake (Table 2). The vaccinations that were studied were diverse, most papers discussed vaccinations for influenza (n = 7), hepatitis B virus (HBV) (n = 6), hepatitis A virus (HAV) (n = 5) and meningococcal disease (n = 5). Regarding age, three papers reported that younger people had a higher uptake (43, 45, 49). However, for influenza vaccination this was the opposite: older travellers were more likely to be vaccinated for seasonal influenza (52, 57). Gender was not a significant predictor of vaccination uptake in any of the studies. Education level was studied by three papers (43, 52, 56). Two found this determinant to be positively associated with (intention to) obtaining recommended vaccinations (53, 56). Seven studies reported travel purpose in relation to vaccination uptake, but the results were diverse. One study concluded vaccination uptake was highest if the reason of travelling was business or backpacking (45). However, work-related travel was associated with lower uptake in another study (OR = 0.39, [0.17–0.92]) (52). Travellers visiting friends and relatives (VFR) had a lower uptake in two studies (49, 54), but two other studies found no association (45, 50). Six papers studied the relation between travel duration and vaccination uptake. Two studies showed that uptake was significantly lower when people travelled longer (49, 53), while one found that it was higher (for rabies only) (54) and three studies found no difference (44, 45, 52).

Table 2. Overview of determinants of vaccination uptake in travellers.

Characteristics and determinants	Balaban, 2013 (16)	Barasheed, 2014 (17)	Duffy, 2013 (18)	Frew, 2017 (19)	Goodman, 2014 (20)	Herbinger, 2011 (21)	Heywood, 2016 (22)	Igreja, 2019 (23)	Lammert, 2016 (24)	Paudel, 2017 (25)	Pavli, 2019 (26)	Pfeil, 2010 (27)	Selcuk, 2016 (28)	Tan, 2017 (29)	Tashani, 2016 (30)	Wiemken, 2015 (31)	Yanni, 2010 (32)
Type of travellers																	
Hajj	x	x													x		
VFR							x							x			
Backpackers				x													
VPD																	
Influenza	x	x										x	x	x		x	x
Men					x						x		x	x		x	
Pneu														x	x	x	
HAV							x			x			x	x		x	
HBV				x		x	x						x	x		x	
DTP/Tdap													x	x	x	x	
MMR										x				x		x	
VZV/HZV														x		x	
YF													x	x		x	
JE			x											x		x	
Rabies													x	x		x	
Typhoid fever										x				x		x	
Vaccines in general								x	x								
Determinants																	
Predisposing factors																	
Age			↓	=	↓				↓			↑	=	=		=	↑
Gender: male				=	=				=			=		=		=	
Education level			=										↑			↑	
Travel purpose: VFR					=				↓	=				↓			
Travel purpose: business					↑							↓					
Travel duration				=	=				↓			=	↓	↑			

Information factors																			
Internet			^	^		^	^					^							^^
TV/radio							ˇ												
Primary HCW (GP)		^	^		^	^	^^					^			^^				^^
Specialist HCW (travel clinic)			^		^	^	^												^
Family/friends		^	^	^		^	^												^
Travel organization		^^	^																
Cognitive determinants																			
Awareness		^	^^		^^	^^	^^								^				
Perceived knowledge				=	↑	^^					↑				^^				
Perceived risk of infection	↑	^	^	=	^^				^^			=	^^		^^				^^
Perceived risk of vaccination								ˇˇ	ˇ						ˇˇ				ˇ
Attitude		^		=			^	^^							^^	=			
Social Influence/norm		^^			^	^^		^^				^			^^	=			
Self-efficacy																			
Intention to behaviour																=			
Barriers																			
Costs							ˇ		ˇ					=	ˇ				
Time		^					^												
Promotors																			
Reminder							^												

The following symbols are used: x applicable; = no significant difference; ↑ significant positive association (tested by multivariate analysis); ↓ significant negative association (tested by multivariate analysis); ↑ significant positive association (tested by chi-square, univariate analysis or correlation coefficient); ↓ significant negative association (tested by chi-square, univariate analysis or correlation coefficient); « (double caret pointing upwards) significance was not tested, but determinant was positively linked to vaccination uptake in ≥50% of the population; « (double caret pointing downwards) significance was not tested, but determinant was negatively linked to vaccination uptake in ≥50% of the population; ‹ (caret pointing upwards) significance was not tested, but determinant was positively linked to vaccination uptake in ≥10% of the population; ‹ (caret pointing downwards) significance was not tested, but determinant was negatively linked to vaccination uptake in ≥10% of the population. * determinants were studied in relation to intention to be vaccinated instead of vaccination uptake. The following abbreviations are used (in alphabetical order): CD = Crohn's Disease; DTP = diphtheria, tetanus, poliomyelitis; GP = general practitioner; HAV = hepatitis A virus; HBV = hepatitis B virus; HCW = healthcare workers; HIV = human immunodefiency virus; HSCT = hematological stem cell transplantation; HZV = herpes zoster virus; IBD = inflammatory bowel disease; IS = immunosuppressants; JE = Japanese encephalitis; Men = meningococcal disease; MMR = measles, mumps, rubella; Pneu = pneumococcal disease; Tdap = tetanus, diphtheria, acellular pertussis; SOT = solid organ transplantation; VFR = travellers visiting friends and relatives; VZV = varicella zoster virus; YF = yellow fever.

Information Factors

No clear relationship between information sources and vaccination uptake was reported. However, eight studies reported a role for the GP, of which three said that the GP was very influential (47, 54, 55, 57).

Cognitive Determinants

Of all the cognitive determinants studied, perceived risk of infection was most frequently described in relation to vaccination uptake (n = 10). Only one study found a significant positive relation (OR 1.74 (95% CI 1.14–2.62)) (41), and another five reported this factor to play a role in the majority of the study population. Although not often tested for significance, "not feeling at risk of the disease" was a common explanation of a lot of travellers for not receiving the recommended vaccinations. Perceived risk of vaccination was sparsely discussed (n = 4).

Social influence, which comprises mostly trust and recommendations of healthcare providers in this selection of studies, was reported in seven papers and was recognised as important by the majority of the study population in four papers.

Attitude was described in six papers, and was not found to be significant in two of them (44, 56); reliance on natural immunity was mentioned three times as a reason to reject vaccination (42, 48, 55). Awareness was also discussed in six papers; although it was not tested for significance, 13–73% mentioned unawareness of the availability of the vaccination (or unawareness of the recommendation of the vaccination) as an important reason for non-uptake (42, 43, 45-47, 55).

Five studies reported on knowledge of VPD; two found a significant positive relation between knowledge and vaccination uptake (45, 51), one found no relation (44).

Barriers and Facilitators

Reported barriers could be classified in costs and lack of time. Costs were the most described; however, it played a modest role in explaining non-uptake and differed per vaccination. For instance, for influenza vaccination uptake costs were mentioned to play a role in less than 7% of travellers, while for HBV (12%), Japanese encephalitis (35%) and pneumococcal vaccination (38%) concerns about costs were much higher. In two papers lack of time was given as part of the explanation of non-uptake in more than 10% of the study population (42, 47). One paper described that 3%–24% of travellers require a reminder to complete their vaccination series (47).

Vaccination Uptake Among Immunocompromised Patients

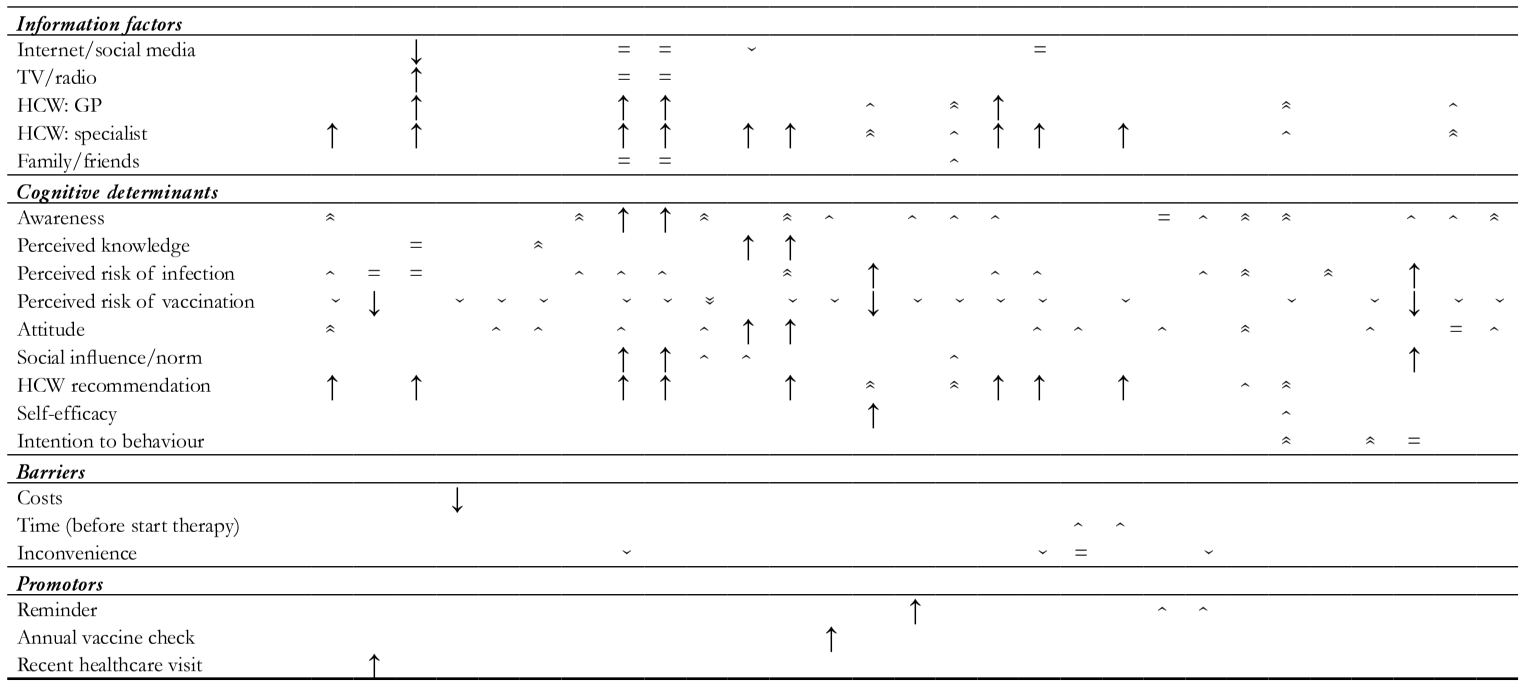
Twenty-nine articles concerning ICP were included. Most of these studies were cross-sectional (*n* = 23), but four were prospective (with a follow-up moment) and two retrospective (Table 1). Studies were performed among European (*n* = 23), American (*n* = 3) and Canadian (*n* = 3) populations. Sixteen studies involved patients with auto-immune diseases, of which four studies focussed completely on patients with inflammatory bowel disease. The vaccination uptake of HIV patients was studied in three papers. Four papers studied populations with solid tumours, six papers studied patients who received haematological stem cell transplantation (HSCT) and three papers investigated patients who received a solid organ transplantation (SOT). Almost all papers addressed the influenza vaccination uptake (*n* = 25) and many also included the uptake of pneumococcal vaccinations (*n* = 13). Influenza vaccination rates varied from 6–79% and pneumococcal vaccination rates from 2–54%. Lowest rates were reported in Polish inflammatory bowel disease (IBD) patients (85) and highest in American rheumatic patients (80). In ICP, health behaviour models were cited slightly more than in the travellers population. Two studies were based on the (HBM) and another three studies used KAP surveys.

Predisposing Factors

Most studies (17 out of 24 that studied age) found a positive association between age and vaccination uptake (Table 3). Especially for influenza vaccination, older patients tend to be more compliant with vaccination guidelines in the studied year. Only in one study a negative association was found (OR 0.02, 95% CI (0.01–0.57)) (71, 132). Most studies report that gender and education level are not significant predictors of vaccination uptake in ICP, with a few exceptions. Three studies showed in a multivariate analysis that males had a higher uptake. Two studies showed a negative association between uptake and education level, while one showed a positive association. In five studies, the use of strong immunosuppressive medication was positively associated with vaccination uptake, whereas in two studies the association was negative and in three there was no association. Generally, ICP with comorbidities in their medical history tend to have a higher uptake in four (63, 64, 67, 79) out of seven studies. One study reported a negative association (61, 133) and two found no significant difference (58, 77). All five papers that included vaccination history (for the same or another vaccination), concluded that there was a positive association between vaccination uptake in the past and current uptake (59, 68, 71, 72, 77).

Table 3. Overview of determinants of vaccination uptake in ICP.

Characteristics and determinants	Akin, 2016 (33)	Althoff, 2010 (34)	Battistella, 2019 (35)	Chehab, 2018 (36)	Chin-Yee, 2011 (37)	Gagneux-Brunon, 2019 (38)	Haroon, 2011 (39)	Harrison, 2017 (40)	Harrison, 2018 (41)	Lachenal, 2010 (42)	Loubet, 2015 (43)	Loubet, 2018 (44)	Malhi, 2015 (45)	Miller, 2018 (46)	Mouthon, 2010 (47)	Narula, 2012 (48)	Nguyen, 2017 (49)	Poeppl, 2015 (50)	Price, 2019 (51)	Restivo, 2017 (52)	Ruiz-Cuesta, 2016 (53)	Sadlier, 2015 (54)	Sandler, 2016 (55)	Savage, 2011 (56)	Struijk, 2015 (57)	Teich, 2011 (58)	Urun, 2013 (59)	Waszczuk, 2018 (60)	Wilckens, 2011 (61)
Risk groups																													
Auto-immune (IS treatment)				x			x		x		x	x	x		x	x	x				x	x	x	x		x		x	x
HIV		x				x		x																					
Solid tumors	x																	x	x								x		
HSCT					x					x	x			x				x	x										
SOT											x									x					x				
Vaccines																													
Influenza		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x		x	x	
Pneu				x		x	x				x	x	x				x					x	x	x	x		x	x	
Men				x									x																
HBV						x							x								x				x			x	
HAV						x							x								x				x				
DTP/Tdap				x																					x				
MMR																					x								
VZV/HZV													x								x		x						
Vaccines in general	x																									x			x
Determinants																													
Predisposing factors																													
Age	=	↑	↑→	↑	↑	↑	↑	↑	↑	↑	↑	=	=	↓	↑	↑	↑	↑	↑	=		↑			=	=	↑		
Male gender	=		↑→					=	=		↑	=	=	=	=	=	↑		=	=		↑			=				
Education level	↓	=	→	=							=	=	=	=			↑			=	↑	=			=	=	=	=	
Use of (strong) IS				↑→					↑		↓	↑	↑			↓	=					=				=			
Comorbidities	=			→		↑	↑			↑										=		↑							
Vaccination history		↑									↑			↑	↑					↑									



The following symbols are used: x applicable; = no significant difference; ↑ significant positive association (tested by multivariate analysis); ↓ significant negative association (tested by multivariate analysis); ↑ significant positive association (tested by chi-square, univariate analysis or correlation coefficient); ↓ significant negative association (tested by chi-square, univariate analysis or correlation coefficient); « (double caret pointing upwards) significance was not tested, but determinant was positively linked to vaccination uptake in ≥50% of the population; « (double caret pointing downwards) significance was not tested, but determinant was negatively linked to vaccination uptake in ≥50% of the population; ‹ (caret pointing upwards) significance was not tested, but determinant was positively linked to vaccination uptake in ≥10% of the population; ‹ (caret pointing downwards) significance was not tested, but determinant was negatively linked to vaccination uptake in ≥10% of the population. The following abbreviations are used (in alphabetical order): CD = Crohn's Disease; DTP = diphtheria, tetanus, poliomyelitis; GP = general practitioner; HAV = hepatitis A virus; HBV = hepatitis B virus; HCW = healthcare workers; HIV = human immunodefiency virus; HSCT = hematological stem cell transplantation; HZV = herpes zoster virus; IBD = inflammatory bowel disease; IS = immunosuppressants; JE = Japanese encephalitis; Men = meningococcal disease; MMR = measles, mumps, rubella; Pneu = pneumococcal disease; Tdap = tetanus, diphtheria, acellular pertussis; SOT = solid organ transplantation; VFR = travellers visiting friends and relatives. VZV = varicella zoster virus, YF = yellow fever.

Information Factors

Thirteen studies investigated where ICP retrieve their information from. In general, gathering information from online media sources was somewhat associated with a lower vaccination uptake, while receiving information from HCW resulted in a higher uptake (60, 66).

Cognitive Determinants

Perceived risk of vaccination was the most frequently mentioned cognitive determinant, being discussed in 21 of the 29 articles. In all three papers that tested for significance, a negative correlation with vaccination uptake was found, meaning that a higher perceived risk of a vaccine results in a lower uptake. But also that a lower perceived risk, reflected for example by trust in the effectivity of this specific vaccine, increases the uptake. Fear for side-effects or deterioration of their disease caused by the vaccination were mentioned often. Another concern that was often expressed was the doubt of effectivity of vaccination, due to either the immunogenicity of the vaccine or due to the compromised state of the patients' immune system. Distrust was reported more often for influenza than for other vaccinations (80).

Awareness of either the availability of or the indication for a vaccination was also widely discussed (n = 17). While only found to be significantly correlated twice, this determinant played a role in the majority of the study population in seven papers. Because ICP often mention vaccination not being proposed as a reason for non-uptake, this determinant is related to the information factors, knowledge, and HCW recommendation.

Attitude, covering the attitude to vaccinations in general, was mentioned in 14 studies and was found to be positively correlated twice in multivariate analysis. The effect of a favourable attitude to vaccinations in general was larger on uptake of influenza (adjusted odds ratio (aOR) 3.4 (95% confidence interval (CI) 1.2–9.5)) than on uptake of pneumococcal vaccination (aOR 1.7 [95% CI 0.8–3.5]) (69). Perceived risk of infection was mentioned equally often as attitude (n = 14) and was also positively associated with uptake, in two of the four studies that tested for significance (71, 84).

Although knowledge was only addressed in four papers, in two out of the three articles that tested for significance a positive correlation was found. Recommendation of an HCW was studied in 12 out of the 29 papers and a significant correlation was found in all eight papers that performed statistical analysis. In addition, a frequently reported reason for not being vaccinated was that vaccination was not offered or recommended, which we included under awareness.

Self-efficacy was reported in two papers. One reported that more than 10% of unvaccinated ICP were unsure of how to arrange to receive the vaccines (81), while another reported that patients who find it easier to attend a GP for vaccination, have a higher intention to get vaccinated ($p < 0.001$) (71). Regarding intention to behaviour, one high-quality study expressed that 80% of their IBD study population expressed to be willing to receive all of the recommended vaccinations, while only 9% had ever received a pneumococcal vaccination and only 28% was vaccinated against influenza at the time of participation in the study (134). In another study with 17% influenza and 4% pneumococcal vaccination uptake, the intention to be vaccinated next year was also high and not significantly different between the vaccinated (89%) and unvaccinated group (80%) (84).

Barriers and Facilitators

Cost was only mentioned as a barrier in one paper that found a significant negative correlation with uptake (61). Lack of time ($n = 2$) and the inconvenience of another appointment ($n = 4$) were more often given as reasons for declining vaccination.

Vaccination Uptake Among Healthcare Workers

In HCW, influenza vaccination uptake is most widely studied. In 35 articles out of the 44, seasonal influenza vaccination was the only vaccine studied, with uptake varying between 9% (88) to 97% (mandatory policy) (121). Most studies were conducted in Italy ($n = 8$), followed by France ($n = 5$) and the USA ($n = 5$). All but one were designed as cross-sectional surveys, with sample sizes ranging from 77 (101) to 32,808 (116). Seven studies mentioned the use of a theoretical model for their study, which includes the HBM (113), the TPB (114), the risk perception attitude framework (118), the Triandis model of interpersonal behaviour (106), the cognitive model of empowerment (124) or mixtures of different models (104) (Table 1).

Predisposing Factors

Thirty-six articles studied at least one predisposing factor in relation to vaccination uptake (Table 4). Of the 30 articles that studied age, 22 found that older healthcare workers had a significantly higher uptake. On the other hand, in the case of hepatitis B (109, 120) and measles (103, 107), younger HCW's had higher compliance. In the 27 papers that studied gender, being male was associated with higher vaccination uptake in 13 studies. Five papers mentioned a significantly higher uptake in women, one for rubella only (122), and another for hepatitis B only (107). Occupation was studied in relation to vaccination uptake in 18 articles. Sixteen papers showed that physicians had a significantly higher uptake than other HCW. This also complies with the significant positive association between education level and uptake that was found in five papers.

Presence of a chronic disease resulted in significantly higher uptake in seven studies. In three other studies investigating this factor, no association was found. Having children at home was studied in nine papers, but six found no significant role for this factor in vaccination uptake. Good vaccine compliance in the past turned out to be an excellent predictor of uptake in all 11 studies investigating this factor.

Information Factors

The role of information sources in vaccination uptake was studied in six articles. When information was gathered from evidence-based sources, uptake was significantly higher in all five studies that investigated this source. On the other hand, uptake was lower when information was retrieved from social media, television, or radio (88, 117). Only one study found that gaining information from colleagues was associated with a higher uptake (103).

Cognitive Determinants

Perceived risk was the most frequently described determinant in HCWs. More specifically, perceived personal risk of infection reflects the perceived risk to contract the VPD, including the perceived susceptibility to get infected and the perceived severity of the disease if contracted. In 33 out of 35 papers mentioning perceived risk of infection, a significant positive relation was found between this determinant and vaccination uptake (n = 13), or these reasons were mentioned in a considerable part of the study group (n = 20). Furthermore, in 18 papers a high perceived risk to infect patients was given as a reason for vaccination uptake. Perceived risk (vs. benefit) of vaccination was mentioned in 34 papers. Fifteen studies reported a significant negative relation between perceived risk and uptake, indicating that high perceived risk or low perceived benefit of the vaccination resulted in lower uptake. Additionally, five papers mentioned that this determinant played a role in the majority of the study population. Adequate knowledge of recommendations, effectiveness, and side-effects of vaccinations was significantly positively associated with uptake in 11 papers; in four studies, no significant association was found. Attitude towards vaccination was studied in 22 articles. In half of them, a significant positive association with vaccination uptake was found. Social influence (encouragement of colleagues, managers, family) was analysed in almost half of the studies (n = 15). In only one study no association was found (91), but the others showed either a significant (n = 8) or considerable (n = 6) positive relation with vaccination uptake. Specific for HCW are the social arguments 'I got vaccinated because it's my duty as an HCW' or 'as an HCW, I have a role in the prevention of epidemics/spread of diseases', that we collected under the term 'professional norms'. This determinant was positively associated with uptake in all 15 studies focusing on this factor; in seven out of 11 studies that tested for

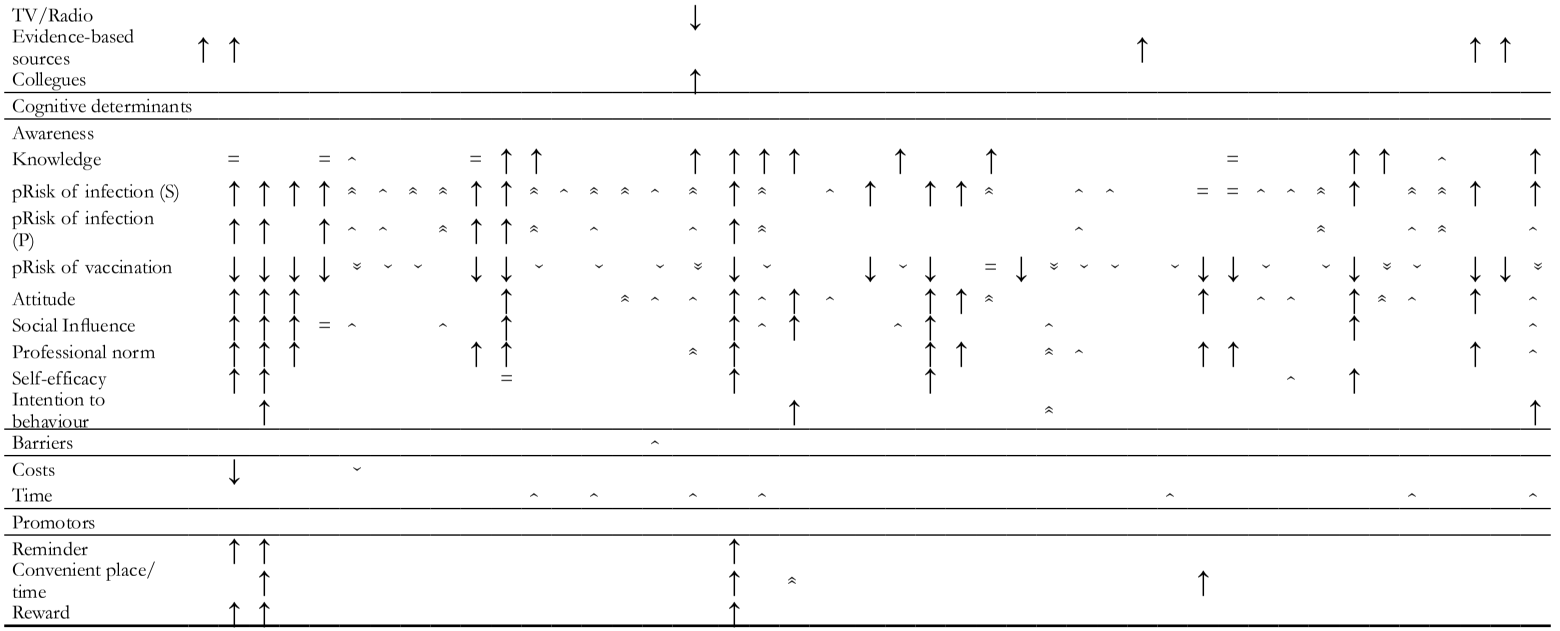
significance, this factor remained a strong predictor for uptake in multivariate analysis.

Barriers and Facilitators

In comparison with the previous determinants, barriers and facilitators are relatively less studied. Of the barriers, time-related factors were mentioned most frequently and played a considerable role (>10%) in hindering uptake in seven studies. Costs turned out to be no barrier. The fact that the vaccines were free of charge even appeared to be a reason for uptake in two studies (87, 92). On the other hand, facilitators stimulating uptake were getting a reminder (n = 3), convenient time/place of distribution (n = 4), and getting a reward (n = 3). However, in none of the studies were the potential rewards specified.

Table 4. Overview of determinants of vaccination uptake in HCW.

Characteristics and determinants	Akan, 2016 (62)	Asma, 2016 (63)	Boey, 2018 (64)	Bonaccorsi, 2015 (65)	Castilla, 2013 (66)	Ciftci, 2018 (67)	Costantino, 2019 (68)	Dedoukou, 2010 (69)	deSante, 2010 (70)	Dominguez, 2013 (71)	Durando, 2016 (72)	Ehrenstein, 2010 (73)	Giese, 2016 (74)	Gramegna, 2018 (75)	Gutknecht, 2016 (76)	Hagemeister, 2018 (77)	Harrison, 2016 (78)	Hopman, 2011 (79)	Hulo, 2017 (80)	Johansen, 2012 (81)	Kalemaki, 2020 (82)	Karlsson, 2019 (83)	Kisic-Tepavcevic, 2017 (84)	Lehmann, 2015 (85)	Maridor, 2017 (86)	Napolitano, 2019 (87)	Nowrouzi, 2014 (88)	Pielak, 2010 (89)	Prematunge, 2014 (90)	Quan, 2012 (91)	Rabensteiner, 2018 (92)	Real, 2013 (93)	Rebmann, 2012 (94)	Scatigna, 2017 (95)	Surtees, 2018 (96)	Taddei, 2014 (97)	Tanguy, 2011 (98)	Vallée-Tourangeau, 2018 (99)	Verger, 2016 (100)	Virseda, 2010 (101)	Wicker, 2010 (102)	Wilson, 2019 (103)	Wilson, 2020 (104)	Zhang, 2011 (105) & 2012 (106)
VPD																																										
Influenza	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
HBV																																										
DTP/Tdap																					x						x								x								
MMR																	x				x						x								x								
VZV/HZV																											x								x								
Vaccines in general																	x				x																						
HCW																																											
Physician	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x			x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Nurses	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Other HCW			x	x		x	x	x		x	x	x					x	x	x				x	x											x	x						
Determinants																																											
Predisposing factors																																											
Age	←	←	←	←	←	←	←	←	←	←	←	‹				=	↓↑	←	←	=	↓↑		→	←	←	=	←					=	→	=						←	←	=	=
Gender: male	←	=	←	←	←	→			←	←	←	←				←	=	←	←		→			=	=	=	→				←	=	→	=					←	←	=	=
Education level						←					←																																
Occupation: physician	←		←	←	←	←	‹	←			‹					←		←	←				←	←	←	=				←					←								
Work experience (years)	←	←	←			←											=	←	←	=			→	←			←	=			=											
Chronic disease	←	←	←	←	←						=						=	←	←	=																						
Children living at home	=	=	←	=	←				=							=																=										
Vaccination history					←	←		←	←	←										←				←	←	←				←			←						←			
Information factors																																											
Social media	→																															→										



* One scale (MoVac-flu scale) was used for following determinants: knowledge, attitude and self-efficacy. The following symbols are used: x applicable; = no significant difference; ↑ significant positive association (tested by multivariate analysis); ↓ significant negative association (tested by multivariate analysis); ↑ significant positive association (tested by chi-square, univariate analysis or correlation coefficient); ↓ significant negative association (tested by chi-square, univariate analysis or correlation coefficient); ↓↑ significant association, for one vaccine positive, for the other negative; « (double caret pointing upwards) significance was not tested, but determinant was positively linked to vaccination uptake in ≥50% of the population; « (double caret pointing downwards) significance was not tested, but determinant was negatively linked to vaccination uptake in ≥50% of the population; ‹ (caret pointing upwards) significance was not tested, but determinant was positively linked to vaccination uptake in ≥10% of the population; ‹ (caret pointing downwards) significance was not tested, but determinant was negatively linked to vaccination uptake in ≥10% of the population. pRisk = perceived risk. pRisk of infection (S/P): S = self; P = patient. The following abbreviations are used (in alphabetical order): CD = Crohn's Disease; DTP = diphtheria, tetanus, poliomyelitis; GP = general practitioner; HAV = hepatitis A virus; HBV = hepatitis B virus; HCW = healthcare workers; HIV = human immunodefiency virus; HSCT = hematological stem cell transplantation; HZV = herpes zoster virus; IBD = inflammatory bowel disease; IS = immunosuppressants; JE = Japanese encephalitis; Men = meningococcal disease; MMR = measles, mumps, rubella; Pneu = pneumococcal disease; Tdap = tetanus, diphtheria, acellular pertussis; SOT = solid organ transplantation; VFR = travellers visiting friends and relatives. VZV = varicella zoster virus, YF = yellow fever.

DISCUSSION AND CONCLUSIONS

Our review of the currently available literature shows that there are clear differences in determinants that play a role in vaccination uptake in travellers, ICP, and HCW. For travellers, low perceived risk of infection and low awareness of vaccination recommendations are most accountable for low uptake. For ICP, awareness of the indication of vaccination plays an important role, together with receiving vaccination recommendations from their treating physician. ICP have a high perceived risk of vaccination, due to not only fear for general side-effects but also concerns about potential consequences for their illness. For HCW, perceived risk of (the severity of) infection for themselves and for their patients together with perceived benefits of vaccination contribute most to their vaccination behaviour.

Regarding predisposing factors, there is a clear positive relationship between age and influenza vaccination uptake in all risk groups. This could be explained by the additional indication older people have for influenza vaccination. However, for other vaccinations, this relationship is either inverted or non-existent. Higher vaccination uptake was seen in males in HCW and ICP, which could be associated with the fact that females worry more about vaccine safety and efficacy than males (135). Indeed, more side-effects are reported by females, while on the other hand, from a biological perspective, females typically mount higher antibody responses (135). Although we did not find a clear relationship between education level and vaccination uptake in the risk groups, in HCW the uptake was markedly higher in physicians compared to other HCW. Overall, vaccination history seems to be an excellent universal predictor of future vaccination uptake, probably due to unaltered cognitive determinants.

Regarding cognitive determinants, the greatest diversity between risk groups was found in awareness. In ICP, almost two-thirds of the studies mentioned limited awareness, compared to one-third in travellers and none in HCW. With their education and occupation, it seems quite obvious that HCW are aware of the opportunities and indications for vaccinations. The fact that ICP seem less aware than travellers might have to do with travellers taking an active decision to go abroad realizing that they have to prepare themselves, while patients get passively diagnosed with a disease, and are more dependant of the HCW for information provision. In all groups, HCW as a source of information has a positive effect on uptake. The strong relationship between HCW recommendations and vaccination uptake in ICP (reaching odds ratios up to 53 (77) and 187 (69)), underline the importance of positive attitudes towards vaccination in HCW themselves (136, 137).

In general, knowledge has a positive influence on uptake in all risk groups. However, since several studies showed no relation between knowledge and uptake (44, 60, 87, 91, 96, 120), improving education alone will probably not be sufficient to increase uptake. In all groups, the perceived susceptibility and severity of diseases on one hand and the perceived effectiveness and risks of vaccinations on the other hand are important determinants predicting uptake. Especially ICP and HCW express concerns about the safety and effectiveness of vaccines particularly for influenza vaccination (63, 69). And although the effectiveness of influenza vaccination varies with the coverage of circulating strains each year, another part of the perceived lack of effectiveness could also be explained by the lack of protection for other common cold viruses that can cause influenza-like symptoms (138). Travelers seem to have low risk perceptions for the diseases they could be vaccinated for as well as for the potential negative effects of vaccination. Despite the high morbidity and mortality of some VPD such as yellow fever, hepatitis B, and influenza, in all risk groups, some participants stated they preferred natural immunization or were against vaccinations in general. Remarkably, attitudes differ for specific vaccinations, for instance, people tend to have a more positive attitude towards pneumococcal vaccination in comparison to the seasonal influenza vaccination (80). Interestingly, the mistrust of ICP and HCW towards the vaccinations produced by the pharmaceutical industry seems disproportionate to therapeutics manufactured by the same pharmaceutical companies (65, 75, 97, 103). Here, the difference between prevention and treatment might play a role, where the latter provides a more direct and visible effect. Another possible reason for the negative general attitude towards vaccination, also described in decision making for childhood vaccinations (139), is the increasing tendency for self-empowerment towards personal health decisions. In this view, individuals stand up against imposed policies and want to make their own decisions, which could also be judged by peers as independent and smart decision making (139, 140). At the same time, sources that are being used to make personal health decisions, such as the internet, contain a lot of negative stories (141).

Practical barriers and facilitators play a limited role in vaccination uptake compared to the other determinants. In all three groups, a reminder is an important facilitator and (lack of) time an important barrier. Especially for HCW, this factor is interesting. Physicians report this factor most frequently (98). They do not only experience lack of time to get vaccinated, they also feel that lack of time impedes their duty to recommend vaccinations to their patients (142). Again, as HCW recommendations are strongly positively associated with uptake, not only in the other risk groups, but also for HCW themselves (by colleagues for example) (91, 105), removing this barrier can result in achieving optimal care for all groups.

Only 16 of the 90 articles that were analysed in this review were based on a health behaviour model. Many of those found determinants which contributed to vaccination uptake to a greater or lesser extent (71, 89, 104, 118, 124). Interventions that focus on a single determinant, such as knowledge, repeatedly proved to be ineffective in the past (91), while multifactorial cognitive intervention strategies are effective to improve uptake (143, 144). Therefore, all determinants that play a role have to be taken into account. Predisposing factors could be used to target specific subgroups and personalize uptake strategies (118). Facilitators and barriers could be added or taken away to increase vaccination uptake. But, most importantly, interventions need to address cognitive determinants. Interventions that increase awareness and risk perception of infectious diseases are more effective than those decreasing risk perceptions of vaccination by providing scientific information (145). Social norms can be influenced in the case of hierarchical relationships, for instance, the employer will have an effect on the vaccination decision of HCW and HCW will impact ICP's decisions. Therefore, multifactorial interventions are needed that address the most important cognitive determinants. As these include awareness and risk perceptions, reminders and incidence data could help. Reminders for travellers could be disseminated in general media before holidays, while for ICP patient associations and HCW could play a role. To improve risk perceptions for the infections, cases of vaccine-preventable diseases should be made public. To decrease risk perceptions of negative effects of vaccinations (e.g., adverse events) new studies should compare the number of influenza-like illnesses in vaccinated and non-vaccinated groups. Furthermore, social norms can be included by making the decisions of vaccination uptake public. For example, in HCW trials have been implemented to test the effects of providing a pin that vaccinated HCW may wear that is saying "deliberately vaccinated", which could affect both colleagues and patients (146).

Vaccination decisions of travellers and ICP are less well studied than those of HCW. Additionally, data on uptake of vaccinations other than influenza are limited. As the available data show large differences in determinants predicting uptake of influenza versus other vaccinations, further studies are required regarding the uptake of recommended vaccinations for diseases other than influenza. Reaching a more comprehensive understanding of vaccination uptake in different risk groups for the different vaccinations that are indicated, interventions can be developed based on evidence. Moreover, this understanding could help with the implementation of new vaccines for certain risk groups, for instance when a novel SARS-CoV-2 vaccine will be recommended for HCW.

A number of limitations have to be taken into account when interpreting the results of this review. First, articles were only included if they discussed any cognitive determinants that were possibly related to vaccination uptake. This resulted in the exclusion of papers

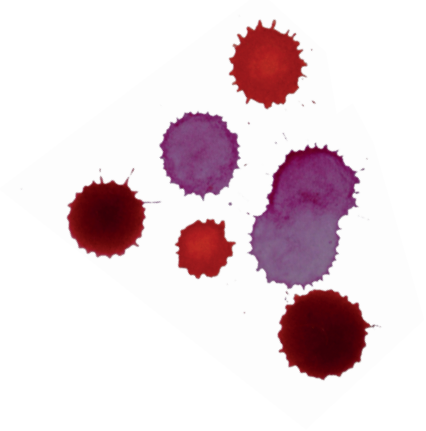
that looked only, although thoroughly, into predisposing factors. Secondly, there was a high level of heterogeneity in the determinants reported, as studies used various health behaviour models as a framework for their studies, and many did not even use a model but just reported results of questionnaires with either open-ended or multiple-choice questions. Furthermore, the influence of determinants on vaccination uptake was measured with different statistical analyses, which also contributed to the high heterogeneity of the data. Therefore, we choose to report the significance and direction of the association, instead of the magnitude. In addition, we choose to compare three different risk groups that we think are important, thereby we could not discuss all determinants in depth. Finally, included studies were based on self-reported vaccination behaviour. Therefore, we have to take into account a certain level of social desirability and recall bias.

To our knowledge, this is the first review that provides a comprehensive overview of health behavioural determinants explaining vaccination uptake in three different risk groups, namely travellers, ICP, and HCW. We showed that there is a large diversity of determinants that affect uptake to a greater or lesser extent. Therefore, we argue that future studies and interventions should be based on multifactorial health behaviour models, especially for travellers and ICP as only a limited number of such studies is available yet.

Supplementary materials are available online at https://www.mdpi.com/2076-393X/8/3/480/s1.

CHAPTER 2

Protecting Travellers



Chapter 2.1

Adherence to travel health guidelines in Dutch Families - The Dutch travel Vaccination Study (DiVeST)

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ABSTRACT

This Dutch travel Vaccination Study (DiVeST) aimed to study adherence or compliance to Dutch travel health guidelines in travelling families and to identify risk groups to provide better advice and protection for international travellers.

Between 2016 and 2018, family members who travelled to Eastern Europe or outside Europe during the preceding year were recruited via Dutch secondary schools. The vaccination status of the travellers was assessed using questionnaires and vaccination records and hepatitis A virus antibody concentrations in dried blood spot (DBS) eluates. Subgroups of travellers with lower adherence to guidelines were identified.

Of the 246 travellers that participated in this study, 155 (63%) travelled to destinations for which the HAV vaccination was recommended. Of these 155 travellers, 56 (36%) said they visited a pre-travel clinic, and 64 of them (41%) showed a valid HAV vaccination in their vaccination records. Of the 145 travellers with available DBS eluates, anti-HAV antibodies were detected in 98 (68%) of them.

We found that adherence to travel health guidelines, in terms of HAV vaccination, was suboptimal. According to our results, specific attention should be paid to children, persons visiting friends and relatives and those who travel relatively short distances.

INTRODUCTION

The dynamic field of travel medicine has undergone some serious changes during the past 10 years. Currently, in many Western European countries, the total number of people travelling internationally is equal to, or even exceeds, the respective number of inhabitants in those countries (147). And not only has the number of travellers increased, the distance that these people travel has also increased significantly. Remote areas are becoming more accessible, while flights are becoming more and more affordable. Nowadays, the accessibility of air traffic makes it possible to travel to the other side of the world in a matter of hours. A few decades ago this would have taken several days (148). The growing number of people able to travel now goes hand-in-hand with an increased exposure to infectious diseases. Furthermore, the ease of international travel and the wide availability of "last-minute" trips nowadays can make people unaware of the necessity to take pre-travel precautions.

Travellers are increasingly at risk of exposure to pathogens that they have never encountered before. The risk of infection varies greatly per country and so can the appropriate preventive measurements recommended before travelling. International travel health guidelines are available and the vaccinations recommended will depend on travellers' health, risk behaviour, length of stay, and, most importantly, travel destination. Furthermore, recommendations may differ between various international guidelines, reflecting differences of risk assessments between countries, vaccination programmes and national policies.

The Dutch National Coordination Centre for Travellers' Health Advice (Landelijk Coördinatiecentrum Reizigersadvisering, LCR) has developed guidelines to optimally protect travellers living in the Netherlands (149). These guidelines are updated annually. However, based on several survey studies, it is clear that about one third of Dutch travellers are unaware of the risk of contracting infectious diseases when visiting countries in which these diseases are endemic (150, 151). Focusing on risk destinations that are relatively close to home for example, such as Croatia, Turkey and Morocco, up to 64% of Dutch travellers are unaware of the recommended vaccinations (150). Travellers visiting friends and relatives (VFR) are particularly at risk, as they often make the journey without getting pre-travel advice, then spend a longer period of time abroad, often in close contact with the local inhabitants. This puts them at greater risk of contracting infectious diseases (152, 153). Young travellers also constitute a significant risk category, as many parents assume that their children are sufficiently protected by the Nationwide Immunization Program (NIP). This includes the diphtheria, tetanus and polio (DTP) vaccination, but

not the other frequently recommended travel vaccinations, such as hepatitis A (154). Given that survey-only research is prone to contain bias, we studied adherence to travel health guidelines adding serological data.

Serosurveillance data is widely available for NIP and some other infectious diseases, but these data only reflect the immune status of the general population (155). Furthermore, plenty of data about specific outbreaks is also available (156-158). However, information about the vaccination coverage in the healthy travelling populations, based on seroprevalence of vaccine-preventable diseases (VPD), is limited. Prior survey studies provide an indication of the frequency of pre-travel consultations. However, this is not representative of the number of unprotected travellers, as frequent travellers can be protected by vaccinations received in the past. The duration of protection offered by vaccines can range from a year to lifelong (149). Besides, individuals who were born in an endemic country or who have lived abroad for a certain period could very well be protected by childhood vaccinations or natural infection, which means they would be in compliance with recommendations found in travel health guidelines when travelling to certain destinations (159). In other words, not visiting a travel clinic prior to travel does not automatically mean that someone is travelling unprotected. Moreover, travellers can sometimes forget that they have received vaccinations (160) or vaccination records can be lost, all of which means that data from survey-only studies can be unreliable. Therefore, in carrying out the Dutch travel Vaccination Study (DiVeST) our objective was to do unbiased research into: (1) the vaccination status, and (2) the seroprotection rate of travelling families living in the Netherlands. Given that we did not want to rely solely on vaccination registration, because of potential loss of valuable information we strived to increase the reliability of the vaccination status data by collecting dry blood spots. This, so as to obtain insights in the presence of antibodies. Given that all travellers between the ages of 12 and 16 attend secondary school (161) it stands to reason that families travelling to any destination, by any mode of transport and with any opinion about the need to be vaccinated, can be reached via secondary schools. This made these schools a highly suitable source of data for this study.

The hepatitis A virus (HAV) vaccination is a widely advised travel vaccination - recommended according to the LCR guidelines in 184 out of the 227 (81%) defined destinations for which a vaccination is recommended (149). Furthermore, the HAV vaccination is not included in the Dutch NIP. Therefore, using HAV as a representative VPD, HAV vaccination status and HAV seroprotection rates would provide valuable insights in the adherence to travel health guidelines in healthy Dutch travelling families (162-164). Within the context of DiVeST, it was our intention to determine the proportion

of Dutch travelling family members that adhere to travel health guidelines and identify risk groups among those that don't adhere. The overriding objective of all this was to provide better advice to international travellers and offer protection to more of them.

MATERIAL AND METHODS

Study population

This cross-sectional study was conducted in secondary schools throughout the Netherlands between September 2016 and December 2018. Travellers, consisting of school personnel and students and their family members who visited an Eastern European or non-European country in the preceding year were recruited. Eastern Europe is in this study defined as Albania, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech, Estonia, Greece, Hungary, Kosovo, Latvia, Lithuania, Macedonia, Moldova, Montenegro, Poland, Romania, Russia, Serbia, Slovakia, Slovenia, Turkey, and Ukraine. To ensure we recruited a sufficient number of travellers who should have been vaccinated according to Dutch travel health guidelines, travellers to Western European countries were excluded. Thirty-seven schools were approached, based on their respective locations (population density), type of school (public or denominational) and education level. After receiving permission and confirmation of participation in the study from school principals, we visited the applicable schools on plenary evenings or approached students' families via e-mail. Once participants had given written consent, anonymised demographic data, health data and vaccination and travel history were collected with OpenClinica, an electronic data-management application. Where available, copies of vaccination records were obtained. Additionally, dried blood spots were collected to ascertain vaccination status.

Sample collection

Following a finger prick (with a BD Lancet Device), capillary blood was collected on filter paper cards (Whatman™ Protein Saver™ 903™), dried for at least two hours and stored in foil bags with a small packet of desiccant for a maximum of three weeks at room temperature. Subsequently, these foil bags were placed in a freezer at minus 80 degrees Celsius until they could be tested.

Elution of DBS samples

The filter paper cards were thawed and dried blood spots (DBS) were punched from these cards with a 12.7 mm or 6 mm diameter paper-hole punch. A 12.7 mm diameter spot was considered to contain 26 microlitres of serum and a 6 mm spot 5 microlitres. The spots were 1:6 eluted in phosphate buffered saline (PBS) with 2% fetal bovine serum (FBS) and

incubated overnight on a rotating device in a room kept at 4 degrees Celsius. The next day the supernatant was transferred to a cone vial and the paper spots were squeezed to get the maximum volume out of the DBS. Eluates were centrifuged prior to testing.

Laboratory testing

Given that the standard clinical test for HAV serology is the anti-HAV chemiluminescent immunoassay (CLIA), this test was used for the first screening of the eluates from all the DBS cards that were properly filled. The test was performed following the instructions of the manufacturer (DiaSorin LIAISON®). The limit of detection of this assay is a HAV antibody concentration of 18 micro international units per millilitre (mIU/ml), with a range of 15.5-21.5 mIU/ml. However, because of the 1:6 dilution factor, we had to maintain a cut-off of 108 mIU/ml for the CLIA of this study with a range of 93-129 mIU/ml.

Generally, used cut-offs range from 10-33 mIU/ml (165, 166), but it is known that during the maintained protective periods of vaccines titres are much higher than the cut-offs.. The concentration of HAV antibodies is at least 100 mIU/ml in 95% of the population in the first six months after primary vaccination, at least 1,000 mIU/ml in 95% of the population up to 15 years after secondary vaccination and 1,000-10,000 mIU/ml up to nine years after natural infection (167-169).

With a cut-off of 108 mIU/ml, the CLIA might not have been able to detect all positive samples, especially the ones there longer than 6 months after primary vaccination. Therefore, we subsequently tested all negative and equivocal eluates in the CLIA with a commercial enzyme-linked immunosorbent assay (ELISA) (DiaSorin® ETI-AB-HAVK PLUS). Also, because the CLIA needs an input volume of at least 240 microlitres per sample, the eluates from DBS cards that were sparsely filled were tested with the ELISA, as the input needed for this is only 50 microlitres. The ELISA was carried out according to the instructions of the manufacture, with additional dilutions of the calibrator. This resulted in a cut-off value of 10mIU/ml, leading to a cut-off of 60 mIU/ml due to the dilution factor. Therefore, equivocal samples (values 20% from the cut-off) were also considered positive (>48 mIU/ml). Thus, we considered DBS eluates negative if both the CLIA and ELISA gave a negative result, or if the ELISA gave a negative result and the sample was not tested with the CLIA. DBS eluates were considered positive if either the CLIA or the ELISA gave a positive or an equivocal result. So, samples that were tested negative or equivocal with the CLIA, but gave a positive or equivocal result in the ELISA, were considered positive. This was because the cut-off of the ELISA was closer to the correlate of protection (Supplemental Figure 1).

Definitions

Travellers were categorised according to the pre-travel HAV vaccination recommendation of the LCR as this would pertain to the (last) trip they made to an Eastern European country or a country outside Europe.

- PTA+ (pre-travel advice positive): travellers who visited a destination for which a pre-travel HAV vaccination is recommended.
- PTA- (pre-travel advice negative): travellers who visited a destination for which a pre-travel HAV vaccination is NOT recommended.
- PTA+/- (pre-travel advice depends): travellers who visited a destination (South Africa or Israel) for which a pre-travel HAV vaccination is only recommended under certain circumstances.

Data analysis

The study population was described using descriptive statistics. Categorical variables were compared between categories with chi-square tests and continuous variables with Mann-Whitney tests. The following baseline variables were analysed: sex, age, education level, nationality and immunocompromised state. Additionally, the following travel characteristics and travel-related behaviour were also analysed: destination, travel distance, length of stay and purpose of travel. Pre-travel recommendations were compared with travel clinic visits, self-reported HAV vaccination, vaccination records and serological status with chi-square tests. Additionally, a logistic regression model was used to calculate whether travel distance could predict vaccination status. Data analyses were performed with IBM SPSS statistics 25 and RStudio. 95%- confidence intervals were maintained and a p-value of <0.05 was considered significant.

Ethics

The study protocol was approved by the Medical Ethical Research Committee of the Erasmus Medical Centre (MEC-2015-538). Furthermore, the study was carried out in accordance with the declaration of Helsinki.

RESULTS

Our study population consisted of 246 travellers from 188 families; 30% were children and 55% were females. The study participants were mainly from municipalities in the Netherlands that were highly populated in 2015 (170). A relatively large proportion of the study population was highly educated with 49% of students attending VWO (the

Dutch equivalent of pre-university education) (171).

As shown in Table 1, 63% of travellers fell into category PTA+. Their pre-travel advice would have included HAV vaccination, given their destination and date of travel. Of these travellers, 9% went to Croatia, where HAV vaccination was only recommended if they had travelled before February 2017, due to a subsequent update of the LCR guidelines. Furthermore, 7% of the study population were categorised as PTA+/- because they travelled to South Africa or Israel, two countries for which the pre-travel advice only included the HAV vaccination if travellers stayed under unhygienic circumstances. In category PTA- there were 73 travellers, representing 30% of the study population. Table 1 shows that the education level of both children and parents differs significantly between the categories of travellers, with higher education levels being more strongly represented in the PTA+ group. P-values were comparable when PTA+/- travellers were excluded.

Table 1. Travellers' characteristics, categorised by pre-travel health advice

	PTA+ n=155 (63%)	**PTA- n=73 (30%)**	**PTA+/- n=17 (7%)**	**Total n=246° (100%)**	**p-value**
Sex					0.348
Female	36 (49.3)	90 (58.1)	11 (64.7)	137 (55.9)	
Age (in years)					0.909
11-18	44 (28.4)	24 (32.9)	5 (29.4)	73 (29.8)	
>18-35	7 (4.5)	3 (4.1)	2 (11.8)	12 (4.9)	
>35-65	102 (65.8)	45 (61.6)	10 (58.8)	157 (64.1)	
>65	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.4)	
Education level (child)*					0.031
VMBO	28 (18.1)	17 (23.3)	0 (0.0)	45 (18.4)	
HAVO	46 (29.7)	13 (17.8)	7 (41.2)	66 (26.9)	
VWO	76 (49.0)	35 (47.9)	8 (47.1)	119 (48.6)	
**Highest level of education (parent) ** **					0.036
Secondary school	9 (8.2)	2 (4.2)	1 (8.3)	12 (7.1)	
MBO	10 (9.1)	15 (31.9)	1 (8.3)	26 (15.4)	
HBO	40 (36.4)	12 (25.5)	5 (41.7)	57 (33.7)	
WO	46 (41.8)	18 (38.3)	5 (41.7)	69 (40.8)	
Nationality					0.099
Other than Dutch or dual	16 (10.3)	4 (5.5)	0 (0.0)	20 (8.2)	
Medical history					0.392
Immunocompromised	3 (1.9)	0 (0.0)	0 (0.0)	3 (1.2)	

***** VMBO stands for pre-vocational secondary education, HAVO stands for secondary general education and VWO stands for pre-university education level. ** MBO stands for senior secondary vocational education and training, HBO stands for higher professional education and WO stands for university level (172).

°For one participant the travel destination was not clear, so he could not be included in one of the PTA categories.

In Table 2, we compared the vaccination-related behaviour of the three categories of travellers. As you would expect, more PTA+ than PTA- travellers said they received pre-travel advice prior to their last trip (36% as opposed to almost 3%). Similarly, according to their vaccination records, more PTA+ than PTA- travellers were able to prove they were protected against HAV (41% as opposed to 16%). Despite the smaller numbers, according to their vaccination records, PTA+/- travellers showed higher percentages than PTA+ travellers for both receiving pre-travel advice and HAV protection. However, it must be added here that many participants did not know their HAV vaccination status (29% for PTA+/- and 97% for PTA-) and vaccination records were often not available (34% for PTA+ and 48% for PTA-).

Table 2. HAV vaccination status compared with HAV vaccination pre-travel advice in guidelines

	Received pre-travel advice			Self-reported HAV vaccination			HAV protected according to vaccination records			DBS eluate anti-HAV positive*			TOTAL (%)
	No (%)	Yes (%)	? (%)	No (%)	Yes (%)	? (%)	No (record) (%)	Yes (%)	? (%)	No (%)	Yes (%)	? (%)	
PTA+	98 (63.2)	56 (36.1)	1 (0.7)	21 (13.5)	69 (44.5)	65 (41.9)	38 (24.5)	64 (41.3)	53 (34.2)	47 (30.3)	98 (63.2)	10 (6.5)	155 (100)
PTA-	69 (94.5)	2 (2.7)	2 (2.7)	1 (1.4)	1 (1.4)	71 (97.3)	26 (35.6)	12 (16.4)	35 (48.0)	44 (60.3)	22 (30.1)	7 (9.6)	73 (100)
PTA+/-	5 (41.7)	12 (70.6)	0 (0.0)	4 (23.5)	8 (47.1)	5 (29.4)	2 (11.8)	9 (52.9)	6 (35.3)	8 (47.1)	8 (47.1)	1 (5.9)	17 (100)
p-value	<0.001			<0.001			0.002			<0.001			

*DBS eluate anti-HAV positive means a positive or equivocal result with the CLIA or ELISA. DBS eluate anti-HAV negative means a negative result with the CLIA and ELISA, or a negative result with the ELISA and not tested on CLIA.

Therefore, we chose to compare compliance with guidelines of the PTA+ group on the basis of the serological status of travellers. From the 155 PTA+ travellers, 145 DBS eluates were available (Table 2). In these samples, we found that 98 of the 145 (67%) had anti-HAV antibodies (DBS+), compared to 22 of 66 (33%) in PTA- travellers and 8 of 16 (50%) of the PTA+/- travellers ($p<0.001$).

Of the 47 PTA+ DBS- travellers, 35 (74%) had not visited a travel clinic and 37 (79%) of them said that they were not aware that vaccinations were advised for their destination. Vaccination records of these 47 PTA+ DBS- individuals showed that 20 travellers (43%) indeed had no proof that they were (still) protected against HAV, but according to their

vaccination records 9 travellers (19%) were HAV protected during their trip. From the 18 cases in which DBS eluates were missing, 5 (28%) claimed to have been HAV vaccinated. In the PTA+ category without DBS analysis, 2 out of 10 (20%) self-reported to be HAV vaccinated. Therefore, even in the most optimistic of scenarios, 23% of PTA+ individuals travelled unprotected.

To distinguish the risk category of PTA+ travellers lacking anti-HAV antibodies (DBS-), we compared baseline and travel characteristics of DBS+ and DBS- travellers in this category (Table 3). Significantly more often children were DBS- than DBS+ (37% as opposed to nearly 23%, respectively). However, when using age as a continuous variable, this significant difference disappears. However, the proportion of PTA+ children who were DBS- was 45% (17/38 available DBS). Furthermore, the reason behind the journey for DBS- travellers was more often visiting friends or relatives (VFR), as opposed to regular holidays or business trips. Also, PTA+ DBS- individuals travelled to closer destinations more frequently. A logistic regression model showed that travel distance can be a significantly predictor of serological status (DBS+ or DBS-). PTA+ travellers who covered a distance of more than 5,000 kilometres (as the crow flies) were more likely to be DBS+ than those who travelled shorter distances to their destinations (OR=2.89, p=0.00421). In 45% of cases, PTA+ DBS- participants travelled to destinations in Eastern Europe or North Africa. Although we had to take into account that the reported numbers are small, none of the travellers to Eastern Europe and the Middle East sought advice from a pre-travel clinic and neither did 86% of travellers to North Africa. Of the PTA+ individuals travelling to these regions, 58, 50 and 33%, respectively, were DBS- (Figure 1).

Table 3. Characteristics of seronegative and seropositive PTA+ travellers

	PTA+ DBS- travellers (%) n = 47	**PTA+ DBS+ travellers (%)** n = 98	**Total PTA+ travellers (%)** n = 145	**Chi-square** p-value
Age groups (in years)				0.042
11-18	17 (37.0)	22 (22.5)	39 (27.1)	
>18-35	0 (0.0)	6 (6.1)	6 (4.2)	
>35-65	28 (60.9)	70 (71.4)	98 (68.1)	
>65	1 (2.2)	0 (0.0)	1 (0.7)	
Education level (child)*				0.942
VMBO	8 (17.8)	20 (21.1)	28 (20.0)	
HAVO	13 (28.9)	28 (29.5)	41 (29.3)	
VWO	24 (53.3)	47 (49.5)	71 (50.7)	
Duration of travel (days)				0.488
0-7	10 (21.3)	12 (12.2)	22 (15.2)	
>7-28	33 (70.2)	76 (77.6)	109 (75.2)	
>28-56	4 (8.5)	9 (9.2)	13 (9.0)	
>56	0 (0.0)	1 (1.0)	1 (0.7)	
Purpose of travel				0.011
Holiday	28 (60.9)	73 (74.5)	101 (70.1)	
VFR	10 (21.7)	13 (13.3)	23 (16.0)	
Business	4 (8.7)	12 (12.2)	16 (11.0)	
Other	4 (8.7)	0 (0.0)	4 (2.8)	
Destination				0.013
Eastern Europe	15 (31.9)	11 (11.2)	26 (17.9)	
**South/Central America	8 (17.0)	14 (14.3)	22 (15.2)	
North Africa	6 (12.8)	12 (12.2)	18 (12.4)	
Central Africa	4 (8.5)	11 (11.2)	15 (10.3)	
Middle East	4 (8.5)	4 (4.1)	8 (5.5)	
Asia	10 (21.3)	46 (46.9)	56 (38.6)	

* VMBO stands for pre-vocational secondary education, HAVO stands for secondary general education and VWO stands for pre-university education level. **Eastern Europe in this table includes the following PTA+ countries: Albania, Belarus, Bosnia, Bulgaria, Macedonia, Montenegro, Romania, Russia, Slovenia and Turkey.

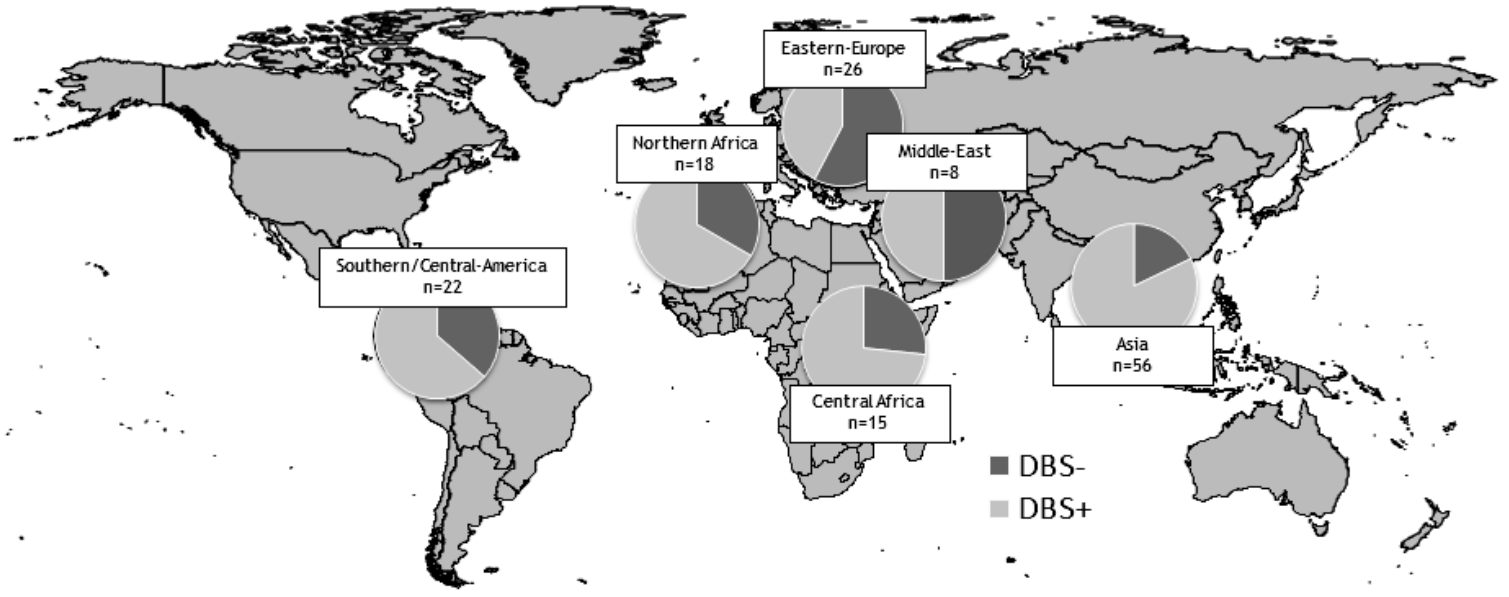


Figure 1. Adherence to travel health guidelines per PTA+ region

*Missing data was excluded

Eastern Europe includes the following PTA+ countries: Albania, Belarus, Bosnia, Bulgaria, Macedonia, Montenegro, Romania, Russia, Slovenia and Turkey. South/ Central America includes Brazil, Colombia, Costa Rica, Cuba, Mexico, Nicaragua, Panama and Suriname. North Africa includes Egypt, Morocco, Gambia and Senegal. Central Africa includes Ghana, Kenya, Mauritius, Namibia, Uganda and Tanzania. The Middle East includes Armenia, Georgia, UAE, Iran, Israel, Jordan and Kazakhstan. Asia includes Cambodia, Hong Kong, India, Indonesia, Japan, Korea, Laos, Mongolia, Myanmar, Nepal, New Caledonia, Sri Lanka, Thailand and Vietnam.

DISCUSSION AND CONCLUSION

According to the serological data from this study, only 67% of Dutch travellers comply with travel health guidelines. Of all the travellers visiting a region for which a HAV vaccination was recommended (PTA+), only 36% received pre-travel advice prior to their trip, while only 41% had proof of valid HAV vaccination in their records. In line with these findings, in a third of all PTA+ instances, we could not find HAV antibodies in dry blood spot analyses, suggesting a lack vaccination protection. Non-adherence to travel health guidelines was associated with being a child, visiting friends and relatives and/or only travelling a short distance.

As expected, in the surveys that we conducted and in the records that were available to us, there proved to be a relatively large amount of missing vaccination data. Recall bias will have played a role here, even though we tried to include travellers returning a maximum of one year after their journey. We therefore increased the reliability of our data by adding serological evidence of vaccination by analysing DBS eluates. The fact that 9 out of 47 PTA+ travellers who were DBS- could show proof of being HAV protected during travel but had no detectable antibodies, could be explained by the fact that their vaccination was up to 32 weeks prior to travel and the DBS sampling was up to 12 months after they returned. In other words, at the moment of DBS sampling their HAV antibody concentrations could have decreased (probably after the primary vaccination) and the vaccination was no longer valid, although it would have offered the necessary protection during their journey. Also, the cut-off used in this study (48 mIU/ml) is slightly higher than the commonly used correlates of protection (10-33 mIU/ml). This could also explain this inconsistency, if the antibody titre dropped just below our detection rate but was still above the correlate of protection. Finally, people could belong to the small group (approximately 5%) of non- or low-responders to the HAV primary vaccination (173, 174).

Low adherence to travel health guidelines would seem to be mainly a matter of unawareness of the vaccination recommendations (160), particularly when it comes to travelling shorter distances. As destinations in Eastern Europe seem relatively close to home, travellers probably underestimate the risk of contracting an infectious disease (175). Together with Asia, Eastern Europe is the travel region where the most morbidity attributable to vaccine-preventable diseases occurs (176). However, the guidelines-compliance rate of travellers to Eastern Europe was only 37% - while for these travellers HAV is one of the most preventable VPDs (176, 177). Asia is the most visited region among the DiVeST population and 82% of travellers who go there comply with travel health guidelines.

However, the number of people travelling to Turkey and other countries quite close to the Netherlands but outside Europe, such as Morocco and other North African countries, was relatively high. This is probably due the number of VFR travellers going to those countries. VFR travellers also seem to have a lower risk perception, as do travellers who travel for holiday or business purposes (160). However, it must be added here that VFR travellers face certain limitations when it comes to pre-travel precautions, such as the cost of consultations and vaccinations and a possible language barrier restricting their access to travel health advice (178). It could be argued that VFR travellers might be protected by natural immunity. However, in our study population, anti-HAV seropositivity in VFR travellers was lower than in non-VFR travellers.

Rates of non-compliance with travel health guidelines obtained from the DiVeST were surprisingly consistent with the results of Dutch survey-only studies. One third of travellers were unaware of the risks of contracting infectious diseases while travelling to endemic regions worldwide, while the same could be said for 64% of those travelling to closer risk destinations (150, 151). Survey-only research from seven other Western countries was also in line with our results, with 67% reporting that they have been vaccinated. However, 70% of travellers sought pre-travel advice, which was much higher than in our study population (160). Given that we had a relatively high rate of fully vaccinated people (of all vaccination records showing any form of HAV vaccination, 80% of them showed proof of having received the complete vaccination series), it might be the case that our population comprised more experienced travellers (160). A survey study from nine European airports, reported a rate of non-compliance in travellers to developing countries of at least 44% for HAV vaccination. This higher rate might be explained by a higher rate of VFR (22%) than in our study (16%) (179). The HAV seroprevalence rate of 27% reported in the general Dutch population aged 15-61, largely corresponds with the 30% seropositivity in our PTA- group (180). However, this pre-existing immunity also implies that the seroprevalence rate of 67% in the PTA+ population might be an overestimation of the adherence to travel health guidelines.

Children, VFR and short-distance travellers were mentioned as risk groups before, among solo travellers and last-minute travellers (181-184). These groups must be addressed with clear, bespoke information to increase their adherence to travel health guidelines and thus decrease travel-related morbidity (176). Moreover, solutions must be found to solve barriers faced by VFR travellers. In our opinion, awareness could best be created via public channels shortly before the start of holiday seasons and, to trigger the awareness of the relevant target groups, specific (short-distance) destinations, travel purposes and age groups should be mentioned (185, 186). The fact that adherence to travel health

guidelines in children is lower than in adults, can be related to insufficient knowledge about the recommendations and availability of preventive measures for these age groups or fear for side-effects (183). Some argue that in very young children hepatitis A is a very mild disease and therefore vaccination is not needed. However, Dutch travel health guidelines recommend to vaccinate children older than 5 (all participants in this study), because even if they are asymptomatic, they will be infectious, putting naïve contacts at risk upon their return (36, 187).

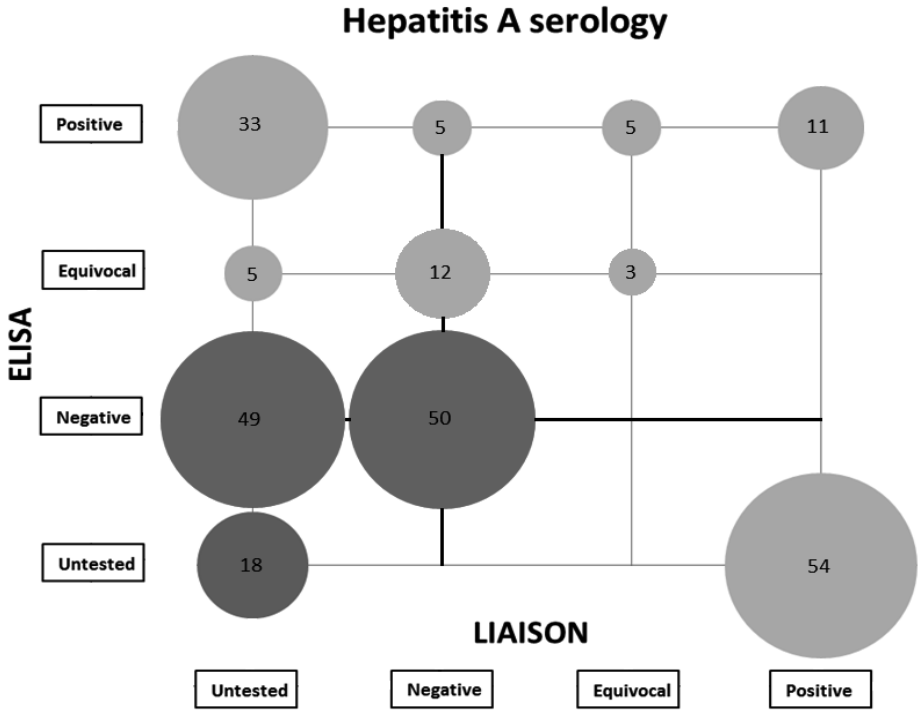
While in this study we only focused on the vaccination status and seroprevalence of HAV, it should only be seen as a representation of adherence to guidelines. Travel health advice provided in guidelines is not restricted to vaccination recommendations; it also includes recommendations regarding chemoprophylaxis and risk behaviour related to other travel-related aspects, such as food and water consumption, sexual behaviour and protection against mosquito bites. An extensive study by EuroTravNet has shown that pre-travel consultations were associated with reduced morbidity when it comes to travel-related infectious diseases (188). The long-term protection afforded by vaccinations can explain why travellers often do not visit a travel clinic prior to their next trip. However, as travellers do not visit a travel clinic prior to each journey, prevention other than vaccination, like pre-travel health advices, hygiene and advice regarding risk behaviour, might also be forgotten in the long run. Another point of attention is making general healthcare providers aware of risks of travel-related infections for travellers to destinations on short-distance, as short-distance travellers in particular are likely to consult their general practice instead of a specialized travel clinic for pre-travel health advice (175, 189).

Covering travellers from all over the Netherlands, including all people who did and did not consult a travel clinic, the DiVeST represents a broad travelling population. Although the study excluded elderly and childless couples, it includes people who travelled with all modes of transportation, in contrast with airport surveys. This is highly relevant because air travel comprises only 57% of all international travel (147). Shorter-distance travellers are not only more likely to travel by land, they are also more likely to be unvaccinated. In this study, members of the same family were included, so as to recruit a larger number of travellers. Although most participants were single-family members, this might still have induced some selection bias. However, the vaccination status of parents and children of the same family can differ, due to natural immunity and misperceptions of protection induced by the NIP.

Furthermore, the study population had a greater proportion of highly educated people than the general population. However, as there is no significant difference in education

level between the PTA+DBS- and PTA+DBS+ (Table 3), there is no reason to expect the adherence rate to be different. Seen in the context of all international travellers, the study population comprised relatively few VFR travellers - 16% as opposed to 27% of international travellers (147). Furthermore, non-NIP vaccinated individuals were less willing to participate and were thus underrepresented. This implies that 67% adherence to travel health guidelines is more likely to be an overestimation than an underestimation.

In conclusion it can be said that only two-thirds of Dutch travellers adhere to travel health guidelines. Because their protection is suboptimal, during international travel children, VFR and short-distance travellers are particularly vulnerable to contracting travel-related infectious diseases like hepatitis A. According to our results, these subgroups constitute excellent target groups on which to focus in raising the necessary awareness and thereby reducing travel-related morbidity.



Supplemental Figure 1.
blue means untested. The size of the circle represents the number of samples, which is also quantified by the number in the circle.

Chapter 2.2

Measles seroprevalence among Dutch travelling families

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Submitted

ABSTRACT

While measles vaccination is widely implemented in national immunisation programmes, measles incidence rates are increasing worldwide. Dutch inhabitants who were born between 1965-1975 may have fallen between two stools, lacking protection from a natural infection, and having missed the introduction of the measles vaccination schedule. With this study we aim to find the measles seroprevalence in travellers born between 1965-1975, compared to those born before 1965 and after 1975.

Families travelling to Eastern Europe or outside Europe during the preceding year were recruited via Dutch secondary schools between 2016 and 2018. Their vaccination status was assessed using questionnaires, vaccination records and measles serology in dried blood spot (DBS) eluates. Measles virus antibody concentrations were determined with an ELISA (EUROIMMUNE®) and a subset was retested with a focus reduction neutralization assay (FRNT).

In 189 (79%) of the 239 available DBS eluates, the ELISA could detect sufficient measles virus-specific IgG antibodies. Of the negative samples that were retested with FRNT, 85% remained negative, resulting in an overall seroprevalence of 82% [95% CI 76-86]. Children had a lower seroprevalence (72%) than adults (87%). Travellers born between 1965-1975 were protected in 89%.

In this study, we report a measles seroprevalence of 82% among Dutch travelling families. Remarkably, seroprevalence rates were lowest in children (12-18 years) instead of travellers born between 1965-1975. Although a fraction of people without detectable antibodies may be protected by other immune mechanisms, these data suggest that measles (re) vaccination should be considered for travellers to endemic regions.

INTRODUCTION

Globally, measles cases are on the rise (31, 190). Measles, being a vaccine-preventable virus infection, is an important cause of childhood mortality and can induce neurological complications and long-lasting immune suppression (191). Incidence rates are increasing as a consequence of declining vaccination coverage rates driven by factors such as health care access and vaccine hesitancy (190). Numerous countries are experiencing measles outbreaks. Not only regions barely connected to Europe, but also popular holiday destinations, like Thailand, and high-income countries like the United States of America, are affected by measles virus. Also, in the European region, we see increases up to 300% compared to one year earlier (192). As measles outbreaks are currently happening in countries were no other vaccinations are recommended for, travellers may not be aware of the need of being sufficiently protected against measles.

With measles being one of the most contagious infectious diseases of humans (31), unprotected travellers are at increased risk with respect to these global outbreaks. A Swedish study reported 31 measles cases in a cumulative 500 million days of travel, mainly to other European countries and the Asian continent (193). Besides the morbidity a measles virus infection can cause for the individual traveller, an infection in a traveller can also contribute to the spread of measles virus (194). We have to be careful to prevent a global measles outbreak being the next public health emergency of international concern (195).

Since its availability in 1960s, live-attenuated measles vaccines have been incorporated in national immunisation programmes (NIPs) worldwide (196). In the Netherlands, measles vaccination was included in the NIP in 1976. At that time, all infants born in 1975 received a single vaccination, infants born in 1978 and later got a measles vaccination twice. Later, in 1987 the combined measles, mumps and rubella (MMR) vaccine was introduced. In the current NIP, children receive MMR vaccinations at the ages of 14 months and 9 years. Individuals born before 1965 are considered immune due to natural infection because of high measles endemicity at that time and empirical proof that the majority was found seropositive for measles (197, 198). Therefore, Dutch travel health guidelines recommend measles vaccination to every traveller born after 1965 who did not experience measles nor has a history of measles vaccination when they plan to visit a risk destination. Individuals born between 1965 and 1975 are considered at higher risk not being immune for measles (198). If the immune status is not clear, serology can be performed, or direct measles vaccination can be considered (197). Different serological assays are available to determine the level of antibodies. It is important to note that an individual is considered protected for measles if the concentration of antibodies that

neutralize measles virus is higher than 120 mIU/ml (199). However, subjects without detectable neutralizing antibodies may still be protected based on cellular immunity.

Since 1987, Dutch infants get vaccinated with two doses MMR vaccine: one at the age of 14 months (MMR-1) and another one at nine years (MMR-2) (200). In 2019, the reported vaccination coverage for MMR-1 was 92.9% among two-year olds in the Netherlands (201). Based on the combined immunity in older adults from natural infection during childhood, the high immunogenicity of the live-attenuated measles vaccine and the recurrent outbreaks in small, unvaccinated subpopulations, the overall measles seroprevalence in the Netherlands reached 95.7% (data from 2006-2007) (202).

At this time of measles resurgence, travel clinics have to pay special attention to measles protection (203). Therefore, we studied the current seroprevalence rate among Dutch travellers, and aimed to find risk factors for lacking measles protection. We build upon a previous study in which clinical data and dry blood spot samples were collected in a cohort of 246 Dutch travelling family members (204). As we expected to find a lower seroprevalence rate in individuals born between 1965 and 1975, this cohort was perfectly suitable, as it included many parents of school-going children, who were of that age category.

MATERIAL AND METHODS

Study population

For this cross-sectional study, we visited secondary schools throughout the Netherlands between September 2016 and December 2018. We recruited students (12 years and older), their family members, and school employees who had travelled to an Eastern European or non-European country in the preceding year. These destinations were chosen as inclusion criteria because of the vaccination recommendations in the Dutch travel health guidelines. This cohort was originally designed to assess the adherence to hepatitis A travel health guidelines (204).

Data collection

After participants (and their parents or representatives if the participant was 12-18 years old) had given written informed consent, they were asked to fill out questionnaires (containing demographics, medical history, and travel and vaccination history), share a copy of their vaccination records if available and donate blood by a finger prick. An electronic data-management application (OpenClinica®) was used to collect all this coded

information. Filter paper cards (Whatman™ Protein Saver™ 903™) were used to collect capillary blood. After drying the cards for at least two hours, they were packed in foil bags with a small packet of desiccant. They were stored for a maximum of two weeks at room temperature and subsequently in a freezer at minus 80 degrees Celsius until tested.

Elution of DBS samples

Filter paper cards were thawed and dried blood spots (DBS) were punched from these cards with a three millimetre diameter paper-hole punch. This spot size was considered to contain 1.5 microlitres (µl) of serum (205, 206). The spots were transferred to an uncoated round-bottom 96-wells plate (Greiner®) and eluted in 150 µl sample buffer from the kit which will be described in the next section, resulting in a 1:101 serum dilution and incubated for one hour at 37 degrees Celsius. If the remaining DBS was not sufficient, the 1:6 eluates from the previous study on this cohort (204) were used and further diluted with the sample buffer from the ELISA kit to 1:101 as well. These remaining samples were once eluted 1:6 in phosphate buffered saline supplemented with 2% fetal bovine serum and stored thereafter at -80 degrees Celsius. Total IgG concentrations were measured in seronegative DBS eluates (with the human IgG ELISA, Cusabio) to confirm that a minimal level of IgG was present.

Laboratory testing

Enzyme linked immunoassay

Measles IgG concentrations were measured before in DBS samples, using the enzyme immunoassay (EIA) Dade Behring Enzygnost (207). Unfortunately, this EIA was not available anymore at the time of this study. Therefore, we measured concentrations of anti-measles virus immunoglobulin G (IgG) with the EUROIMMUN® Anti-Measles Virus enzyme linked immunoassay (ELISA), also validated for the use of DBS specimens. EUROIMMUN® reports that neither the sensitivity nor specificity was impaired by the use of DBS specimens and that the correlation coefficient between DBS and serum was 0.992 (n=12).

One hundred µl of the DBS eluates was transferred to the ELISA plate. The test was performed following the instructions of the manufacturer. According to the recommendations of the EUROIMMUN manual, an antibody concentration lower than 150 IU/l conferred a negative result comparable to non-protective antibody concentrations as determined by the PRNT (<120 IU/l). Values between 150 and 200 IU/l were considered equivocal and higher than or equal to 200 IU/l as positive.

Virus neutralization assay

Due to the relatively low sensitivity of measles ELISAs (208-210) we decided to retest all negative and equivocal samples with a focus reduction neutralization test (FRNT). The FRNT is a simplified neutralisation test based on the gold standard PRNT (211). As the FRNT has not been used before on filter paper samples, we first performed a validation study of 20 paired serum and DBS samples in duplicate showing excellent results up to a sensitivity and specificity of 100% (manuscript in preparation).

The FRNT was performed as described previously (212), with some modifications due to the start dilution of the DBS. Shortly, 48 µl of the 1:6 DBS eluates and 1:12 of the WHO 3rd international standard containing 3000 mIU/ml were transferred to the first row of V-bottom plates of which the subsequent rows were filled with 24 µl of DMEM (Gibco Invitrogen, USA) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich, USA), further referred to as D10F. Twofold serial dilutions were made by serial transfer of 24ul. Subsequently, 3200 $TCID_{50}$ of recombinant measles virus strain Edmonston, modified to express EGFP ($rMV^{rEdt}EGFP$) was added to each well (resulting in a 1:8 serum dilution in the first row). Plates were incubated for two hours at 37 degrees for neutralization. Subsequently, the virus-serum dilutions were transferred to Vero-humanCD150 (213) monolayers - that were seeded four days prior to the start of the assay - and incubated for another four hours. Thereafter, virus-serum dilutions were replaced by 50 µl of D10F supplemented with 200µM fusion inhibitory peptide (FIP: Zd-Phe-L-Phe-Gly-OH, Bachem, Heidelberg, Germany) to prevent cell-to-cell spread of the virus. After 48 hours of incubation, single infected EGFP-positive cells could be observed by fluorescence microscopy. Monolayers were washed twice with DPBS (lacking calcium and magnesium) (Lonza BioWhittaker, Switzerland). Cell layers were fixed with 2% paraformaldehyde for 30 minutes at room temperature and washed once again before the EGFP spots were scanned and counted with CTL ImmunoSpot® analyser (CTL, Bonn, Germany). Neutralizing antibody levels were calculated based on the serum dilution that reduced the number of infected cells by 50% (ND50), and expressed in mIU/ml based on the result of the international standard. Both serum and DBS were tested in duplicate and the geometric mean titres were used as final result. Based on WHO recommendations, we considered an antibody level lower than 120 mIU/ml as negative, between 120 and 200 mIU/ml as equivocal and higher than or equal to 200 mIU/ml as positive.

Data analysis

The study population was described using descriptive statistics. Subgroups were compared with chi-square tests in case of categorical variables and independent T-tests or Mann

Whitney test in case of continuous variables. Correlations between variables were calculated with either Pearson or Spearman correlation coefficients. IBM SPSS statistics 25 was used to perform data analyses. A p-value of <0.05 was considered significant and 95%-confidence intervals were maintained.

Ethics

The study protocol was approved by the Medical Ethical Research Committee of the Erasmus Medical Centre (MEC-2015-538). Furthermore, the study was carried out in accordance with the declaration of Helsinki.

RESULTS

Of the 246 travellers that were recruited between September 2016 and December 2018, 30% was a child (12-18 years old), 55% was female and 8% had a (partly) other nationality than Dutch. In this study population, 97% was vaccinated following the NIP. Individuals were divided to birth cohorts according to their assumed measles immunity, resulting in 25 individuals (11%) born before 1965, 112 (47%) born between 1965 and 1975, and 100 (42%) born after 1975 (Table 1).

Measles vaccination recommendations

Countries where measles vaccination was recommended for, and travellers from this cohort travelled to, included: Armenia, Bosnia Herzegovina, Cambodia, Dubai, Egypt, Gambia, Georgia, Ghana, Hongkong, India, Indonesia, Iran, Japan, Kenia, Laos, Morocco, Mongolia, Montenegro, Myanmar, Namibia, Nepal, New Caledonia, Romania, Senegal, South-Africa, Sri Lanka, Tanzania, Thailand, Uganda, United Arabic Emirates, Vietnam. Among these, Indonesia (n=20), Thailand (n=19), South-Africa (n=14) and Morocco (n=11) were the most popular destinations.

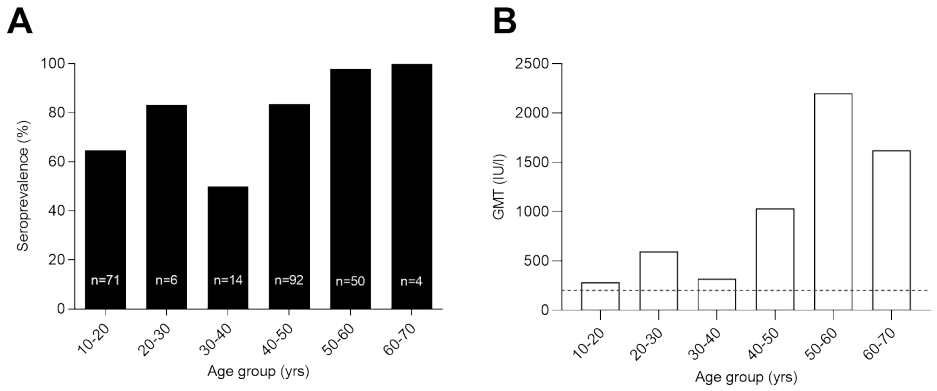


Figure 1. Seroprevalence rates and geometric mean titres per age group
The A panel shows the rate of travellers with a measles antibody titre >200 IU/l. The B panel shows the geometric mean of the measles antibody titre (IU/l) with the cut-off set at 200 IU/l.

Measles seroprevalence

DBS were collected from 239 travellers and the EUROIMMUN® ELISA was performed on fresh eluates in January 2020. In 29 cases, we had to use old eluates that had a 1:6 dilution and we diluted them with sample buffer to a 1:101 serum dilution. The total seroprevalence of measles antibodies - measured with ELISA and using the cut-off of ≥200 mIU/ml - in this travellers cohort was 79%. The seroprevalence in the travellers born before 1965 was 100%. In the children (all older than 12), we found a seroprevalence rate of only 67%, compared to 84% in all adults (chi-square, p=0.002). When we divided the study cohort in smaller age groups, we found the lowest seroprevalence rates and the lowest titres in those aged younger than 40 at the time of sampling (Figure 1).

In line with the higher seroprevalence rates in older participants, we report a weak correlation between the two continuous variables measles titre and age (Spearman R=0.563 [95% CI 0.466-0.646], p<0.0001). As is shown in Table 1, we did not find any other baseline characteristic being related to the lower seroprevalence but age.

Looking into the travel-related characteristics, we found no significant differences between the seronegative and seropositive group. Out of the 119 travellers who visited a destination where measles vaccination was recommended for, only 95 (80%) was considered protected on basis of their antibody levels. This seroprotection rate was comparable to the total cohort of travellers. Also, receiving pre-travel advice or receiving a measles vaccination were equally reported between groups (Table 2). A previous study reported the seroprevalence rate of hepatitis A virus (HAV) in the same travellers cohort (204). When we combined these data, we found no significant

correlation between HAV specific and measles-specific antibodies (p=0.2991). We also tested total IgG concentrations in 44 of the 50 eluates that were tested either negative or equivocal and found that the average level was 12.6 g/l [95% CI 11.4-13.8 g/l], which is within the normal 7-16 g/l range for a healthy population aged 12 years and older (214).

Table 1. Travellers' baseline characteristics, categorised by immune status

	Measles seronegative* n = 50	**Measles seropositive** n = 189	**Total** n = 239	**p-value (chi-square)**
Traveller				0.002
Child (%)	24 (48.0)	48 (25.4)	72 (30.1)	
Sex				0.407
Female (%)	30 (60.0)	101 (53.4)	131 (54.8)	
Born in year				0.000
<1965	0 (0.0)	25 (13.3)	25 (10.5)	
1965-1975	14 (28.0)	98 (52.4)	112 (47.3)	
>1975	36 (72.0)	64 (34.2)	100 (42.2)	
Nationality				0.426
(Partly) other nationality than Dutch	2 (4.0)	16 (8.5)	18 (7.5)	
NIP				0.588
Yes	48 (96.0)	178 (96.8)	227 (96.6)	
Education level (child)				0.131
VMBO	7 (14.0)	38 (20.1)	45 (18.8)	
HAVO	15 (30.0)	47 (24.9)	62 (25.9)	
VWO	22 (44.0)	96 (50.8)	118 (49.4)	
missing	6 (12.0)	8 (4.2)	14 (5.9)	
Education level (adult)				0.346
MBO	5 (19.2)	21 (15.1)	26 (15.8)	
HBO	5 (19.2)	51 (36.7)	56 (33.9)	
WO	14 (53.8)	54 (38.8)	68 (41.2)	
missing	2 (7.7)	13 (9.4)	15 (9.1)	

NIP = national immunisation programme; VMBO = preparatory vocational and general secondary education; HAVO = advanced general secondary education; VWO = pre-university education; MBO = senior secondary vocational education and training; HBO = higher professional education; WO = university.* Including equivocal results.

Table 2. Travellers' vaccination status, categorised by immune status

	Measles seronegative* n = 50 (%)	Measles seropositive n = 189 (%)	Total n = 239 (%)	p-value (chi-square)
Measles vaccination recommended for their destination				0.776
No	26 (52.0)	94 (49.7)	120 (50.2)	
Yes	24 (48.0)	95 (50.3)	119 (49.8)	
Visited travel clinic for pre-travel advice				0.466
No	38 (76.0)	130 (68.8)	168 (70.3)	
Yes	12 (24.0)	56 (29.6)	68 (28.5)	
Missing	0 (0.0)	3 (1.6)	3 (1.3)	
Reported measles vaccination				0.846
No	11 (22.0)	49 (25.9)	60 (25.1)	
Yes	1 (2.0)	4 (2.1)	5 (2.1)	
Missing	38 (76.0)	136 (72.0)	174 (72.8)	
Proof of measles vaccination in records				0.428
No	17 (34.0)	74 (39.2)	91 (38.1)	
Yes	1 (2.0)	10 (5.3)	11 (4.6)	
Missing	32 (64.0)	105 (55.6)	137 (57.3)	

* Including equivocal results.

Out of the 37 samples that were found to be negative in the ELISA (<150 IU/l), 20 were retested with the FRNT (the ones that had enough volume of DBS eluate left) in June 2020. Of these 37 samples, 18 (49%) were children. Of these 20 retested samples, 9 (45%) were children.

Out of the 14 samples that were found to be equivocal in the ELISA (150-200 IU/l), 5 were retested with the FRNT. As an extra control, we also retested 7 positive samples (≥200 IU/l). All retested seropositive samples in the ELISA (titre range 252-4735 IU/l), were also positive in the FRNT. All samples with equivocal results in the ELISA (titre range 173-191 IU/l) tested negative in the FRNT. Two samples that were negative in the ELISA showed equivocal results in the FRNT (165 and 141 IU/l). Of all the samples that were found to be negative in the ELISA and were retested with the FRNT (n=20, ELISA titre range <50-134 IU/l), 85% (95% CI 64-95) was negative in the FRNT as well. One sample became positive (424 IU/l).

We assume, based on the combined ELISA and FRNT data, that 85% of the seronegative individuals measured with the ELISA are truly seronegative. Consequently, we report an overall seroprevalence rate of 82% [95% CI 76-86] (instead of 79) for Dutch travelling

families. For children the seroprevalence rate was 72% [95% CI 61-81] (instead of 67) and for adults 87% [95% CI 84-93] (instead of 84). For the birth cohort (1965-1975), considered at risk for measles, the seroprevalence rate was 89% [95% CI 82-94] (instead of 88).

DISCUSSION AND CONCLUSION

In this study, we report an overall measles seroprevalence of 82% among Dutch travelling families. Remarkably, seroprevalence rates were lowest in children 12-18 years old (who had received two MMR vaccinations), with only 72% being seroprotected for measles. In contrast, the travellers who are generally considered at risk due to their year of birth between 1965 and 1975 (197) had a higher seroprevalence of 89%.

The overall seroprevalence we found in this study was lower than expected. As 97% of our study cohort reported to be vaccinated following the NIP and the vaccination response after two doses of MMR is 96% (215) the predicted seroprevalence was 93%. Moreover, a national serosurveillance study (named PIENTER) that is performed in the Netherlands every decade, reported a measles seroprevalence of 95.7% (95% CI 95.1-96.2) among 7900 Dutch inhabitants of all ages living throughout the Netherlands in 2006-2007 (202). However, that study population included a higher proportion of older inhabitants and could therefore have found a higher seroprevalence than the 82% we report. Another Dutch study performed in healthcare workers (aged 18-52) found a measles seroprevalence of around 90%, tested with three commercial immunoassays (EIAs) (209). However, when they retested these samples with the PRNT, the rate increased to 99% (209).

A number of seroprevalence studies performed in other high-income countries reported data similar to our results. An American cross-sectional seroprevalence study reported a discrepancy between the immunity rates reported by national seroprevalence studies (96%) and those found by them (86%) (216). Specifically in travellers, a large retrospective study in Australia reported lacking serological evidence of protection against measles in 8% of the 683 travellers (217). They also noticed higher rates of seronegative results in those born after 1982 (15%) (217). In addition, low measles seroprevalence rates in young people were reported by other European studies (218-220). In a study among health care workers (HCW) in the United Kingdom, a mean seroprevalence of 88% was found. Remarkably, they also found a decrease in measles seroprevalence with the more recent year of birth. HCW born before 1960 had a seroprevalence rate of 99%, and those born after 1990 only had a rate of 74% (used serological assay not reported)

(218). A French study in HCW reported an seroprotection rate (CAPTIA anti-measles IgG > 90 IU/l) of HCWs younger than 30 years old was 87% compared to 96% older than 30 (220). In an Italian study, seroprevalence rates between 73-79% were found in people aged 19-36 years, while seroprevalence rates ranged from 82-99% in those older than 37 years (measured with LIAISON XL) (219). On the other hand, in a German paediatric population, only 9% of the 14-17 years old had a negative measles titre (Siemens Enzygnost anti-measles IgG titre <150 IU/l) (221). Despite the considerable number of studies available, it is difficult to compare the results. The composition of the study populations and their age distribution have a high heterogeneity and many different assays are used, which are often EIAs with suboptimal sensitivity (209).

The increasing vaccination hesitancy in the last decade could play a role in the lower seroprevalence rates we found in younger travellers. The high compliance to the NIP that was reported by participants could have given a more optimistic reflection of the measles vaccination coverage than in reality due to social desirability in the questionnaires. However, we were unable to verify the vaccination status by inspection of vaccination records. Also, because NIP vaccinations are mostly registered separately from the travel vaccination records. The Dutch National Institute for Public Health and the Environment reports a vaccination coverage in the Netherlands for the first measles vaccination of 97.4%, while for the second it was only 92.0% (cohort 2005, reporting year 2016) (222). In theory, people in this cohort could have missed one or both measles vaccination, either by accident or by choice, without reporting.

Furthermore, travellers could have decided to antedate the first measles vaccination for their children, when travelling to a destination with a high measles incidence before the age of fourteen months. Early measles vaccination is known to give a lower serological response and a decrease in antibodies on the long-term (223), and therefore only recommended if needed. This suboptimal response at younger age is partly explained by the inhibitory effect of maternal antibodies that new-borns received passively (224). Although sparse data on vaccination history was available to check this, we expect this effect to have played a limited role in our cohort.

Another potential explanation for our findings is primary vaccine failure. However, it seems highly unlikely that this would fully explain the low seroprevalence rate, as many studies showed excellent immunogenicity of the live-attenuated trivalent MMR vaccine (215, 225). Moreover, as we are not the first to report low seroprevalence rates, and different vaccine strains are used throughout the world, we do not expect that this will play a major role in explaining low seroprevalence rates in younger people. Furthermore,

no significant difference in vaccine effectiveness was found between two commonly used measles vaccines containing either the Schwarz or the Edmonston-Zagreb measles strain (215).

A more probable explanation for the low seroprevalence rate in younger people might be waning vaccine-induced immunity (226). In the younger age groups, we observed lower measles antibody concentrations, while older travellers showed higher titres due to natural infections. Naturally, lower titres are more prone to dropping below the cut-off than higher titres. However, Dine et al (227) have shown that in 92% protective titres persisted 26 to 33 years after vaccination.

And still, although a virus neutralizing antibody level of 120 IU/l is commonly recognized as a correlate of protection, the evidence for this threshold is not conclusive (199, 225, 228). Titres below this level do not necessarily imply susceptibility to a full-blown measles virus infection. In some vaccinated people, measles virus infections associated with mild symptoms have been described (229), defined as breakthrough infections (230). Measles has an incubation time of approximately two weeks, which allows a secondary immune response to accelerate viral clearance and (partially) prevent disease. Although data on pre-infection titres of these people are often lacking, one could argue that mild infections might occur due to suboptimal levels of neutralizing antibodies. In addition to less severe disease, lower viral loads have been reported in vaccinated people as compared to measles virus infection of naive individuals (230). So even if the level of measles neutralizing antibodies does not reach the 120 IU/l, vaccinees could still be (partly) protected. Here, cellular immunity probably plays a mitigating role. Therefore, it would be interesting to study the role of T-cells in the measles immunity in subsets of seronegative populations like ours.

If there is waning immunity, as implied by some researchers (226), one could consider administering a booster vaccination to seronegative individuals. However, the booster effect provided by a third dose of MMR vaccine and timing thereof are still uncertain (215). Therefore, it is important to aim for the highest possible compliance to the two-doses MMR in national immunization programmes and perform check-ups to find out if every willing individual has been properly vaccinated (231).

The CDC stated that in the United States, the majority of measles cases are seen in international travellers (232). It therefore remains important to optimally protect travellers, to prevent measles disease in this group, and to prevent measles outbreaks in the home countries caused by unprotected travellers. A consult at a travel clinic provides an

opportunity to check if the travelling individual, especially in case of a child, has received both MMR vaccines, and if not to catch up with the vaccination scheme. Therefore, and as travellers often do not have their complete vaccination history available, it is important for travel clinics to get insight in NIP registrations. With decreasing vaccination coverage rates and outbreaks of vaccine-preventable diseases on the rise, we think it might be valuable to get uniform digitalized vaccination registrations, so that travel nurses and doctors can support completion of the NIPs (233).

Since this study was originally designed to study the protection against hepatitis A in travellers, clinical data on measles vaccination were not collected specifically. As childhood vaccinations are normally reported in birth vaccination records instead of a traveller's vaccination booklet, we had to report missing data. Also, as already mentioned, the data that we collected via questionnaires can be subject to social desirability and memory bias, what could have led to an overestimation NIP coverage.

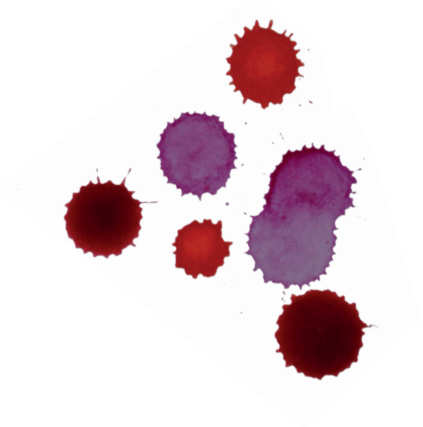
As we collected DBS instead of serum, which allowed us to sample travelers from all over the Netherlands, including children, the concentration of antibodies is based on an estimation of the amount of serum. This could have led to underestimation of the titres if the amount of serum in the dried whole blood was lower than expected. However, the measles seronegative samples had equally often high levels of HAV-specific antibodies as the measles seropositive samples which argues against underestimation. Another point of concern is the time of storage for the DBS. As the period between sampling and testing ranged from one to four years, there could be decay of antibodies. However, due to fast storage after drying (mostly within one day, but maximum 14 days) and storage at -80 degrees Celsius we expect this effect to be marginal (234). However, as the total IgG concentrations in our DBS eluates were similarly distributed as in serum in an average population (aged 12+), these arguments are unlikely to explain the low seroprevalence found. As a virus neutralization assay for measles is time consuming and was not performed before on filter paper samples, we decided to analyse the sample set with an ELISA validated for DBS samples. In general, ELISAs perform well compared to the PRNT (235). However, due to the unexpectedly low seroprevalence in the youngest age group, we decided to retest a subset of (negative) samples with the FRNT. By using this virus neutralization assay, we could substantiate our conclusions. However, we could not retest all our negative samples with the FRNT due to the small volume left. Also, the FRNT still is a surrogate for the gold standard (PRNT) (199). Although earlier studies reported good agreement between the FRNT and PRNT (211), there still might be issues with the sensitivity, which could lead to underestimation of the true seroprevalence. Therefore, it would be good to verify our conclusions in serum samples with PRNT and to check the

measles vaccination status of pediatric travelers carefully awaiting these results.

With this study, we showed an unexpectedly low seroprevalence rate for measles among Dutch travelling families. Based on our data, a focus on the individuals born between 1965 and 1975 seems unjustified. More attention should be given to compliance to NIP in travelling children.

CHAPTER 3

Protecting Healthcare Workers



Chapter 3.1

Evaluation of the hepatitis B vaccination programme in medical students in a Dutch university hospital

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ABSTRACT

Healthcare workers (HCW) are at increased risk of contracting hepatitis B virus (HBV) and are, therefore, vaccinated pre-exposure. In this study, the HBV vaccination programme for medical students in a university hospital in the Netherlands was evaluated. In the first part, the effectiveness of the programme, which consisted of a vaccination with Engerix-B® at 0, 1, and 6 months, was retrospectively evaluated over 7 years (2012-2019). In the second part of this study, we followed students (the 2019 cohort) who had previously been vaccinated against HBV vaccination (4-262 months prior to primary presentation) in order to investigate the most efficient strategy to obtain an adequate anti hepatitis B surface antigen titre. In the latter, titre determination was performed directly during primary presentation instead of giving previously vaccinated students a booster vaccination first. The vaccination programme, as evaluated in the retrospective first part of the study, was effective (surpassed the protection limit of 10 IU/L) in 98.8 percent (95% CI [98.4-99.2]). In the second part of our study, we found that 80 percent (95% CI [70-87]) of the students who had previously been vaccinated against HBV were still sufficiently protected and did not require a booster vaccination. With this strategy, the previously vaccinated students needed an average of 1.4 appointments instead of the 2 appointments needed with the former strategy. This knowledge is important and can save time and resources in the process of occupational HBV vaccination of HCW.

INTRODUCTION

Healthcare workers (HCW) are at increased risk of contracting the hepatitis B virus (HBV) from infected patients. HBV is a highly contagious virus, transmitted by body fluids. HCW usually obtain the virus during medical interventions, for example needle stick injuries (236). The clinical syndrome of a HBV infection varies from asymptomatic to fulminant liver failure: 30 percent of cases present as mild disease with fever and jaundice. HBV causes chronic disease in about 10 percent of the cases, disproportionately affecting new-borns and children. Furthermore, chronic hepatitis leads to liver cirrhosis, liver failure or hepatocellular carcinoma in approximately 25 percent of the cases (237). Estimations indicate that in the European Union (EU), there are nearly 5 million chronic HBV cases of which 80 percent is likely undiagnosed (238). In fact, the World Health Organization (WHO) estimates that globally almost 90 percent of people chronically infected are unaware of their infection (239). Most acute infections in Europe are sexually transmitted; however, 16 percent are caused by nosocomial transmission (240).

To mitigate this risk, HBV vaccination for HCW is recommended in all EU countries, although exact policies differ (238, 241). Lifelong protection against HBV is presumed when the concentration of antibodies against the HBV surface antigen (anti-HBs) is above 10 IU/L (242). In 1991, the WHO recommended including HBV vaccination in national immunisation programmes (NIP) (243). In 2011, the Dutch government implemented this HBV vaccination initiative using a hexavalent vaccine and is now provided thrice in the first year of a child's life. This vaccine provides protection for diphtheria, pertussis, tetanus, poliomyelitis, Haemophilus influenzae type B (Hib), and hepatitis B (244). Before 2011, only children who fell in special risk groups (children of HBV infected mothers and children with trisomy 21) received HBV vaccination (244).

HCW without a history of HBV vaccination are requested to follow a standard HBV vaccination schedule at time points 0, 1, and 6-12 months, with a measurement of anti-HBs titre one month after the last vaccination (245). In less than 10 percent of HBV vaccinated individuals, no antibody response (≤10 IU/L) is seen (246). In those cases, a second series of 3 vaccinations is usually administered with one month intervals (245). Male gender, increased age, higher BMI, smoking, and concomitant disease are associated with this risk of non-responding (247). In case HCW received their vaccinations at some point in the past (mostly for travel purposes) the strategies to achieve adequate anti-HBs titers differ (241). Clinical discussions with other Dutch vaccination centres showed that some centers, including our own vaccination centre (Erasmus MC, University Medical Centre Rotterdam), administer booster vaccinations during the first visit of

the vaccination clinic, while others determine anti-HBs titers directly. After a literature search about this subject, clinical questions arose around the necessity of administering a booster vaccination and if timing of the original immunization series should impact this decision (241-243). From August 2019 onwards, we no longer administered a booster vaccination but immediately determined the anti-HBs titre to establish the best strategy for this subgroup.

In this study, we aim to evaluate the efficacy of the HBV vaccination programme of first year medical students of the Erasmus University Medical Centre, the largest university hospital in the Netherlands in order to ensure the best strategy for the future. First, we determined the efficacy of the vaccination policy over the past 7 years (2012-2019) after both primary HBV vaccination series and booster vaccination. Secondly, we evaluated the newly implemented policy to first test immunological memory in previously vaccinated students in order to find the best strategy to ensure protection against HBV in these future HCW.

METHODS

Study setting

This study was conducted at the in-house vaccination clinic of a large university hospital in the Netherlands (Erasmus MC, University Medical Centre Rotterdam) responsible for the occupational vaccinations of all medical students. These students are vaccinated during their first year, in order to ensure adequate protection before starting their clinical internships. For the retrospective part of this study, we included all students that were vaccinated between May 2012 and November 2019 and reviewed their laboratory results. Students who did not complete their vaccination series, including titre determination; students with a known chronic HBV positive carrier status; and students with a known severe allergic reaction to any (component of) hepatitis B vaccination, have been excluded. Students were informed about the main side effects of vaccination or venipuncture in advance. Documentation regarding the students' vaccination history was not recorded on individual level in the majority of cases. According to protocol, anti-HBs levels had been determined in all students to ensure protective titres (245). When this titre was insufficient (≤10 IU/L) or low (≤100 IU/L), hepatitis B surface antigen (HBsAg) and anti-HB-core (anti-HBc) levels were determined additionally to exclude active HBV infection (245, 248).

A new vaccination policy for previously vaccinated students has been implemented in our

centre since August 2019. Before this date, students received a full vaccination series if they had not received HBV vaccinations before and were administered a booster when the vaccination series was completed more than 3 months ago. After implementation of the new protocol, blood was collected from students who pre-viously received a complete HBV vaccination series prior to eventual administration of booster dose. These students form a separate cohort in this study. Baseline characteristics (age, gender, date of last HBV vaccination, and type of HBV vaccination) were collected from both groups.

Due to the study design, this study was not subjected to review according to the Dutch Medical Research Involving Human Subjects Act (WMO). The study complied with the Netherlands Code of Conduct for Scientific Practice from the Netherlands Federation of University Medical Centres.

Vaccinations

Students presenting at our clinic are generally vaccinated with a monovalent, recombinant HBsAg vaccination named Engerix-B® (GlaxoSmithKline). A full series consists of 3 doses of 20 ug HBsAg per dose was given at 0, 1, and 6 months (249). One dose of Engerix-B (GlaxoSmithKline) was administered as booster vaccination as well. In case of non-response, another series of Engerix-B® was given with intervals of 1 month (at month 7, 8 and 9 since start of first series). An additional option in case of non-response is the administration of Fendrix® (GlaxoSmithKline) which consists of 20ug HbsAg with the adjuvant AS04C. Other options for HBV immunization are Ambirix® and Twinrix Adult® (GlaxoSmithKline) - both combined hepatitis A and B preparations (249). However, these combination vaccines were not routinely used for primary or booster vaccination in our clinic.

Laboratory tests

Laboratory tests were conducted with a chemiluminescent microparticle immunoassay (CMIA, Architect, Abbott) before 2015 and with a chemiluminescence immunoassay (CLIA-K, Liaison®, DiaSorin) from 2015 onwards. The interpretation of the anti-HBs titres does not differ between these 2 manufacturers, as these measurements are set internationally (IU/L). Both HBsAg and anti-HBc are determined qualitatively using signal-to-cutoff ratio's (S/CO).

Data analysis

Hepatitis B serology results were retrieved from the local laboratory information management system LabTrain® (Bodegro, v3.48.1.6). In this cohort, vaccination data was collected from the patient registry systems BaseNet and Vaccinatieregister® (version

2020.07.06.220-85). IBM SPSS Statistics 25 was used for data analysis. We used descriptive statistics to calculate means, standard deviations (SD), percentages and confidence intervals (CI). In the second part of this study, we used chi-square tests for categorical data and Mann-Whitney U tests for nominal data. Spearman's rank correlation tests were used to measure correlation between titres and time since last vaccination. P-values smaller than 0.05 were considered significant.

RESULTS

Retrospective case series

During the study period, a total of 2925 students were vaccinated at our clinic. After excluding missing data regarding baseline characteristics, the mean age of these students was 19.5 years (2.0 SD), and 34 percent (95% CI [32-36%]) was male. In the 2933 students where HBV serology was performed, 2888 (98.8 percent, 95% CI [98.4-99.2]) demonstrated to have sufficient anti-HBs titres (>10.0 IU/L) after their first vaccination series or booster vaccination (Figure 1). The mean age of these responders was 19.4 years [CI 19.4-19.5] which is comparable to the mean age of the non-responders (20.4 years [19.1-21.8], p=0.205). In the non-responder group, 40 percent consisted of male compared to 33 percent in the responder group (p=0.346).

Out of the 34 non-responders (anti-HBs ≤10 IU/L), 2 students (6%) tested HbsAg and anti-HBc positive. These results were reported to the Municipal Health Services as obliged by the Dutch law and the students were referred to the appropriate healthcare facilities. Of the remaining 32 non-responders, 8 students did not show up for their second series during this study period. Of the 24 students that received a second series (7, 8, and 9 months) of Engerix®, 21 (88%) showed sufficient titres. The remaining 3 students received a booster vaccination of Fendrix® after which 2 students responded (>10 IE/L). The student who did not respond was referred to the occupational specialist.

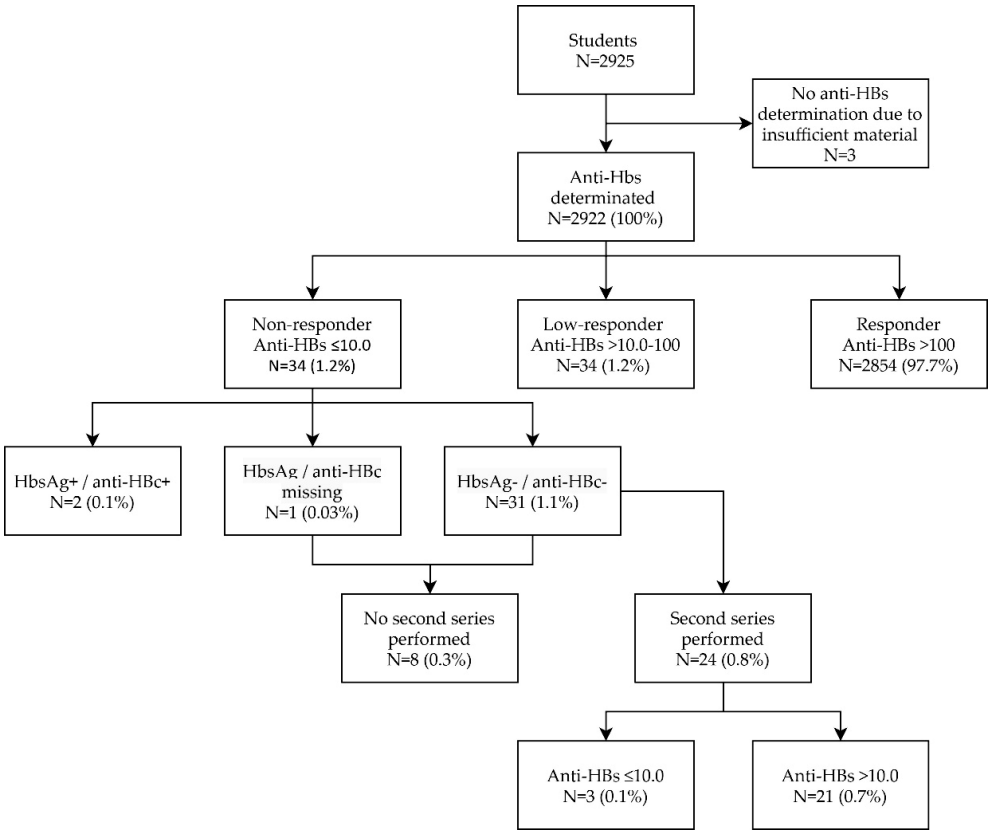

Figure 1. Evaluation of the occupational vaccination of students from May 2012 till November 2019

Necessity of booster vaccination

From August 2019 onwards, 352 students visited the vaccination clinic. Eighty of them (22.7%) previously completed a HBV vaccination series and blood was collected independently of the date of their last vaccination (range 4-262 months). Sixty-four (80%, CI [70%-87%]) still had a protective level of antibodies (>10.0 IU/L). In this group, the average duration between the last vaccination and the titre determination was significantly shorter compared to the group (n=16) with an anti-HBs titre ≤10.0 IU/L, with 79 (95% CI [65-94]) versus 122 (95% CI [90-153]) months respectively (p=0.018, Table 2, Figure 2). Gender and mean age did not differ between these groups. The direct responders were often vaccinated with the bivalent vaccines Twinrix® and Ambirix® whereas Engerix® was given more frequently in the other group. However, no significant difference in vaccination scheme between these 2 groups was found (p=0.067). A significant negative correlation (Spearman r=-0.36, p=0.001) was found between the

level of anti-HBs antibodies and the time elapsed between the last vaccination and the date of blood collection for HBV serology.

Table 1. Baseline characteristics of students who did not receive a booster although their last HBV vaccination was more than three months ago

	Titre ≤10 IU/L (n=16)	Titre >10 IU/L (n=64)	Sign.
Age, yrs (SD)	18.5 (1.2)	18.4 (0.8)	0.960[b]
Female (%)	12 (75)	47 (73)	0.899[a]
Months since last HBV vaccination (SD)	122 (64)	79 (60)	0.018[b]
Vaccination scheme			
Ambirix® (%)	8 (50)	37 (58)	
Twinrix® (%)	1 (6)	8 (13)	
Engerix® (%)	5 (31)	7 (11)	0.067[a]
Unknown (%)	2 (13)	12 (19)	

[a]Chi-square, [b]Mann-Whitney U test. SD: standard deviation.

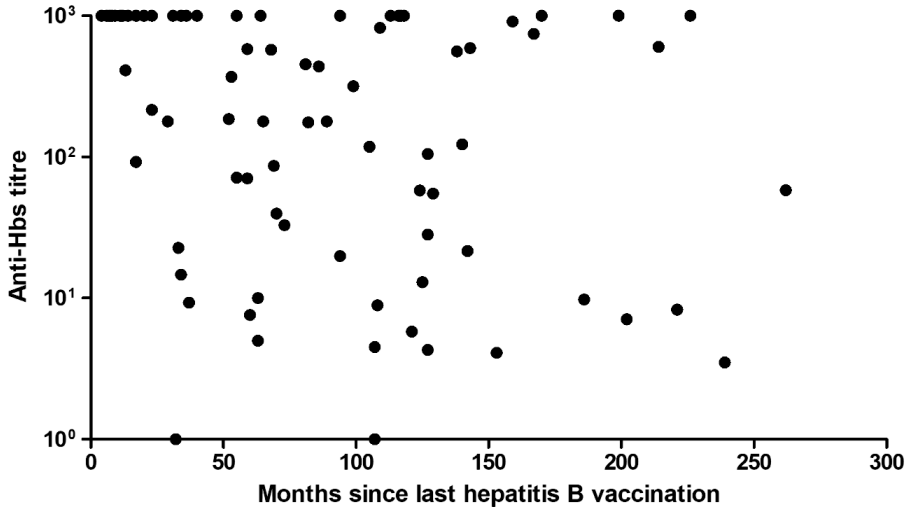

Figure 2. Anti-HBs titre (IU/L) as a function of months passed since the last hepatitis B vaccination. Minimum value of anti-HBs titer is ≤1.0 whereas the maximum value is ≥1000 IU/L. An anti-HBs titre >10.0 IU/L is considered protective.

Although 80 percent of this cohort (the direct responders) only needed 1 appointment for blood collection, 20 percent needed 2 additional appointments: 1 for a booster vaccination and another 1 month post-vaccination for another blood collection. In order to identify the most efficient and convenient strategy, we elaborated 5 different scenarios

(Table 2) using the data from this cohort. In scenario A, our policy before August 2019, all previously vaccinated students received a booster at their first appointments. In scenario B, our policy since August 2019, titre determination was performed directly in all previously vaccinated students. Booster vaccinations were only given when the anti-HBs titre was ≤10 IU/L. In the other hypothetical scenarios, policy at first appointment depended on time passed since the last HBV vaccination. For example, in scenario C, 49 students who completed their vaccination series more than 5 years ago (n=49) received booster vaccination at their first appointment. The other 31 underwent titre determination first, of which 2 were insufficient. The average number of appointments per student was 1.7. We calculated the same for the cut-off at 10 years (scenario D) and at 15 years (scenario E). According to our data, the most efficient strategy to ensure sufficient protection in this cohort consists of direct determination of anti-HBs titres at first appointment (scenario B). Hereby, it can also be considered to give a booster vaccination to students that completed their original series more than 15 years ago, as this is just as efficient (scenario E).

Table 2. Number of booster vaccinations and/or titre determinations per appointment in five different scenarios.

Scenario	Appointment	1	2	3	Total	Mean no. of appointments per student
A	Booster vaccination	80	0	-	160	2
	Titre determination	0	80	-		
B	Booster vaccination	0	16	-	112	1.4
	Titre determination	80	0	16		
C	Booster vaccination	49	2	-	133	1.7
	Titre determination	31	49	2		
D	Booster vaccination	23	8	-	119	1.5
	Titre determination	57	23	8		
E	Booster vaccination	8	12	-	112	1.4
	Titre determination	72	8	12		

A. Every student receives a booster vaccination before blood is collected for a titre determination. B. Blood collection for a titre determination is directly performed in all students during first appointment. A booster vaccination is only provided to students with titres <10 IE/L. C. Students receive a booster at first appointment if their last vaccination is more than 5 years ago. Blood collection was performed directly at first appointment in the students who received their last vaccination in the previous 5 years. D. Same as C, but with a cut-off of 10 years for the last vaccination. E. Same as C, only with a cut-off of 15 years for the last vaccination.

DISCUSSION

In this study, we found that almost 99 percent of the students that presented at our centre between 2012 and 2019 had protective anti-HBs titres. Furthermore, we found long-lasting protective anti-HBs titres in 80 percent of the students who completed a primary HBV vaccination series in the past. This new policy turned out to be more efficient compared to the previous old policy, which dictated the administration of a booster vaccination prior to a titre check.

Adequate HBV vaccination induces a protective level of antibodies in more than 95 percent of healthy infants, children, or adolescents and is considered to provide lifelong protection (246). Therefore, generally in immunocompetent subjects, administration of a booster is considered unnecessary after HBV vaccination (246, 250). The higher rate found in our cohort could be explained by the fact that our 7-year cohort includes not only subjects that recently completed their primary vaccination series, but also subjects receiving a booster vaccination when they previously completed their original series. As the rate of previous vaccinated students was 22.8 percent in the cohort vaccinated after August 2019, we can assume this proportion was about as high in the years before, which may explain our high rate of seroprotection.

Although the newly implemented policy will prevent many unnecessary booster vaccinations in the upcoming years, the plans for Dutch occupational vaccination clinics will change in 2029. By then, the first generation of students that are HBV vaccinated under the NIP are expected to start their medical studies. In order to be prepared for this new situation, we evaluated our data and compared this to studies from countries that had implemented HBV in their NIP at an earlier stage. In 1991, Italy was one of the first countries to add HBV to their NIP (250). Italian studies have shown that insufficient anti-HBs levels are relatively more present in individuals vaccinated during infancy than in individuals vaccinated at an older age, even when corrected for years past since vaccination (250-253). For example, in 2 studies evaluating anti-HBs titres in young adults, 20 years after their infant vaccinations, only 32 and 37 percent showed titres above 10 IU/L (253, 254). As we and other researchers (250, 253) have found that there is a negative correlation between the level of anti-HBs antibodies and the time elapsed after the last vaccination, waning anti-HBs titres are a probable explanation of these differences.

However, a lack of antibodies in the bloodstream of vaccinated individuals does not imply absence of immunity. Previous studies have shown that in subjects whose antibodies

decayed, sufficient titres are detected within several days after in vitro B-cell stimulation with HbsAg (248). Since the mean incubation time of a natural infection is 60-90 days (237), this secondary immune response will prevent these subjects from contracting a clinical relevant infection. Furthermore, HBV vaccination does also evoke a T-cell response, which has been demonstrated in previous studies both in vivo (255) and in vitro (256-258). Ideally, previously vaccinated subjects with insufficient anti-HBs titres should be tested for the presence of HBV specific B- and T-cell memory without the use of a booster vaccination (256). Nevertheless, this is a costly and time consuming method compared to the administration of a booster vaccination.

As 95 percent of the vaccinated individuals normally respond to primary HBV vaccination series and the rest may rely on herd immunity, anti-HBs determination is not performed routinely in immunocompetent subjects. However, as HCW have a higher individual risk, post-vaccination antibody testing is recommended to ensure adequate immunological priming (243, 259). Future HCW, vaccinated under the NIP, will lack proof of an effective primary vaccination series and, as such, titre determination seems inevitable. Since previous studies have shown that these titres are insufficient in more than half of the cases (253, 254), switching back to the old strategy (giving booster vaccination to all students at first appointment) should be reconsidered from 2029 onwards.

This research has some limitations that have to be taken into account. First, in our 7-year cohort, the vaccination history per student was lacking. Therefore, we could not specify the number of individuals that did receive a full vaccination series versus the people that only received a booster. Second, our separate cohort of previously vaccinated students was too small to allow scientific rigor. Furthermore, their primary vaccination series were given relatively recently. Because titre determination is not routinely performed after HBV vaccination outside healthcare settings, no anti-HBs levels determined after the primary series were available of this cohort. A comparison between the peak antibody levels one month after the original series and the actual anti-HBs level could have provided more information regarding the potential of their immune response. Lastly, as policies between countries are very different (260), the results of this study are not universally applicable. However, several European countries have not yet implemented universal childhood HBV vaccination (Denmark, Finland, Iceland, and Sweden) or implemented HBV vaccination in 2017 (Norway and the United Kingdom), so our results can be of guidance for them as well (261).

In conclusion, we showed that 98.8 percent of our 7-year cohort of future HCW had protective anti-HBs titres after either a primary vaccination series for HBV or a booster

vaccination. Thereby, we found that 80 percent of previously vaccinated students had sufficient anti-HBs levels without receiving a booster. With this data, we conclude that until 2029 the most efficient strategy to ensure protection in previously vaccinated students is to directly draw blood to determine the anti-HBs level. After 2029, when individuals who were HBV vaccinated under the NIP will start studying medicine, this policy has to be revised. Future alternative immunological methods to verify successful HBV vaccination other than anti-HBs titres could be helpful to prevent many potentially unnecessary booster vaccinations in future HCW.

Chapter 3.2

Dried blood spot cards: A reliable sampling method to detect human antibodies against rabies virus

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ABSTRACT

Although preventable by vaccination for more than a century, rabies virus still causes numerous fatalities every year. To determine antibody levels in humans, blood collected with a finger prick and applied on dried blood spot (DBS) cards is an alternative for venipuncture. The use of DBS is specifically valuable in remote areas, as it is easy to perform, store and transport. Therefore, the technique is frequently used for epidemiological studies of tropical diseases. Up to present, determination of rabies virus antibody levels on human DBS has not been validated.

We evaluated the use of human DBS for rabies serology and analyzed 99 pre- or post-vaccination serum and DBS samples with a fluorescent antibody virus neutralization test (FAVNt), which is the gold standard to detect protective antibody levels, and a Bio-Rad Platelia Rabies II ELISA. Sensitivity and specificity of DBS eluates tested with the FAVNt were 97% and 92%, respectively and 87% and 96% when tested with the Platelia-II ELISA. Antibody levels measured in serum with the FAVNt, correlated best with antibody levels measured in DBS with the FAVNt (R=0.88).

This is the first study that applied DBS for reliable detection of human antibodies against rabies virus. Both the FAVNt and Platelia-II ELISA demonstrated an acceptable performance on DBS, providing opportunities for rabies serology in remote areas. This technique could drastically ease studies evaluating (novel) rabies vaccination strategies and monitoring persisting immunity in humans at risk, living in rabies endemic regions.

INTRODUCTION

Despite the availability of safe and effective vaccines, rabies virus (RABV) causes approximately 59.000 fatal infections every year (262). Pre-exposure prophylaxis (PrEP) is indicated when the risk of exposure to rabies is high and can go unnoticed (e.g. occupational exposure), when fast access to post-exposure prophylaxis is limited (e.g. in remote areas); and when it is difficult to control rabies in the animal reservoir (263, 264). For healthy individuals PrEP exists of two vaccinations with an interval of seven days, after which an individual normally is considered to be lifelong protected (264, 265). However, in individuals with occupational exposure, protective immunity should be checked every one to two years (264). Protective immunity is defined by the World Health Organization (WHO) as a rabies virus neutralizing antibody (RVNA) concentration ≥0.5 IU/ml detected in serum using the rapid fluorescent focus inhibition test (RFFIT) (266) or the fluorescent antibody virus neutralization test (FAVNt) (262, 267). These gold standard assays are reliable but expensive, time-consuming and require qualified biosafety level 3 laboratories with trained employees. An enzyme-linked immunosorbent assay (ELISA) is a less demanding alternative for human rabies serology and is therefore easier to implement in low-resource settings (268). The Bio-Rad Platelia Rabies II ELISA, measuring binding of antibodies to the virus' glycoprotein, showed a good performance in previous human studies, when compared to the FAVNt (269). Nevertheless the ELISA should be considered as a surrogate to determine immunity against rabies, given that it measures all antibodies that bind the RABV glycoprotein, in contrast to the FAVNt that specifically measures functional virus neutralizing antibodies.

Both FAVNt and ELISA are usually performed on serum, but blood collected with a finger prick on dried blood spot (DBS) cards can be a valuable alternative, specifically when specimens are collected in remote areas. DBS cards can be easily obtained and transported, facilitating on-site sampling (27). DBS cards have been used for decades for various purposes, from early neonatal screenings for metabolic diseases (28) to molecular or serological diagnosis of infectious diseases and therapy monitoring (27, 270, 271), but it has not yet been applied for detection of RVNA in human populations (272). The only reported experience with rabies serological assays on filter paper cards, is a study performed in foxes and raccoons. However in this study thicker Mini Trans-Blot Filter Paper cards were used, they reported promising results (273). The ease of DBS sampling makes it an appropriate tool in monitoring immunity against rabies virus, surveillance or other types of studies in resource-limited areas in support of the global agenda to end human rabies transmitted by dogs (264). The minimal invasiveness of DBS cards eases the collection of samples in settings outside medical

care facilities, which is crucial as 80 percent of human rabies infections occur in rural areas (274). Furthermore, it is a commonly used technique in young children. Forty percent of the victims are under 15 years of age (274), in whom venipuncture can be difficult to perform. In this study, we evaluated the performance of both the FAVNt and the Platelia-II ELISA on DBS eluates in comparison to serum samples and present promising results.

METHODS

Sample collection

Forty-eight students that were rabies vaccinated between 2015-2018 were selected from the Vaccination Cohort (COVA) biobank that was carried out at the Travel Clinic of Erasmus MC. As the rabies vaccination guidelines for travelers changed during the study period, either three or two intramuscular vaccinations (with Verorab® or Rabipur®) were given on days 0, 7 (and 21-28) (265). Upon informed consent for participation in the COVA biobank study, students were sampled pre- and (1-30 months) post-vaccination, resulting in 99 paired venipuncture and DBS (blood directly aspirated from the serum separating tube and applied on a Whatman® Protein Saver™ 903™ Card) samples. After two to three hours drying at room temperature, each DBS card was stored in a foil-barrier zip lock bag with a desiccant sachet and kept for a maximum of two weeks at room temperature (275). Serum was centrifuged and kept at four degrees Celsius for a maximum of four weeks. Serum and DBS cards were stored respectively at -20 or -80 degrees Celsius until testing, because of the prolonged time period between collection and testing (234).

FAVNt

Elution of DBS in order to reach the highest serum concentration was optimized during pilot studies and resulted in an elution of 4 DBS card punches in 520 microliter PBS with 2% FBS during the study (275, 276). DBS punches contained 26 microliter serum each [18], resulting in a serum dilution of 1:6 (104 µl serum in 520 µl PBS+2%FBS). Eluates were incubated overnight at four degrees Celsius on a rotating device (276). The next day, eluates were heat-inactivated for 60 minutes at 56 degrees Celsius. Eluates were centrifuged at 3000 rpm for five minutes to remove any debris that can be formed during elution (275), before being processed for the FAVNt as described by Cliquet et al, 1998 (267). Serum samples were heat-inactivated prior to use in the FAVNt and processed simultaneously with the eluates (267). Microtiter plates were read blinded. The lower limit of detection of serum RVNA with the FAVNt was 0.06 IU/ml, which resulted in

a lower detection limit of 0.36 IU/ml of the DBS eluates due to the dilution factor. The upper limit of quantification is 13.77 IU/ml.

Platelia-II ELISA

During our pilot studies, we found that the elution protocol that was used for the FAVNt, resulted in background signal in the Platelia-II ELISA. Therefore, a separate, optimized protocol was used for Platelia-II ELISA. First, individual punches were eluted in 858 microliter PBS with Surfact-Amps™ Detergent Sampler (to result in a starting serum dilution of 1:34), while shaking overnight at four degrees Celsius. The next days, eluates were incubated for 90 minutes with 1716 microliter Blocker™ Blotto Blocking Buffer (to result in a final serum dilution of 1:100, which is the recommended dilution for the ELISA) and were centrifuged afterwards.

Microplates were pre-treated with the blocking buffer for 90 minutes to avoid aspecific binding (277). Thereafter, the Platelia-II ELISA was performed quantitatively following the protocol of the manufacturer using the threshold of positivity set on 0.5 IU/ml, as described by Wasniewski et al (272). Serial dilutions of the positive control serum (containing 4 IU/ml) were included for quantification of the results. Additional serial dilutions in sample buffer of 1:500 and 1:1000 for each serum and DBS sample allowed us to measure titer values up to 40 IU/ml.

Statistical methods

Based on a previous vaccination study in students (278) and on the composition of our study cohort (19 pre-vaccination samples and 80 post-vaccination samples taken 1-30 months post-vaccination), the expected prevalence of antibodies was 80 percent, which is comparable to situations in which monitoring or evaluation of vaccination strategies are performed. For this study, we aim for a specificity of at least 90 percent, as with the high expected prevalence of antibodies in this vaccinated study cohort, the positive predictive value will be high. A sample size calculation was performed, with a power of at least 0.80 and a p-value smaller than 0.05, and resulted in an sample size of approximately 100 (279). Coefficients of variance (CV) were calculated to define the repeatability and a value <20 percent was considered as acceptable. GraphPad Prism version 5.0 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com) was used for statistical analysis and to create figures.

Ethics

This study was performed on samples from the Vaccination Cohort (COVA) biobank. Due to the biobank format, the work has been exempted from medical ethical approval

requirements by the medical ethical research committee of the Erasmus MC (MEC-2014-398). Written informed consent has been obtained from every participant and data was analyzed anonymously. The study has been conducted according to the principles of the declaration of Helsinki (59th version, WMA General Assembly, Seoul, October 2008).

RESULTS

The FAVNt and Platelia-II ELISA results on DBS eluates were compared to the gold standard, which is serum tested by FAVNt (serum-FAVNt), and applying the WHO recommended threshold of protection (0.5 IU/ml). When using the serum-FAVNt, the RVNA titers of the 80 post-vaccination serum samples tested ranged from 0.17 to 13.77 IU/ml (S1 Data), with an average of 3.38 and a standard deviation of 3.23 IU/ml. The RVNA titers of 19 pre-vaccination serum samples were all 0.06 IU/ml. The FAVNt on the paired DBS eluates (DBS-FAVNt) showed a sensitivity and specificity of respectively 97.3 percent (95% CI 90.6-99.7) and 92.0 percent (95% CI 74.0-99.0) when compared to serum-FAVNt. Only two false positives (with RVNA concentrations of 0.60 and 0.60 IU/ml) and 2 false negatives (0.36 and 0.42 IU/ml) were found.

The DBS-ELISA showed a sensitivity of 86.5 percent (95% CI 76.6-93.3) and a specificity of 96.0 percent (95% CI 79.7-99.9) compared to the gold standard serum-FAVNt. The sensitivity increased to 91.6 percent (95% CI 82.5-96.8) when the DBS-ELISA was compared with serum-ELISA. Moreover, the specificity increased to 100 percent (95% CI 87.7-100.0).

The intra-assay variation of serum-FAVNt in our WHO reference laboratory is assessed during yearly WHO proficiency testing. With a mean CV of 2.9 percent (WHO proficiency report 2019 (280), n=14, tested in triplicate), the assay has an excellent performance. To assess repeatability, five DBS samples were eluated and tested in triplicate in separate plates for both the FAVNt and Platelia-II-ELISA. The average CV for DBS-FAVNt was 11.6 percent (RVNA titers range 0.5-7.92 IU/ml). For the DBS-ELISA the mean CV is 18.1 percent. In serum, the Platelia-II-ELISA manual reports a reproducibility of less than 10 percent.

Because the concentration of RABV (neutralizing) antibodies is being correlated to the duration of protection (281), we investigated the quantitative performance of both assays on DBS eluates. The antibody concentrations measured in DBS eluates and sera correlated significantly when measured with either FAVNt or Platelia-II ELISA (Figure 1).

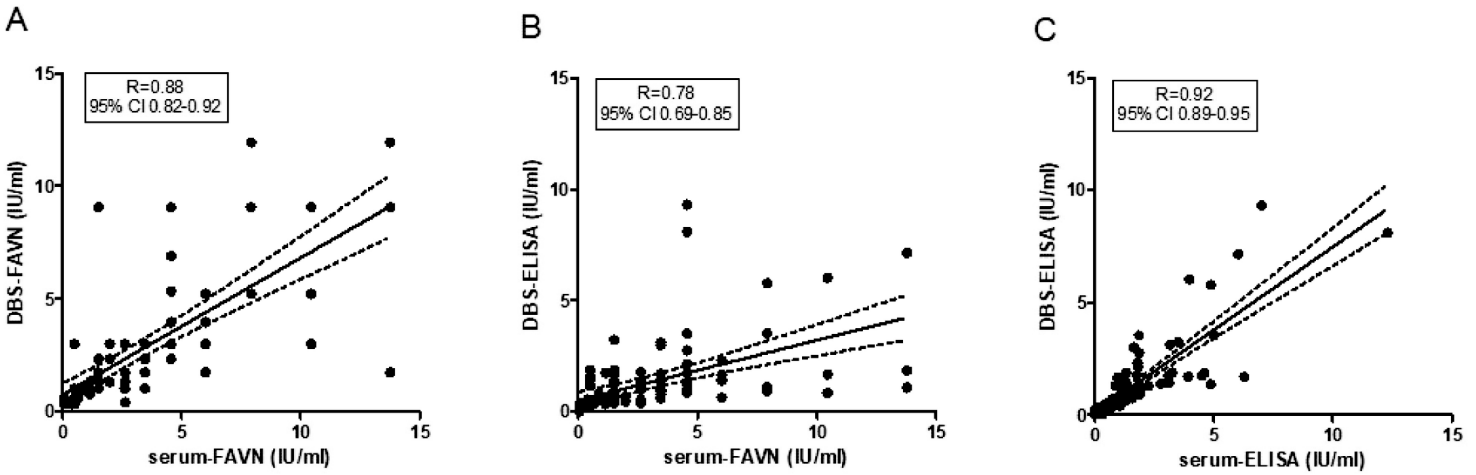


Figure 1. Correlation of quantitative results of the FAVNt and the Platelia-II ELISA performed on DBS and serum. Panel A shows the correlation between the RVNA titer of DBS-FAVNt and the RVNA titer of undiluted serum measured with the FAVNt. Panel B shows the antibody titer of DBS-ELISA compared to the RVNA titer of undiluted serum measured with the FAVNt. Panel C shows the antibody titer of DBS-ELISA compared to the antibody titer of serum-ELISA. R represents the Spearman correlation coefficient. CI is confidence interval. Dashed lines represent the of 5% confidence bands of the best fit line (represented by solid lines)

However, the DBS-FAVNt results corresponded better to serum-FAVNt (Spearman R = 0.88, Figure 1A) than the DBS-ELISA (Spearman R = 0.78, Figure 1B). The titers in the DBS-ELISA correlated best with serum-ELISA titers (Spearman R = 0.92, Figure 1C).

Finally, the agreement between the quantitative results on DBS and serum-FAVNt was determined by Bland-Altman plots (Figure 2). RABV antibody concentrations were on average 0.33 IU/ml lower in DBS-FAVNt versus serum-FAVNt (Figure 2A), and 1.48 IU/ml lower in DBS-ELISA versus serum-FAVNt (Figure 2B). DBS-ELISA antibody levels were on average 0.32 IU/ml lower than in serum-ELISA (Figure 2C). All three plots however show that the assays on DBS eluates are reliable when RVNA concentrations are low (<3.0 IU/ml).

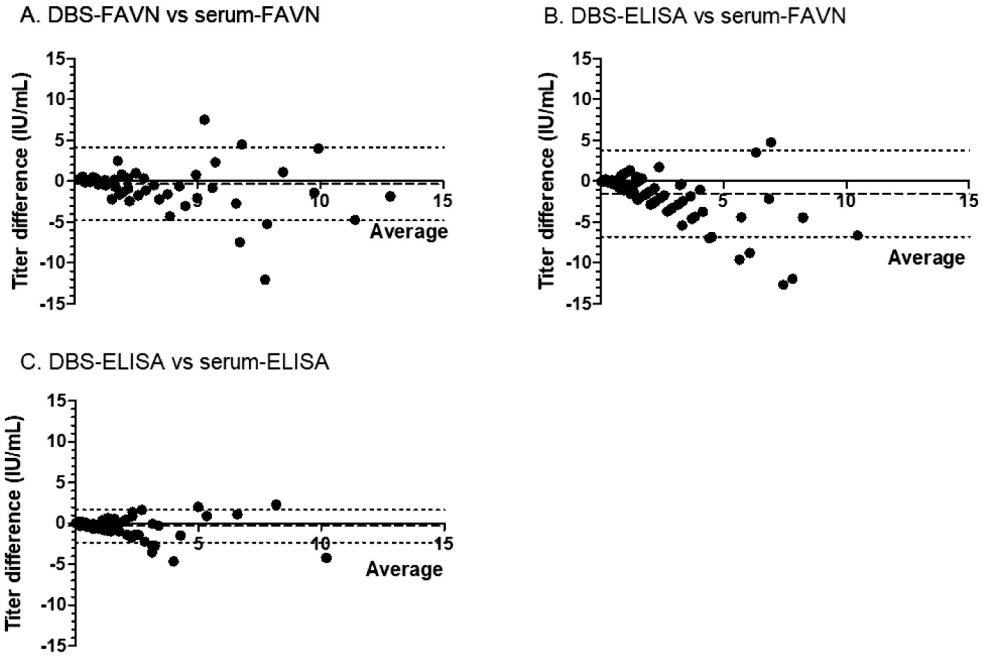


Figure 2. Bland-Altman plots of the mean titer of DBS and serum plotted against the difference between both values. Panel A shows the average antibody titer (IU/ml) of the DBS and sera measured with the FAVNt on the x-axis and the value (IU/ml) of the DBS minus the value of the serum on the y-axis. Panel B shows the average value of the DBS-ELISA and the serum-FAVNt on the x-axis and the value of the DBS-ELISA minus the value of the serum-FAVNt on the y-axis. Panel C shows the mean antibody level of the DBS and sera measured with the Platelia-II-ELISA on the x-axis and the value of the DBS minus the value of the serum on the y-axis. The long-dashed lines close to y=0 represent the mean bias, the short-dashed lines represent the 95% limits of agreement (mean bias ±1.96 times the SD).

DISCUSSION

In this study, we show for the first time that blood collection on DBS can be used for the detection of either neutralizing or binding antibodies against rabies virus in humans. When compared to the WHO gold standard in rabies serology, in which RVNA are measured on serum, the use of DBS eluates in the FAVNt performed best. The high sensitivity assures that more than 97 percent of the individuals who are protected against rabies will be detected, whereas the specificity shows that 92 percent of the negatives will be detected. Besides the detection of protective immunity in populations at high risk for (unnoticed) exposure, like veterinarians, medical and nursing hospital staff and laboratory workers (264, 282), it can be applied as a tool in monitoring immunity against rabies virus in resource-limited, endemic areas. Given that RABV has a case-fatality rate approaching 100%, reliably monitoring of post-vaccination titers is of utmost importance. Both the DBS-FAVNt and the DBS-ELISA have a high specificity, and can be used to determine which individuals at risk are in need of revaccination. Although the DBS-ELISA performs better than the DBS-FAVNt in terms of specificity, and could therefore be used as large scale screening method, the FAVNt with its complementary high sensitivity, prevents unnecessary administration of booster vaccinations.

The most probable reason for a higher correspondence between the DBS-FAVNt and serum-FAVNt than the DBS-ELISA and the serum-FAVNt, lies in the fact that both assays measure antibodies with different characteristics. The Platelia-II ELISA detects all antibodies that bind rabies glycoprotein, whereas the FAVNt measures the fraction of functional, neutralizing RABV antibodies (262). However, the fact that RVNA are directed against the rabies glycoprotein, the protein that contributes to protection (283), makes the Platelia-II ELISA a suitable alternative.

The Bland-Altmann plots demonstrated that the assays on DBS eluates are reliable when RVNA concentrations are low. This reduces the impact on decision making for the administration of booster vaccines, as larger deviations are seen at higher (>3.0 IU/L) RVNA levels. The fact that the levels of RABV antibodies were slightly lower in DBS compared to serum in all three comparisons, is likely due to a minor overestimation of the amount of serum in certain DBS eluates. This could also explain the false negative results in DBS.

A DBS elution of 1:6 was the lowest feasible elution in our pilot studies, which resulted in a lower limit of detection of DBS-FAVNt of 0.36 IU/ml, which is close to the recommended threshold of protection (0.5 IU/ml). One could consider to choose a

slightly higher test cut-off when using DBS in the FAVNt, as the two false positive results were both only 0.6 IU/ml. In case a RVNA titer measured in DBS is considered negative when equal to or lower than 0.6 IU/ml, the specificity will increase up to 100 percent. As a consequence, the sensitivity will slightly decrease and become 94.6 percent, which is still acceptable.

The repeatability of the FAVNt (mean CV = 3%) and the Platelia-II ELISA (reproducibility <10%) when testing sera were high. When using DBS eluates in the assays, the performance characteristics decreased to a mean CV of 12 for DBS-FAVNt and 18 for DBS-ELISA. An important factor in the increased variation is the amount of blood, and as a result the amount of antibodies, on the DBS cards. Therefore, adequate instructions when performing a finger prick and filling DBS cards, is crucial. In addition, cautious interpretation of antibody levels obtained by the use of DBS cards is of importance; the assay variation and cut-off should be determined carefully prior to application of the assays.

Although this is the first report in which the use of DBS is applied in human rabies serology, DBS were previously studied in other viral infections (27). Studies are difficult to compare, due to the specific characteristics of antibodies and the differences in serological assays and elution protocols. A successful example of the use of DBS is in early detection of hepatitis B and C infections in resource-limited countries, which is now possible and recommended by the WHO. This advances treatment initiation and can finally prevent chronic complications in endemic regions (270, 284). A quantitative meta-analysis in which the performance of DBS was assessed reported pooled estimates of a sensitivity of 98 percent and specificity of 99 percent. Other studies showed the use of DBS for the detection of measles-specific IgG concentrations (285), and anti-hepatitis A virus antibodies (164). The latter showed that measuring antibodies in post-vaccination samples decreased the sensitivity to 77-98 percent (depending on the time elapsed post-vaccination), suggesting that the assay is less sensitive in the lower range, which we did not observe. The application of rabies serology differs largely from testing high levels of naturally acquired, neutralizing antibodies against measles and hepatitis A virus. In rabies serology the aim is to detect protected individuals, mostly upon vaccination, although DBS sampling can also be used to further investigate natural, non-lethal rabies exposure. Whereas rabies is generally seen as an inevitable fatal disease, this idea is challenged by some studies that found rabies antibodies in serum of unvaccinated populations living in endemic areas (286). The studies are limited - as populations with high exposure to possible rabies-infected wildlife are often hard to reach - and heterogeneous due to the variance in serological assays and used cut-offs (287). DBS sampling provides solutions for

both issues, and could therefore contribute to the understanding of the immunopathology of rabies in seropositive populations. Here, the DBS-FAVNt has the preference, because the high sensitivity assures that most seropositive individuals are detected.

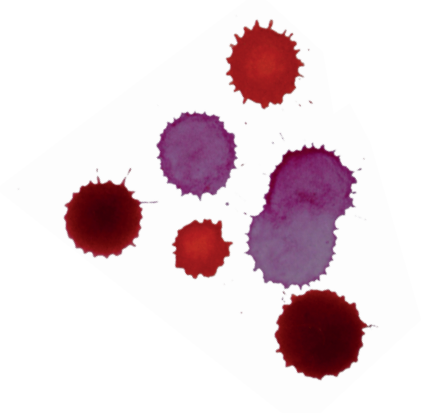
The WHO strives to end human deaths from dog-mediated rabies by 2030 (274). Elimination programs are usually evaluated by seroepidemiology and disease surveillance data. As rabies cases are often not diagnosed in endemic areas, disease surveillance is very insensitive to monitor elimination programs for rabies. Seroepidemiological studies can provide insight in vaccination status of risk groups and serosurveys could determine risk groups with suboptimal protection (282, 288). Furthermore, serology is important as an endpoint in clinical trials of newly developed vaccines, different vaccination schedules or other administration routes. These developments are ongoing in rabies prevention strategies, as the availability and costs of the currently used rabies vaccines in endemic regions cause difficulties (262). Determination of either rabies neutralizing or binding antibody levels – performed on easily obtained DBS – can be very supportive in large-scale evaluation of new vaccination strategies in endemic regions.

In conclusion, we demonstrated that DBS sampling provides accurate results in RABV-vaccinated and non-vaccinated adult travelers from a European country. As blood collection on DBS eases sampling and transportation, it is a promising alternative for rabies serology in children and in remote settings. Therefore, performance will need to be evaluated specifically in endemic regions and risk populations. With these promising results, DBS provides opportunities for evaluation of rabies vaccination trials and monitoring of persistence of immunity against rabies in humans in high-risk areas.

Supporting data can be found at https://doi.org/10.1371/journal.pntd.0008784.

CHAPTER 4

Protecting Immuno-compromised Patients



Chapter 4.1

Dutch healthcare professionals' opinion on vaccination and education to prevent infections in immunocompromised patients. A mixed-method study with recommendations for daily practice.

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Vaccine, 2019

ABSTRACT

The fast-growing population of immunocompromised patients (ICP) is more vulnerable to infectious diseases, demanding strategies to protect them. These strategies seem inconsistent in available guidelines and in practices. We aim to evaluate healthcare professionals' (HCP) opinions on vaccination to reduce the number and severity of infections in ICP.

A mixed-method study, with an exploratory sequential design, was performed. Medical specialists from various departments in a tertiary care center in the Netherlands were invited for semi-structured interviews to explore their perspective on preventive care of ICP. Topics that play a substantial role in daily practice for ICP were translated into a survey to gain insight into what extent opinions were generalizable to Erasmus Medical Center.

Surveys were completed by 689 HCP (43% of the invitees), 269 of them treated at least two ICP weekly on average and were considered eligible for further analysis. Quantitative data showed that according to 80 percent of HCP, preventive care for ICP can be improved. Education was chosen by 40 percent as the most important intervention to reduce the number and severity of infections. Vaccinations were valued as important by seventeen percent of HCP. Except for influenza, vaccinations were not regularly discussed during routine consultations. Difficulties to administer vaccinations were experienced by 75 percent of HCP.

According to our respondents, education is the most promising intervention to reduce the number and severity of infections in ICP. To reach a higher vaccine uptake, we recommend HCP to address vaccinations more frequently during consultations and to search for solutions to alleviate barriers to vaccinate.

INTRODUCTION

Continuously evolving treatment strategies for chronic diseases result in limited disease burden and better survival rates in patients. Part of these diseases or their treatments result in an immunocompromised state, defined as a diminished function of the immune system. This makes the heterogeneous group of immunocompromised patients (ICP) more vulnerable to infectious diseases (289, 290). Several pharmacological and non-pharmacological strategies are available to decrease the number and severity of infections.

While most pharmacological approaches rely on their product characteristics, successful immunization relies both on the patients' immune system and on vaccine characteristics. An interaction between numerous cells, receptors and cytokines is required to mount an effective immune response (291, 292) – hampered in ICP. Furthermore, vaccine-safety should be considered, especially for live-attenuated vaccines – as in ICP these vaccines might induce serious adverse events (293).

Strategies to prevent infections in ICP, such as vaccination and education, are often mentioned in (international) guidelines (294). These guidelines are usually concentrated to a specific disease or treatment and might differ on international, national and hospital level. In practice, this results in heterogeneous patient management. Moreover, awareness of the immunocompromised state in both healthcare professionals (HCP) and ICP, as well as availability of the vaccination status, seems suboptimal (295-297).

With this mixed-method study, we assess opinions of HCP on strategies to prevent infections in ICP and corroborate these findings in a tertiary care center. This involves HCP from multiple departments as well as HCP with various levels of experience and interactions with ICP. By addressing possible improvements from the HCP perspective, we formulate suggestions and recommendations to optimize preventive care for this vulnerable group of patients.

METHODS

Study Design and Population

This mixed-method study was conducted from December 2015 to December 2017 in the Erasmus Medical Center (EMC), Rotterdam. This 1320 bed hospital is considered as the largest tertiary center in the Netherlands (298). We used an exploratory sequential design, consisting of semi-structured interviews with medical specialists, followed by

a hospital-wide survey containing closed-ended questions. The interviews were used to discover key topics that play a role in preventive care for ICP. Subsequently, using surveys, we tested whether colleagues share these opinions (299). In this study, HCP are defined as a comprehensive term for medical specialists, residents (both in training and not in training), physician researchers as well as nurse practitioners (NP) and nursing consultants (NC).

Qualitative data collection and analysis

We invited medical specialists from various departments (Supplementary table 1), who are actively involved in preventive care for ICP, by e-mail for interviews held between December 2015 and April 2016. One researcher (WJ, ♂) conducted the semi-structured interviews while another researcher (LD, ♀) made field notes. Both researchers are medical doctors with an additional position at the in-house travel clinic. After informed consent was signed and the purpose of the study was introduced, 30-minute interviews were held in interviewees' or researchers' offices and audio was recorded. A topic list, based on clinical experience, guidelines and literature (297, 300-303), was used to assess: field of work of HCP; characteristics of ICP within their department; availability and usage of guidelines; practices with regards to prevention of infections, in particular vaccination and education; presence of any barriers to vaccinate; and suggestions for improvements in preventive care. After data saturation was reached, data was transcribed ad verbatim by either LD, MH, WJ or KW. Transcripts were read and key themes were manually labeled by LD and WJ (open coding). Overarching ideas were discussed in the research group, and subcategories were generated for a broader understanding of the key categories by constant comparison (axial coding) (304).

Quantitative data collection and analysis

Recurrent themes from the qualitative part of the study were used as a framework for the major topics in the survey with closed-ended questions. The main topics of the survey were: characteristics of ICP; vaccination practices; preventive care; knowledge and awareness of the immunocompromised state; usability of guidelines; and management of travel plans. A draft survey was piloted with one medical specialist (EG), three residents, two NPs and a medical student. We used LimeSurvey (305), an online survey tool, to invite 1723 HCP that possibly treat ICP (refer to Supplementary table 1 for a list of invited departments). The survey was set-up using unique one-time use invites and was available from November 27, 2017 until December 20, 2017. To increase the response rate, we requested interviewed HCP to bring the survey to the attention of their colleagues and we sent out two reminders.

Baseline characteristics of all respondents were recorded. To select a study population that was representative for HCP that routinely treat ICP, successive data was collected from respondents who, on average, treated two or more ICP weekly. Data was analyzed with IBM SPSS Statistics 24 (306) and represented with Graphpad Prism 5 (307).

Descriptive methods were used to summarize the survey findings. To assess whether differences in opinions exist between specific groups of HCP (e.g. nurses versus medical doctors), we used a Mann-Whitney test. P-values <0.05 were considered significant.

Ethics

Written informed consent was obtained from all interviewed HCP. Data obtained in this research was stored on a local drive that was only accessible to LD and WJ. Referrals to natural subjects were coded. LimeSurvey data was stored on Erasmus MC servers. A statement of implicit informed consent at return of the survey was included on the first page of the survey. In consultation with the Medical Ethical Research Committee of Erasmus MC, this study was exempted from review according to the Dutch Medical Research Involving Human Subjects Act (308). No plausible harm to participants could arise from this study. The study complied with the Netherlands Code of Conduct for Scientific Practice from the Netherlands Federation of University Medical Centers (309).

RESULTS

The qualitative component of this study resulted in twelve interviews with middle aged medical specialists, seven males and five females. A third of the interviewees was already acquainted with the researchers. Fifteen specialists were invited. Non-participation (n=3) was due to limited affinity with the topic. Two specialists referred to a colleague. The interviews resulted in four main topics being: characteristics of HCP and ICP; daily practices and responsibilities; travel opportunities and precautions; suggestions for improvement. The quantitative component resulted in a response rate of 40 percent (n=689) (baseline characteristics shown in Supplementary Table 2). As shown in Figure 1, our main results comprised data of HCP treating more than two ICP weekly (n=269).

We first describe the qualitative results including quotations of medical specialists, followed by the quantitative results supported with figures.

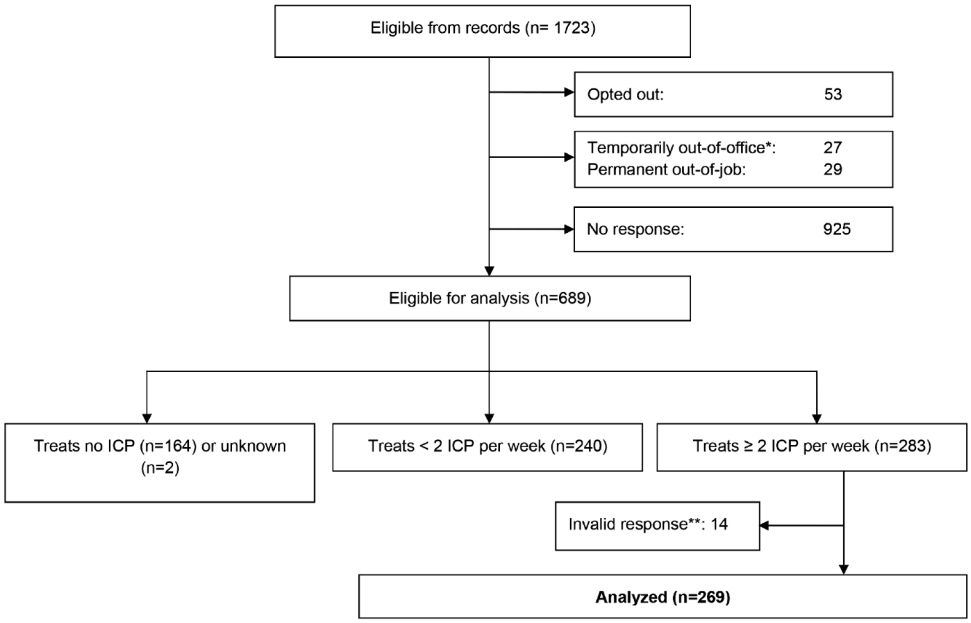

Figure 1. Flowchart according to CONSORT statement for quantitative component
Consolidated Standards of Reporting Trials (CONSORT) flowchart delineating the number of eligible healthcare professionals for the quantitative component of this mixed-method study. ICP = Immunocompromised patient(s) * due to holiday, maternity or sick leave, etc. ** no data available regarding ICP

Qualitative results

Characteristics of HCP and ICP

Interviewees described heterogeneity in the immunocompetence of their patient populations. Some medical specialists stated that they often diagnose and initiate treatment in immunocompetent patients; others stated that they treat patients using immunosuppressive therapies (e.g. biologicals), initiated in another, often secondary care, center.

"More than half of our immunocompromised patients become immunocompromised by the medication we start." (ZS004)

Daily practices and responsibilities

Interviewees iterated the fact that ICP have to adapt to a substantial amount of information with regards to therapy, preventive measures and life rules. This education is provided predominantly during consultations, complemented by guidebooks and newsletters.

Several medical specialists mentioned the complementary role that is appointed to NP and NC for educating ICP:

"We implemented a dedicated consultation hour with an experienced resident in training. A NP is involved to take care of the follow up" (ZS007)

One of the preventive measures stated is vaccination, preferably done during a screening period, ahead of surgical interventions and/or start of immunosuppressive therapy. Most interviewees questioned the effectiveness and the extent of protection of vaccinations:

"… everyone struggles with the same question: is it [the sum of vaccinations administered to ICP] too much or too little?" (ZS003)

Interviewees underlined the need to consider to vaccinate their patients. Almost all specialists stated that they advise ICP to get a yearly influenza vaccination at their general practitioner. Some raised questions about whose role and responsibility it is to administer and register vaccinations.

"The treating physician should take the responsibility for vaccinations that are medically indicated, in case of traveling: it's the patients' responsibility." (ZS005)

Travel opportunities and precautions

Interviewees mentioned that the quality of life of their patients has improved over the last years, increasing possibilities and willingness to travel. In some situations, treating physicians discourage patients to travel outside Western countries, particularly due to the risk of travel-related infections. Occasionally, patients ask their physician for travel advice, while others travel to tropical countries without prior notice.

"We see quite some second or third generation people [with a history of migration] travelling to visit their grandparents without notification. I consider it as a tropical journey, in their opinion it's not, it remains a risk." (ZS008)

Suggestions for improvement

To the opinion of interviewees, there is room for improvement:

"Infections are a problem, progress remains to be made." (ZS001)

Mentioned areas of concern in preventive care are: accessibility of specialized vaccination clinics; frequency of interdisciplinary consultations; expense coverage of vaccinations; limited evidence and recommendations on vaccinations and antibiotic prophylaxis in guidelines. Some medicals specialists are not informed about the overall vaccination status:

> "… the vaccination status of my patient? I got no clue!" (ZS005)

Quantitative results

Characteristics of HCP and ICP

Relevant characteristics of HCP are given in Table 1. The quantitative results concerning heterogeneity of immunocompromised states at first contact with HCP correspond to the interview data. More than half of the patients were considered to be moderately (score 3) to severely (score 4) immunocompromised at first presentation, as scored on a five point Likert scale, ranging from not immunocompromised to worst stage of immunosuppression. Thirteen percent of the patients were considered to be not immunocompromised at their first presentation.

We asked HCP to evaluate their own knowledge and the knowledge of their patients about strategies to prevent infections in ICP. The HCP scored themselves a mean score of 7 (SD 1.66) and patients a mean score of 5 (SD 1.89) on a 1-10 scale (1 = very bad and 10 = excellent).

Table 1. Baseline characteristics of HCP (who treat > 2 ICP per week) and ICP per department

	Internal Medicine	Pediatric/ Neonatology	Acute Medicine	Pulmonology	Neurology/ Neurosurgery	Gastroenterology	Surgery	Dermatology	Cardiology	Rheumatology	Other	
HCP age												**Mean**
Mean (yrs)	38,6	42,8	37,5	38,2	40,2	39,2	42,2	39,9	43,1	44,3	42,9	40
HCP gender												**Total**
Female	58 (68,2)	39 (78,0)	14 (53,8)	11 (50,0)	12 (63,2)	13 (72,2)	8 (66,7)	7 (63,6)	5 (62,5)	5 (71,4)	7 (70)	179 (66,5)
Profession, n (%)												**Total**
Residents, physicians (in training)	34 (40,0)	8 (16,0)	10 (38,5)	7 (31,8)	7 (36,8)	7 (38,9)	2 (16,7)	3 (27,3)	1 (12,5)	1 (14,3)	2 (20)	82 (30,5)
(Physician) researchers	2 (2,4)	1 (2,0)	0	0	2 (10,5)	3 (16,7)	0	1 (9,1)	0	0	0	9 (3,3)
Medical specialists	31 (36,5)	28 (56,0)	12 (46,2)	7 (31,8)	6 (31,6)	6 (33,3)	6 (50,0)	7 (63,6)	4 (50,0)	3 (42,9)	6 (60)	116 (43,1)
NP/NC	18 (21,2)	13 (26,0)	4 (15,4)	8 (36,4)	4 (21,1)	2 (11,1)	4 (33,3)	0	3 (37,5)	3 (42,9)	2 (20)	62 (23,0)
Years after graduation												**Mean**
Mean (yrs)	13,5	16,9	11,6	13,3	15,6	13,9	17	15,6	18,8	21,3	18,9	15
Patients' age, n (%)												**Total**
<18 yrs (%)	1 (1,2)	50 (100)	5 (19,2)	0	1 (5,2)	0	0	0	1 (12,5)	0	0	58 (21,6)
18-35 yrs (%)	0	0	0	1 (4,5)	2 (10,5)	6 (33,3)	0	0	0	1 (14,3)	4 (40)	14 (5,2)
35-65 yrs (%)	69 (81,2)	0	16 (61,5)	16 (72,7)	14 (73,7)	11 (61,1)	8 (66,7)	7 (63,6)	7 (87,5)	6 (85,7)	5 (50)	159 (59,1)
>65 yrs (%)	15 (17,6)	0	5 (19,2)	5 (22,7)	2 (10,5)	1 (5,6)	4 (33,3)	3 (27,3)	0	0	1 (10)	36 (13,4)
Nr. (% of total participants)	85 (31,6)	50 (18,6)	26 (9,7)	22 (8,2)	19 (7,1)	18 (6,7)	12 (4,5)	11 (4,1)	8 (3,0)	7 (2,6)	10 (3,7)	269 (100)

NP = nurse practitioners, NC = nurse consultants.

Daily practices and responsibilities

The majority of respondents (71%) agreed it is the treating physician and their team's responsibility to administer vaccinations in case they consider it as a part of adequate treatment for the ICP. As shown in Figure 2, the majority of HCP (84%) are aware of guidelines containing recommendations regarding vaccinations and other preventive strategies that are applicable to their work field. In contrast to the qualitative results, to less than fifteen percent of respondents, the available guidelines are insufficient to be used in preventive care for ICP.

A comprehensive overview of the reported frequency of discussing vaccinations at distinct departments is represented in Figure 3. In line with the qualitative data, influenza is the most frequently discussed vaccine and is addressed in the consultations of 70 percent of respondents. When HCP consider vaccinating their ICP, up to 75 percent indicated that they experience difficulties. Most important barriers to vaccinate were timing issues (42%), logistical obstacles (30%) and financial problems (19%). Timing issues included short time to start of immunosuppressive therapy or transplantation.

Concerns of limited interdisciplinary consultations raised by the interviewees, were not shared by the surveys' respondents, as 84 percent was satisfied with the frequency of consultations with other HCP regarding care for ICP (176 out of 209). Most HCP consulted their colleagues weekly to monthly (28%) or monthly to few times a year (32%).

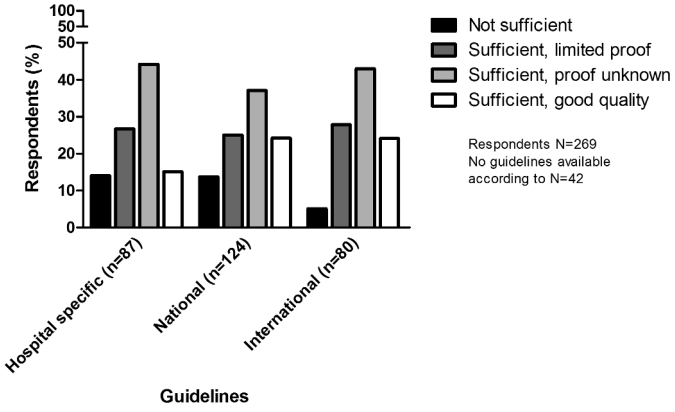

Figure 2. Availability and opinion of HCP on guidelines that discuss preventive measures
Availability and opinion of HCP on hospital specific, national and international guidelines that discuss preventive measures to prevent infections in ICP. According to 42 respondents, no guidelines addressing care for ICP were available to them. One answer per guideline category was allowed.
HCP = Healthcare professional(s); ICP = Immunocompromised patient(s)

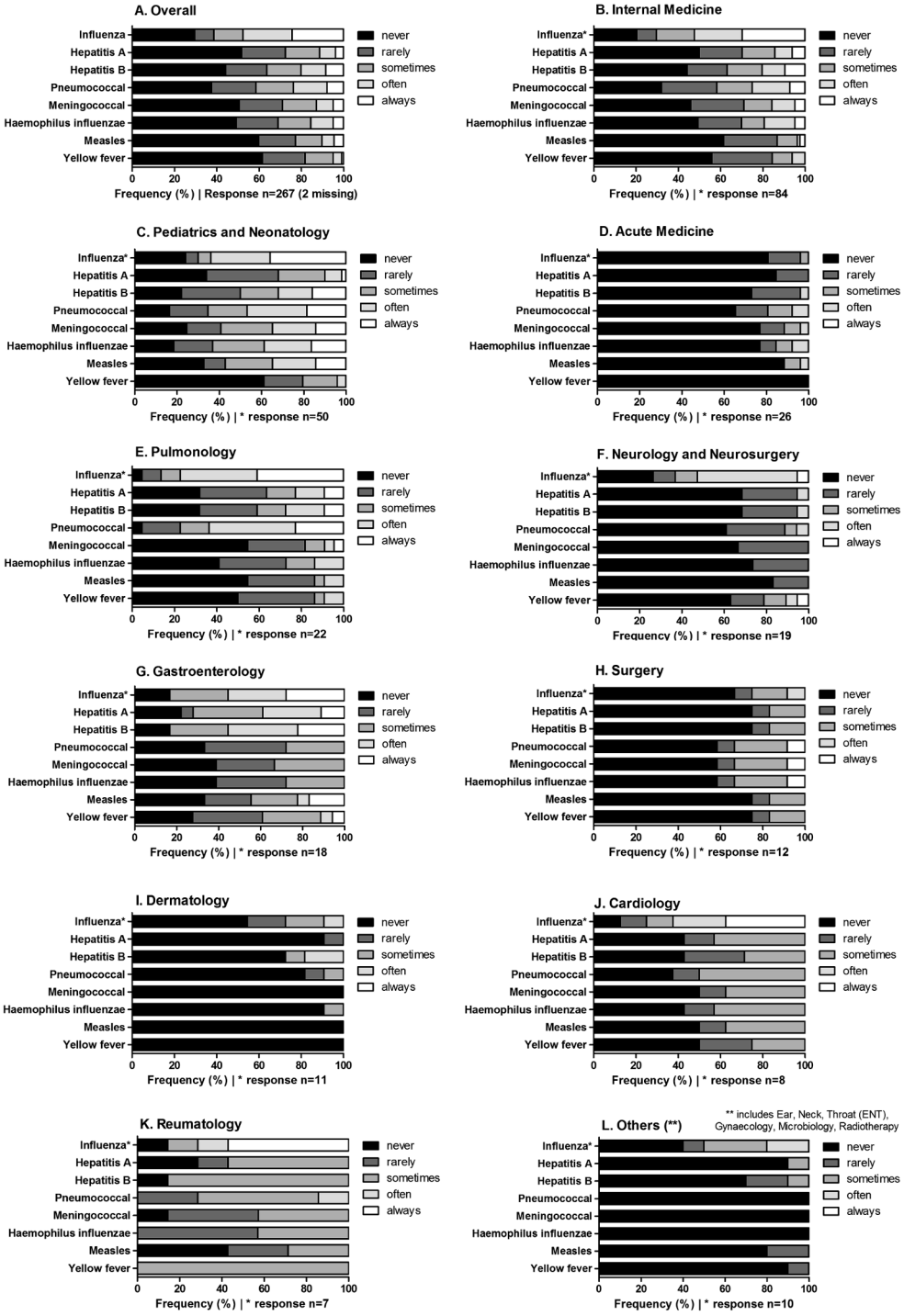

Figure 3. Frequency of discussing vaccinations during consultations
The frequency of discussing vaccinations by HCP during patient consultations, represented as overall (A) and per department (B-L). The number of responses, represented by the responses for influenza vaccine, is shown below the graphs.

Travel opportunities and precautions

The majority of HCP (65%) indicated to regularly discuss travel plans during their consultations. The topic seems to be discussed most frequently at the departments of Rheumatology, Gastroenterology and Pulmonology and least frequently at the departments of Surgery, Dermatology and Acute Medicine (Supplementary Figure 1). Nurses reported to discuss travel plans more often than doctors do (p=0.001). The follow-up actions of HCP in case ICP informed them about their travel plans are summarized in Figure 4. The majority of respondents indicated to refer their patients to a specialized travel clinic.

Suggestions for improvements

In line with the qualitative data, 80 percent of HCP agreed that there is room for improvement with regards to the prevention of infections in ICP (data available from 248 HCP). Up to 40 percent agreed that education is the most important tool to reduce the number and severity of infections in ICP. To other respondents, refining infection control in the hospital (16%); usage of (prophylactic) medication (13%); or vaccination (13%) is of most importance. According to seventeen percent, the infections they face are not preventable; two percent answered that there are no or few infections.

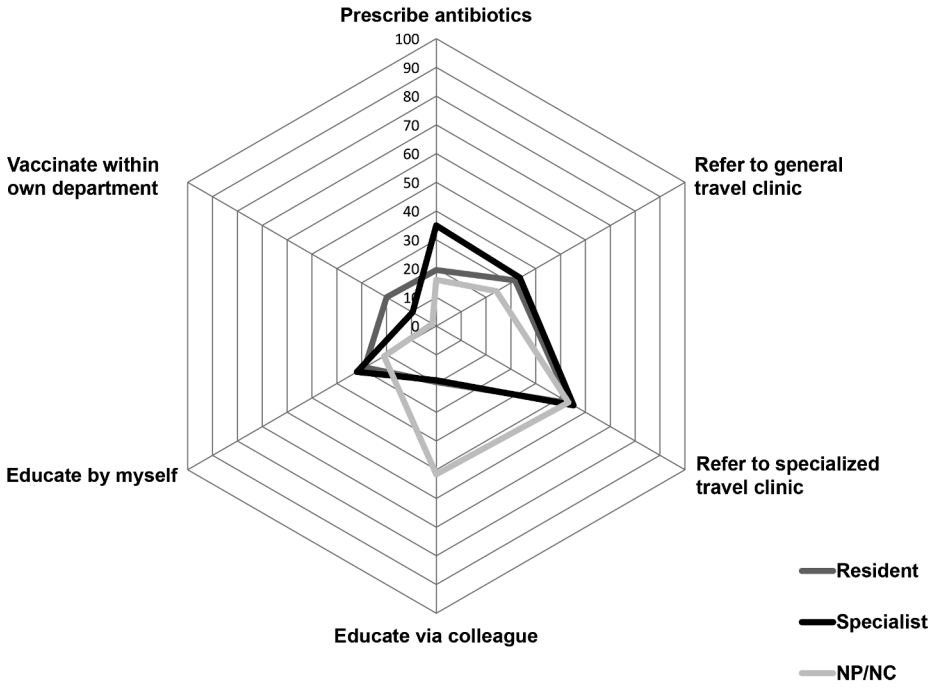

Figure 4. Infographic follow-up actions of HCP if ICP has travel plans
Infographic showing follow-up actions of HCP if their ICP indicate they have travel plans. Results are shown per type of profession. HCP = Healthcare professional(s); ICP = Immunocompromised patient(s); NP/NC = nurse practitioner/nurse consultant.

DISCUSSION AND CONCLUSION

This mixed-method study showed that according to the majority of HCP, there are opportunities to further enhance prevention of infections in ICP. Education, as method to optimize knowledge, was chosen by forty percent as the most promising method. Accordingly, HCP graded the knowledge of their patients as insufficient. In addition, one out of seven HCP agreed that ICP could benefit from vaccinations as method to prevent infections. Despite being recommended in guidelines and valued as important, vaccinations are not discussed by default during consultations. Moreover, even when recommended in guidelines, many HCP experience timing issues and other barriers to vaccinate their ICP.

The agreement of HCP from different specialties on the added value of education as method to prevent infections is striking. Vaccination as strategy seems less important to HCP, which might be explained by the limited evidence of vaccine effectiveness in specific populations, as reported in the interviews. Furthermore, the limited ability to prevent a wide range of infections might play a role. Addressing behavior by education therefore seems to transcend the effects that vaccinations induce by means of preventing a smaller number of infectious diseases. On one hand, individualized education is time consuming, while on the other hand, the heterogeneity of ICP is a complicating factor in the provision of general applicable information. Limited frequency of discussing vaccinations during consultations might be due to short time. In addition, difficulties in timing, logistics and finances, could withhold HCP to discuss vaccinations and act accordingly.

In comparison to studies that tested knowledge of HCP with closed-ended questions (297, 310), the HCP in this study rated themselves 'rather good' on knowledge about methods to prevent infections. The variation in the reported frequencies of discussing vaccinations seems in line with studies where immunization histories indicated vaccination rates of 24 to 70 percent for recommended vaccinations (300). Several strategies are known to reduce the number or severity of infections in ICP. For example, in post-transplantation patients, both influenza vaccination and early antiviral treatment reduced the severity of influenza infections (311). In asplenic patients, pneumococcal vaccination, sufficient knowledge level about risks and the usage of prophylactic antibiotics, were associated with a reduction of overwhelming post splenectomy infections (312).

Previous studies showed that vaccine uptake increases both in case HCP promotes vaccination and if the ICP has sufficient knowledge on recommended and contraindicated vaccinations (313, 314). Since the frequency of discussing vaccinations and the estimated knowledge of ICP were low in our study, this warrants further attention.

Based on our findings we suggest addressing the following:

First, and according to the data of this study most importantly: integrating a method of education in preventive care of ICP could support reducing the number and severity of infections in this patient population.

Second, efforts should be made to reach a higher vaccine uptake for vaccinations that are known to contribute in the prevention of infections for ICP, by discussing them more frequently during consultations. Especially, during the first HCP visit, still a substantial part of ICP is not yet immunocompromised, and is at that time more likely to mount an effective immune response to vaccination.

In order to increase knowledge and achieve a higher vaccine uptake in ICP, the following interventions are suggested: telephone support programs by HCP (315); targeted information campaigns (68); solid online information resources (316); electronic health record patient portal messages (317, 318); involvement of pharmacists as educators (319); automated telephone communication (320). The optimal strategy to educate ICP remains to be investigated.

Third, we propose to actively track down barriers to vaccinate and solve them in a multidisciplinary approach, incorporating HCP, policy makers, insurance companies and other funding suppliers. Timing issues, which can only partly be resolved due to possible short intervals between diagnosis and start of immunosuppressive therapy, could diminish if acted upon in secondary and primary care centers. We suggest healthcare centers to support HCP by alleviating logistical barriers such as starting a dedicated vaccination clinic or appoint HCP in a consulting role for ICP and vaccination advice.

In addition, we want to stress the importance for HCP to be aware of risks involved with international travel. Travel-associated risks can be integrated in patient education, as increasing quality of life in ICP due to evolved treatment strategies might increase the willingness to travel (321). Studies showed that up to two-third of ICP travelled to high risk destinations while being immunocompromised, while only 55 to 69 percent sought pre-travel advice (302, 322).

For this study, some limitations have to be taken in mind when interpreting the results. The study was conducted in a single tertiary care center, limiting the external validity to other centers. For the interviews, we only invited medical specialists, for their clinical experience as senior HCP. This approach has left the opinions of nurses and residents

unnoticed, partly redressed by involving them in pilot-testing of the surveys. Internal validity was strengthened by inviting all HCP that possibly treat ICP; it however resulted in small number of responses for some departments. We therefore chose to display the HCP as one group, highlighting remarkable results per subgroup.

Following HCP's opinions, education is considered as most important strategy to reduce the number and severity of infections in ICP. Improved levels of knowledge of ICP about their immunocompromised state; risks for infections; and recommended and contraindicated vaccinations could contribute to the prevention of infections. The optimal method to deliver education is an important topic to elaborate on in future research. In parallel, solving issues that retain HCP to vaccinate could increase the frequency of discussing vaccinations and the vaccine uptake. As the immunocompromised population is increasing, we encourage HCP to contribute to integrative approaches that implement education, vaccinations and other measures to prevent infections.

Supplementary data can be found at https://doi.org/10.1016/j.vaccine.2019.01.075.

Chapter 4.2

High immunogenicity to influenza vaccination in Crohn's disease patients treated with ustekinumab

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Vaccines (Basel), 2020

ABSTRACT

Influenza vaccination can be less effective in patients treated with immunosuppressive therapy. However, little is known about the effects of ustekinumab, an anti-IL-12/23 agent used to treat Crohn's disease (CD), on vaccination response. In this prospective study, we assessed immune responses to seasonal influenza vaccination in CD patients treated with ustekinumab, compared to CD patients treated with anti-TNFα therapy (adalimumab) and healthy controls. Humoral responses were assessed with hemagglutinin inhibition (HI) assays. Influenza-specific total $CD3^+$, $CD3^+CD4^+$ and $CD3^+CD8^+$ T-cell responses were measured with flow cytometry. Fifteen patients treated with ustekinumab, 12 with adalimumab and 20 healthy controls were vaccinated for seasonal influenza in September 2018. Seroprotection rates against all vaccine strains in the ustekinumab group were high and comparable to healthy controls. Seroconversion rates were comparable, and for A/H3N2 highest in the ustekinumab group. HI titres were significantly higher in the ustekinumab group and healthy controls than in the adalimumab group for the B/Victoria strain. Post-vaccination T-cell responses in the ustekinumab group were similar to healthy controls. One month post-vaccination proliferation of $CD3^+CD8^+$ T-cells was highest in the ustekinumab group. In conclusion, ustekinumab does not impair immune responses to inactivated influenza vaccination. Therefore, CD patients treated with ustekinumab can be effectively vaccinated for seasonal influenza.

INTRODUCTION

Patients with inflammatory bowel disease (IBD) are frequently treated with immunomodulatory or immunosuppressive medication. Due to these therapies and the underlying inflammatory disease, they are at risk of more severe complications of infectious diseases (323). Influenza causes significant morbidity and mortality in the general population (324) and the incidence of severe influenza is even higher in IBD patients, as demonstrated by higher rates of hospitalization (5.4% in IBD patients vs. 1.9% in healthy controls) (325). Vaccination against influenza reduces the risk of infection in immunocompromised patients (326). However, influenza vaccination may be less effective in patients treated with immunosuppressive therapies (327-329) and immunological mechanisms of the impaired vaccination response in IBD patients are often poorly understood (330).

Over the past decades, immunomodulatory and biologic therapies for the treatment of Crohn's disease (CD) and ulcerative colitis (UC) have become widely available. Adalimumab is a frequently prescribed anti-TNFα agent that is administered subcutaneously and has proven efficacy for CD since 2006 (331). Use of anti-TNFα agents and immunomodulators, especially when used combined, is associated with a lower serological response to influenza vaccination in both children and adults with IBD (327-329, 332-335). This is explained by the involvement of TNFα in B-cell and T-cell interactions to achieve adequate antibody production (336, 337). Ustekinumab, a human monoclonal antibody directed against the p40 subunit of interleukin (IL)-12 and IL-23 that normally binds to the interleukin-12 receptor β1 (IL-12Rβ1) of Th1 and Th17 cells, has more recently been approved as a treatment option for moderate-to-severe CD (331) and UC (338). Although ustekinumab is effective and the safety profile reassuring (339, 340), infections remain feared complications and preventive measures including annual influenza vaccination is currently advised by the European Crohn's and Colitis Organisation (ECCO) guidelines (341). Yet, little is known about the effects of ustekinumab on the immune responses to vaccinations.

Ustekinumab selectively inhibits IL-12 and IL-23 and thereby mainly Th1 and Th17 cell development (342). However, IL-12Rβ1–mediated signaling via STAT3 and probably also STAT4, affected by ustekinumab treatment, plays a role in the generation of T follicular helper (T_{FH}) cells (343). As T_{FH} cells are important for the B-T cell interaction to generate high-affinity antibodies, humoral responses may be compromised (344). In this study, we aim to investigate the humoral and cellular immune response after the inactivated 2018-2019 trivalent influenza vaccination (TIV) in adults with CD treated with ustekinumab (UST) compared to those treated with adalimumab (ADA) and healthy controls (HC).

MATERIALS AND METHODS

Study Design and Population

We performed a prospective study on a selected cohort from a vaccination biobank in the Erasmus Medical Centre. All adult CD patients treated with either ustekinumab or adalimumab who wished to receive the seasonal influenza vaccination in September 2018 were asked to participate in the biobank study and were included following written informed consent. Healthcare workers who were offered the influenza vaccination for their occupation were selected from the biobank after age and sex matching to the CD patients, and included as healthy controls.

Data Collection and Analysis

At baseline, informed consent forms were signed and medical history was collected from participants and electronic patient files. Medical IBD history was classified using Montreal classification.(345) We collected medication use including dose at moment of vaccination. Ustekinumab was routinely injected in a dose of 90 mg every eight weeks or 12 weeks and adalimumab in a dose of 40 mg once every two weeks, defined as standard dose. More frequent injections were classified as escalated dose. Blood sampling was performed prior to the administration of the TIV. The 2018/2019 inactivated TIV (Influvac®; Abbott biologicals®) contained 15 microgram of HA antigen of each of the following influenza virus strains: A/Michigan/45/2015 (H1N1)pdm09-like virus; A/Singapore/INFIMH-16-0019/2016 (H3N2)-like virus; and B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage), and was administered intramuscularly in the deltoid. Patients were followed-up at one (T1), three (T3) and nine months (T9) post-vaccination. During each patient visit blood samples were collected in a BD Vacutainer® Serum Separating Tubes II Advance and a BD Vacutainer® Cell Preparation Tube. Within 24 hours after collection, serum samples were centrifuged and stored at -20 °C until further use. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient Ficoll separation and thereafter washed with phosphate buffer saline (PBS). Subsequently, PBMCs were counted and frozen in mononuclear cell medium with 10% dimethyl sulfoxide (DMSO) at a minimum of 2×10^6 mononuclear cells per ampule. These samples were stored overnight in Nalgene® Mr. Frosty™ Freezing Containers (Thermo Scientific) at -80°C and transferred to liquid nitrogen thereafter.

Laboratory Assessments

Hemagglutination Inhibition Assay

To assess antibody responses against the influenza virus vaccine strains, a hemagglutinin inhibition (HI) assay was performed simultaneously on all available serum samples, using a standard protocol (346, 347). Briefly, sera were pre-treated with Vibrio cholerae neuramidase (dilution of 1:5 of an in-house produced cholera filtrate), by incubation overnight at 37 °C and heat-inactivation for one hour at 56 °C. Nonspecific agglutination in sera was eliminated, if present, by incubating 15 parts of the serum-cholera filtrate mixture with one part 100% turkey erythrocytes for one hour at 4 °C. Due to the pre-treatment steps, a starting serum dilution of 1:10 was used for all experiments. Three hemagglutinin antigens, each representing a strain of virus contained in the vaccine, were added and twofold serial dilutions were made up to 1:20,480. The highest dilution of antiserum that was still able to block agglutination between test influenza viruses and 1% turkey erythrocytes was considered the HI titre.

T-cell Proliferation Assay Using Flow Cytometry

Six doses of 2018/2019 inactivated TIV vaccine were dialysed (3 ml) with a slide-a-lyzer (Thermo Scientific) for contaminant removal to avoid interference in the T-cell proliferation assay. The amount of purified membrane glycoprotein subunit was analysed with a bicinchoninic acid (BCA) assay (Thermo Scientific™, Pierce™) and compared to undialysed vaccine content. If there was no difference in the amount of protein between dialysed and undialysed vaccine, we assumed no membrane protein was lost. PBMCs were thawed at 37^{0}C and washed twice with IMDM (Gibco Invitrogen, USA), supplemented with 2 mM L-glutamine, 100 U/ml penicillin (Lonza BioWhittaker, Switzerland) and 100 μg/ml streptomycin (Lonza BioWhittaker, Switzerland) (PSG) and 10% heat-inactivated fetal bovine serum (HI-FBS; Sigma-Aldrich, USA), further referred to as I10F. Subsequently, PBMCs were incubated with 50 U/ml Benzonase (Merck Millipore, USA) in I10F for 30 minutes at 37 °C, washed once and cultured over night at a density of 1–3 × 10^5 cells/well in RPMI-1640 supplemented with HI-FBS and PSG, further referred to as R10F. The next day, cells were washed once with PBS and labelled with 600 nM CFSE (in PBS) for 5 minutes at 37 °C. Afterwards, PBMCs were washed with R10F, plated at a density of approximately 1.5 × 10^5 cells per well in R10F and cultured for five days. Per donor and time point three wells were left unstimulated, while three wells were stimulated with 100 ng/well of the dialysed purified membrane glycoprotein subunit preparations of the 2018/2019 TIV (348). Concanavalin A (ConA) was used as a positive control at a concentration of 5 μg/ml. Five days after stimulation PBMCs were stained for CD3, CD4 and CD8. Briefly, cells were washed once with PBS

containing 2mM EDTA and 0.05% BSA (FACS buffer) and then stained for 15 minutes at 4^0C in FACS buffer with the following monoclonal antibody-fluorochrome conjugates: CD3/APC Cy7 (1:50 dilution, BD Pharmingen), CD4/V450 (1:50 dilution, BD Horizon) and CD8/PE-Cy7 (1:25 dilution, eBioscience). After staining, cells were washed twice with FACS buffer and flow cytometry was performed with a BD FACSLyric™ flowcytometer (BD Bioscience, USA).

Outcomes and Parameters

Functional antibody responses were assessed with the HI assay. The assay was performed in duplo and geometric mean titres were calculated. For calculation purposes, HI titres <10 were adjusted to 1. From these results, the following outcomes were calculated: (1) seroprotection rate: the percentage of participants per study group with an antibody titre above 40, which is considered the best surrogate correlate of protection (349); (2) seroconversion rate: the percentage of participants in the study group that had at least a fourfold increase of the post-vaccination antibody concentration when compared to the pre-vaccination antibody concentration; (3) geometric mean titres (GMT) per time point per study group. We corrected for high pre-vaccination antibody titres, using a log10 transformation of GMTs and a linear regression formula described by Beyer and colleagues (350), which results in a 'reset' of pre-vaccination antibody titres to zero. Data were back log transformed to show interpretable results.

Cellular responses were assessed by the proliferation of influenza specific $CD3^+$, $CD4^+$ and $CD8^+$ T-cells. Stimulation indexes (SI) were calculated by dividing the percentage of proliferated cells in stimulated samples by the percentage of proliferated cells in unstimulated samples per donor, time point and T-cell subset (total CD3, CD4 or CD8).

Data Analysis

FACS data was analysed with FlowJo version 10.6.1. Gating strategies used for analysis are shown in Figure S1. We set the mean background of proliferation in unstimulated samples to 1.5 and applied the same gating strategy to stimulated samples. SPSS version 24 was used for data analysis. A Shapiro-Wilk test was used to assess normality of distributions. Comparison of parametric continuous variables between the three groups was done using one-way ANOVA and of non-parametric continuous variables using Kruskal-Wallis tests. Comparison of non-parametric continuous variables between two groups was done using Mann-Whitney U tests. Fisher's exact tests were used for comparing differences in categorical variables. To prevent finding significances due to multiple testing, we only performed testing between two groups when the comparison between three groups showed a p-value of <0.10. A p-value <0.05 was considered significant. Outliers

were detected with the Tukey's box-plot method which defines outliers as being outside the interquartile interval (Q1 – 1.5·IQR, Q3 + 1.5·IQR). Missing data were excluded per variable. GraphPad Prism version 5.0 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com) was used to create figures.

Ethical Considerations

All subjects gave their informed consent for inclusion before they participated in the study. This study has been exempted from medical ethical approval requirements by the Medical Ethical Research Committee of the Erasmus Medical Center on November 13, 2017, due to the biobank format of the Vaccination Cohort study (COVA study, MEC-2014-398). The study has been conducted according to the principles of the declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013).

RESULTS

Forty-seven subjects were enrolled in this study between September 2018 and November 2018. We studied the 2018-2019 TIV vaccine response of 47 individuals in three different study groups: 15 CD patients using ustekinumab, 12 CD patients using adalimumab, and 20 healthy controls with influenza vaccination history. Demographic baseline characteristics were comparable between the three groups and described in Table 1. The average median age of the total study population was 39 years (IQR 29-50) and 57 percent was female. Median duration of use of adalimumab was 32 months, and 19 months for ustekinumab ($p = 0.022$). In the ustekinumab group, one patient was injected every seven weeks and one patient every six weeks. In the adalimumab group two patients were injected weekly, two patients every 10 days and one every four weeks. Three patients in the ustekinumab group additionally used an immunomodulator (thiopurines or methotrexate) compared to two patients in the adalimumab group. Montreal classification, use of co-medication and influenza vaccination history did not differ significantly between the three groups.

Table 1. Baseline characteristics.

	UST	ADA	HC	Difference between groups
	n = 15	*n* = 12	*n* = 20	Sig. (p-value)
Gender				
Female, *n* (%)	11 (73.3)	5 (41.7)	11 (55.0)	0.260†
Pregnant, *n* (%)	1 (9.1)	2 (40.0)	0 (0)	0.079†
Age				
Median, years (IQR)	36 (26-56)	45 (28-59)	36 (29-49)	0.688‡
Country of birth				
Netherlands, *n* (%)	13 (86.7)	11 (91.7)	19 (95.0)	0.808†
BMI				
Mean, kg/m^2 (SD)	24.5 (4.6)	25.3 (5.2)	24.0 (4.3)	0.723§
Lifestyle				
Smoker, *n* (%)	5 (33.3)	1 (8.3)	3 (15.0)	0.201†
Alcohol, *n* (%)	9 (60.0)	7 (58.3)	19 (95.0)	**0.016**†
Duration of CD				
Median, years (IQR)	15 (9-25)	14 (8-35)	NA	0.845¶
Disease Location				
Small intestine (L1)	3 (20.0)	2 (16.7)		
Large intestine (L2)	1 (6.7)	1 (8.3)		
Small and large intestine (L3)	8 (53.3)	7 (58.3)		
L3 + upper GI	3 (20.0)	2 (16.7)	NA	1.000†
Disease Behaviour				
Non-stricturing, non-penetraring (B1)	4 (26.7)	4 (36.4)		
Stricturing	7 (46.7)	6 (54.5)		
Penetrating	4 (26.7)	1 (9.1)	NA	0.666†
Perianal disease	4 (26.7)	3 (25.0)	NA	1.000†
Duration medication,				
Median, months (IQR)	13 (5-19)	32 (15-82)	NA	**0.022**¶
Dose medication				
Standard dose	13 (86.7)	7 (66.7)		
Escalated dose	2 (13.3)	4 (33.3)	NA	**0.357**†
Immunosuppressive co-medication* *n* (%)				
None	9 (60.0)	7 (58.3)		
Low dose corticosteroids	2 (13.3)	3 (25.0)		
High dose corticosteroids	1 (6.7)	0 (0.0)		
Methotrexate	2 (13.3)	0 (0.0)	NA	0.643†
Thiopurines	1 (6.7)	2 (16.7)		
Influenza vaccine history, *n* (%)				
never before	3 (20.0)	3 (25.0)	6 (30.0)	
once before (2017)	0 (0.0)	2 (16.7)	2 (10.0)	
twice before (2016, 2017)	1 (6.7)	0 (0.0)	1 (5.0)	
thrice before (2015–2017)	0 (0.0)	1 (8.3)	1 (5.0)	
more than thrice before	5 (33.3)	5 (41.7)	4 (20.0)	0.537†
at least once, but not 2017	6 (40.0)	1 (8.3)	6 (30.0)	

Percentages within study groups. T-tests were used to calculate differences between continuous variables, chi-square tests were used for categorical variables. UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. CD = Crohn's Disease, NA = not applicable. * used while vaccinated or during the 3 months before. Low-dose corticosteroids = prednisone <10mg/day or budesonide (<9mg/day). High-dose corticosteroids = prednisone ≥10mg/day (at least 14 consecutive days or 700 mg total). † Fisher's exact test, ‡ Kruskal-Wallis test, § one-way ANOVA, ¶ Mann-Whitney U test.

Humoral Immune Response

Seroprotection Rates

Pre-vaccination seroprotection rates for all three strains were not significantly different between the groups (Table 2). Seroprotection rates to the H3N2 strain one month post-vaccination were 100 percent in all three groups, and remained 100 percent three months post-vaccination in healthy controls and the ustekinumab group. In the adalimumab group, seroprotection rates were lower three months post-vaccination compared to the other two groups, reaching borderline significance (81.8%, $p = 0.056$). Seroprotection rates to the H1N1 strain were higher than 90.0 percent one month post-vaccination and at least 78.6 percent three months post-vaccination for the three study groups and did not differ significantly (Table 2). Pre- and post-vaccination titres were lowest to the B/Victoria strain, especially in the adalimumab group (T1 and T3: 63.6%), however there was no significant difference between study groups.

Table 2. Seroprotection rates per study group (% HI-titers ≥1:40).

		UST	ADA	HC	Overall	UST vs. HC	UST vs. ADA	ADA vs. HC
		%	*%*	*%*	*p-value*	*p-value*	*p-value*	*p-value*
A/H3N2	**T0**	71.4	75.0	90.0	0.328			
	T1	100	100	100	1			
	T3	100	81.8	100	0.056	1	0.183	0.118
A/H1N1pdm09	**T0**	57.1	58.3	55.0	0.982			
	T1	91.7	90.0	100	0.379			
	T3	78.6	90.9	90	0.561			
B/Victoria	**T0**	42.9	33.3	60.0	0.311			
	T1	92.3	63.6	85.0	0.170			
	T3	92.9	63.6	75.0	0.202			

UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. Significances were calculated with Fisher's exact tests.

Seroconversion Rates

Seroconversion rates to the H3N2 strain were significantly different in the three groups at three months post-vaccination (T3: $p = 0.014$, Table 3) and borderline significant at one month post-vaccination (T1: $p = 0.064$). The ustekinumab group had higher seroconversion rates compared to the adalimumab group (T3: $p = 0.015$, Table 3) and the healthy controls (T1: $p = 0.038$, T3: $p = 0.035$, Table 3). Seroconversion rates to the other influenza vaccine strains in the three study groups were highest in the ustekinumab group and lowest in the adalimumab group, although this reached no significance.

Table 3. Seroconversion rates per study group (% ≥4-fold increase).

		UST	ADA	HC	Overall	UST vs. HC	UST vs. ADA	ADA vs. HC
		%	%	%	*p-value*	*p-value*	*p-value*	*p-value*
A/H3N2	**T0-T1**	69.2	27.3	30.0	0.064	**0.038**	0.100	1
	T0-T3	71.4	18.2	30.0	**0.014**	**0.035**	**0.015**	0.676
A/H1N1pdm09	**T0-T1**	75.0	40.0	50.0	0.288			
	T0-T3	50.0	36.4	45.0	0.863			
B/Victoria	**T0-T1**	61.5	27.3	35.0	0.227			
	T0-T3	50.0	27.3	30.0	0.520			

UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. Significances were calculated with Fisher's exact test.

Antibody Titres

The post-vaccination antibody titres in the ustekinumab group were comparable to those of the healthy controls. In the adalimumab group, geometric mean titres (GMT) were lower compared to the other two groups for all influenza vaccine strains at both T1 and T3, except for the H1N1 strain three months post-vaccination (Table 4, Figure 1). This reached significance for the B/Victoria strain at both T1 and T3, when comparing the three groups (T1: $p = 0.031$ and T3: $p = 0.028$, Table 4, Figure 2,) and specifically the ustekinumab and adalimumab group ($p = 0.028$ and $p = 0.009$, Table 4) respectively.

As pre-vaccination titres in the ustekinumab group were significantly lower than in the healthy controls and the adalimumab group ($p = 0.013$), we studied antibody titres after correction for high pre-vaccination titres in the latter two (Table 4). Post-correction antibody titres at T3 for the H3N2 strain were significantly lower in the adalimumab group compared to healthy controls and the ustekinumab group ($p = 0.041$, Table 4). For the B/Victoria strain, post-correction antibody titres were significantly higher for both T1 and T3 in the ustekinumab group compared to the other two groups (T1: $p = 0.014$, T3: $p = 0.015$, Table 4).

Cellular immune Response

T-cell proliferation was studied per group, per time point and per T-cell subset (example shown in Figure S2). In general, stimulation indexes showed a pattern of increased proliferation from baseline to T1 and T3 (with the exception of $CD3^+CD8^+$ response in healthy controls) and a decrease between T3 and T9 (with the exception of the $CD3^+$ and $CD3^+CD8^+$ response in the ustekinumab group) (Figure 3). In all three groups, baseline $CD3^+$ and $CD3^+CD4^+$ responses were low (mean SI <1.36). However, $CD3^+CD8^+$ baseline responses were relatively high (mean SI >1.49). When comparing time points and T-cell subsets, no significant differences were found between the three study groups. However, when we compared the groups one by one, we found a significant higher $CD3^+CD8^+$

response one month after vaccination for the ustekinumab group compared to healthy controls (p = 0.025).

Overall, 95% confidence intervals were large and a few donors from all groups showed exceptional high responses (Figure 3). When excluding these outliers, stimulation indexes were significant different between the three groups one month post-vaccination in the $CD3^+CD8^+$ subset (p = 0.031) in favour of the ustekinumab group (UST vs. HC, p = 0.009) (Figures S3 and S4).

Table 4. Geometric mean antibody titres (GMT) per study group per time point.

		UST	ADA	HC	Overall	UST vs. HC	UST vs. ADA	ADA vc HC
A/H3N2	***GMT***				***p-value***	***p-value***	***p-value***	***p-value***
	T0	26	59	163	**0.013**	**0.008**	0.252	0.586
	T1	437	215	474	0.171			
	T3	372	132	427	0.071			
	Post-correction GMT							
	T1	203	75	141	0.159			
	T3	132	35	85	**0.041**	0.396	**0.025**	**0.036**
A/H1N1pdm09	***GMT***							
	T0	15	18	26	0.856			
	T1	195	127	184	0.786			
	T3	80	101	120	0.905			
	Post-correction GMT							
	T1	107	60	91	0.261			
	T3	27	29	33	0.947			
B/Victoria	***GMT***							
	T0	12	9	17	0.337			
	T1	129	30	90	**0.031**	0.073	**0.028**	0.306
	T3	111	26	62	**0.028**	0.125	**0.009**	0.220
	Post-correction GMT							
	T1	53	13	31	**0.014**	**0.043**	**0.005**	0.197
	T3	42	10	21	**0.015**	**0.036**	**0.006**	0.227

UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. GMT = geometric mean antibody titre. Post-correction GMT = transformed post-vaccination GMTs corrected for high pre-vaccination titres. Significance between GMT and post-correction GMT values was calculated with a Kruskal Wallis test. If Kruskal-Wallis test showed a significant difference, differences between separate groups were calculated with Mann-Whitney U-tests.

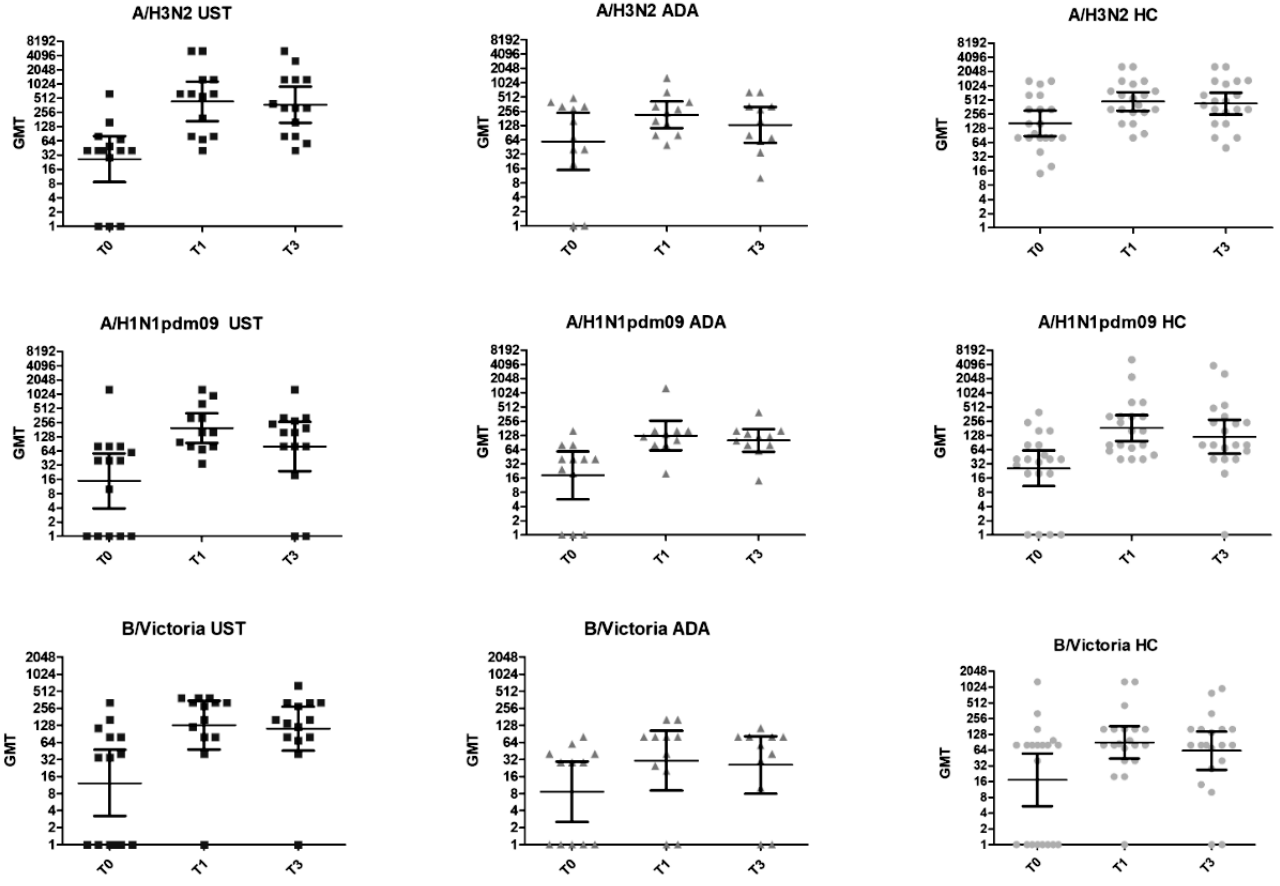

Figure 1. HI titres for each participant to influenza A/H3N2, A/H1N1pdm09 and B/Victoria vaccination per strain and study group. T0 = pre-vaccination, T1 = one month post-vaccinatipr=-yujhn, T3 = three months post-vaccination, T9 = nine months post-vaccination. UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. Geometric mean titres (GMT) and 95% confidence intervals are shown.

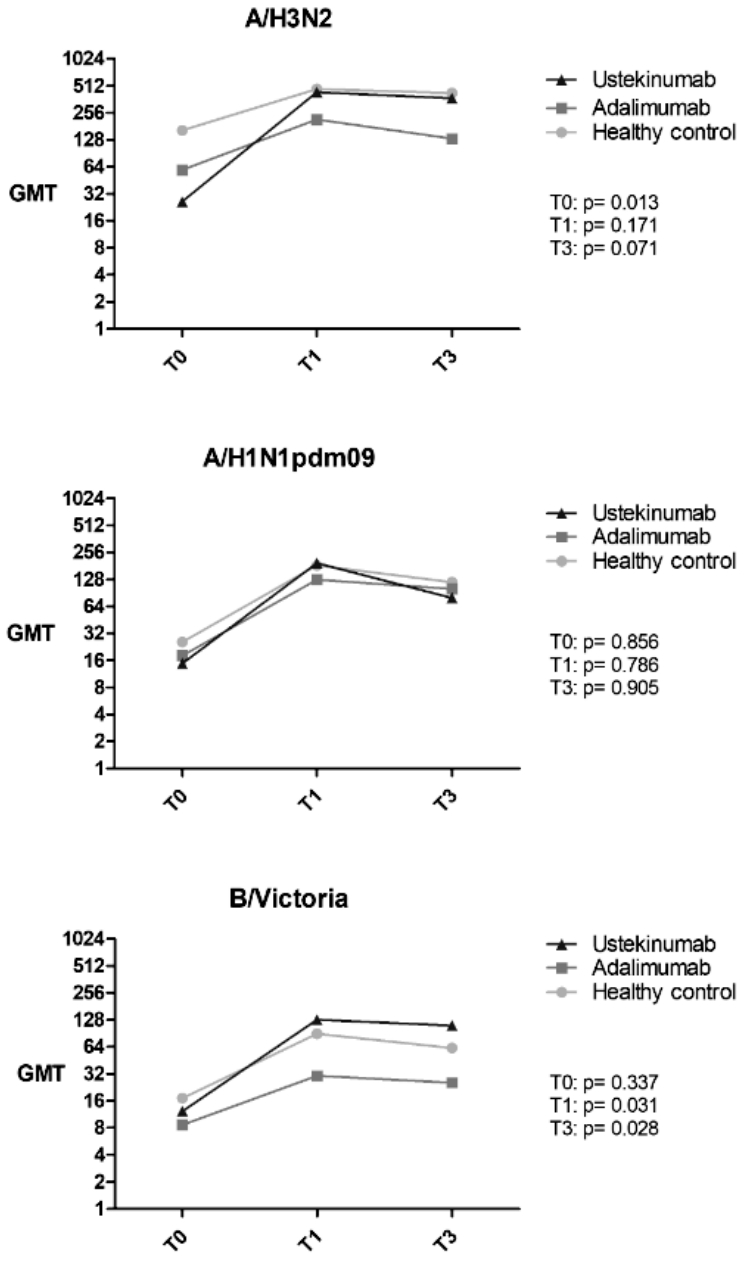

Figure 2. Dynamics of geometric mean HI titres (GMT) to influenza A/H3N2, A/H1N1pdm09 and B/Victoria vaccination according to study groups.
Comparisons between groups were tested using Kruskal Wallis tests. A p-value <0.05 indicates statistical significance.

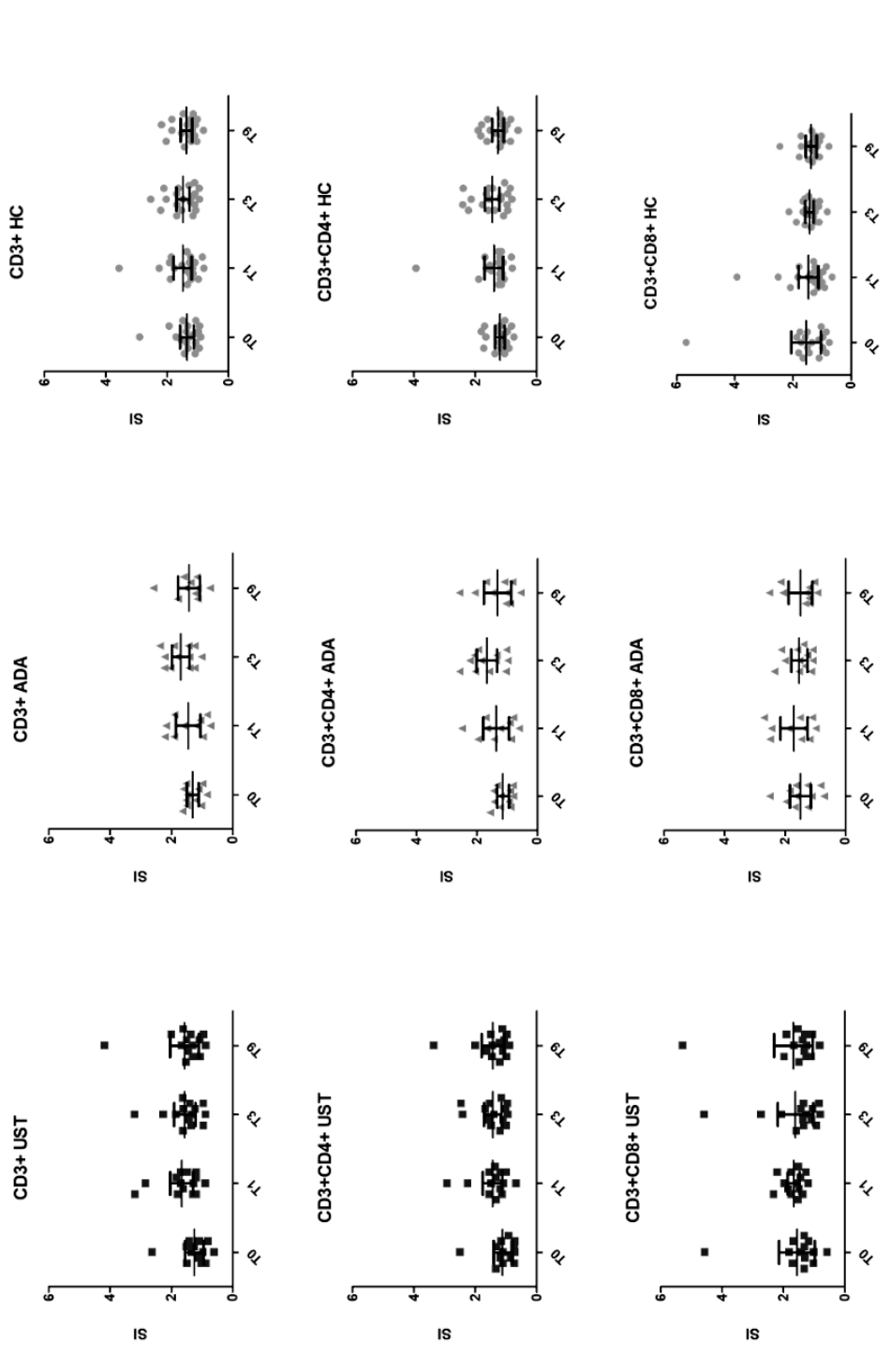

Figure 3. Stimulation indexes for each participant in the three study groups per T-cell subset. T0 = pre-vaccination, T1 = one month post-vaccination, T3 = three months post-vaccination, T9 = nine months post-vaccination. SI = stimulation index. UST = ustekinumab group, ADA = adalimumab group, HC = healthy controls. 95% confidence intervals are shown.

Correlation Between Humoral and Cellular Immune Response

To assess a possible relationship between the humoral and cellular immune responses, we calculated correlations between HI assay titres (GMTs for the three different vaccine strains) and the stimulation indexes (for the three different subsets of T-cell populations) (Figure S5). The highest Spearman correlation coefficient was found between the GMTs for the H1N1 strain and the SI for the $CD3^+$ T-cells ($R = 0.278$, $p = 0.002$).

DISCUSSION

Influenza vaccination is recommended in IBD patients according to international guidelines, however, immunomodulatory or immunosuppressive treatment may impair vaccine responses. This prospective cohort study showed that B-cell as well as T-cell responses to inactivated TIV in patients with CD during ustekinumab treatment were maintained and not impaired compared to healthy controls. Patients treated with ustekinumab had comparable seroprotection rates post-vaccination as healthy controls and better sustained seroprotection rates to the H3N2 strain than patients treated with adalimumab. Seroconversion rates were also higher in the ustekinumab group compared to healthy controls and the adalimumab group at three months post-vaccination for the H3N2 strain. After correction for high pre-vaccination titres using a linear regression formula described by Beyer et al (350) post-correction post-vaccination titres were significantly higher in the ustekinumab group compared to the adalimumab group and healthy controls for the B/Victoria strain. Cellular immune responses in the ustekinumab group were not impaired either. The $CD8^+$ T cell response one month post-vaccination was even significantly higher than in healthy controls.

To our knowledge, this is the first study that shows the immune response to vaccination in CD patients treated with ustekinumab. Our results are in line with a previous study in psoriasis patients treated with ustekinumab. This study showed no differences in the immune response to pneumococcal or tetanus toxoid vaccinations in patients treated with ustekinumab compared to controls (351). In another study, higher antibody responses to hepatitis B virus vaccination were found in patients treated with ustekinumab compared to patients treated with infliximab or adalimumab (352). Immune response to influenza vaccination in patients treated with ustekinumab have not been reported yet. Our results indicate that blocking IL-12 and IL-23 does not influence immune responses to vaccination as has been previously hypothesized (353). T_{FH} cells could still be generated, as studies in IL-12Rβ1-deficient adults have shown that the level of T_{FH} cells was not reduced in the absence of IL-12Rβ1 (354). Alternatively, if the generation of T_{FH} cells is impaired

due to the effect of a lacking signal to IL-12Rβ1 on the STAT3 (and 4) pathway, extra follicular T helper cells might take over T_{FH} cells functions (343, 355).

Although measured against different influenza strains, the HI assay responses in our healthy controls were comparable to those in previous studies, or even higher (356, 357). Higher GMTs can be explained by the influenza vaccination history in our study population. Seroconversion rates might be lower than in non-immune populations due to high pre-vaccination titres. Although antibody titres only increase slightly after repeated annual influenza vaccination, they still prevent laboratory proven influenza infections (357). Several previous studies have shown decreased immune responses to influenza vaccination in IBD patients using anti-TNFα agents (328, 329, 333-335). This is in line with our results from the HI assay, but not reflected by our T-cell proliferation data.

We found no previous studies on cellular responses after influenza vaccination in adult IBD patients. In children with IBD it was shown, in line with our data, that lymphocyte proliferation in general and after stimulation with tetanus antigen and adenovirus antigen was not impaired by several immunosuppressive therapies (358). For T-cell proliferation assays in liver transplant recipients who were vaccinated for seasonal influenza higher SI indexes in healthy controls and patients were reported compared to our data (348). However, due to the use of a thymidine assay to measure the influenza-specific T-cell response at that time, the results might not be comparable to our flow cytometry results. A study investigating T-cell responses after influenza vaccination reported short-lived $CD4^+$ T cell responses when PBMCs were stimulated with live (attenuated) virus strains (357). This is in contrast with our data showing that the T-cell response was still high (or even highest) three months post-vaccination.

In this era of new therapy targets and personalized treatment, immune response to vaccination might be an extra aspect influencing the choice of therapy, in addition to commonly weighed factors such as effectiveness, safety and costs. Combination therapy with anti-TNFα agents and an immunomodulatory agent is more effective for the treatment of CD than monotherapy, most likely due to both suppression of immunogenicity and an additive effects of the two drugs to reach disease remission (359). However, this combined strategy is also associated with a higher risk of infections (360) and may have a negative impact on immune responses to vaccination (327, 333, 334). Several ways to improve the influenza vaccination response during anti-TNFα therapy have been investigated. A booster vaccination failed to show better protection rates (329, 361) and timing relative to infliximab infusion neither showed to affect serological protection (335). Recently, a study found that four times higher dose vaccination resulted in higher antibody responses

to influenza vaccination compared to the standard dose, without leading to more adverse effects (362). Yet, 'high dose' vaccination is currently only recommended by American guidelines for patients aged 65 years or older (363). Current evidence does not support the use of immunomodulatory agents combined with ustekinumab (339). Similar to our results in the ustekinumab group, a recent study showed that immune responses to influenza vaccination in patients treated with vedolizumab, a monoclonal antibody against the α4β7 integrin, were not altered either (362). Interestingly, the immune response to an enterally administered vaccine was impaired during treatment with vedolizumab, possibly reflecting the gut-selective action of this therapy (364).

The ECCO recommends routine influenza vaccination of patients on immunomodulators (341). However, reported influenza vaccination uptake rates among IBD patients are low (28 to 61%) (69, 295, 323, 365), amongst others due to concerns about effectiveness and their unawareness of the recommendation (69, 295, 323). With our results, we provide evidence for high immunogenicity of influenza vaccination in CD patients treated with ustekinumab. As vaccination check-ups and active vaccination recommendations by treating physicians or supportive nurses are associated with improved vaccination uptake (295, 365), we strongly support involved nurses and physicians to recommend annual influenza vaccination to their patients treated with ustekinumab. This advice is similar for CD patients treated with adalimumab, because even though anti-TNFα treatment is associated with a lower serological response, the $CD4^+$ and $CD8^+$ T-cell responses showed to be non-inferior in this study.

Few limitations need to be taken into account with the interpretation of our results. First, this study is hampered by a small sample size and the number of patients on combination therapy with an immunomodulatory agent was too small to do a subgroup analysis. Since lowest influenza vaccine responses in IBD patients are reported in patients using combination therapy with an anti-TNFα agent and an immunomodulatory agent (327, 333, 334), this would have been an interesting addition. However, since immunomodulatory use was equally distributed amongst the patient groups, this will not have affected their comparison. The heterogeneity in the dose of medication in both adalimumab and ustekinumab users at moment of vaccination might have influenced the results. Furthermore, in this study CD patients were treated with adalimumab. Although anti-TNFα agents are comparable in efficacy and side effects, vaccine responses may differ, and cannot be generalized to other anti-TNFα agents than adalimumab. There was a significant higher baseline GMT for the A/H3N2 strain in healthcare workers compared to the ustekinumab group. Since the influenza vaccination history was comparable between the three study groups, this might be explained by a higher exposure to influenza in

healthcare workers (366). By using a linear regression formula we were able to correct for this possible confounder. Also, since the composition of influenza vaccinations change annually it is hard to compare our results one by one with previous and future studies. While we broadly examined influenza-specific immune responses by studying both humoral and cellular responses, in-depth details remain to be elucidated. The HI assays showed that functional antibodies are present in ustekinumab-treated patients, but we cannot conclude anything about the isotypes of the antibody response. With the T-cell proliferation we showed comparable proliferation of influenza-specific $CD4^+$ and $CD8^+$ T cells in all study populations, but the effector functions of $CD4^+$ and $CD8^+$ T cells are still unknown. Therefore, the performance of an intracellular cytokine staining would have been of additional value. Lastly, we compared immunological outcome measures between groups and could therefore not directly draw conclusions about morbidity due to influenza infections in these cohorts.

CONCLUSIONS

In this study, we demonstrated that CD patients treated with ustekinumab have adequate B- and T-cell responses to influenza vaccination. Therefore, our data support the plea for influenza vaccination in CD patients treated with ustekinumab to protect them from severe infections.

Supplementary materials are available online at https://www.mdpi.com/2076-393X/8/3/455.

CHAPTER 5

Prevention by Education

Chapter 5.1

Experience with a multinational, secondary school education module with a focus on prevention of virus infections

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ABSTRACT

Worldwide, virus infections are responsible for many diseases in terms of morbidity and mortality. Vaccinations and therapies are only available for relatively few virus infections and not always where they are needed. However, many viruses can be prevented by knowing their transmission route and thereby prevent infection. In the context of this study we measured the effects of a secondary school education module, named Viruskenner, on knowledge, attitude and risk behavior as these relate to virus infections. A non-randomized intervention study was conducted between April and August 2015 to assess the effect of this two-month education module on knowledge, attitude and behavior of 684 secondary school students in the Netherlands, Suriname and Indonesia. For the Netherlands, a control group of a further 184 students was added. Factor analysis was performed on questions pertaining to attitude and behavior. Comparative analyses between pre- and post-test per country were done using multiple linear regression, independent sample T-tests and one-way ANOVA. These showed a significant increase in knowledge about virus infections and the prevention of infectious diseases among the Dutch and Surinamese groups, while a trend of increased knowledge was evident among the Indonesian participants. The Dutch control group showed an overall decrease in knowledge. Regression analyses showed that there was a significant interaction effect between participation and time on knowledge, attitude and awareness and behavior and risk infection. Attitudes improved significantly in the intervention group. Pearson correlation coefficients between knowledge, attitude and behavior were found to be positive.

INTRODUCTION

Viruses are responsible for almost half of all emerging infections worldwide and are among the most emerging pathogens (367-369). Most virus infections are not treatable with antivirals and neither are they preventable with vaccines. Therefore, education plays a key role in raising awareness for infectious diseases and preventing the spread of virus infections (370). A population that is aware of the different ways a virus can be transmitted and does know how to embed effective preventive methods in daily life, can protect themselves against virus infections. This principle is based on the Knowledge, Attitude, Behavior (KAB) model, showing that increased knowledge can change people's attitudes and lead to behavioral change (371-373). A foundation for health-related attitudes and behavior is laid in early stages of life. Following many theories, the likelihood of changing attitude is high in adolescence (374). Also, the World Health Organization (WHO) states with their Health Promoting School Framework that schools are a good environment to start promoting health (375). Five years ago, a consortium of scientists and teachers developed a school-based education module regarding virus infections, named Viruskenner. This module aimed to teach students how to prevent virus infections. The module started with one secondary school in the Netherlands and evolved to a project with eight different secondary schools in the Netherlands. In 2014 the first Surinamese school joined and in 2015 the first Indonesian school. These two countries were already involved with the organizing institute by an international collaboration on emerging infectious disease population studies, facilitating easy communication and logistics. The Viruskenner module was extensively evaluated by independent researchers in the early years of the project. The conclusions of these evaluations led to improvement of the module and the questionnaires used. For example, in 2012 the concept of students being coached by an infectious disease expert was introduced. When becoming an international education module, it was interesting to see the impact of Viruskenner in different countries on knowledge, attitudes and behavior as they relate to virus infections, and find out which educational factors play a role in these changes. Educational programs that address infectious diseases are quite common, although most education is focused on a specific infection or a group of infections, particularly HIV and other sexually transmitted infections (STIs). A recent systematic review and meta-analysis evaluated 64 school-based sex education programs in middle- and low-income countries. Most of these programs (55 out of 64) focused on comprehensive sex education, with the remaining nine focusing on sexual abstinence. About half of the studies (33) were included in the meta-analysis and showed an overall positive effect on HIV-related knowledge, condom use, the initiation of sexual intercourse, the number of sexual partners and self-efficacy (376). Although HIV is among the virus infections that place the highest burden on society, it is

not the only virus that significantly impacts global health. Besides HIV, lower respiratory tract infections (influenza, for example) and diarrheal diseases (like norovirus) also belong to the 10 leading contributors to the global burden of disease (377). Furthermore, arthropod-borne diseases (like dengue) have a very high incidence (378). Remarkably, virus infections other than HIV are less frequently addressed in education modules. For example, only a few trials were carried out to measure the impact of an educational intervention for viral hepatitis, human papillomavirus, dengue, and influenza (379-383).

Most of the educational interventions that were analyzed showed positive results in improving knowledge and attitude pertaining to the subject of the intervention. Given the success of education programs about HIV, education modules about other virus infections that have global impact might also work. We developed an education module that focuses on multiple viruses with different transmission routes and all with global impact: HIV, dengue, hantavirus, chikungunya, MERS coronavirus, HPV, norovirus, viral hepatitis, measles, and influenza. We studied the efficacy and success of the education module in three countries, Netherlands, Suriname, and Indonesia, each differing in culture, circulating viruses and infection pressure. The education module aims to effectively increase knowledge, attitudes and behavior regarding several virus infections in each of these different circumstances.

MATERIALS AND METHODS

Participants and Setting

Schools in three countries participated in this non-randomized intervention study: four in the Netherlands, a high income country in Europe; one in Suriname, an upper middle income country in Latin-America; and one in Indonesia, a lower middle income country in Southeast-Asia (384). The effect of the education module was measured per country, by comparing the results of a pre-test and a post-test. The situations per country, for example culture and school system, were too different to make a fair comparison between countries. However, the target group for the education module is the same in each country and the concept of the module and measurements were as comparable as possible.

Secondary schools in the Netherlands, Suriname and Indonesia had been invited to apply to participate in the education module with their 10th grade students (generally aged 14 or 15). All schools were well-known public schools for students with an above average socio-economic status. The school in Suriname that participated had about 840 students and was located in Paramaribo, the capital and largest city of the country in inhabitants.

The Indonesian school that participated was located in Surabaya, the second largest city in the country. This was a senior high school (grade 10 to 12) and had about 1200 students. The four schools in the Netherlands ranged in number of students from 1600 to 2400 and were from different regions but all in the Dutch urban agglomeration, including one school from Amsterdam, the capital city of the Netherlands.

The 10th grade is the final stage of the junior high school in the Netherlands, which means that all students have, until then, followed the same subjects and have expressed their interest in the choice for a special curriculum. For example a beta scientific curriculum, which includes the following subjects: biology, physics, chemistry and mathematics.

In the Netherlands the schools that were invited to participate were all schools that offer students an option for Technasium, which is an elective course for students interested in beta scientific subjects (385). The participating students had all chosen this special curriculum with additional technical courses. Information about the module was disseminated via the project website and the Technasium network coordinator. A control group for the Dutch intervention group was selected at one of the participating Dutch schools. Thus, although they had not opted for the Technasium curriculum they do have a similar background and social environment. School curricula are defined differently in each country. In the Netherlands students choose a profile and we defined 'nature and science' and 'nature and health' as scientific profiles. In Suriname, students can choose biology, and we defined this as a scientific profile. Indonesian students can choose between a social profile (IPS) and a science profile (IPA). We defined IPA as a scientific profile. In both Suriname and Indonesia schools that matched most closely, in terms of grade and education level, with the Dutch intervention group were invited to participate in the study. Of the Dutch schools two were pre-university education level (known in the Netherlands as VWO) and two were mixed pre-university education level and advanced general secondary education (known in the Netherlands as HAVO). The Surinamese participants were from one VWO school, which is comparable with the Dutch VWO education level. These Surinamese participants can therefore be seen as pre-university education level (386). The Indonesian participants were from one SMA school, which is comparable with HAVO in the Netherlands and internationally known as advanced general secondary education (387). The Dutch control group, consisted of students with pre-university education level and advanced general secondary education level.

Design of the intervention

The Viruskenner education module is based on the "learning-by-doing" principle. Students are challenged to create a prevention tool for a specific virus infection. By involving students in real-life science-based problems and stimulating active learning (searching for information, test possible solutions and present their idea) a high impact can be achieved (388-390). In each country, the two-month module started with a national opening day, during which all participants of that country were introduced to the field of infectious diseases and viruses by means of four short lectures from experts in the field of virology, public health and infectious diseases. An optimal learning effect can be reached by bringing students in contact with experts (391). So, in all countries one or two Dutch experts from the department of Viroscience in the Erasmus Medical Center in Rotterdam were assigned to a class to coach them during the project. The students were supposed to work in groups of four to six students in competition with the other groups (388, 392). Each group worked on one of the viruses of the subject list including HIV, dengue, hantavirus, chikungunya, MERS coronavirus, HPV, norovirus, viral hepatitis, measles, and influenza. Students developed a prevention tool to disseminate this knowledge among their peers and, in doing so, help prevent virus infections that impact local or global health. During the three national final days (one in each country), the best groups per class, selected by the teachers and coach during a school final, presented their results and final product to their peer students and a jury. This independent jury was selected per country and based on proven expertise in virology, communication strategy and/or overall creativity. In each country, the jury chose two winners: the most informative presented prevention tool and the most creative prevention tool.

The study was conducted between April and August 2015. A pre-test was performed one or two days before the start of the module to assess their basic knowledge, attitude and behavior; a post-test five to seven days after its completion to let the information settle in their memory and give the students some time to evaluate their attitude and behavior a few days after the final day. Other measurement instruments were used to get additional information (Figure 1).

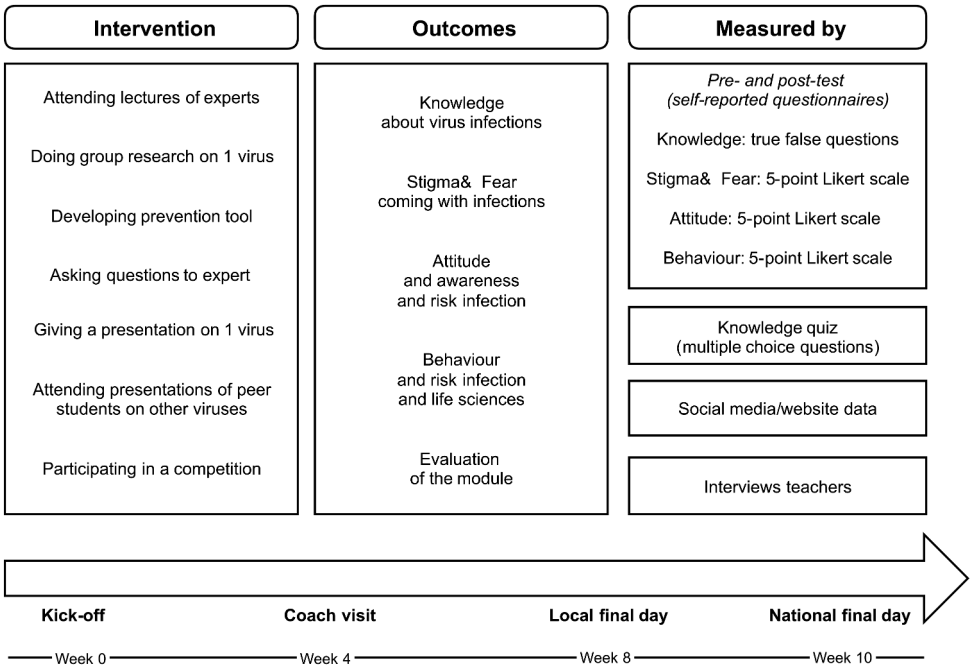

Figure 1. Study overview
Overview of all elements of the intervention and the outcomes that are measured by different instruments. The timeline shows the most important events during the project.

During the intervention students could use the modules' website (www.viruskenner.nl) and other supportive resources, like a YouTube channel and a Facebook page (all in Dutch and English and available for all participating countries), to find more information on the project and on virus infections and to disseminate information about their prevention tools (393-395).

Instruments

The effect of the education module was measured by the KAB model. Given that there was no validated instrument to assess knowledge, attitude and behavior regarding several viral infections, we used a self-designed questionnaire (supplementary material). The questionnaire was based on five years' experience with the education module. A team comprising two senior virologists, a communication scientist, and an education expert developed the questionnaire, which was refined after a pilot with a group of 60 students from a school similar to the participating schools. The questionnaires used for the Dutch and Surinamese schools were in Dutch. The questionnaires for the Indonesian students were first translated into English by a Dutch researcher and then into Indonesian by a native speaker of the language.

The pre- and post-test questionnaires addressed five areas: (1) socio-demographic factors; (2) stigmata and fear; (3) attitude and behavior; (4) knowledge on viruses and infectious diseases in general; and (5) the opportunity to write down questions or comments about the questionnaire or module. The post-test had an additional category: (6) perceptions of the project.

A principal component analysis (PCA) with varimax rotation was performed for the attitude and behavioral questions on the results of the pre-test questionnaires, as suggested in literature (396). Varimax was the preferred rotation because this results in a small number of factors per variable and a small number of variables per factor. This is the most popular type of rotation because it makes the interpretation of the data more reliable and easier (397). One of the behavior items ('I do not use a condom when I have sexual intercourse') was excluded because of more than 10 per cent missing values. The remaining missing values were randomly spread over the sample population. The sample size was big enough to delete these cases list wise. The Kaiser-Meyer-Olkin (KMO) value is a statistic that measures how much two random variables correlate. A KMO value greater than 0.8 represents a small partial correlation which makes a factor analysis more useful. In this study the KMO value was 0.849, which means there were relatively compact patterns of correlations and the factor analysis would provide reliable components (398). The number of extracted factors was based on the objective and interpretability criteria mentioned in Schönrock-Adema et al: (1) the scree test; (2) eigenvalues >1.5; (3) >5% of the variance explained by all factors; and (4) interpretability. However, the criterion of eigenvalue >1.5 led to only two components, which was not interpretable. Therefore, we set the norm of an eigenvalue back to greater than one (Kaiser's criterion) (399, 400). The principal component analysis with varimax rotation finally resulted in four components. The reliability per component was calculated by Cronbach's alpha (Table 1). Internal consistency for the components 'attitude and awareness' and 'behavior and life science' was above 0.7 and therefore acceptable. The components 'attitude and risk infections' and 'behavior and risk infection' should be interpreted with caution, because of the diversity of the constructs (401).

An additional instrument to measure knowledge was a live multiple-choice quiz, which was implemented at the end of the final day. In the Netherlands and Suriname portable electronic devices (keypad and software from Interactive Voting System®) were used by the students to answer 40 knowledge questions. In Indonesia, these portable electronic devices were not available, so the knowledge quiz was done by voting with colored papers, therefor recording these results was not possible.

Table 1. Attitudes and behavior

Component	Construct	Nr of items	Example item	Cronbach's Alpha
1	Attitude & awareness	3	I think it is important to know about viruses	0.797
2	Attitude & risk infection	5	I think getting a tattoo is a risk	0.572
3	Behavior & risk infection	4	I protect myself against mosquito bites when I go to a tropical country/into the forest	0.491
4	Behavior & life sciences	5	I watch science programs or documentaries on TV	0.725

To obtain more information about factors that influenced the impact of the education module, teachers of all four participating schools in the Netherlands were interviewed when they had completed the education module. The aim of this additional qualitative component was to determine possible confounders which might have influenced the difference in outcomes between the pre- and post-tests and to find out whether the teachers noticed increased knowledge or improved attitude and/or behavior among their students. Although the teacher interviews were carried out in the Netherlands only, the module was evaluated in each country. In Suriname and Indonesia, the project was evaluated with the local organizing teams but not per individual teacher. In the Indonesian and Surinamese culture hierarchy is strong and extensive evaluation uncommon. Therefore, the teachers preferred a general evaluation with the head of the school. However, we do feel these interviews were less helpful because the heads of the schools were not closely involved in the project. The Dutch teacher interviews were semi-structured and took about 30 minutes each. Questions that were asked were 'how was the contact with the coaches?' and 'what have the students learned during the project?', for example. Teachers were interviewed in their classrooms after the classes had filled out the post-test questionnaire.

Finally, user data from the website and social media were analyzed after the completion of the module to find out which supportive resources were most popular during the education module.

Outcomes

The primary outcomes in this study were knowledge, attitude and behavior and stigmata and fear. Stigmata and fear, attitude, behavior were measured on a 5-point Likert scale. All these outcomes ranged from 1 (strongly disagree) to 5 (strongly agree). Stigmata and fear were measured with two questions and mean values were calculated. The outcome 'stigmata' was used in this study to describe a negative thought regarding people with

a HIV infection. The stigma was expected to be high before the module started. By gaining knowledge the stigma could be decreased. The outcome 'fear' in this study aims to measure how afraid people are to get infected in case of a large outbreak, at the time of this study Ebola was the best example. The attitude and behavior questions were subdivided into four components by the factor analysis and the mean score per component was calculated. The first component was attitude and awareness and this component represents how aware students are of virus infections and how important they consider them. The second component was attitude and risk infection and this one shows how students evaluate the risk for getting an infection. The third component, behavior and risk infection, showed what students would do in case of a risk for infection. The fourth and last component of the factor analysis was behavior and life sciences and represented what students do to gain information about viruses and related science (Table 1). Unstandardized coefficients (B) as outcomes of the regression analysis showed which factors contribute significantly to these four attitude and behavior components and to knowledge.

The outcome knowledge represented the student's knowledge regarding infectious diseases in general and the viruses in specific that were included in the education module. Knowledge was measured in the questionnaire by means of the responses to 32 questions, which had to be answered with 'true' or 'false'. Each correct answer resulted in one point, an incorrect answer in zero points. The mean percentage of all knowledge questions that were answered correctly was calculated per group and ranged from zero to hundred per cent. Knowledge outcomes from the quiz were calculated in percentages. As a secondary endpoint, the perceptions of the students about their participation in the project were evaluated. Ten statements measured if students enjoyed working on the project and if they thought the project was informative. This was scored on a 5-point Likert scale and ranged from 1 (strongly disagree) to 5 (strongly agree).

Data management and analysis

The questionnaires were read by the open source optical mark recognition program SDAPS. Correct reading was checked manually by two different persons. All data was imported into one database and analyzed with IBM SPSS version 21. All questionnaires in which less than 90 per cent of the knowledge questions had been answered were excluded. Cases that showed a variance equal to zero in the Likert scale questions were excluded for analysis on these outcomes. Items with more than 10 per cent missing values were deleted. In all analyses $p<0.05$ was considered significant.

Descriptive analyses were performed to calculate the frequencies of students' characteristics and Pearson's chi square test was used to identify significant differences between the

characteristics of the groups in the pre- and post-test. Correlations between knowledge, attitude and behavior were calculated for all students in the intervention group, with a Pearson coefficient.

The average knowledge per country in the pre-test and post-test situations was compared by an independent sample T-test. Effect sizes were calculated with Cohen's d. Effect sizes greater than or equal to 0.30 were considered medium, and those greater than or equal to 0.50 as large (402).

Multiple linear regression analysis was used to find factors that influenced the knowledge, attitude and behavior outcomes. Time point (pre- and post-test) and participation (intervention and control group) and the interaction between these two variables were added as independent variables, as well as gender, age, education level, school and country. Tolerance values were computed to assess multicollinearity. Values below 0.2 were viewed as potentially problematic (396).

Stigmata and fear were compared between pre- and post-test with a one-way ANOVA. The sample size allowed us to calculate differences between the components of the factor analysis in pre-test and post-test per country with a one-way ANOVA test. Perceptions of the project were measured only after the module had finished. Means and standard deviation were summarized per country.

Ethics

The study was carried out in accordance with the Declaration of Helsinki. According to Dutch law, this study was exempt from medical ethical approval requirements. The Technasium Network in the Netherlands approved this study to be performed at the Dutch Technasium schools and informed the students and parents. In Suriname and Indonesia, the headmasters of the schools approved conducting the Viruskenner module and evaluations at their schools and informed the students and their parents. Participation was voluntary and anonymity was guaranteed.

RESULTS

Participants and setting

In 2015, a total of 684 (out of 738) secondary school students participated in the Viruskenner education module. Two of the participating schools in the Netherlands, dropped out (54 of a total of 260 Dutch students, representing 20.7 per cent of them) because of non-completion of the module and evaluation program. One school dropped out because the teacher got sick after the kick-off and the other school could not attend the final day because it clashed with another school activity that day (Figure 2). In Suriname, 158 students participated and there was no dropout. This was also the case in Indonesia, where all 320 students completed the education module.

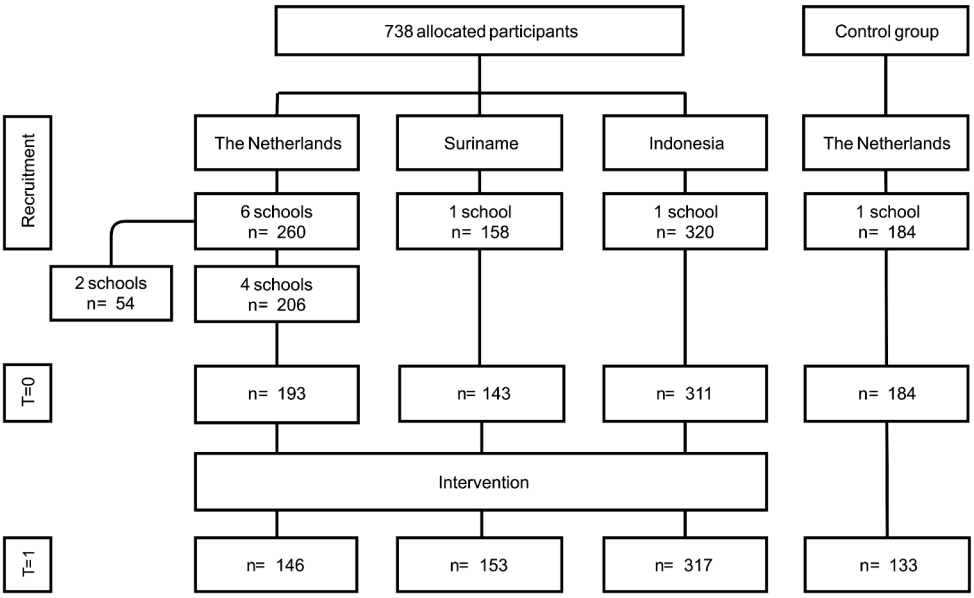

Figure 2. Study flowchart
T=0 represents the pre-test and T=1 represents the post-test after 10 weeks.

Response rates for the Netherlands were 95.6 per cent of participants for the pre-test and 70.9 per cent for the post-test. In Suriname these percentages were 90.5 and 96.8 per cent respectively and for Indonesia 97.2 and 99.1 per cent respectively. The control group had a response rate of 100 per cent for the pre-test and 73.4 per cent for the post-test.

Table 2 presents the pre- and post-test characteristics of the module participants from all three countries and the control group. In all groups, except the Surinamese group, the age category in the post-test was significantly higher than in the pre-test. However, for gender and education the characteristics did not show any significant differences between the pre- and post-test per country. In Indonesia the preference for science was significantly higher in the post-test than in the pre-test.

On average, in the Netherlands most of the participating students were male, while in Indonesia they were mostly female. Generally speaking, in the Netherlands the students from both the control group and the intervention group were significantly younger than average. The pre-test showed that on average more students attended pre-university education in the Netherlands, Suriname and in the control group. In Indonesia all students attended advanced general secondary education. In the post-test the percentage of pre-university education students in the Dutch intervention group rose to 65.3 per cent. In the control group this percentage decreased to 64.1 per cent.

In both the pre-test and post-test, the amount of participants from the intervention group in the Netherlands and Suriname that chose scientific profiles was not significantly different from the average. The control group consisted of less students with scientific profiles than average and Indonesia had more students with science-related profiles.

Table 2. Students' characteristics

	Dutch intervention group			Dutch control group			Suriname			Indonesia		
Time point	T=0	T=1	p (χ^2)	T=0	T=1	p (χ^2)	T=0	T=1	p (χ^2)	T=0	T=1	p (χ^2)
Gender (%)	193 (100)	144 (100)		182 (100)	133 (100)		142 (100)	153 (100)		308 (100)	317 (100)	
Boy	140 (72.5)	100 (69.4)	0.535	95 (52.2)	69 (51.9)	0.955	62 (43.7)	70 (45.8)	0.718	129 (41.9)	130 (41.0)	0.825
Girl	53 (27.5)	44 (30.6)		87 (47.8)	64 (48.1)		80 (56.3)	83 (54.2)		179 (58.1)	187 (59.0)	
Age (%)	193 (100)	145 (100)		183 (100)	132 (100)		143 (100)	153 (100)		311 (100)	316 (100)	
<14	-	-		2 (0.0)	3 (0.0)		-	-		-	-	
14	100 (51.8)	55 (37.9)	0.040	78 (42.6)	27 (20.5)	<0.001	1 (0.0)	0 (0.0)	0.693	4 (0.0)	2 (0.0)	<0.001
15	92 (47.7)	89 (61.4)		97 (53.0)	89 (67.4)		22 (15.4)	20 (13.1)		129 (41.5)	64 (20.3)	
16	1 (0.0)	1 (0.0)		6 (0.1)	13 (9.9)		52 (36.4)	59 (38.6)		175 (56.3)	233 (73.7)	
>16	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		68 (47.6)	74 (48.4)		3 (0.0)	17 (0.1)	
Education (%)	193 (100)	144 (100)		184 (100)	128 (100)		143 (100)	153 (100)		311 (100)	317 (100)	
HAVO	73 (37.8)	50 (34.7)	0.559	50 (27.2)	46 (35.9)	0.099	0 (0)	0 (0)	-	311 (100)	317 (100)	-
VWO	120 (62.2)	94 (65.3)		134 (72.8)	82 (64.1)		143 (100)	153 (100)		0 (0)	0 (0)	
Science	190 (100)	146 (100)		184 (100)	133 (100)		142 (100)	150 (100)		310 (100)	316 (100)	
Non-science	44 (23.2)	39 (26.7)	0.719	107 (58.2)	73 (54.9)	0.215	29 (20.4)	31 (20.7)	0.959	55 (17.7)	36 (11.4)	0.024
Science	144 (75.8)	105 (71.9)		77 (41.8)	60 (45.1)		113 (79.6)	119 (79.3)		255 (82.3)	280 (88.6)	
Don't know	2 (0.0)	2 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	

The given percentages have been calculated from the number of students for which data is available for that variable. The percentages have been rounded off to one decimal place. Pearson chi-square was used to calculate differences per country and the control group between pre- and post-test. T=0 represents the pre-test and T=1 represents the post-test after 10 weeks. Education represents the level of education, in which HAVO stands for advanced general secondary education and VWO stands for pre-university education level. Science represents the interest of the students, measured by their (preferred) choice of curriculum.

Correlations between knowledge score and attitude and behavior

Pearson's coefficients showed a positive and significant correlation between the knowledge scores and all four components regarding attitude and behavior (Table 3). Knowledge was most-strongly correlated with attitude and awareness (r=0.20). Students who scored higher on attitude and awareness also scored higher on behavior regarding risk of infection (r=0.47) and behavior regarding life sciences (r=0.51).

Table 3. Pearson correlation coefficients between knowledge, attitude and behavior

		1	2	3	4	5
1	Knowledge	1				
2	Attitude & awareness	0.20**	1			
3	Attitude & risk infection	0.07*	0.18**	1		
4	Behavior & risk infection	0.14**	0.47**	0.20**	1	
5	Behavior & life sciences	0.14**	0.51**	0.15**	0.38**	1

The correlation coefficients shown have been calculated from all values in the intervention groups at both the pre- and post-test. * $p<0.05$ ** $p<0.01$ *** $p<0.001$

Knowledge

During the project the answer to one of the 32 knowledge questions changed, due to the MERS epidemic in South-Korea. Because of the confusion surrounding this question we decided to exclude it from the analysis. Analyses per country showed differences in achieved knowledge (Figure 3), with mean knowledge increasing in all three participating countries. For Suriname and the Netherlands this increase was significant ($p<0.001$). The overall effect size (Cohen's d) for all intervention groups was 0.43, which represents a medium effect. At 0.77, the effect size for Suriname was the highest. The effect size for the Netherlands was 0.52, which also represents a large effect (402). For example, in the Netherlands the percentage of correct answers on the statement 'Dengue is a virus infection that is transmitted by a tiger mosquito' raised from 71 per cent correct in the pre-test to 90 per cent correct in the post-test (the correct answer is true). The score for 'If someone is infected with HIV this person has AIDS' raised from 76 per cent to 83 per cent (the correct answer is false because AIDS is a syndrome in which the immune system is suppressed and opportunistic infections can cause illness, which can be prevented in HIV infected individuals by taking antiretrovirals).Although in some other questions the percentage of correct answers differs only one percentage point between the pre- and post-test. The mean percentage of correct answers on a few questions declined. The mean total knowledge in the control group decreased significantly ($p=0.032$).

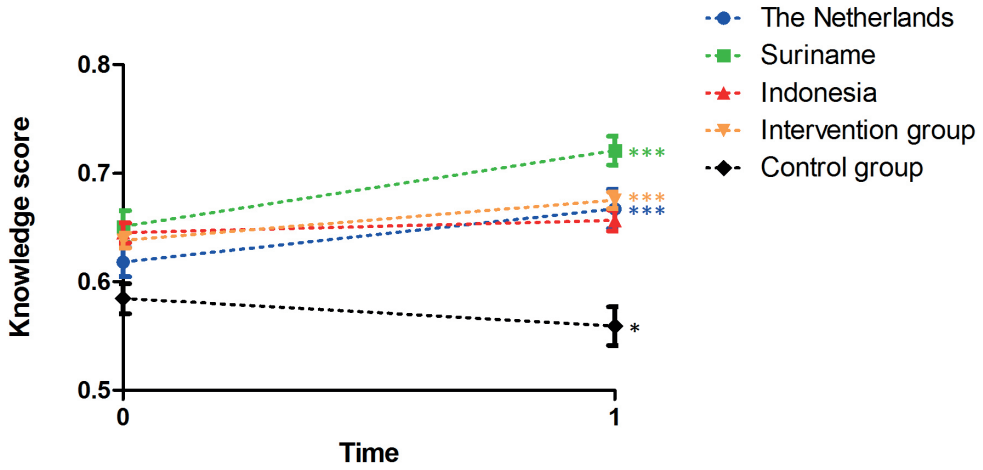

Figure 3. The impact of the Viruskenner on students' knowledge
The knowledge of the participating and non-participating students per country before and after the intervention is represented by the mean percentage of the true/false questions in the questionnaire that were answered correctly. The blue line represents the Netherlands, without the control group. The orange line represents all intervention groups, so from the Netherlands, Suriname and Indonesia. *p<0.05 **p<0.01 ***p<0.001

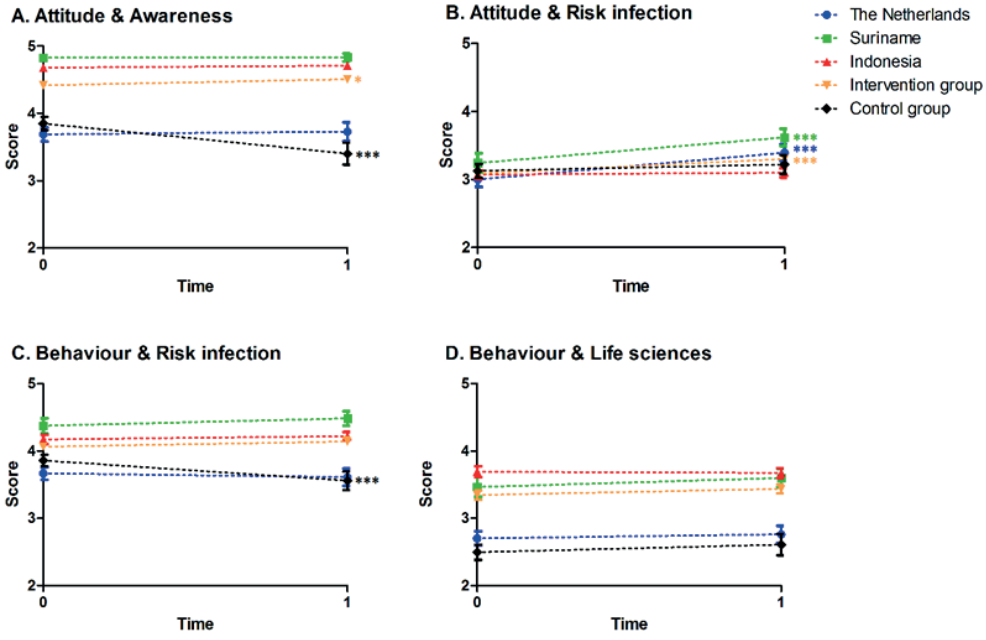

Figure 4. The impact of the education module on students' attitude and behavior
The graphs illustrate the changes per country in the four different components of the attitude and behavior questions that were answered, on a 5-point Likert scale. A higher score represents a more positive attitude or healthier behavior. Panel A shows the score for the attitude and awareness component, panel B for attitude and risk infection, panel C for behavior and risk infection and panel D for behavior and life sciences. The blue line represents the Netherlands, without the control group. The orange line represents all intervention groups, so from the Netherlands, Suriname and Indonesia. *p<0.05 **p<0.01 ***p<0.001

In the multiple regression analyses the variable participation (control group or intervention group) contributed significantly ($p<0.001$; $B=0.078$) to the knowledge outcome. The variable time point (pre- or post-test), however, did not. Most information about the impact of the module on knowledge is given by the interaction between participation and time point, which was significant ($p<0.001$; $B=0.053$). Other variables that contributed significantly to knowledge were gender, age older than 16, and the school (Table 4). The mean tolerance of all variables in the regression analyses is 0.3. Although this suggests that there is some multicollinearity between predictors, this value is no reason for concern.(396) The data from the knowledge quiz showed that the Netherlands had a mean score of 70.7 per cent, with Suriname scoring 59.6 per cent.

Table 4. Multiple regression analysis

	Knowledge	Attitude & awareness	Attitude & risk infection	Behavior & risk infection	Behavior & life sciences
	B (SE)	B (SE)	B (SE)	B (SE)	B (SE)
Participation	0.078 (0.011)***	-0.170 (0.074)*	-0.012 (0.089)	-0.037 (0.080)	0.264 (0.096)**
Time point	-0.015 (0.011)	-0.380 (0.070)***	0.099 (0.084)	-0.288 (0.076)***	0.139 (0.091)
Participation x time point	0.053 (0.012)***	0.430 (0.078)***	0.113 (0.093)	0.310 (0.084)***	-0.104 (0.101)
Girl	0.012 (0.005)*	0.148 (0.031)***	0.025 (0.037)	0.210 (0.033)***	-0.052 (0.040)
Age <14	-0.039 (0.063)	-0.004 (0.342)	0.252 (0.408)	-0.097 (0.369)	-0.099 (0.441)
Age 14	0.007 (0.007)	-0.061 (0.049)	-0.047 (0.058)	0.011 (0.052)	-0.098 (0.063)
Age 16	-0.013 (0.007)	-0.019 (0.044)	-0.035 (0.053)	0.161 (0.048)**	0.087 (0.057)
Age > 16	-0.031 (0.011)**	-0.100 (0.069)	-0.092 (0.082)	0.201 (0.074)**	-0.103 (0.089)
School 2 NL	-0.054 (0.013)***	0.225 (0.084)**	-0.091 (0.101)	-0.080 (0.091)	-0.051 (0.109)
School 3 NL	-0.055 (0.020)**	0.173 (0.130)	0.266 (0.155)	-0.127 (0.140)	0.159 (0.168)
School 4 NL	-0.052 (0.016)**	0.030 (0.105)	-0.034 (0.125)	-0.363 (0.114)**	-0.005 (0.136)
Suriname	0.021 (0.012)	1.086 (0.081)***	0.213 (0.096)*	0.421 (0.087)***	0.624 (0.104)***
Indonesia	-0.006 (0.015)	1.120 (0.070)***	-0.016 (0.115)	0.233 (0.105)*	1.135 (0.125)***
VWO	0.019 (0.011)	0.212 (0.070)**	0.153 (0.084)	0.009 (0.076)	0.430 (0.091)***

* $p<0.05$ **$p<0.01$ ***$p<0.001$
SE = standard error, B = unstandardized coefficient. Participation: The control group has a value of 0 and the participants have a value of 1. Time point: the pre-test has a value of 0 and the post-test has a value of 1. Participation x time point is the interaction between the two values. For all other variables, participants for whom that variable applies have a value of 1, and the others a 0.

Stigmata and fear

The first question regarding stigmata and fear was 'I don't want to mix with people who have HIV' and the second one was 'I am afraid that I will get infected by Ebola'. Generally speaking, the module participants' answers did not change significantly. However, the results per country showed a significant decrease in Suriname on both questions ($p=0.009$; $ES=0.31$ and $p=0.001$; $ES=0.42$ respectively), other countries showed no significant differences in separate analyses, neither did the control group.

Attitude and behavior regarding virus infections and life sciences

Figure 4 shows the changes in the four components regarding attitude and behavior. In the intervention group (all countries combined) attitude and awareness increased significantly ($p=0.028$; ES=0.12) and so did attitude and risk infection ($p<0.001$; ES=0.30). Behavior and risk infection increased, but with the chosen p-value of 0.05 the increase was on the borderline of significance ($p=0.062$; ES=0.11). Behavior and life sciences also increased with borderline significance ($p=0.060$; ES=0.10). In the control group attitude and awareness and behavior and risk infection decreased significantly ($p<0.001$; ES=0.67 and $p<0.001$; ES=0.51 respectively). Attitude and risk infection and behavior and life sciences both showed a slight, but non-significant increase.

Although attitude, and even behavior, in the intervention group seemed to increase, in the sub-analysis per country we only found a significant increase in attitude and risk infection for the Netherlands and Suriname (Figure 4).

The multiple regression analysis showed that as main effects, participation and time point both contributed significantly to attitude and awareness. We also found significant interaction between participation and time point for this outcome ($p<0.001$; B=0.430). The independent variables gender, school 2, education level and countries also contributed significantly to attitude and awareness. For the attitude and risk infection outcome only Surinamese students had higher scores ($p<0.05$; B=0.213). For behavior and risk infection the main variable participation was not significant. But time point was, and it had a negative effect ($p<0.001$; B=-0.288). The interaction between these two resulted in a significantly positive effect ($p<0.001$; B=0.310). Being older, the school and the country also contributed significantly, to behavior and risk infection. Behavior and life sciences was influenced by participation in the module, however time point had no significant effect and the interaction was not significant either. Countries and education level, however, did contribute significantly to behavior and life sciences (Table 4).

Appreciation of the project

Generally speaking, the students enjoyed participating in the education module and said that it taught them a lot about infectious diseases. The score on the statement 'I enjoyed working on the project Viruskenner' was measured on a scale from one (totally disagree) to 5 (totally agree). The mean score in the Netherlands was 3.2, in Suriname 4.37 and in Indonesia 3.93. In total 90 per cent of all students that participated gave a score of 3 or higher. Supplementary Table 5 reports how they answered the other evaluation questions.

Teacher interviews

The first and second school participated in the project for five and four years respectively, but the head teacher of the first school was involved for the first time. The project was completely new for the third and fourth schools. The first school allowed the most time for students to work on the project, six hours a week for eight weeks. The fourth school allowed five hours a week for eight weeks. The second and third schools allowed five and four hours a week respectively, for six weeks in each case. In the first three schools the students had no other lessons about viruses during the project period; only in school four did the biology teacher pay some extra attention to them. None of the teachers let the students prepare for the kick-off, but the teacher in the fourth school told them to read the manual. In the first and fourth schools the students themselves decided on the composition of the collaboration groups and chose their subject of preference. Students in the second school also decided their group composition, but straws were drawn to allocate the subjects. The teacher in the third school divided the students and subjects over the groups randomly. All teachers reported that contact with their coaches during the project was good, although it has to be said that there were some communication problems with the teachers in the second school. And while school number three's teacher said that the contact with the coach was very helpful and amicable, he added that the students got to learn more about the world of scientific research, and that this aspect might have been emphasized even more. Another remark was made by a teacher in the first school, who said the website should be promoted for learning purposes more frequently and contact with the coaches could be more intensive. All teachers responded positively to the question: 'What do you think the students learned from the module?' The teacher of the first school said he thought students are now more aware of the worldwide impact of infectious diseases. He even remarked that during the break on the final day he noticed that more students washed their hands after going to the bathroom. The teacher in the second school insisted that students are now more focused on viruses in the news, such as Ebola or MERS, and that there is a gap between knowing and doing. Finally, a teacher in the fourth school concluded that during the completion of the post-test questionnaires he got the distinct impression that the students learned a lot.

User data online resources

Although the education was mainly face to face, online supportive resources were available to increase the educational impact. The graph in S1 shows the use of the website and social media in time (393).

DISCUSSION

After adjusting for age, sex, education level, school and country, Viruskenner proved to be an effective education module for increasing the knowledge of young people in the Netherlands and Suriname of virus infections, according to this non-randomized intervention study. With all limitations of this study design taken in mind we describe a positive correlation in knowledge, attitude and behavior in the participating secondary school students. Participation had a positive effect on attitude and awareness. This effect was higher among females and higher-educated students. Knowledge, and behavior and risk infection were higher in female and older students (16+). And while the attitude components increased in the intervention group, the behavior components only showed an increasing trend. There was no significant effect of participation on attitude & risk infection, but there was on behavior and risk infection. This might be explained by the positive effect in the control group for attitude and risk infection but negative for behavior and risk infection. It might, for example, be due to there being less motivation in the control group to fill in the questionnaires. The education module had less impact on students' knowledge in Indonesia.

The somewhat limited impact on Indonesian participants could be explained by their lower level of involvement (403). All students in the Netherlands and Suriname developed a prevention tool and prepared a presentation. However, the evaluations found that in Indonesia only a selection of the students did. Additionally, in Suriname (and partly in the Netherlands) family members were invited to attend the final day. In Indonesia this was not possible due to the limited space. The engagement of families could well have had a positive effect. Another striking fact in Indonesia was the relatively high scores for attitude and awareness and behavior, in both pre- and post-tests. The same was true for Suriname, which might point to cultural differences with the Netherlands. Collectivistic countries, like Suriname and Indonesia, tend to give more socially desirable answers to questionnaires than individualistic countries like the Netherlands (404). Overall, most students of all countries enjoyed working on the project. Whereas most outcomes in the intervention group showed a positive trend or change, in the control group knowledge, attitude and awareness and behavior and risk infection decreased. These students did not differ significantly in gender, education level or profile between pre- and post-test. The decreased outcomes might be explained by reduced motivation in doing the same test twice.

As far as we know this is the first study to evaluate an education module on several viruses in several continents. The heterogeneity of the study population increases the external

validity of the study. Comparing the results of the same education module in different countries gave insights in the importance of educational factors on the impact. In each country the pre- and post-tests were compared. However, a limitation of the study is that only the Netherlands had a control group. The control group consisted of more students that had chosen a non-science curriculum than the intervention group. However, there was no significant difference in knowledge score between the non-science and science students in the control group. Although science students scored higher on attitude and awareness and behavior and life science questions.

Although the time that schools spend on the project differed, no direct relationship was found between the hours spent and the results achieved. Making the results translatable to schools that could participate to the module and would spend at least four hours per week during six weeks. Due to logistics, randomization of schools was not possible. For a maximum effect it is important to enclose the project in the curriculum, so schools were chosen by a curriculum in which it would fit, as it is in the Netherlands with Technasium. Multiple participating schools per country would have added value as it would have enabled us to perform a proper multilevel analysis, instead of a multiple linear regression analysis. Furthermore, it might be good to measure any balancing measure, for example the mean grades, to determine if there are no unanticipated harms to scores on other subjects in school, due to the time the students spent on the project. Although unanticipated harms are expected to find less when the project is totally embedded in the curriculum. The questionnaires were composed with accuracy in Dutch (the national language in the Netherlands as well as in Suriname). The ones that were used in Indonesia were translated to Bahasa Indonesia without back translation. Self-reported questionnaires are useful to measure knowledge changes. However, self-reported attitude and behavior have to be interpreted with caution. The effect was little and could even be due to overestimation. The effect of participation in the module on knowledge however was large in two out of three countries. Measuring a long-term effect in these countries as well would be of additional value.

A clear effect on knowledge, but a negligible or non-existent effect on attitude & behavior is common in educational research. Several studies pertaining to HIV or sex education show that knowledge increased after participation in an education module (376, 405, 406). We only found a few studies in which peer-education did not increase knowledge (407, 408). The literature about stigmata, awareness and attitude is inconclusive. Some studies conclude that awareness can be increased or that attitudes can be changed, while others conclude that the effects on these is limited (409-414) (407, 408, 415-417).

According to the available literature, behavior is the most difficult part to measure and to improve. Because most studies about virus education evaluate HIV prevention programs, condom use, or the intention to use condoms, is what was measured the most frequently. In self-reported questionnaires this outcome improved significantly in some studies, which is promising. However, in others it did not (376, 407, 411, 413, 418). We found two studies, both conducted in Africa, that tested behavioral change based on the prevalence of virus infections. In them, participants' blood samples were tested for HIV and HSV antibodies, before and three to eight years after an education module or compared with a control group. However, no significant differences were evident in infection rates (419, 420). So what factors play a role in making an educational intervention effective in changing behavior? We found some studies that based their intervention on the Health Promoting School Framework of the WHO proved to be successful in changing health-related behavior (421). Important elements of this framework which were applied in these studies were: implementation of the intervention in the school curriculum; involvement of the school environment in the project and involvement of family and society in the intervention (421, 422). The Viruskenner education module was implemented in the Technasium curriculum in the Netherlands, but was not part of the curriculum in Suriname and Indonesia. The family and society were involved in the project, particularly in the Netherlands and Suriname. However, stronger involvement of the school environment and ethos in prevention of infectious diseases might increase the impact of the intervention on attitude and behavior in all countries. This might be reached by additional interventions like hand washing posters in the sanitary facilities or selling machines for mosquito nets in the schoolyard for example. Knowledge that is not translated into behavior change would not make a difference in numbers of virus infections. So adjustments to the Viruskenner module are needed to have a greater impact on attitude and behavior. Active learning has the best chance of being successful if every individual student participates. Students' families have to be closer involved and a sharper focus on infection prevention in school environments is needed. Increasing knowledge is a great first step, because it correlates with attitude and behavior. However, significant improvements in attitude and behavior must be reached to have a possible impact on infection rates. Therefore, further exploration of contributing elements of education modules that reached behavioral changes would be very useful.

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Chapter 5.2

Empowering Dutch and Surinamese children to prevent viral infections: implications from an international education module

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ABSTRACT

Viral infections have a large share in human morbidity and mortality. Next to vaccinations and hygiene measures, health education plays a role in preventing infections. Social scientists argue that empowerment should be included in health education, as increasing knowledge is insufficient to achieve sustainable behaviour change. Within the international education module 'Viruskenner', primary school students learn how to prevent virus infections by identifying health risks and developing interventions. This qualitative formative study explored to what extent Viruskenner creates conditions in which empowerment processes can arise and take place in the Netherlands and Suriname. Indicators of empowerment, as defined in literature and placed in the attitude, social influence, and self-efficacy model, were assessed during semi-structured interviews (n = 24) with students, parents, teachers and facilitators. We conclude that Viruskenner is successful in creating conditions for empowerment processes to arise and take place, specifically in attitude and self-efficacy. According to interviewees the module raised students' motivation, skills and confidence to take action to improve health behaviour. Educators played a stimulating role in the participatory setting in both countries, while content relevance and community involvement differed between the Netherlands and Suriname. These outcomes could improve this module and possibly other health education programmes.

INTRODUCTION

Globally, viral infections are causing significant morbidity and mortality in humans (367). Viruses causing diarrheal diseases, like rota- and noroviruses, and respiratory diseases, like adeno- and rhinoviruses contribute to childhood morbidity (423, 424). Furthermore, new emerging infectious diseases, like Zika, Ebola and SARS-CoV-2, have shown to immensly affect public health (425). As for most infections vaccines are unavailable, health education can play an important role in prevention (426).

In this light, education module 'Viruskenner' was developed by Cirion Foundation in 2011 and is anno 2019 running at primary and secondary schools in four different countries with diverse cultures, climates and epidemiology of infectious diseases. Viruskenner's primary goal is creating awareness, increasing knowledge and changing attitudes and risk behaviours among students. Ultimately, the module, voluntary implemented into the school curriculum, aims to foster behaviour changes that contribute to the decline of morbidity and mortality due to infectious diseases.

Evaluated with the Knowledge, Attitude and Behaviour (KAB) model, the 2015 edition of Viruskenner proved to be effective in increasing knowledge about viruses and their transmission routes. In addition, potential effects on attitude were found, but no behaviour changes could be measured (427). It is known that health education interventions mainly relying on knowledge transmission, have largely failed to show significant health behaviour changes (428). Social and economic factors are often overlooked in health-related fields as behavioral medicine and health psychology (429) while these are factors that have mainly been shown to affect health indirectly. Behaviours such as smoking, diet and exercise were clearly related to employment and income levels (430, 431). Therefore popular health theories include social and economic components, such as the Sense of Coherence theory (SOC) from Antonovsky (1987) which reflects a coping capacity of people to deal with everyday life stressors and consists of three elements: comprehensibility, manageability and meaningfulness. In all three components social and economic factors are taken into account to manage good health (432). A more comprehensive theory is the Social Cognitive Theory (SCT) which posits that learning occurs in a social context with a dynamic and reciprocal interaction of the person, environment, and behaviour (433). Without taking into account social and economic factors, and the setting's situation and needs, health education programmes have been unsuccessful in reaching the desired behaviour change (428). Therefore, social and economic factors should also be included while evaluating health education programmes. Following the World Health Organization (WHO) health promotion framework (434), significant and sustainable behaviour change is realized by

including a more specific process, namely empowerment: improving and supporting people's abilities to gain control over and improve their health (24). Social and economic resources are foundations for these.

Theoretical framework

Starting from a broad perspective, empowerment has evolved into a multilevel construct and connects the well-being of individuals and groups with the broader social and political context (435-437). The connection and interaction between the various levels influence individuals within the micro level, referred to as psychological empowerment. This is classified in intrapersonal, interpersonal and behavioural elements (436, 438), which is important in the setting of an education module because students are individuals within a group (class, school, community).

Empowerment is also part of a critical paradigm. Brazilian educator Paulo Freire, who introduced 'empowerment' in the fifties, saw the concept in the view of oppressed people in that era (438). The fact that powerlessness is linked to disease raises the importance of empowerment in health promotion (439). Nowadays, Freire's critical pedagogy is particularly applied to vulnerable groups, instead of oppressed people. Still, it uses the same elements to empower people, namely dialogue, reflection, and action to be able to change one's own situation (438, 440). Participatory and dialogical approaches replace hierarchical relations between professionals and target groups, shedding a new light on the contemporary interaction between teachers and students (24, 441, 442).

From an educational perspective, Freire's theory has become important in health education, as a recently adopted goal is to engage people to become active and in control of their lives (443, 444). Health educators stimulate co-learners (e.g. students) to move from an individual responsibility to a social responsibility to act (445). The goal of social action in health education is to promote community development and change hierarchical relationships, ultimately giving people a greater voice in their community decision-making regarding health improvement (446).

Resulting from the above, it can be concluded that empowerment consists of many components that need to interact to contribute to behaviour change. Several models provide an overview of these interactions. In 1988, de Vries introduced the attitude, social influence and self-efficacy (ASE) model, which is well-known for predicting behaviour change and reflects how motivation explains behaviour (23). Also, this model fits well with the main goal of health promotion according to the WHO (24), which is - in addition to knowledge transmission - raising a positive attitude, skills and confidence needed to

take action to improve health. This makes the ASE model the most appropriate model to structure the empowering elements retrieved from the different perspectives described in the theoretical framework.

The popularity of empowerment has increased in the field of health promotion, amongst not only theoreticians and researchers but also professionals in practice (447). Evaluations of health education programmes, in for example prevention of HIV, showed that empowerment is critical in taking control over one's life and thereby improving health (439, 448). Additionally, schools are an appropriate setting to reach youth and help improve health from a young age onwards (449). Thus, when it comes to successful health promotion, facilitating empowerment in education programmes is essential to achieve substantial and sustainable health behaviour in both individuals and communities (428).

Therefore, the purpose of this qualitative research is to study to what extent Viruskenner creates conditions in which empowerment processes can arise and take place. With this in-depth analysis, we aim to find elements of empowerment that make health behaviour change possible, within Viruskenner specifically and in the field of health education generally.

Table 1. Indicators of success derived from three perspectives on empowerment

Perspectives on empowerment	Explanation of theories	Components for empowerment	Indicators of success	Classification in ASE model
Broad perspective	* Several levels of empowerment can be distinguished: the community level (macro), organizational level (meso) and the individual (micro) level (Zimmerman, 1995). * These levels are interrelated and interdependent (Jacobs, Braakman, & Houweling, 2005). * The connection and interaction between the various levels influence a person within the micro level. This is referred to as psychological empowerment classified in an intrapersonal, interpersonal and behavioural element (Zimmerman, 1995; Boumans, 2012).	1) intrapersonal: the belief in one's own capacities, confidence and the will to influence one's own personal situation	Confidence	E
			Influence personal situation	I
		2) interpersonal: the critical awareness of social opportunities, norms, tools and skills to utilize and mobilize these resources	Critical awareness of opportunities	S
			Critical awareness of skills	E
		3) behavioural: the community involvement, social participation and development of constructive behaviour to make choices and handle new situations	Community involvement	S
			Social participation	S
Critical perspective	* In critical pedagogy, empowerment is characterized by dialogue, reflection, and action in order to extend the current boundaries of one's own situation, also called praxis (Freire, 1971). * Freire's theory on empowerment is critical in enabling increased control over one's life and health (Crossley, 2001; Mohajer & Earnest, 2009; Wallerstein & Bernstein, 1988). * Boumans (2012) adds that empowerment has become associated with individuals, as it provides a guideline to strengthen one's own opportunities and self-reliance. * Boumans (2012) also argues that in welfare, a focus is needed on creating contexts in which individual and collective empowerment processes can arise and take place, instead of active interventions. This change can be achieved through interaction between the individual, social and community level.	1) praxis: the continuous interaction between reflection and action to promote change, on an individual and community level	Praxis	A
		2) the means to enable people to fulfil and shape life-domains (e.g. presenting strategies, collaboration initiatives, tools)	Means to enable people	E
		3) focus on room for dialogue and initiative-taking while providing support.	Role of educators (dialogical process)	B/S
Educational perspective	* Freire proposes a structured dialogue approach in order to achieve human liberation in which everyone should participate as a co-learner to create a jointly-understood reality (Freire, 1971). * Matthews (2014) proposes the co-learner model: all parties are co-learning partners. It is assumed that it takes time, over a long period of acting and reflecting, for members of the group to participate equally. * Educators promote their own growth while promoting growth of others (Matthews, 2014). * By promoting community development and changing power relationships, people get a greater voice in their community decision making (Matthews, 2014).	1) involving all members in identifying a problem (health risks) as well as in critical thinking on its social context	Identifying health risks	A
		2) praxis: continuous interaction between reflection and action	Praxis	A
		3) structuring of the dialogical process by educators to maintain listening, dialogue and action within the group	Role of educators (dialogical process)	B/S
		4) taking people's daily life and issues as a starting point for motivation	Daily life and issues	A
		5) promoting transformative social action should be the main goal throughout the entire programme instead of a mere activity	Promoting social action	I
		6) health educators should want to commit themselves to communities over a longer time.	Role of educators (dialogical process)	B/S

METHODS

The education module

Viruskenner is an international education module that primary and secondary schools can voluntarily add to their curriculum (often implemented in biology classes). The eight week module consists of half-day lectures followed by a research assignment. Students work together in small groups to identify health risks and to develop an intervention to prevent a virus infection. They are guided by teachers and coaches who visit the schools for evaluation sessions. The project is finalized with an interactive final day where students present their ideas and take part in a quiz (Supplementary Figure 1).

Study design

We performed a qualitative study with a deductive, directed-content analysis (DCA), using relevant theories on empowerment in health education as guidance for this formative evaluation research (450).

Study population

Two primary schools participated in the education module, one in Suriname and one in the Netherlands. The school in Suriname is one of the about 350 primary schools in the country and was selected to participate in Viruskenner as it delivers Dutch education. The school participating in the Netherlands was located in a smaller city near the capital and is one of the almost 900 primary schools in that province. We chose to evaluate the module in these primary schools, as these settings were best defined and most comparable between countries. In both countries Dutch is the first spoken language.

To get a complete overview of the project and its empowerment processes, four types of interviewees were included: students [S], parents [P] and teachers [T] from either the Netherlands [n] or Suriname [s], and facilitators of the project [F]. Firstly, five students were invited per school, , representing their class of approximately 20 students, as well as their parents and teachers. Students were randomly selected by the teachers and their parents and teachers were contacted via telephone or face-to-face. The two facilitators who played the most important roles in the Viruskenner module, were invited face-to-face as well.

Research instrument

In preparation for the interviews, we explored the concept of empowerment and distinguished a broad, critical and educational perspective. Within these perspectives, several influential theories in the field of pedagogy and health education exist. Following

DCA, we used these theories to identify key components and translated these into 'indicators of success' (Table 1). Subsequently, we structured these within the ASE model (Figure 1). For example, praxis, the continuous interaction between reflection and action to promote change, was one of the empowering elements described by Freire. This indicator of success is structured under the attitude component of the ASE model since attitude is based on a person's expectations of consequences and beliefs about a certain behaviour, and includes the corresponding evaluations. A certain overlap between the indicators could be found.

In accordance with DCA, we developed two topic lists prior to the interviews, one for students and their parents and another for teachers and facilitators, based on the defined 'indicators of success' (Supplementary Data 2). Through a discussion with the co-authors on the topic list, face-validity was confirmed (451).

Data collection

The semi-structured interviews were conducted between March and May 2017, during the mid-term of the project. Interviews were held individually in separate rooms at the schools, and had a duration of about 30 minutes each. Interviews with the facilitators were held in their work environment, after the final day in Suriname and before the final day in the Netherlands. A female researcher (KS) carried out all interviews face-to-face.

Data analysis

Interviews were recorded with a mobile voice recorder and transcribed verbatim (KS). Hereafter, the data from the interviews was coded with a-priori codes and analysed using Nvivo (KS). A thematic analysis was performed in which relevant comments on each of the indicators of success (as mentioned in Table 1) were grouped. Finally, from the grouped data a matrix was created to extract the results. We aimed to achieve an adequate level of data saturation on all topics. The merged results showed the presence of indicators of success in each subset of interviewees, complemented by quotes of participants. Original quotes were translated from Dutch to English and are listed in Supplementary Data 3.

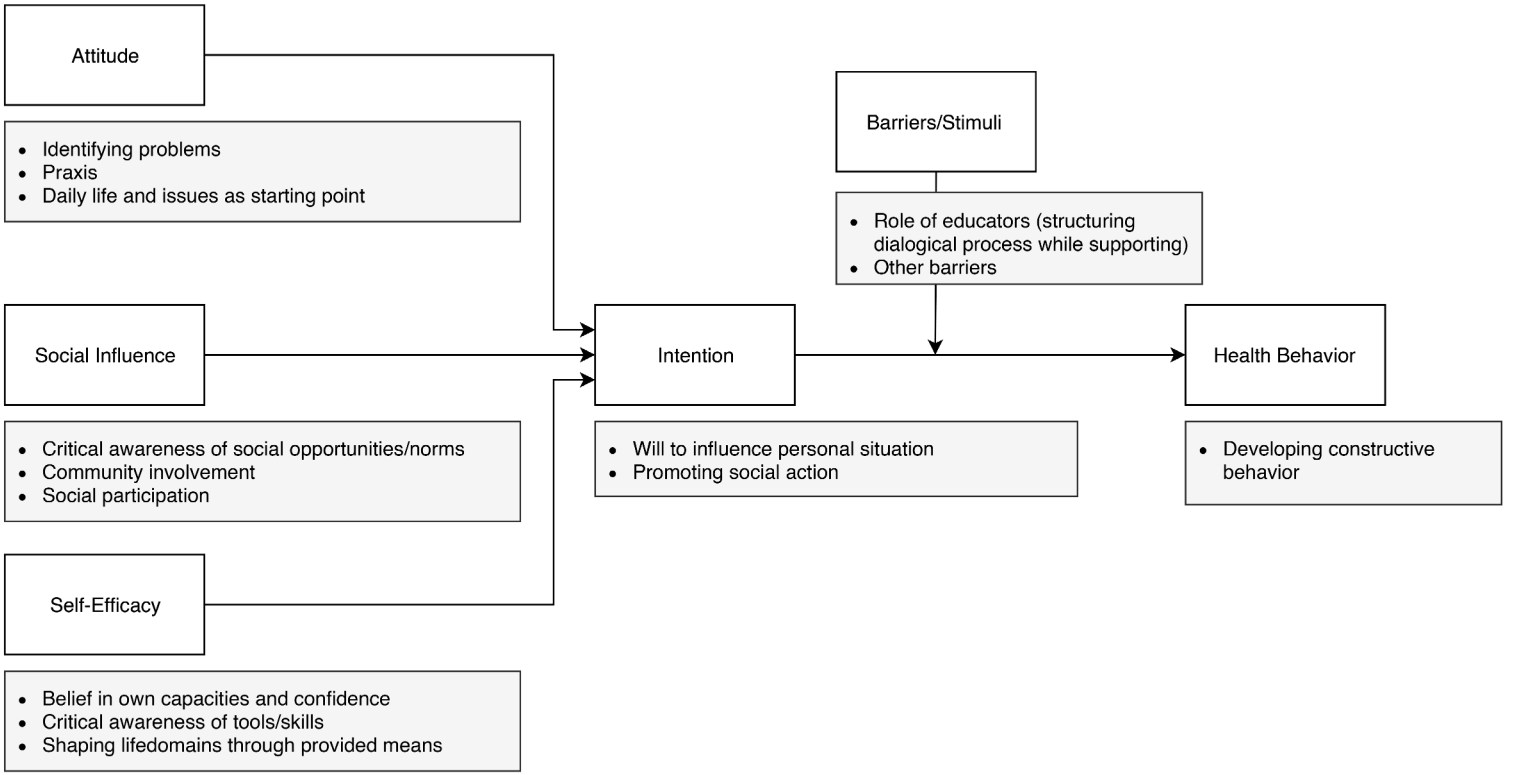

Figure 1. Indicators of success for empowerment organized within the ASE model
The white frames are the components of the ASE model. The grey frames are the indicators of success, which were formulated based on the theoretical background and literature available on the concept of empowerment.

RESULTS

In Suriname, three girls, two boys and their mothers were interviewed. In the Netherlands, two girls, three boys and three of their mothers participated in this study (two declined due to availability). From each country, two female teachers were interviewed. The two facilitators who were interviewed were male (Table 2). Data saturation was reached on most indicators of success, except barriers/stimuli on which insufficient inquiries were made due to its addition to the ASE model in a later stage (452). The presence of the indicators of success in Viruskenner, according to the interviewees, is given in an overview in Supplementary Data 4. We will discuss the indicators of success one by one, based on the structure of the ASE model.

Table 2. Baseline characteristics

Respondent	Suriname, yrs	Gender	Respondent	The Netherlands, yrs	Gender
1	Student, 12	F	1	Student, 11	M
2	Student, 12	F	2	Student, 12	M
3	Student, 12	M	3	Student, 11	M
4	Student, 11	F	4	Student, 12	F
5	Student, 12	M	5	Student, 11	F
1	Parent (girl, 12)	F	1	Parent (boy 1, 11)	F
2	Parent (girl, 12)	F	2	Parent (boy, 12)	F
3	Parent (boy, 12)	F	3	Parent (girl, 12)	F
4	Parent (girl, 11)	F			
5	Parent (boy, 12)	F			
1	Teacher	F	1	Teacher	F
2	Teacher	F	2	Teacher	F
1	Facilitator*	M	2	Facilitator*	M

In the results section, all respondents are indicated with their abovementioned number and [n] for the Netherlands and [s] for Suriname. Students are referred to with S, parents with P, teachers with T and facilitators with F. * Facilitators organized the project in both countries.

Attitude

Students involved in identifying health risks

In the Netherlands, all interviewees agreed that students played a role in identifying health risks and how they preferred to approach and fulfil the assignment. Students reported that besides being assigned to a group and a certain virus, they were free to choose how to research the virus, how to report and prevent the health risk they identified, and how to present this solution. This was consistent with the experience of both teachers and

parents. In Suriname, students, parents, and teachers indicated that the approach to report was more fixed. A student stated:

"We got four chapters from our teacher and then we could think of two extra chapters (…), namely 'the symptoms' and 'what is a virus'." [Ss1]

Facilitators in both countries implemented the same assignment; however, results showed that the teachers played different roles in the implementation. A facilitator elaborated:

"They (students) tend to follow what teachers or coaches have given as examples. But in theory, they have total freedom in which direction they go with their prevention tools, (…) or maybe a role play, or posters or drawings." [F1]

Praxis

Students and teachers in both countries strongly agreed that the coach evaluation session was helpful and gave depth to the project and the students' learning process. Because of this perceived positive outcome, all teachers and facilitators urged for more in-depth reflection moments during the project. A teacher suggested:

"I truly saw what my children got out of it, especially because it was not us who told them how to do it, but because an expert is in the class room and stops at every group to provide feedback. It would make such a difference if that would happen again." [Tn1]

Student's daily life and issues as a starting point

Most interviewees in the Netherlands reported a discrepancy between the topics and the students' daily life and issues. A student elaborated:

"Well, I only knew HIV at first (...) Other than that not many viruses occur here, it's not that dangerous, but it is in other countries where they have Zika and dengue." [Sn5]

This was different for Suriname as all interviewees recognized that certain infections are more common in their environment (e.g. dengue, Zika), even though there was a sense of taboo surrounding some of the virus infections. One of the facilitators elaborated on the visit of an HIV-infected woman who answered questions during a group presentation, and how this made a major impact on the fellow students and everyone else present. The students emphasized that it is important to break taboos around HIV, as the infection can be prevented and treated, while patients are often not compliant due to taboos. A facilitator stated:

"(…) and that was beautiful because there you saw children of 12 breaking through all taboos. And they also dared to mention, use words, which make you think, yes that's what it's called and that's how it is." [F2]

Social influence

Critical awareness of social opportunities and norms

All students but two agreed that they were stimulated to consider other people's positions and norms on the group work, the problem definition, and suggested solutions. This was confirmed by parents and teachers in both countries. Facilitators mentioned that due to the participatory format, students were placed in a position to consider social norms and negotiate. A parent explained:

"They had to talk about it (the assigned virus) in class and present it. And then she came home one day and she and her father also talked about it which had never happened before, that they talked about such a topic as hepatitis." [Ps3]

This corresponded with a situation in the Netherlands where a group faced challenges in their collaboration. A student explained:

"I didn't know at first what to do and how to state it. I didn't really want to but then I saw the others presenting and they (my group) helped with how to say it and I just presented in front of the class." [Sn2]

Moreover, interviewees reported students as a group became more enthusiastic about the topic and started to see the relevance.

Community involvement

Students, parents, teachers and facilitators in both countries reported limited community involvement. Besides the closely involved school and family, students only sometimes mentioned Viruskenner to other family members and/or friends. Furthermore, students, parents and teachers in Suriname reported that parents or other family members sometimes helped out with the project, but they did not attend Viruskenner activities in school. This was the other way around for the Netherlands: students and parents reported that family was not involved throughout the project but that they did attend the final day. Still, facilitators recognized the importance of community involvement:

"I think it's so important to involve people from all fields, who are evenly enthusiastic and believe in it. Because that's what supports the project (…) that you see and serve the same relevance and purpose." [F2]

Social participation

All interviewees in both countries experienced that social participation was stimulated in the project, although one student experienced some difficulties working in a group. A student stated:

"It was fun because you got to know each other better, that's why I became such good friends with her (fellow group member). We already spoke to each other but after the assignment we really became closer." [Ss4]

Self-efficacy

Belief in one's own capacities and confidence

All interviewees in the Netherlands and Suriname reported that students felt they gained more confidence throughout the project, not in the least because they had to develop certain skills to finish the project successfully. For example, a student said the following about her research skills:

"I've become better at writing reports. (…) I learned a lot from her, she helped me to look things up in an easier way, on websites. So I looked up more information, so I know more about the virus now." [Sn3]

Critical awareness of tools and skills

Interviewees agreed that students developed important skills. Students recognized they learned to collaborate and give presentations. Parents, teachers and facilitators added research skills, critical thinking and problem solving onto this. They mainly attributed this to the participatory format of Viruskenner. A facilitator explained:

"The impact is big, especially because you make the kids the experts. You can do it in a passive way and say 'watch out, don't do this'. That is mainly negative, but in this case they can do something themselves to prevent something. As I said, they become experts." [F2]

In line with this, the other facilitator elaborated:

"They got that information at the kick-off and have worked on it for six weeks, and then had to present it themselves on the final day. I think what we emphasize, the route of transmission and how you can stop it – which is not that hard to understand – that knowledge (about the assigned virus) is remembered the groups." [F1]

Provided means to gain control

Most students and all parents in both countries reported that students had been provided with sufficient tools, ideas and support to complete the assignment in an autonomous way. Teachers and facilitators agreed that all students used adequate and sometimes surprising strategies to let the project succeed. A facilitator gave an example:

"One way or another, probably with the help of teachers and maybe also parents, they managed to give the perfect presentation. Where besides content, it was also the context, by involving a patient and they did that very well." [F2]

Intention

When abovementioned attitude, social influence and self-efficacy are positively aligned with each other towards the desired behaviour, this can develop an intention to behave in that way.

Will to influence own personal situation

Most interviewees in both countries mentioned it is difficult to predict whether students would willingly change their personal situation. All students reported they would put certain information into practice to a limited extent. A student explained:

"Yes it's important because now I know it's a disease on the liver, and I didn't know it was a liver disease, so I have to be careful with my liver." [Ss5]

In accordance, a teacher expected:

"(…) washing hands, they will do that, they already do that. But for the children whose parents are traveling to, you know, South America, or they are coming along, they will remember and tell their parents, 'oh be careful, we have to do something about Zika, or dengue'." [Tn1]

Promote social action

Whether the prevention tools of students would promote social action in the future, was reported in terms of hopes and wishes of the stakeholder. A teacher explained her hope:

"So they know, hey, I was the one doing that and they did something with it in the adult world (…) if the ideas of these children are used in a playful way, you might be able to reach more children. You reach adults often via the message of children." [Ts1]

Accordingly, parents also expressed their hopes the students would spread gained information:

"Other than that, I expect they will do a couple of things for prevention. HIV, hantavirus. Norovirus for sure, by washing your hands and that kind of stuff. It seems simple but it is of great importance. Yes, I hope they tell that to others, that they spread the word." [Pn3]

Barriers/Stimuli

Stimulating role of educators

All interviewees reported that educators (both teachers and facilitators) established an equal basis with the students as well as stimulate the dialogical process and initiatives from the students. A teacher stated the following about how she structured the dialogical process within a group, maintaining an equal level with the students:

"There was one group in my class that didn't perform that well because one boy didn't do that much. I called them together to talk about this (…) Soon it became clear he didn't know what to do exactly and the others said 'oh but we know what you can do.' And I didn't have to get involved at all which was great." [Tn2]

When asked about their willingness to commit to Viruskenner over a longer period of time, Dutch teachers were hesitant because of their heavy workload. They were willing to organize a reflective moment for the future students to look back and learn from the achievements of the previous Viruskenner students. Both teachers in Suriname were willing to be involved in Viruskenner for a longer period of time and could help to roll out the project within their country. A teacher suggested:

"If I take a Suriname (regulated) school as an example, there you have 30 children in one class room, so you can create more groups, or expand the groups, or deal with more viruses. Oh yes, for sure, if you would want to appoint me to bring this to schools in Suriname, I would do that with pleasure. No I'm serious, I'm serious!" [Ts2]

Other barriers

Especially in Suriname, students, parents, and teachers agreed that the extent of the project put pressure on the families of participating students. They all mentioned that finding a location to work together was challenging, as they live far away from each other.

A teacher elaborated:

"A few parents sent me a WhatsApp so often. 'Miss, do they have to stay at school again today?' 'Yes dear, they have to stay, and thanks for your flexibility to pick them up later'." [Ts2]

Both facilitators explained that the assignment might be in need of more refinement for a primary school setting, as it was originally designed for secondary schools. They insisted that for the younger students, it is important to keep the project playful and that nonetheless, their projects and presentations this year exceeded expectations as usual. A more in-depth evaluation of possible other barriers could not be found.

DISCUSSION

Viruskenner is successful in creating conditions in which empowerment processes can arise and take place. Interviewees greatly appreciated the continuous reflection elements in the project – representing the attitude component of the ASE model – and even urged for more. Social influence in the education module appeared to be limited to family and school. Community involvement was less present. Concerning self-efficacy, interviewees agreed that Viruskenner helped students to gain more confidence, develop important skills and complete the assignment with their student group. On intention towards desired behaviour, limited but hopeful expectations were reported. Furthermore, we conclude that educators play a key role in empowerment by establishing an equal basis with the students and stimulating a dialogue.

Praxis was mainly present during the evaluation sessions, when coaches met with each student group and asked questions about their work to motivate them and support self-

reflection. The fact that these moments were highly appreciated by interviewees and that they demanded more moments of reflection underlines the importance of the presence of the attitude component in the education module. A qualitative study that evaluated the presence of empowering elements in an education module regarding healthy lifestyle, also underscored self-reflection as an activity to enable active learning in groups (453). However, how continuous reflection on the praxis is performed varies considerably. Guidance and supervision are key contributors to reflection (454). This implicates that involving interactive, reflective practices in education modules, like evaluation sessions with coaches, is relevant to reach learning objectives. It follows that educators play an important role in guiding the reflective process, as will be discussed later.

Another element of the attitude component that was considered relevant was taking the daily life of students as a starting point. Here, we found striking differences between the Netherlands and Suriname. In Suriname, it became clear there was more relatability of the students, parents and teachers to the topics of Viruskenner, as the viruses covered in the 2017 edition were more prevalent in Suriname than in the Netherlands. This is important as content relevance is associated with the development of emotions and ultimately increased motivation (446, 455). Health-related interventions that aim to empower people could benefit taking people's daily life into account in their design and implementation.

Regarding social influence, two important findings should be elaborated on. Firstly, critical awareness of social opportunities and norms and social participation were present in Viruskenner. The fact that all students had become more enthusiastic about the topic 'viruses' illustrates how important the social norm is. Yet, in light of the second remarkable finding that community involvement did not extend beyond the micro level, it becomes clear that the social norm is especially strong within the school context, as here most social participation took place. Following the Health Promotion School's framework, involving the community in health education programmes is important to stimulate the desired behaviour and shift from individual empowerment to community empowerment (445, 456). In a randomized trial in Tanzania, researchers found a significant reduction of HIV incidence in female sex workers that participated in a community-based HIV prevention project (457). It follows here that future editions of Viruskenner and other health-related interventions – aimed at aiding people to become empowered – should take into account the meso level, e.g. the households, neighbours and the media, in order to strengthen the social norm and participation and increase the chance of success.

Exploring self-efficacy, interviewees mentioned that during the project students developed confidence, autonomy and participative skills, such as presenting, collaborating and problem-solving. These skills seem to fit well with the so-called '21st century skills' that have recently become important in innovative learning (458). In line with the literature on self-efficacy, confidence, autonomy and participative skills are important in achieving psychological empowerment (23, 436). In Kenya, a participatory setting was evaluated in student-teachers that taught health education. In this 'participatory action research' project they found that the participatory setup offers opportunities for educational development, even in a somewhat disempowering hierarchical context (459). One could conclude this could happen even more so in an empowering context such as Viruskenner. This gives support for other (health-related) interventions to transform into a participatory setting to become more successful.

Educators were found to establish a more equal basis with the students as well as to structure the dialogical processes during the project, which is in line with Matthews (2014). It appears that educators play a stimulating role in creating the participatory setting. Teacher variables such as non-directivity, and encouraging critical thinking and learning are found te be above-average correlated with self-esteem, participation and positive motivation in students (460). Since the role of educators is of great importance for the participatory setting, experienced and qualified educators seem to be essential for the success of projects like Viruskenner. In our findings, it became clear that the stimulating role of educators cannot be seen as a separate element of the model because it influences all three ASE elements, as they guide reflection moments, help create a participatory setting and could play a role in involving the community.

We performed a qualitative study which builds on the results of the earlier published quantitative evaluation of this health education project (427). It provides an in-depth analysis on the experience of four kinds of interviewees from two different settings. Furthermore, the approach to empowerment from a broad theoretical framework, responds to the complex nature of empowerment as a concept. Even though empowerment is rather difficult to evaluate, it can be demonstrated as an important promotor of health (443). Quantitative studies could benefit from the structured ASE model to evaluate empowerment by taking surveys at different time points and on a larger scale.

The interviews and analysis have been conducted by an individual researcher which may affect the internal validity of the research. Yet, since peer feedback was given on the topic lists, it can be expected that this will not have a major impact on the results. For future studies, investigator triangulation can be applied by involving multiple researchers in the

study to strengthen the internal validity (451).

Additionally, Viruskenner is a school project which targets primary and secondary school students. In this research we focused on primary school students, which make the results generalizable to participating students from primary schools. Outcomes still need to be studied for secondary school students.

The education module Viruskenner was developed from practice. With the insights from Doornekamp et al. (427) and this study, it came to light that some adjustments of the programme could increase the impact on health behaviour. The reflection sessions with teachers and coaches should be expanded, community involvement should be promoted, and the content of the module needs to be relevant for the context in which the module is running.

In conclusion, Viruskenner is successful in creating the conditions needed for empowerment processes to arise and take place. Guided and interactive reflection, a participatory setting and community involvement appeared to be the most important empowering elements in this education module. Educators played a key role in stimulating students' empowerment. These elements should be taken into account in the development and design of future editions of Viruskenner and other health promotion programmes. This may contribute to improving health behaviour with regards to prevention of virus infections.

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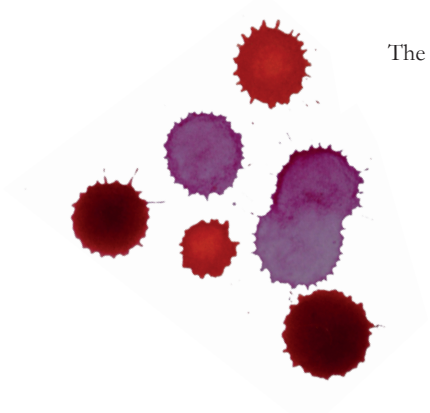
CHAPTER 6

Summarizing Discussion



Partly based on:

The fight against Ebola – lessons learned from the epidemic in Western Africa.
L. Doornekamp, E.C.M. van Gorp, M.P.G. Koopmans
Tijdschrift voor Infectieziekten, 2019

Combatting the Ebola outbreak in DR Congo anno 2019:
the role of available vaccinations and treatments.
L. Doornekamp, E.C.M. van Gorp, M.P.G. Koopmans
Nederlands Tijdschrift voor Medische Microbiologie, 2019

Vaccinations have had a significant contribution to the enormous decline of infectious diseases. Still, the world's hope for many major infectious diseases like HIV, tuberculosis, and malaria is pinned on vaccines. Also during outbreaks of novel infections all eyes are on vaccine developers, like we have seen during the severe acute respiratory syndrome coronavirus (SARS-CoV-2) pandemic. Novel vaccines are continuously developed using modern technologies and new scientific insights. However, there is little chance that vaccines will be available for all viruses that may threaten human's health worldwide, due to properties of individual viruses, absence of appropriate animals models, ethical dilemmas in trials and economic reasons. Therefore, it is important to focus on broader prevention strategies and include strategies to target health behaviour. This is also of importance when vaccines are in place, as recent years incidences of VPD were rising (461). During outbreaks of VPD, 59 to 93 percent of the individuals infected are estimated to be intentionally unvaccinated (5). Therefore, it is important to not only study vaccine efficacy, but also determinants that play a role in vaccination uptake and other behavioural determinants.

To improve the prevention and control of virus infections in risk populations, it is necessary to clarify the gaps in protection. Within this thesis we investigated how well three selected risk populations, namely travellers, ICP and HCW, are protected for VPD and which determinants play a role herein. The overarching aim was to provide information needed to intervene in the right places to reach optimal protection against virus infections in these risk populations.

Main findings

After the general introduction, **Chapter 1.2** gives a comprehensive overview of the socio-behavioural determinants that affect vaccination uptake in travellers, ICP and HCW. In travellers, a low perceived risk of infection and little awareness of vaccination recommendations are the most frequently reported explanations for suboptimal vaccination uptake. Also ICP have limited awareness of vaccination indications. This partially explains why their treating physicians have that much impact with their vaccination recommendations. ICP have a high perceived risk of the consequences of vaccination, mostly due to fear for possible deterioration of their underlying illness. HCW may provide education and take away ICP's concerns about side effects of recommended vaccines. When HCW make their own vaccination decisions, perceived risk of the severity of infection and the perceived benefits of vaccination play a key role. This study underlined that knowledge is not a single determinant, neither the most important factor in changing behaviour. Many other cognitive determinants have to be taken into account when aiming for higher vaccination uptake rates. Of specific interest is the finding that these

determinants have proven to be subgroup specific.

Chapter 2 focusses on the first defined risk group: "Travellers". This chapter is based on the results of the DiVeST study, a cross-sectional study started in 2015 to get an idea of the compliance of travelling families to recommended vaccinations in current travel health guidelines. Secondary schools throughout the Netherlands were visited to finally collect questionnaires, copies of vaccination records and blood from 246 travellers (both students and their parents). Seroprevalence rates of two VPD were determined in the collected DBS. First, **Chapter 2.1** presents the results of adherence to travel health guidelines represented by the compliance to hepatitis A vaccination. The questionnaires revealed that only 36 percent of all travellers visiting a region for which a HAV vaccination was recommended received pre-travel advice. In the available vaccination records, only 41 percent had proof of valid HAV vaccination. The dry blood spots confirmed the lack of protection in a large part of the cohort, by showing that only two-thirds of all travellers visiting a region for which HAV vaccination was recommended were seroprotected. Higher rates of non-adherence were found in paediatric travellers, in travellers who were visiting friends and relatives and in those travelling a short distance (<5000km). Likely explanations here are limited awareness for the risk of infectious diseases at destinations nearby, as also found in Chapter 1.2.

In the same travellers cohort measles seroprotection rates were studied. Measles vaccine is included in the Dutch NIP but evidence of effective protection is getting more important due to the increasing incidence of measles in recent years. **Chapter 2.2** showed a measles seroprevalence of 82 percent among Dutch travelling families, which was higher than the seroprevalence for hepatitis A, but lower than expected, as measles vaccination is included in the NIP. Also here, children (12-18 years old in this study) were at additional risk with only 72 percent being seroprotected. While the travellers born between 1965 and 1975 who are generally considered at risk as they could have fallen between two stools (being born after measles was endemic in the Netherlands and before the introduction of measles vaccine in the NIP), had a higher seroprevalence of 89 percent. As 97 percent of the study cohort reported to be vaccinated according to the NIP, we first excluded that the low seroprevalence was explained by a low sensitivity of the ELISA that was used. Therefore, a subset of the seronegative samples was retested with a virus neutralization assay. Eighty-five percent of these seronegative samples remained negative. Another explanation of the low seroprevalence in younger travellers could be waning immunity. We did not measure T-cell immunity while it has been observed that measles specific humoral and cellular immunity do not correlate and that vaccinated seronegative subjects can be still protected from measles (462). Consequently, we discussed if better correlates

of protection can be determined. Measuring T-cells solely may give lack of information. Moreover, memory T-cells may reside in lymphoid tissues, complicating demonstration of immunity in peripheral blood components (463).

In **Chapter 3**, the vaccination status of HCW was assessed and suggestion to improve their protection are discussed. A widely recommended vaccination for HCW is hepatitis B, a blood borne virus. Determination of the antibody titre post-vaccination is widely recommended in HCW, to detect and follow-up non-responders. In **Chapter 3.1** we first evaluated the hepatitis B vaccination policy in future HCW in a large university centre in the Netherlands and secondly investigated sustained immunity after prior vaccination. Maintaining the policy to provide a full three doses series of Engerix® with a titre determination a month thereafter and a booster vaccination prior to the titre determination if the last vaccination was more than 3 months ago, we found that only 1.3 percent of the 2922 future HCW had an anti-HBs level ≤10 IE/L. Of these 34 non-responders, 2 (6%) turned out to be chronic HBV carriers (HBsAg and anti-HBc positive). Of the 24 students that were provided a second full series of HBV vaccinations, 12.5 percent still did not respond, but 2 out of 3 did after one extra shot with a hepatitis B vaccination with an additional adjuvans (Fendrix®). Adopting a new policy, we found out that an estimated 23 percent (n=80) of new students were already fully vaccinated for hepatitis B (4-262 months ago). At first presentation blood was collected from these students (without providing a booster) showing that 80 percent still had sufficient anti-HBs levels. This small follow-up study suggests that only 20 percent of students who were previously vaccinated do require a booster. This provides new insights for vaccination policies in many hospitals, limiting unnecessary booster vaccinations and redundant vaccination clinics visits. Unfortunately, these results cannot be extended to the period after 2029, when medical students who were HBV vaccinated in the NIP will present as medical students. The average time elapsed between the last vaccination and blood collection in our cohort was 7 years, while literature showed that if this time period expands to 18 years, only 32-37 percent still has protective antibody levels for HBV (254, 464).

A less frequently recommended but very important occupational hazard for specific HCW, like certain laboratory workers, is rabies. As rabies is a nearly 100 percent fatal disease it is very important to assure protection to HCW that are exposed to this virus. Guidelines recommend determination of rabies virus neutralizing antibodies (RVNA) after primary vaccination series to detect non-responders and after a certain period of time to detect waning immunity. **Chapter 3.2** shows that reliable detection of human rabies virus neutralizing antibodies is possible on DBS eluates. As rabies is mainly prevalent in remote areas, DBS can provide a solution to check if HCW at risk are effectively vaccinated. We

used the gold standard fluorescent antibody virus neutralization test (FAVNt) to compare RVNA levels in DBS eluates and serum. The FAVNt showed a sensitivity and specificity of respectively 97 and 92 percent. When adjusting the cut-off of RVNA levels on DBS to 0.6 IU/ml instead of the 0.5 IU/ml in serum recommended by the WHO, the specificity even increases to 100 percent while the sensitivity slightly decreases and becomes 95 percent. This makes DBS a very valuable tool to check the immunity of medical and nursing staff, laboratory workers and veterinarians that are occupationally exposed to rabies. As their exposure is higher and can go unnoticed, confirmed protection is desirable and prevents the application of expensive immunoglobulins post-exposition. Moreover, this collection method for rabies serology may be used for evaluation of (novel) vaccination strategies. Secondly, we also compared antibody levels in DBS eluates with an easier to perform ELISA (Platelia-II ELISA) with RVNA levels in serum-FAVNt, resulting in a sensitivity and specificity of respectively 87 and 96 percent. With the lower sensitivity, the Platelia-II ELISA does not have the preference, as this may result in unnecessary administration of booster vaccinations, while the shortage and expenses of rabies vaccination programmes are among the limiting factors in the control of this disease (465).

In **Chapter 4** we shift the focus from HCW to ICP. **Chapter 4.1** presents the results from a mixed-methods study among Dutch HCW to get insight in their opinions on prevention of infectious diseases in ICP. Eighty percent of HCW that participated in the survey agreed that there are opportunities to further enhance prevention of infections in ICP. Education was chosen as the most promising tool by 40 percent, while only 14 percent of HCW selected vaccinations. Although not further investigated in this study, we consider the fact that education can address behaviour that may prevent distinct infections, while vaccination may only prevent a few, as a likely explanation for this. On the other hand, three quarter of HCW indicated that they experience barriers to provide vaccinations to their ICP, dealing with timing (to start immunosuppression), logistics and finances, which can also withhold them from choosing vaccinations as promising method to prevent infections. Also, HCW's attitudes to vaccination were not thoroughly studied here, but can play a role (108). However, 70 percent of HCW reported that influenza vaccination is discussed during consultations, which is the highest rate of all vaccines that were questioned.

As we learned from Chapter 2.1, the information provision or recommendations of HCW regarding vaccination have the highest impact on vaccination uptake in ICP. Of all specific vaccines recommended in ICP apart of the NIP, the uptake of influenza is reported highest (466). However, questions remain regarding the efficacy of the seasonal inactivated influenza vaccine in ICP. In **Chapter 4.2** the influenza vaccination response

is broadly studied in 15 Crohn's disease (CD) patients treated with the anti-IL-12/IL-23 agent ustekinumab. As both the humoral and cellular immune response post-vaccination were unimpaired compared to the 20 healthy controls included, we concluded that ustekinumab-treated vaccinees are well-protected against influenza. Also here, we did not find a correlation between the strength of the B- and T-cell responses. We even found that humoral responses in CD patients treated with adalimumab were impaired, while influenza specific $CD4^+$ and $CD8^+$ T-cell responses were intact. So, one could say, even if protective antibodies cannot be demonstrated, vaccinations might still reach their goal.

Finally, **Chapter 5** sheds a light on the role of education in prevention of infectious diseases. **Chapter 5.1** presents an evaluation of the knowledge, attitudes and behaviour acquired by participation in a multinational, secondary school education module ("Viruskenner") with a focus on prevention of virus infections. The results of this non-randomized intervention study showed an increase in knowledge after participation in this module, which was significant in two of the three participating countries. Attitudes also slightly but significantly increased with participation in the module (attitude and awareness: $p = 0.02$, ES = 0.12; and attitude and risk infection $p < 0.001$, ES = 0.30). Students who scored higher on attitude and awareness also scored higher on behaviour regarding risk of infection ($r = 0.47$). So although a direct effect on behaviour was not found, an association between knowledge, attitude, awareness, risk perception and behaviour was shown.

The same education module was performed in primary schools in the Netherlands and Suriname and we evaluated which components of the ASE model contribute to the success of this module. **Chapter 5.2** shows that empowerment processes arise and take place during participation. Students and their parents and teachers reported that participation raised motivation, skills and confidence to take action to improve health behaviour. Educators played a stimulating role in the participatory setting. Content relevance and community involvement were found to be important and should be improved. Involvement of the social context is also discussed in Chapter 5.1. Social involvement is also part of the Health Promoting School Framework of the WHO and a component of the many health behavioural models that exist, like 'Social Influence' in the ASE model. The studies in Chapter 5 underlined the importance of this component in interventions.

New insights

In this thesis, we elaborated on seroprevalence rates of various VPD in risk groups, discussed components that play a role in vaccination uptake and efficacy and evaluated existing policies and educational interventions to prevent infectious diseases in risk

groups. Based on these new insights, we state the following:

- Uptake of recommended vaccinations in travellers is suboptimal and may pose threats to public health in home countries upon their return (Chapter 2.1 & 2.2).
- Increasing knowledge is not sufficient to change health behaviour like vaccination uptake (Chapter 1.2 & 5.1 & 5.2).
- Information provision can help to increase awareness of the indication for vaccination in risk groups, however attitudes, perceived risks (of infections and vaccinations) and social influence should be addressed complementary (Chapter 1.2 & 4.1 & 5.1 & 5.2).
- Barriers for HCW to discuss vaccinations in ICP should be alleviated, as information provision and recommendations of HCW have a large impact on ICP's vaccination uptake (Chapter 1.2 & 4.1)
- DBS is a useful alternative sampling tool to collect serum for rabies serology, and should be further investigated with the gold standards of other VPD (Chapter 2.1 & 2.2 & 3.1).
- However the presence of (virus neutralizing) antibodies above a certain level generally implies protection against an infection, the absence of these antibodies do not exclude immunity (Chapter 2.2 & 3.2 & 4.2)
- New diagnostic methods to assess levels of protection for VPD including both humoral and cellular immunity are desirable, although challenging to develop (Chapter 2.2 & 3.2 & 4.2).
- Vaccine efficacy of indicated vaccinations should be investigated in ICP with new immunosuppressive therapies. If humoral responses seem impaired, the role of T-cell immunity should be studied as well (Chapter 4.2).

Further developments and recommendations for practice

To improve the prevention and control of infectious diseases in risk groups, the availability of a vaccine is not sufficient. Vaccines have to be safe and effective in a high proportion of its target groups. By preference, they prime both B- and T-cells and provide long-lasting immunity which can be measured. Furthermore, vaccines must be accessible for risk groups. Practical issues like distribution, costs and timing can impede this. Moreover, even if vaccines meet all of these requirements, risk groups still need to be aware, willing and able to receive them.

The WHO developed a Research & Development (R&D) blueprint for emerging infections, in response to the Ebola epidemic in West-Africa in 2013-2016 (467). That blueprint exists of three pillars: (1) improvement of the coordination of the outbreak response;

(2) accelerating scientific research in vaccines and treatment for infectious diseases; and (3) the development of norms and standards for clinical trials and data sharing. Within the aims of the second pillar, the WHO has designed 'roadmaps' and 'target product profiles (TPP)'. Roadmaps comprise short overviews of the knowledge gaps regarding those infectious diseases and TPP describe the requirements for the development of future diagnostic methods, vaccines and therapies. These lists of requirements differentiate between vaccines for reactive and for prophylactic use and describe desired characteristics like the vaccination schedule, the duration of protection and the acceptance regarding adverse events (467). These characteristics are borne in mind with the development and production of new vaccines. With recent technologies, novel vaccine types become available, like vector-based, DNA, RNA and virus-like particles (VLP) vaccines, which are all employed for a SARS-CoV-2 vaccine. Recombinant vaccines using viral vectors, either replicating or non-replicating, show promising results. Vectors that are being used for these strategies are for instance modified vaccinia virus Ankara (MVA) and adenoviruses (Ad). A viral vector vaccine already on the market is the rVSV-ZEBOV-GP vaccine, a recombinant vesicular stomatitis virus that express a glycoprotein (GP) of Zaire Ebola virus (468). This vaccine played an important role in combatting recent Ebola outbreaks in DR Congo (467). Novel vaccine technologies may not only provide potential for fast development of effective vaccines in outbreak settings, but also new possibilities to develop safe and effective vaccines for ICP, like the non-replicating viral vectors inducing both a humoral and cellular immune response.

However, caution is still advised with the introduction of novel vaccines on the market. The way the H1N1 vaccination was introduced as part of the outbreak response during the influenza pandemic in 2009, might have caused lack of trust in the vaccine and in institutions that may have contributed to a wave of vaccine hesitancy thereafter (469). As was said by the former WHO director-general in her speech after the influenza pandemic in 2009: "We anticipated problems in producing enough vaccine fast enough, and this did indeed happen. But we did not anticipate that people would decide not to be vaccinated." (470).

Also, during the Ebola outbreaks in West-Africa and in DR Congo distrust, low risk perceptions and negative attitudes towards the newly developed vaccines were experienced (468, 471). This does not only happen in outbreak settings but also during implementation of vaccines in the NIP. For example, at the time the human papilloma virus vaccine was introduced in the Netherlands and other countries, people were also sceptical to receive it (472, 473). With the SARS-CoV-2 pandemic going on at the moment of finalizing this thesis, a strategy should be adopted for the introduction to the market of the many

vaccines currently under development. The common demand for a vaccine is high if we believe the media; people are longing for the economy and their societal life to continue. Yet, the willingness to receive a future SARS-CoV-2 vaccine is varying per individual and per country (474, 475). This raises questions about the cognitive determinants that play a role in vaccination uptake amidst a pandemic. Also, the introduction of a vaccine for this large pandemic may change general vaccination attitudes on the long-term, in either a positive or negative way.

Therefore it is of utmost importance to take cognitive determinants into account when developing novel vaccines and introducing them onto the market. Increased cooperation of biomedical researchers, social scientists, epidemiologists, public health specialists and policy makers is needed to optimize prevention of infectious diseases. Luckily, some initiatives already arise (476).

Secondly, even a step further, citizens should be involved in the development of TPP for vaccines. Patient and public involvement (PPI) is becoming more common practice (477), so why not extending it further? Citizens are starting to participate in study designs, data collection and dissemination of findings that concern them, to study matters that are of relevance to them. Empowerment also plays an important role here, as involvement leads to the feeling that they gain control over their future health (24). Incorporating PPI in vaccination development for target groups would have two advantages. On one hand involvement of the public could clarify what factors are of importance to the receivers of vaccines and on the other hand make them feel empowered by being involved in the developmental processes. Moreover, this initiative may bridge the gap between science and society.

In some interventions – like the education module "Viruskenner" – citizens get involved in development of prevention methods for infectious diseases. By being involved, they do not only gain knowledge, but also change their attitude and risk perceptions. The basic idea behind Viruskenner, translated as virus expert, is actively involving students in the fight against infectious diseases. Students get a group assignment to develop a prevention tool for a certain virus infection and investigate its efficacy. By doing so, they get aware of and achieve knowledge about this infectious disease in a social context and may adapt new behaviour to prevent an infection. One of the side projects Viruskenner supported, was the rollout of an educational programme to involve adolescents in information campaigns for HPV vaccination. Here, students changed their mind about the uptake of the HPV vaccine and started discussions with their social environment.

One step further is involving students in large-scale research, by inviting them to collect and analyse data. This principle is called citizen science, defined as "scientific work undertaken by members of the general public, often in collaboration with or under the direction of professional scientists and scientific institutions" (478). Citizen science is a relatively new concept in the field of infectious diseases, which fits in the new vision of empowering people to prevent and control diseases. Simple collection methods, like DBS, may facilitate the role of citizens in surveillance of infectious diseases. Although still some hurdles have to be taken to make it fruitful for both sides (479), the upcoming trend of this collaboration between public and science is promising.

Recommendations for future research

As addressed in the last paragraph, cognitive determinants need to be investigated at the time novel vaccines are developed and introduced on the market. Awareness, attitudes and risk perceptions to these particular vaccines should be measured, so concerns can be addressed in early stages. As cognitive determinants are vaccine- and subgroup specific (amongst others due to variance in perceived risks) (480), they should be addressed separately. In Chapter 1.2 we found that influenza vaccination uptake was studied intensively, especially in HCW. However, limited data was available about determinants that play a role in the uptake of e.g. meningococcal vaccines in ICP. Also, health behaviour models were sparsely employed. Self-designed, non-validated questionnaires were often used, resulting in biased and very heterogenic data. When more solid data becomes available regarding determinants that play a role in uptake in risk groups, the next step will be to design interventions based on these outcomes and evaluate them with help of validated questionnaires based on the determinants of health behaviour models. One of the interventions thought of is a digital vaccination registry, including all vaccinations ever received (e.g. NIP, occupational and travel vaccinations). This digital tool will not only provide better insight in uptake rates but may also suggest indicated vaccinations based on users' reported behaviour, health and occupation. If this registry would be accessible for doctors and nurses, they may quickly see which patients they still have to provide with information regarding vaccinations, favouring uptake in patients. Also, a complete digital vaccination registry accessible by travel clinics, give travel nurses and doctors the opportunity to support compliance to NIP in travellers, which could be helpful for the completion of measles vaccination in pediatric travellers.

Cognitive determinants are not only vaccine- and subgroup specific, but will also differ in time. In an emerging situation, like an epidemic, people may have other considerations to get vaccinated than when a threat is not directly visible. Therefore, it is important to

study cognitive determinants that explain the uptake of a novel vaccine both in emerging and non-emerging situations.

A pandemic, like the one caused by SARS-CoV-2, is a unique chance to investigate expectations and cognitive determinants regarding novel vaccines in many subpopulations and different countries. Many health-behaviour models are available to describe and understand distinct actions of individuals and populations. However, a specific model to explain vaccination uptake is lacking. In Chapter 1 of this thesis, a relatively new model, named the I-Change model, was used and adapted to comprise the determinants that explain vaccination uptake according to available literature. This model combined different established health-behaviour models, but none of these was specific to vaccination behaviour. The WHO proposed the capability-opportunity-motivation-behaviour model (COM-B model) and also adapted this to vaccination behaviour. However, the evidence gathered with the existing behaviour models, could form a basis for the development of a vaccine-specific behaviour model. This model should be applicable to distinct infectious diseases/vaccinations and different target populations, including adult risk populations in populations with a different socio-economic and cultural background. The development of a specific model could be the basis for validated questionnaires that give broad and reliable insight in vaccination behaviour. A validated questionnaire to measure cognitive determinants in (future) adult vaccine recipients should provide valid information both before and after the introduction of a vaccine to monitor cognitive determinants that may change. It should be universal questionnaire applicable to any vaccine, population and country to make it possible to compare data between vaccines, populations, nations and time points, like the Vaccine Confidence Index does for general trust in (childhood) vaccinations (481). More in-depth data, focused on adult vaccinations will not only support the implementation of novel vaccines, but also guide policies for promotion of vaccines already on the market and help designing effective interventions to increase uptake rates. The administration of this questionnaire could even be (recommended or obligatory) implemented in the clinical vaccine trial stages. Although this would be an extra step in the process to license a new vaccine, it should not cause any delay, as this research could be performed simultaneously with phase 3 trials. With this small additional investigation, uptake rates could be increased making the effort of vaccine development more than worth it.

Additionally, opinions of HCW that provide care to ICP should be further studied in other healthcare centres and being extended to primary and secondary care. Explanations for the strong preference for education to decrease infectious disease should be explored. HCW's cognitive determinants regarding vaccinations recommended in their patient

groups should be included. Furthermore, it would be good to link these results to the actual vaccination uptake in their ICP. In light of PPI, patients should be included, either as study participants or as co-researchers for the research design or interpretation of the results.

This thesis included several studies using dry blood spot cards, and included the first report of DBS being used to detect RVNA. As this study provided very promising results, the use of DBS for rabies serology in healthcare workers should further be tested. Sample collection, transport and storage variables should be investigated in settings were this sampling technique would be most useful. Therefore, a follow-up study should be set up in a remote, rabies-endemic country, among (vaccinated) healthcare workers at risk. Peripheral blood should be collected both with a venipuncture to collect serum and a finger prick to collect dry blood spots. These samples should be processed in parallel both locally with an ELISA and after shipment with a FAVNt at a BSL-3 laboratory. Different transport and storage protocols must be included to determine to optimal conditions. As the DBS has proven its usefulness for many other virus infections like HIV, we believe it may help advance protecting individuals at risk against rabies as well.

With the low seroprevalence rates of hepatitis A and measles found in Dutch travellers, it would be interesting to study the incidence of VPD in the Netherlands and the vaccination coverage among travellers who got infected. With VPD being notifiable by law, this should be possible. Also, it might favour vaccination uptake if these cases of VPD would be brought to light. As by appearing in the news, people's awareness may rise and risk perceptions may change.

Furthermore, we concluded in Chapter 2.2 that a large part of (young) travellers was seronegative for measles. Now we found that this population might be more vulnerable than we are aware of, it would be interesting to further study the measles-specific T-cell immunity of a young, measles vaccinated but seronegative population. For that purpose, ideally a cohort should be composed with both children and young adults, who had a vaccination history of none, one or two doses measles vaccine and were either tested seronegative or seropositive for measles. Then serum and peripheral blood mononuclear cells should be collected. The same could be done for hepatitis A, however we had less cues to presume that there might be protection by their cellular immunity.

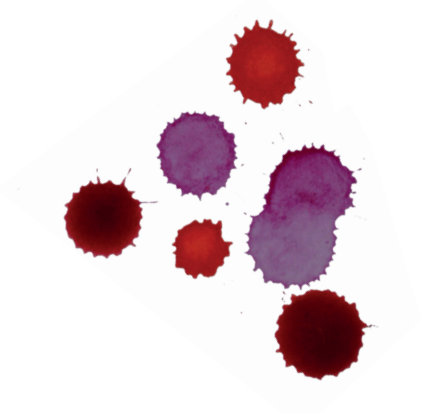
Immune responses to influenza vaccination were studied in depth in CD patients using ustekinumab and showed strong results. However, literature on immune responses to other vaccinations than influenza was scarce in CD patients using ustekinumab. Furthermore,

this research should be extended to study immune responses to influenza vaccination in other patient groups using ustekinumab, for instance in patient diagnosed with psoriasis, to eliminate disease specific reactions. As a consequence of the fast-developing field of biologicals, immune responses to vaccinations in ICP treated with other biologicals, like vedolizumab, still have to be studied.

During the studies described in this thesis, difficulties were encountered measuring protective immunity post-vaccination. For measles and for hepatitis B lower levels of (neutralizing) antibodies were found than expected based on the reported vaccination history. Also, there was no correlation between B- and T-cell immunity responses after influenza vaccination. Therefore, more research is needed to develop convenient laboratory assays that are capable of demonstrating protective immunologic memory after vaccination.

CHAPTER 7

Dutch Summary / Nederlandse Samenvatting



Hippocrates zei het duizenden jaren geleden al: voorkomen is beter dan genezen. De toenemende aandacht voor preventieve zorg heeft de samenleving al veel leed bespaard. Vaccinaties hebben geleid tot een enorme afname van mortaliteit en morbiditeit door infectieziekten. En nog steeds vervullen ze een belangrijke rol in de publieke gezondheid. Dat blijkt ook uit de dringende vraag naar vaccins tijdens een pandemie, zoals door SARS-CoV-2 veroorzaakt. Vaccinaties beschermen het individu tegen infectieziekten, maar dit werkt alleen als vaccins op de juiste wijze worden toegediend en voldoende werkzaam zijn in het individu. Bescherming van een populatie, ook wel groepsimmuniteit genoemd, hangt af van het percentage beschermde individuen en de verspreiding van onbeschermde individuen binnen die groep. Als vaccins niet beschikbaar zijn, is preventie volledig afhankelijk van gezondheidsgedrag. Dit proefschrift richt zich op de preventie van virusinfecties in risicogroepen in de breedste zin van het woord. Zowel de biomedische kant (vaccinaties) als psychosociale kant (gezondheidsgedrag) komen aan bod, omdat alleen een interactie tussen deze twee resulteert in optimale bescherming. Drie groepen volwassenen met een verhoogd risico op het oplopen van een virusinfectie door hun gedrag, werk of gezondheid worden bestudeerd, namelijk reizigers, gezondheidswerkers en immuungecompromitteerde patiënten. Aan iedere risicogroep is een apart hoofdstuk gewijd (hoofdstukken 2, 3 en 4). Dit proefschrift brengt in kaart wat de beschermingsgraad van deze populaties is, hoe onvoldoende beschermde individuen gedetecteerd kunnen worden en wat de oorzaken van suboptimale protectie zijn.

Na de inleiding over preventie van virusinfecties en risicogroepen (**hoofdstuk 1.1**) volgt een overzicht van de beschikbare literatuur over verklaringen voor het wel of niet ontvangen van een aanbevolen vaccinatie door de risicogroepen (**hoofdstuk 1.2**). Bij reizigers speelden twee factoren een grote rol; zij hadden een lage risicoperceptie voor het oplopen van de ziekte en waren zich niet bewust van de vaccinatieadviezen. Immuungecompromitteerde patiënten hadden vooral een hoge risicoperceptie voor mogelijk nadelige effecten van vaccinaties, maar waren zich eveneens vaak niet bewust van de vaccinatieadviezen. Dit hoofdstuk laat zien dat motivaties voor het wel of niet ontvangen van vaccinaties erg uiteenlopen en verschillen per risicogroep. Concluderend liet dit uitgebreide literatuuronderzoek zien dat reizigers, gezondheidswerkers en immuungecompromitteerde patiënten als risicogroepen afzonderlijk benaderd moeten worden. Dit om gericht barrières weg te kunnen nemen en op specifieke determinanten in te kunnen spelen. In volgende hoofdstukken wordt meer in detail ingegaan op biologische en beleidsmatige factoren die preventie van virusinfecties per risicogroep kunnen optimaliseren.

Er komen nog regelmatig reizigers terug uit het buitenland met klachten die mogelijk passen bij een infectie. Niet-wetenschappelijke artikelen en persoonlijke verhalen deden vermoeden dat veel reizigers zich niet bewust zijn van geldende vaccinatieadviezen voor bepaalde bestemmingen en daardoor onvoldoende beschermd op reis gaan. Om dat vermoeden te onderzoeken, is in 2015 een reizigerscohortstudie opgezet die de vaccinatiestatus van gezinnen uit heel Nederland in kaart heeft gebracht. In hoofdstuk 2 zijn de resultaten van deze studie, genaamd DiVeST, opgenomen. In het eerste deel (hoofdstuk 2.1) ligt de focus op de reizigersvaccinatiegraad voor hepatitis A. In het tweede deel (hoofdstuk 2.2) komt de mazelenvaccinatiegraad onder reizigers aan bod.

Hepatitis A is het meest geadviseerde reizigersvaccin in Nederland. Deze vaccinatie, die niet onder het Rijksvaccinatieprogramma (RVP) valt, wordt aanbevolen voor vele reisdoelen, inclusief nabijgelegen bestemmingen. Derhalve is in **hoofdstuk 2.1** de vaccinatiegraad voor hepatitis A representatief beschouwd voor de algemene reizigersvaccinatiegraad. Uit serologische testen, op het bloed afgenomen bij reizigers via een vingerprik, bleek dat slechts tweederde van alle reizigers die vertrokken naar een bestemming waarvoor een hepatitis A vaccinatieadvies gold, voldoende beschermd was. Reizigers met een bestemming in een straal van minder dan 5.000 km van Nederland bleken een grotere kans te hebben onvoldoende gevaccineerd te zijn dan reizigers met een reisdoel verder weg. Daarnaast was de kans groter dat kinderen (12-18 jaar) en reizigers die vrienden of familie bezochten op reis vertrokken zonder voldoende bescherming. De meest waarschijnlijke verklaring daarvoor is dat deze reizigers zich niet bewust waren van geldende vaccinatieadviezen.

Mazelen is een soort ondergeschoven kindje in de reizigersgeneeskunde. Vele reizigers worden als reeds beschermd beschouwd; of door het RVP (indien geboren na 1975) of door de ziekte te hebben doorgemaakt (indien geboren vóór 1965). Echter, reizigers geboren tussen 1965 en 1975 en degenen die afzien van deelname aan het RVP zijn mogelijk onbeschermd. Dit is alarmerend, omdat de mazelenuitbraken, die momenteel in verschillende landen plaatsvinden, die onvoorbereide reizigers kunnen treffen. Dat was de aanleiding om in **hoofdstuk 2.2** de beschermingsgraad van mazelen in het reizigerscohort van de DiVeST te onderzoeken. Gebaseerd op de laboratoriumuitslagen die op 'dry blood spots' (DBS) zijn uitgevoerd, werd een gemiddelde mazelenvaccinatiegraad van slechts 82 procent gevonden. In tegenstelling tot de hypothese dat reizigers geboren tussen 1965-1975 mogelijk minder beschermd waren, werd gevonden dat kinderen een lagere beschermingsgraad hadden (72%) dan dit geboortecohort (89%). Een mogelijke verklaring van deze resultaten kan het niet of slechts gedeeltelijk navolgen van het RVP zijn. Echter, deze resultaten vragen om nader onderzoek. Een belangrijke vraag is of deze

kinderen mogelijk voldoende beschermd zijn middels mechanismen die niet gemeten konden worden in deze studie, zoals bijvoorbeeld T-cel immuniteit.

Hoofdstuk 3 gaat in op het beleid rondom vaccinaties van gezondheidswerkers. De werkzaamheden die zij verrichten - waarbij ze in contact komen met patiënten of hun lichaamsmaterialen - geven risico op het oplopen van via bloed overdraagbare aandoeningen, zoals hepatitis B. In Nederland worden gezondheidswerkers standaard gevaccineerd tegen hepatitis B en wordt met een titerbepaling 4-6 weken na het laatste vaccin van de serie gekeken of er voldoende antistoffen zijn gegenereerd. In **hoofdstuk 3.1** is het hepatitis B vaccinatiebeleid voor toekomstig gezondheidswerkers geëvalueerd. Daaruit bleek dat van de bijna 3.000 studenten slechts 1,3 procent onvoldoende had gerespondeerd op de hepatitis B vaccinatieserie. Verder liet de studie zien dat 80 procent van de studenten die in het verleden (4 maanden tot 22 jaar geleden) gevaccineerd zijn, nog steeds een voldoende hoge antistoftiter had. Deze resultaten geven aanleiding tot het terugbrengen van het aantal boostervaccinaties voorafgaand aan de titerbepalingen.

Laboratoriummedewerkers lopen - naast het risico om hepatitis B op te lopen door contact met patiëntmaterialen - het risico om geïnfecteerd te raken met de virussen waarmee zij in het laboratorium werken. Dit is vooral een risico in de streng beveiligde BSL-3 laboratoria, waar met gevaarlijke virussen zoals rabiës kan worden gewerkt. Omdat een rabiësvirusinfectie vrijwel altijd fataal afloopt, is het uiterst belangrijk medewerkers hier goed voor te beschermen. Dit gebeurt met vaccinatie opgevolgd door - opnieuw - een titerbepaling. In **hoofdstuk 3.2** is een nieuwe methode onderzocht om immuniteit tegen rabiës aan te tonen. In een cohort van 99 volwassenen zijn serum en DBS afgenomen en is met twee verschillende testen - een ELISA en een virusneutralisatietest - gekeken of in beide sampletypen gelijke antistoftiters worden gezien. De DBS liet veelbelovende resultaten zien. Wanneer een afkapwaarde van 0,6 IU/ml wordt gehanteerd (die iets hoger ligt dan de 0,5 IU/ml die door de WHO wordt geadviseerd) was de virusneutralisatietest in staat 100 procent van de gevallen die onvoldoende beschermd zijn (specificiteit) aan te tonen en 95 procent van de gevallen die voldoende beschermd zijn (sensitiviteit). DBS faciliteert een gemakkelijke afname, sample transport en opslag. Daarom biedt het uitvoeren van rabiësserologie op deze materialen kansen om de beschermingsstatus van gezondheidswerkers, in afgelegen gebieden waar rabiës endemisch is, te verbeteren.

Hoofdstuk 4 gaat in op de immuungecompromitteerde patiënten. Om inzicht te krijgen in de dagelijkse praktijk van preventieve zorg voor deze patiënten zijn interviews uitgevoerd en vragenlijsten afgenomen onder hun behandelaren. De resultaten staan in **hoofdstuk 4.1**. Deze laten zien dat 80 procent van de behandelaren mogelijkheden ziet om de

preventie van infecties voor hun immuungecompromitteerde patiënten te verbeteren. Veertig procent van de ondervraagde zorgverleners verkiest educatie boven vaccinatie om dit doel te bereiken. Influenzavaccinatie wordt als de belangrijkste vaccinatie beschouwd en wordt het meest besproken met patiënten.

De werking van influenzavaccinatie in een populatie immuungecompromitteerde patiënten wordt verder bestudeerd in **hoofdstuk 4.2**. In een vaccinatiecohortstudie (COVA) is gekeken hoe patiënten met de ziekte van Crohn, die behandeld worden met het relatief nieuwe geneesmiddel ustekinumab, responderen op influenzavaccinatie. Om dit in kaart te brengen, is vóór vaccinatie en op drie tijdstippen na vaccinatie bloed afgenomen in deze groep en in twee controlegroepen (patiënten met de ziekte van Crohn die behandeld worden met een reeds langer beschikbaar medicijn genaamd adalimumab en gezondheidswerkers). Hieruit bleek dat zowel de humorale als de cellulaire immuunrespons van de patiënten, die behandeld worden met ustekinumab, non-inferieur waren aan die van de controlegroepen. Deze resultaten laten zien dat influenzavaccinatie werkzaam is voor patiënten met de ziekte van Crohn die behandeld worden met ustekinumab.

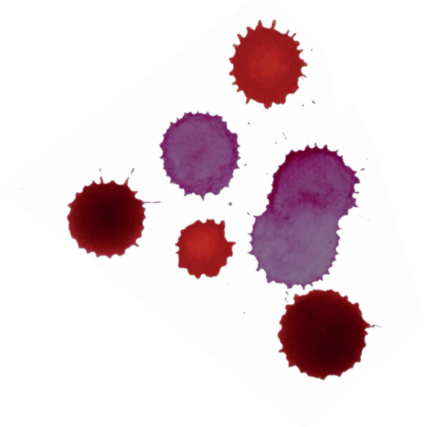
In hoofdstuk 5 wordt een uiterst belangrijk middel in de preventie van virusinfecties besproken: educatie. Om jongeren vroeg te leren hoe ze zichzelf en hun omgeving kunnen beschermen tegen virusinfecties is de internationale educatiemodule 'Viruskenner' opgericht. Deze module daagt scholieren uit om actief mee te denken over preventiemiddelen en het doen van wetenschappelijk onderzoek onder begeleiding van coaches. In **hoofdstuk 5.1** wordt het effect van deelname - op kennis, attitude en gedrag gerelateerd aan virusinfecties - geëvalueerd. Uit pre- en post-interventie vragenlijsten bleek dat de kennis van leerlingen in twee van de drie participerende landen (Suriname en Nederland) na deelname aan de module gestegen was. Ook was er een klein effect te zien op de attitude van leerlingen en werd er een correlatie gevonden met gedrag. Maar het gemeten gedrag veranderde niet significant na deelname aan de module.

Een belangrijk concept voor gedragsverandering is volgens de WHO 'empowerment'. Vanuit dat oogpunt is met de studie, genoemd in **hoofdstuk 5.2**, gekeken in hoeverre dit concept aanwezig is in de educatiemodule Viruskenner. Uit interviews bleek dat deelname aan de educatiemodule bij leerlingen motivatie, vaardigheden en vertrouwen creëerde om gezondheidsgedrag te verbeteren. De coaches speelden hierbij een belangrijke rol. De module kon verbeterd worden door de inhoud aan te passen op de relevantie in de setting waar de module zich afspeelt en door de sociale omgeving van de participanten te betrekken. Deze uitkomsten onderstrepen het belang van de bestaande modellen die deze facetten beschrijven voor gezondheidsgedrag zoals ook besproken wordt in hoofdstuk 1.2.

Tot slot zijn in **hoofdstuk 6** de resultaten van alle studies in dit proefschrift in perspectief geplaatst. Het werk geeft aanleiding tot verschillende vervolgstudies, zoals het verder testen van de DBS toegepast voor rabiësserologie in een endemisch gebied, het bepalen van de T-cel immuniteit bij de jonge reizigers die onvoldoende antistoffen voor mazelen bleken te hebben en het verder ontwikkelen van diagnostiek die meer nauwkeurig die immuunstatus (na vaccinatie) in beeld kan brengen. De artikelen in dit proefschrift laten ook zien dat de introductie van vaccins op de markt verbeterd kan worden, door vooraf cognitieve determinanten van de doelgroep in kaart te brengen. Hiervoor is een gedragsmodel nodig, dat is toegepast op het ontvangen van vaccinaties en dat de basis kan vormen voor een gevalideerde vragenlijst die bruikbaar is in verschillende populaties en voor verschillende vaccinaties. Een mogelijke vervolgstap is dat toekomstige ontvangers van de vaccins betrokken worden bij het ontwikkelingsproces van vaccins. Dit, om de kloof tussen wetenschap en de maatschappij te verkleinen, meer begrip te creëren tussen beide partijen en de introductie doeltreffend te laten verlopen.

CHAPTER 8

Appendix



ABOUT THE AUTHOR

Laura Doornekamp was born in Liempde, the Netherlands, on 15th of May 1991. She attended gymnasium at Jacob Roelandslyceum in Boxtel and graduated cum laude in 2009. She decided to move to Maastricht to start studying Health Sciences at Maastricht University. One year later she changed her mind and decided to switch to study Medicine at the same university. She chose to do her clinical electives at the department of Viroscience in the Erasmus Medical Center in Rotterdam and at the department of Medical Microbiology in Samarinda, Indonesia. She finished her masters with an internship at the department of Internal Medicine and Gastroenterology at the Catharina hospital in Eindhoven, the Netherlands in 2017. After her internship at the Erasmus MC, she was offered a PhD position at the department of Viroscience. Prof. dr. E.C.M. van Gorp and dr. M. Goeijenbier supervised her as a PhD candidate during the period 2015-2020. In 2017, she also started working as physician at the Vaccination and Travel Clinic of the Erasmus MC. In December 2020 she started a new position as resident in Medical Microbiology in Albert Schweitzer hospital in Dordrecht and Erasmus MC in Rotterdam and she will continue her research activities.

PHD PORTFOLIO

PhD student: Laura Doornekamp	PhD period: April 2015 – November 2020
Erasmus MC Department: Viroscience	Promotor: Prof. dr. ECM van Gorp
Research School: MolMed	Co-promotor: Dr. M Goeijenbier

1. PhD training	Year	Workload
General Courses		
Systematic Literature Retrieval (4 parts, note: 8.5)	2015	1.0 ECTS
Biomedical English Writing Course	2015	2.0 ECTS
BROK (Good Clinical Practice) (90% score)	2016	1.5 ECTS
OpenClinica	2016	0.7 ECTS
Research integrity	2018	0.3 ECTS
Basic Flow Cytometry course	2018	0.3 ECTS
BROK herregistratie	2019	0.3 ECTS
IELTS Academic English	2019	1.0 ECTS
Specific courses		
Biomedical Research Techniques (MolMed)	2015	1.5 ECTS
Basismodule Reizigersadvisering	2015	0.8 ECTS
Basismodule reizigersadvisering en -immunisatie voor artsen	2017	3.0 ECTS
Advanced Immunology	2018	4.5 ECTS
Basic course on R	2019	2.0 ECTS
Adobe Photoshop & Illustrator	2020	0.3 ECTS
Adobe InDesign	2020	0.3 ECTS
Seminars and workshops		
Masterclass One Health	2016	0.5 ECTS
NCOH meeting	2018	0.8 ECTS
AMRO meetings	2019	
Presentations		
Invited speaker FIGON: *Knowledge as Antivirus: A new education tool used as prevention* @Dutch Medicine Days	2015	
Poster presentation ECV: *Detection of Human Rabies virus Antibodies on Dried Blood Spot Cards*	2019	
Oral presentation ESCV: *Adherence to travel health guidelines in Dutch families: The Dutch travel Vaccination Study (DiVeST)*	2019	
Invited speaker Vaccinology Symposium: *The race to the licensed Ebola vaccine: where are we now?* – Postponed due to COVID-19 pandemic	2020	
Poster presentation WOHC: *Dried Blood Spot Cards: A Reliable Method to Detect Human Rabies Antibodies*	2020	
National and international conferences		
European Leptospirosis Meeting (ELS) 2015	2015	
Dutch Medicine Days (FIGON) (oral, invited speaker)	2015	
Dutch Annual Virology Symposium	2016, 2019	

ECCMID	2019
ECV (poster presentation)	2019
ESCV (oral presentation)	2019
Vaccinology Symposium (oral, invited speaker)	2020
WOHC (poster presentation)	2020
Other	
Peer reviews PLoS One	2018
Scholarship Prins Bernard Cultuursfonds	2019
Peer reviews The Lancet Rheumatology	2021
2. Teaching	
Lecturing	
Viruskenner kick-offs	2015-2019
Viruskenner (teach the teachers, kick-offs in the Netherlands, Suriname and Indonesia)	2016-2019
Guest lesson secondary schools (Elde College, Beekvliet, STC, Libanon)	2018-2019
Hogeschool Rotterdam – Guest lessons on vaccinations	2018-2020
Infection & Immunity research master	2018-2020
NSPOH modules for nurses and physicians	2019-2020
STOLA One Health workshop	2019-2020
Tropencursus Havenziekenhuis	2019
MEDtalks	2020
Supervising practicals and excursions, tutoring	
Viruskenner coach	2016-2019
Supervising master thesis	
Roos Peerdeman, Marjolijn Hordijk, Lisanne Preusting	2017, 2018
Other	
Minor 'Beyond Borders' Hogeschool Rotterdam	2015

* 1 hour = 0.1 ECTS

LIST OF PUBLICATIONS

International journals

Langerak T, Rooij I, Doornekamp L, Chandler F, Baptista M, Yang H, Koopmans MPG, GeurtsvanKessel CH, Jacobs BC, Rockx B, Adriani K, van Gorp ECM. Guillain-Barré syndrome in Suriname; clinical presentation and identification of preceding infections. Front. Neurol. 2021;12:635753.

Doornekamp L, Comvalius AD, GeurtsvanKessel CH, Slobbe L, Scherbeijn SMJ, van Genderen PJJ, van Binnendijk R, van Gorp ECM, de Swart RL, Goeijenbier M. Measles seroprevalence among Dutch travelling families. *Submitted*

Doornekamp L*, van Leeuwen LPM*, Goeijenbier S, de Jong W, de Jager HJ, van Gorp ECM. Evaluation of the hepatitis B vaccination programme in medical students in a Dutch university hospital. Vaccines (Basel). 2021;9(2), 69. **Authors contributed equally*

Shoker K*, Doornekamp L*, Horjus B, Wagener MN, Aron GI, Goeijenbier M, van Gorp ECM. Empowering Dutch and Surinamese children to prevent viral infections: implications from an international education module. Health Promotion International. 2021, daaa153. **Authors contributed equally*

Doornekamp L, van Leeuwen L, van Gorp ECM, Voeten HACM*, Goeijenbier M*. Determinants of Vaccination Uptake in Risk Populations: A Comprehensive Literature Review. Vaccines (Basel). 2020;8(3). **Authors contributed equally*

Doornekamp L*, Goetgebuer RL*, Schmitz KS, Goeijenbier M, van der Woude CJ, Fouchier R, van Gorp ECM, de Vries AC. High Immunogenicity to Influenza Vaccination in Crohn's Disease Patients Treated with Ustekinumab. Vaccines (Basel). 2020;8(3). **Authors contributed equally*

Doornekamp L, Embregts CWE, Aron GI, Goeijenbier S, van de Vijver DAMC, van Gorp ECM, GeurtsvanKessel CH. Dried blood spot cards: A reliable sampling method to detect human antibodies against rabies virus. PLoS Negl Trop Dis. 2020 Oct 13;14(10):e0008784.

Doornekamp L, GeurtsvanKessel CH, Slobbe L, te Marvelde MR, Scherbeijn SMJ, van Genderen PJJ, van Gorp ECM, Goeijenbier M. Adherence to hepatitis A travel health

guidelines: A cross-sectional seroprevalence study in Dutch travelling families - The Dutch travel Vaccination Study (DiVeST). Travel Med Infect Dis. 2019;32.

Doornekamp L*, de Jong W*, Wagener MN, Goeijenbier M, van Gorp ECM. Dutch healthcare professionals' opinion on vaccination and education to prevent infections in immunocompromised patients: A mixed-method study with recommendations for daily practice. Vaccine. 2019;37(11):1476-83. **Authors contributed equally*

Doornekamp L, Stegers-Jager KM, Vlek OM, Klop T, Goeijenbier M, van Gorp ECM. Experience with a Multinational, Secondary School Education Module with a Focus on Prevention of Virus Infections. Am J Trop Med Hyg. 2017;97(1):97-108.

Langerak T, Yang H, Baptista M, Doornekamp L, Kerkman T, Codrington J, Roosblad J, Vreden SG, De Bruin E, Mögling R, Jacobs BC, Pas SD, GeurtsvanKessel CH, Reusken CB, Koopmans MP, van Gorp ECM and Alberga H. Zika Virus Infection and Guillain-Barre Syndrome in Three Patients from Suriname. Front Neurol. 2016;7:233.

Dutch journals

Doornekamp L, van Gorp ECM, Koopmans MPG. The fight against Ebola - lessons learned from the epidemic in Western Africa. Tijdschrift voor Infectieziekten. 2019;14(1).

Doornekamp L, van Gorp ECM, Koopmans MPG. Combatting Ebola anno 2019: the role of available vaccines and therapies. Tijdschrift voor Medische Microbiologie, 2019; 27(4), 238-246.

GLOSSARY

ADA	adalimumab
Anti-HBc	antibody against the hepatitis B core antigen
Anti-HBs	antibody against the surface antigen
aOR	adjusted odds ratio
ASE	attitude, social norm and self-efficacy
BCA	bicinchoninic acid
BCG	Bacillus Calmette-Guerin
BIM	behavioral intention model
CD	Crohn's Disease
CFSE	carboxyfluorescein succinimidyl ester
CI	confidence interval
CLIA	chemiluminescent immunoassay
CME	cognitive model of empowerment
ConA	Concanavalin A
COVA	Vaccination Cohort
COVID-19	coronavirus disease 2019
CU	colitis ulcerosa
CV	coefficient of variance
DBS	dry blood spot
DMSO	dimethyl sulfoxide
DTaP	diphtheria, tetanus, acellular pertussis
DTP	diphtheria, tetanus and polio
ECCO	European Crohn's and Colitis Organisation
EIA	enzyme immunoassay
ELISA	enzyme linked immunosorbent assay
EMC	Erasmus Medical Center
ERIG	equine rabies immunoglobulins
EU	European Union
FACS	fluorescence-activated cell sorting
FAVNt	fluorescent antibody virus neutralization test
FBS	fetal bovine serum
FDA	food and drug administration
FRNT	focus reduction neutralization assay
GGD	regional public health services
GMT	geometric mean titres
GP	general practitioner
HA	hemagglutinin
HAV	hepatitis A virus

HAVO	advanced general secondary education
HBM	Health Belief Model
HBO	higher professional education
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HC	healthy controls
HCP	healthcare professionals
HCW	healthcare workers
HI	hemagglutinin inhibition
Hib	Haemophilus Influenzae type B
HIM	Health Intention Model
HIV	human immunodeficiency virus
HPV	human papillomavirus
HRIG	human rabies immunoglobulins
HSCT	hematological stem cell transplantation
HZV	herpes zoster virus
IBD	inflammatory bowel disease
I-Change	integrated change
ICP	immunocompromised patients
IgG	immunoglobulin G
IL	interleukine
IPV	inactivated poliomyelitis vaccine
IQR	interquartile range
IS	immunosuppressive treatment
JE	Japanese encephalitis
KAB/P	Knowledge, Attitude, Behaviour/Practices
LCR	Landelijk Coördinatiecentrum Reizigersadvisering (Dutch National Coordination Centre for Travellers' Health Advice)
MBO	senior secondary vocational education and training
Men	meningococcal disease
MMR	measles, mumps, rubella
NC	nursing consultants
NIP	national immunisation programme
NL	the Netherlands
NP	nurse practitioners
OR	odds ratio
PAPM	Precaution Adoption Process Model
PBMCs	peripheral blood mononuclear cells
PBS	phosphate buffered saline
PCV-13	pneumococcal conjugate vaccine including 13 serotypes

PEP	post-exposition prophylaxis
PHC	primary healthcare
Pneu	pneumococcal disease
PPI	patient and public involvement
PPSV-23	pneumococcal polysaccharide vaccine including 23 serotypes
PrEP	pre-exposure prophylaxis
PRNT	plaque reduction neutralization assay
PTA	pre-travel advice
R0	basic reproduction number
RA	rheumatoid arthritis
RABV	rabies virus
RFFIT	rapid fluorescent focus inhibition test
RPA	risk perception attitude framework
RVNA	rabies virus neutralizing antibodies
RVP	Rijksvaccinatieprogramma', see NIP
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SCT	Social Cognitive Theory
SI	stimulation index
SOT	solid organ transplantation
TBC	tuberculosis
TIG	tetanus immunoglobulins
TIV	trivalent influenza vaccination
TNF	tumor necrosis factor
TPB	Theory of Planned Behaviour
TPP	target product profiles
TRA	Theory of Reasoned Action
Triandis	Triandis model of interpersonal behavior.
TTM	Trans-Theoretical Model
UK	United Kingdom
USA	United States of America
UST	ustekinumab
VFR	visiting friends and relatives
VMBO	preparatory vocational and general secondary education
VNA	virus neutralizing antibodies
VPD	vaccine-preventable disease
VWO	pre-university education
VZV	varicella zoster virus
WHO	World Health Organization
WO	university
YF	yellow fever

REFERENCES

1. Bennett JE, Dolin R, Blaser MJ, Mandell GL, Douglas RG. Mandell, Douglas, and Bennett's principles and practice of infectious diseases. Philadelphia, PA: Elsevier/Saunders; 2015.
2. Sadarangani M. Protection Against Invasive Infections in Children Caused by Encapsulated Bacteria. Frontiers in immunology. 2018;9:2674-.
3. Murphy K, Weaver C. Janeway's immunobiology: Garland science; 2017.
4. Korenkov D, Isakova-Sivak I, Rudenko L. Basics of CD8 T-cell immune responses after influenza infection and vaccination with inactivated or live attenuated influenza vaccine. Expert Review of Vaccines. 2018;17(11):977-87.
5. Phadke VK, Bednarczyk RA, Salmon DA, Omer SB. Association Between Vaccine Refusal and Vaccine-Preventable Diseases in the United States: A Review of Measles and Pertussis. Jama. 2016;315(11):1149-58.
6. Gochman DS. Handbook of health behavior research. 3. Demography, development and diversity: Plenum Press; 1997.
7. de-Graft Aikins A, Boynton P, Atanga LL. Developing effective chronic disease interventions in Africa: insights from Ghana and Cameroon. Global Health. 2010;6:6-.
8. WHO. Advocacy, Communication and Social Mobilization for TB control /A guide to developing knowledge, attitude and practice surveys. 2008.
9. Working Group on Monitoring & Evaluation . Knowledge, Attitudes, and Practices (KAP) surveys during cholera vaccination campaigns: Guidance for Oral Cholera Vaccine Stockpile Campaigns. 2014.
10. Taylor D, Bury M, Campling N, Carter S, Garfied, Newbould DJ, et al. A Review of the use of the Health Belief Model (HBM), the Theory of Reasoned Action (TRA), the Theory of Planned Behaviour (TPB) and the Trans-Theoretical Model (TTM) to study and predict health related behaviour change. 2006.
11. Jones CL, Jensen JD, Scherr CL, Brown NR, Christy K, Weaver J. The Health Belief Model as an explanatory framework in communication research: exploring parallel, serial, and moderated mediation. Health Commun. 2015;30(6):566-76.
12. Bandura A. Social Cognitive Theory: ScienceDirect; 1977 [Available from: https://www.sciencedirect.com/topics/immunology-and-microbiology/social-cognitive-theory.
13. Fishbeim M. Theory of Reasoned Action. The International Encyclopedia of Communication2008.
14. Thompson CR. Factors Influencing Maintenance of Physical Activity. In: Thompson CR, editor. Prevention Practice A Physical Therapist's Guide to Health, Fitness, and Wellness. United States of America: SLACK; 2007. p. 47.
15. Kools EJ, Thijs C, Kester AD, de Vries H. The motivational determinants of breast-feeding: predictors for the continuation of breast-feeding. Prev Med. 2006;43(5):394-401.
16. Hoving EF, Mudde AN, de Vries H. Predictors of smoking relapse in a sample of Dutch adult smokers; the roles of gender and action plans. Addictive Behaviors. 2006;31(7):1177-89.
17. Kasten S, van Osch L, Candel M, de Vries H. The influence of pre-motivational factors on behavior via motivational factors: a test of the I-Change model. BMC Psychology.

2019;7(1):7.
18. Hochbaum G, Rosenstock I, Kegels S. Health Belief Model. United States Public Health Service1952.
19. LaCaille L. Theory of Reasoned Action. In: Gellman MD, Turner JR, editors. Encyclopedia of Behavioral Medicine. New York, NY: Springer New York; 2013. p. 1964-7.
20. Prochaska JO, Velicer WF. The Transtheoretical Model of Health Behavior Change. American Journal of Health Promotion. 1997;12:38-48.
21. Ajzen I. The Theory of Planned Behavior Organizational Behavior and Human Decision Processes. 50: Academic Press. Inc.; 1991. p. 179-211.
22. Weinstein ND, Sandman PM, Blalock SJ. The Precaution Adoption Process Model. Health Behavior and Health Education2008. p. 123-47.
23. de Vries H, Dijkstra M, Kuhlman P. Self-efficacy: the third factor besides attitude and subjective norm as a predictor of behavioural intentions. Health education research. 1988;3(3):273-82.
24. Kickbusch I, Nutbeam D. Health promotion glossary: WHO; 1998 [Available from: http://www.who.int/hpr/docs/glossary.html
25. Fine P, Eames K, Heymann DL. "Herd Immunity": A Rough Guide. Clinical Infectious Diseases. 2011;52(7):911-6.
26. Plans-Rubió P. Evaluation of the establishment of herd immunity in the population by means of serological surveys and vaccination coverage. Human Vaccines & Immunotherapeutics. 2012;8(2):184-8.
27. Snijdewind IJ, van Kampen JJ, Fraaij PL, van der Ende ME, Osterhaus AD, Gruters RA. Current and future applications of dried blood spots in viral disease management. Antiviral Res. 2012;93(3):309-21.
28. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics. 1963;32(3):338-43.
29. Poland GA, Whitaker JA, Poland CM, Ovsyannikova IG, Kennedy RB. Vaccinology in the third millennium: scientific and social challenges. Curr Opin Virol. 2016;17:116-25.
30. WHO. Ten threats to global health in 2019 2019 [Available from: https://www.who.int/news-room/feature-stories/ten-threats-to-global-health-in-2019.
31. WHO. New measles surveillance data for 2019: WHO; 2020 [updated April 15, 2019. Available from: https://www.who.int/immunization/newsroom/measles-data-2019/en/.
32. Kohn M, Schaffner W. Vaccinating adults with chronic disease: We can do better. Vaccine. 2017;35(27):3431-2.
33. WHO. Life expectancy and Healthy life expectancy: WHO; 2018 [updated April 6, 2019. Available from: https://apps.who.int/gho/data/view.main.SDG2016LEXREGv?lang=en.
34. Harpaz R, Dahl R, Dooling K. The Prevalence of Immunocompromised Adults: United States, 2013. Open Forum Infectious Diseases. 2016;3(suppl_1).
35. UNWTO. International Tourism Highlights: UNWTO; 2019 [updated July 2019. Available from: https://www.e-unwto.org/doi/pdf/10.18111/9789284421152.
36. Gautret P, Botelho-Nevers E, Brouqui P, Parola P. The spread of vaccine-preventable diseases by international travellers: a public-health concern. Clin Microbiol Infect. 2012;18 Suppl 5:77-84.
37. Haviari S, Bénet T, Saadatian-Elahi M, André P, Loulergue P, Vanhems P. Vaccination of

healthcare workers: A review. Human Vaccines & Immunotherapeutics. 2015;11(11):2522-37.
38. Angus K CG, Purves R, Bryce S, MacDonald L, Gordon R. Systematic literature review to examine the evidence for the effectiveness of interventions that use theories and models of behaviour change: towards the prevention and control of communicable diseases. . Stockholm: ECDC, 2013.
39. Knops-Dullens T, de Vries N, de Vries H. Reasons for non-attendance in cervical cancer screening programmes: an application of the Integrated Model for Behavioural Change. Eur J Cancer Prev. 2007;16(5):436-45.
40. Downes MJ, Brennan ML, Williams HC, Dean RS. Development of a critical appraisal tool to assess the quality of cross-sectional studies (AXIS). BMJ Open. 2016;6(12):e011458.
41. Balaban V, Stauffer W, Hammad A, Afgarshe M, Abd-Alla M, Ahmed Q, et al. Predictors of protective behaviors among American travelers to the 2009 Hajj. J Epidemiol Global Health. 2013;3(4):187-96.
42. Barasheed O, Rashid H, Heron L, Ridda I, Haworth E, Nguyen-Van-Tam J, et al. Influenza vaccination among Australian Hajj pilgrims: Uptake, attitudes, and barriers. J Travel Med. 2014;21(6):384-90.
43. Duffy MR, Reed C, Edelson PJ, Blumensaadt S, Crocker K, Griggs A, et al. A survey of US travelers to Asia to assess compliance with recommendations for the use of japanese encephalitis vaccine. J Travel Med. 2013;20(3):165-70.
44. Frew G, McGeorge E, Grant S, de Wildt G. Hepatitis B: A cross-sectional survey of knowledge, attitudes and practices amongst backpackers in Thailand. Travel Med Infect Dis. 2017;15:57-62.
45. Goodman AL, Masuet-Aumatell C, Halbert J, Zuckerman JN. Awareness of meningococcal disease among travelers from the United Kingdom to the meningitis belt in Africa. Am J Trop Med Hyg. 2014;91(2):281-6.
46. Herbinger KH, Nothdurft HD, Prymula R. Online survey: knowledge about risks, prevention and consequences of infections with HBV among travellers from four European countries. Curr Med Res Opin. 2011;27(3):489-96.
47. Heywood AE, Nothdurft H, Tessier D, Moodley M, Rombo L, Marano C, et al. Pre-travel advice, attitudes and hepatitis A and B vaccination rates among travellers from seven countries†. J Travel Med. 2016;24(1).
48. Igreja R, Barros K, Teodósio R. Attitudes on vaccination among portuguese travelers and brazilian migrants: A pilot study in Portugal. Acta Med Saliniana. 2019;49:1-3.
49. Lammert SM, Rao SR, Jentes ES, Fairley JK, Erskine S, Walker AT, et al. Refusal of recommended travel-related vaccines among U.S. international travellers in Global TravEpiNet. J Travel Med. 2016;24(1).
50. Paudel P, Raina C, Zwar N, Seale H, Worth H, Sheikh M, et al. Risk activities and pre-travel health seeking practices of notified cases of imported infectious diseases in Australia. J Travel Med. 2017;24(5).
51. Pavli A, Katerelos P, Maltezou HC. Meningococcal disease awareness and meningoccocal vaccination among Greek students planning to travel abroad. Int J Adolesc Med Health. 2019;31(3).
52. Pfeil A, Mütsch M, Hatz C, Szucs TD. A cross-sectional survey to evaluate knowledge,

attitudes and practices (KAP) regarding seasonal influenza vaccination among European travellers to resource-limited destinations. BMC Public Health. 2010;10:402.
53. Selcuk EB, Kayabas U, Binbasioglu H, Otlu B, Bayindir Y, Bozdogan B, et al. Travel health attitudes among Turkish business travellers to African countries. Travel Med Infect Dis. 2016;14(6):614-20.
54. Tan EM, Njeru JW, Jacobson DJ, Wilson PM, Chun F, Marcelin JR, et al. Pre-travel Health Care Utilization Among Travelers Who Visit Friends and Relatives. 2017;5(2):53-9.
55. Tashani M, Alfelali M, Azeem MI, Fatema FN, Barasheed O, Alqahtani AS, et al. Barriers of vaccinations against serious bacterial infections among Australian Hajj pilgrims. Postgrad Med. 2016;128(6):541-7.
56. Wiemken TL, Carrico RM, Kelley RR, Binford LE, Peyrani P, Ford KD, et al. Understanding why low-risk patients accept vaccines: a socio-behavioral approach. BMC Res Notes. 2015;8:813.
57. Yanni EA, Marano N, Han P, Edelson PJ, Blumensaadt S, Becker M, et al. Knowledge, attitudes, and practices of US travelers to Asia regarding seasonal influenza and H5N1 avian influenza prevention measures. J Travel Med. 2010;17(6):374-81.
58. Akin S, Dizdar O, Ozisik L, Tanriover MD, Kamisli S, Erman M, et al. Vaccination attitudes among patients with cancer receiving chemotherapy. UHOD Uluslararasi Hematol -Onkol Derg. 2016;26(3):167-72.
59. Althoff KN, Anastos K, Nelson KE, Celentano DD, Sharp GB, Greenblatt RM, et al. Predictors of reported influenza vaccination in HIV-infected women in the United States, 2006-2007 and 2007-2008 seasons. Prev Med. 2010;50(5-6):223-9.
60. Battistella C, Quattrin R, Celotto D, d'Angelo M, Fabbro E, Brusaferro S, et al. Factors predicting influenza vaccination adherence among patients in dialysis: an Italian survey. Hum Vaccines Immunother. 2019;15(10):2434-9.
61. Chehab G, Richter JG, Brinks R, Fischer-Betz R, Winkler-Rohlfing B, Schneider M. Vaccination coverage in systemic lupus erythematosus-a cross-sectional analysis of the German long-term study (LuLa cohort). Rheumatology. 2018;57(8):1439-47.
62. Chin-Yee BH, Monkman K, Hussain Z, Minuk LA. Attitudes toward Vaccination for Pandemic H1N1 and Seasonal Influenza in Patients with Hematologic Malignancies. J Supportive Oncol. 2011;9(4):156-60.
63. Gagneux-Brunon A, Fresard A, Lucht F, Botelho-Nevers E. Vaccine coverage in PLWH: disparities and potential impact of vaccine hesitancy. Hum Vaccines Immunother. 2019;15(2):305-6.
64. Haroon M, Adeeb F, Eltahir A, Harney S. The uptake of influenza and pneumococcal vaccination among immunocompromised patients attending rheumatology outpatient clinics. Jt Bone Spine. 2011;78(4):374-7.
65. Harrison N, Poeppl W, Herkner H, Tillhof KD, Grabmeier-Pfistershammer K, Rieger A, et al. Predictors for and coverage of influenza vaccination among HIV-positive patients: a cross-sectional survey. HIV Med. 2017;18(7):500-6.
66. Harrison N, Poeppl W, Miksch M, Machold K, Kiener H, Aletaha D, et al. Predictors for influenza vaccine acceptance among patients with inflammatory rheumatic diseases. Vaccine. 2018;36(32):4875-9.
67. Lachenal F, Sebban C, Duruisseaux M, Biron P, Blay JY, Ghesquières H. Influenza vaccination

in patients with haematologic malignancies: Analysis of practices in 200 patients in a single center. Bull Cancer. 2010;97(4).
68. Loubet P, Kernéis S, Groh M, Loulergue P, Blanche P, Verger P, et al. Attitude, knowledge and factors associated with influenza and pneumococcal vaccine uptake in a large cohort of patients with secondary immune deficiency. Vaccine. 2015;33(31):3703-8.
69. Loubet P, Verger P, Abitbol V, Peyrin-Biroulet L, Launay O. Pneumococcal and influenza vaccine uptake in adults with inflammatory bowel disease in France: Results from a web-based study. Dig Liver Dis. 2018;50(6):563-7.
70. Malhi G, Rumman A, Thanabalan R, Croitoru K, Silverberg MS, Hillary Steinhart A, et al. Vaccination in inflammatory bowel disease patients: Attitudes, Knowledge, and Uptake. J Crohn's Colitis. 2015;9(6):439-44.
71. Miller PDE, Forster AS, De Silva TI, Leonard H, Anthias C, Mayhew M, et al. Sociodemographic and psychological determinants of influenza vaccine intention among recipients of autologous and allogeneic haematopoietic stem cell transplant: A cross-sectional survey of UK transplant recipients using a modified health belief model. BMJ Open. 2018;8(8).
72. Mouthon L, Mestre C, Bérezné A, Poiraudeau S, Marchand C, Guilpain P, et al. Low influenza vaccination rate among patients with systemic sclerosis. Rheumatology (UK). 2010;49(3):600-6.
73. Narula N, Dhillon AS, Chauhan U, Marshall JK. An audit of influenza vaccination status in adults with inflammatory bowel disease. Can J Gastroenterol. 2012;26(9):593-6.
74. Nguyen MTT, Lindegaard H, Hendricks O, Friis-Møller N. Factors associated with influenza and pneumococcal vaccine uptake among rheumatoid arthritis patients in Denmark invited to participate in a pneumococcal vaccine trial (Immunovax_RA). Scand J Rheumatol. 2017;46(6):446-53.
75. Poeppl W, Lagler H, Raderer M, Sperr WR, Zielinski C, Herkner H, et al. Influenza vaccination perception and coverage among patients with malignant disease. Vaccine. 2015;33(14):1682-7.
76. Price SA, Podczervinski S, MacLeod K, Helbert L, Pergam SA. Understanding influenza vaccination rates and reasons for refusal in caregivers and household contacts of cancer patients. Am J Infect Control. 2019;47(4):468-70.
77. Restivo V, Vizzini G, Mularoni A, Di Benedetto C, Gioè SM, Vitale F. Determinants of influenza vaccination among solid organ transplant recipients attending Sicilian reference center. Hum Vaccines Immunother. 2017;13(2):346-50.
78. Ruiz-Cuesta P, González-Alayón C, Jurado-García J, Iglesias-Flores EM, Barranco-Quintana JL, García-García L, et al. Adherence to a predefined vaccination program in patients with inflammatory bowel disease. Gastroenterol Hepatol. 2016;39(6):385-92.
79. Sadlier M, Sadlier C, Alani A, Ahmad K, Bergin C, Ramsay B. Poor adherence to vaccination guidelines in dermatology patients on immunosuppressive therapies: An issue that needs addressing. Br J Dermatol. 2015;173(1):288-9.
80. Sandler DS, Ruderman EM, Brown T, Lee JY, Mixon A, Liss DT, et al. Understanding vaccination rates and attitudes among patients with rheumatoid arthritis. Am J Managed Care. 2016;22(3).
81. Savage J, Muller F, Ormerod AD. Awareness and uptake of recommended vaccines among

immunosuppressed patients. J R Coll Phys Edinburgh. 2011;41(3):202-5.
82. Struijk GH, Lammers AJJ, Brinkman RJ, Lombarts MJMH, van Vugt M, van der Pant KAMI, et al. Immunization after renal transplantation: Current clinical practice. Transplant Infect Dis. 2015;17(2):192-200.
83. Teich N, Klugmann T, Tiedemann A, Holler B, Mössner J, Liebetrau A, et al. Vaccination coverage in immunosuppressed patients - Results of a regional health services research study. Dtsch Arztebl. 2011;108(7):105-11.
84. Urun Y, Akbulut H, Demirkazik A, Cay Senler F, Utkan G, Onur H, et al. Perception about influenza and pneumococcal vaccines and vaccination coverage among patients with malignancies and their family members. J B U ON. 2013;18(2):511-5.
85. Waszczuk K, Waszczuk E, Szenborn L. Can we better protect patients with inflammatory bowel disease against infections – patient attitude and personal immunization knowledge. Acta Gastro-Enterol Belg. 2018;81(2):257-61.
86. Wilckens V, Kannengiesser K, Hoxhold K, Frenkel C, Kucharzik T, Maaser C. The immunization status of patients with IBD is alarmingly poor before the introduction of specific guidelines. Scand J Gastroenterol. 2011;46(7-8):855-61.
87. Akan H, Yavuz E, Yayla ME, Külbay H, Kaspar E, Zahmacioğlu O, et al. Factors affecting uptake of influenza vaccination among family physicians. Vaccine. 2016;34(14):1712-8.
88. Asma S, Akan H, Uysal Y, Poçan AG, Sucakli MH, Yengil E, et al. Factors effecting influenza vaccination uptake among health care workers: A multi-center cross-sectional study. BMC Infect Dis. 2016;16(1).
89. Boey L, Bral C, Roelants M, De Schryver A, Godderis L, Hoppenbrouwers K, et al. Attitudes, believes, determinants and organisational barriers behind the low seasonal influenza vaccination uptake in healthcare workers – A cross-sectional survey. Vaccine. 2018;36(23):3351-8.
90. Bonaccorsi G, Santomauro F, Porchia BR, Niccolai G, Pellegrino E, Bonanni P, et al. Beliefs and opinions of health care workers and students regarding influenza and influenza vaccination in Tuscany, Central Italy. Vaccines. 2015;3(1):137-47.
91. Castilla J, Martínez-Baz I, Godoy P, Toledo D, Astray J, García S, et al. Trends in influenza vaccine coverage among primary healthcare workers in Spain, 2008-2011. Prev Med. 2013;57(3):206-11.
92. Çiftci F, Şen E, Demir N, Çiftci O, Erol S, Kayacan O. Beliefs, attitudes, and activities of healthcare personnel about influenza and pneumococcal vaccines. Hum Vaccines Immunother. 2018;14(1):111-7.
93. Costantino C, Ledda C, Genovese C, Contrino E, Vitale E, Maida CM, et al. Immunization status against measles of health-care workers operating at three sicilian university hospitals: An observational study. Vaccines. 2019;7(4).
94. Dedoukou X, Nikolopoulos G, Maragos A, Giannoulidou S, Maltezou HC. Attitudes towards vaccination against seasonal influenza of health-care workers in primary health-care settings in Greece. Vaccine. 2010;28(37):5931-3.
95. deSante JE, Caplan A, Shofer F, Behrman AJ. Physician attitudes towards influenza immunization and vaccine mandates. Vaccine. 2010;28(13):2517-21.
96. Domínguez A, Godoy P, Castilla J, Soldevila N, Toledo D, Astray J, et al. Knowledge of and attitudes to influenza vaccination in healthy primary healthcare workers in Spain, 2011-2012.

PLoS One. 2013;8(11):e81200.
97. Durando P, Alicino C, Dini G, Barberis I, Bagnasco AM, Iudici R, et al. Determinants of adherence to seasonal influenza vaccination among healthcare workers from an Italian region: Results from a cross-sectional study. BMJ Open. 2016;6(5).
98. Ehrenstein BP, Hanses F, Blaas S, Mandraka F, Audebert F, Salzberger B. Perceived risks of adverse effects and influenza vaccination: a survey of hospital employees. Eur J Public Health. 2010;20(5):495-9.
99. Giese C, Mereckiene J, Danis K, O'Donnell J, O'Flanagan D, Cotter S. Low vaccination coverage for seasonal influenza and pneumococcal disease among adults at-risk and health care workers in Ireland, 2013: The key role of GPs in recommending vaccination. Vaccine. 2016;34(32):3657-62.
100. Gramegna A, Dellafiore S, Contarini M, Blasi F, Aliberti S, Tosatto R, et al. Knowledge and attitudes on influenza vaccination among Italian physicians specialized in respiratory infections: An Italian respiratory society (SIP/IRS) web-based survey. J Prev Med Hyg. 2018;59(2):E128-E31.
101. Gutknecht P, Winiarski T, Trzeciak BG, Molisz A, Pietrzykowska M, Nowicka-Sauer K, et al. Opinions and behavior of family doctors concerning vaccinating against influenza. Fam Med Prim Care Rev. 2016;18(3):241-3.
102. Hagemeister M, Stock N, Ludwig T, Heuschmann P, Vogel U. Self-reported influenza vaccination rates and attitudes towards vaccination among health care workers: Results of a survey in a German university hospital. Public Health. 2018;154:102-9.
103. Harrison N, Brand A, Forstner C, Tobudic S, Burgmann K, Burgmann H. Knowledge, risk perception and attitudes toward vaccination among Austrian health care workers: A cross-sectional study. Hum Vaccines Immunother. 2016;12(9):2459-63.
104. Hopman CE, Riphagen-Dalhuisen J, Looijmans-van den Akker I, Frijstein G, Van der Geest-Blankert ADJ, Danhof-Pont MB, et al. Determination of factors required to increase uptake of influenza vaccination among hospital-based healthcare workers. J Hosp Infect. 2011;77(4):327-31.
105. Hulo S, Nuvoli A, Sobaszek A, Salembier-trichard A. Knowledge and attitudes towards influenza vaccination of health care workers in emergency services. Vaccine. 2017;35(2):205-7.
106. Johansen LJ, Stenvig T, Wey H. The Decision to Receive Influenza Vaccination Among Nurses in North and South Dakota. Public Health Nurs. 2012;29(2):116-25.
107. Kalemaki D, Karakonstantis S, Galanakis E, Lionis C. Vaccination coverage of general practitioners: a cross-sectional study from Greece. Public Health. 2020;181:110-3.
108. Karlsson LC, Lewandowsky S, Antfolk J, Salo P, Lindfelt M, Oksanen T, et al. The association between vaccination confidence, vaccination behavior, and willingness to recommend vaccines among Finnish healthcare workers. PLoS ONE. 2019;14(10).
109. Kisic-Tepavcevic D, Kanazir M, Gazibara T, Maric G, Makismovic N, Loncarevic G, et al. Predictors of hepatitis B vaccination status in healthcare workers in Belgrade, Serbia, December 2015. Eurosurveillance. 2017;22(16).
110. Lehmann BA, Ruiter RAC, van Dam D, Wicker S, Kok G. Sociocognitive predictors of the intention of healthcare workers to receive the influenza vaccine in Belgian, Dutch and German hospital settings. J Hosp Infect. 2015;89(3):202-9.

111. Maridor M, Ruch S, Bangerter A, Emery V. Skepticism toward Emerging Infectious Diseases and Influenza Vaccination Intentions in Nurses. J Health Commun. 2017;22(5):386-94.
112. Napolitano F, Bianco A, D'Alessandro A, Papadopoli R, Angelillo IF. Healthcare workers' knowledge, beliefs, and coverage regarding vaccinations in critical care units in Italy. Vaccine. 2019;37(46):6900-6.
113. Nowrouzi-Kia B, McGeer A. External cues to action and influenza vaccination among post-graduate trainee physicians in Toronto, Canada. Vaccine. 2014;32(30):3830-4.
114. Pielak KL, McIntyre CC, Tu AW, Remple VP, Halperin B, Buxton JA. Identifying attitudes, beliefs and reported practices of nurses and doctors as immunization providers. J Adv Nurs. 2010;66(7):1602-11.
115. Prematunge C, Corace K, McCarthy A, Nair RC, Roth V, Suh KN, et al. Qualitative motivators and barriers to pandemic vs. seasonal influenza vaccination among healthcare workers: A content analysis. Vaccine. 2014;32(52):7128-34.
116. Quan K, Tehrani DM, Dickey L, Spiritus E, Hizon D, Heck K, et al. Voluntary to mandatory: Evolution of strategies and attitudes toward influenza vaccination of healthcare personnel. Infect Control Hosp Epidemiol. 2012;33(1):63-70.
117. Rabensteiner A, Buja A, Regele D, Fischer M, Baldo V. Healthcare worker's attitude to seasonal influenza vaccination in the South Tyrolean province of Italy: barriers and facilitators. Vaccine. 2018;36(4):535-44.
118. Real K, Kim S, Conigliaro J. Using a validated health promotion tool to improve patient safety and increase health care personnel influenza vaccination rates. Am J Infect Control. 2013;41(8):691-6.
119. Rebmann T, Wright KS, Anthony J, Knaup RC, Peters EB. Seasonal influenza vaccine compliance among hospital-based and nonhospital-based healthcare workers. Infect Control Hosp Epidemiol. 2012;33(3):243-9.
120. Scatigna M, Fabiani L, Micolucci G, Santilli F, Mormile P, Giuliani AR. Attitudinal variables and a possible mediating mechanism for vaccination practice in health care workers of a local hospital in L'Aquila (Italy). Hum Vaccines Immunother. 2017;13(1):198-205.
121. Surtees TC, Teh BW, Slavin MA, Worth LJ. Factors contributing to declination of annual influenza vaccination by healthcare workers caring for cancer patients: An Australian experience. Vaccine. 2018;36(14):1804-7.
122. Taddei C, Ceccherini V, Niccolai G, Porchia BR, Boccalini S, Levi M, et al. Attitude toward immunization and risk perception of measles, rubella, mumps, varicella, and pertussis in health care workers working in 6 hospitals of Florence, Italy 2011. Hum Vaccin Immunother. 2014;10(9):2612-22.
123. Tanguy M, Boyeau C, Pean S, Marijon E, Delhumeau A, Fanello S. Acceptance of seasonal and pandemic a (H1N1) 2009 influenza vaccination by healthcare workers in a French Teaching Hospital. Vaccine. 2011;29(25):4190-4.
124. Vallée-Tourangeau G, Promberger M, Moon K, Wheelock A, Sirota M, Norton C, et al. Motors of influenza vaccination uptake and vaccination advocacy in healthcare workers: Development and validation of two short scales. Vaccine. 2018;36(44):6540-5.
125. Verger P, Collange F, Fressard L, Bocquier A, Gautier A, Pulcini C, et al. Prevalence and correlates of vaccine hesitancy among general practitioners: A cross-sectional telephone survey in France, April to July 2014. Eurosurveillance. 2016;21(47).

126. Vírseda S, Restrepo MA, Arranz E, Magán-Tapia P, Fernández-Ruiz M, de la Cámara AG, et al. Seasonal and Pandemic A (H1N1) 2009 influenza vaccination coverage and attitudes among health-care workers in a Spanish University Hospital. Vaccine. 2010;28(30):4751-7.
127. Wicker S, Zielen S, Rose MA. Obstacles in the Motivation of Health Care Workers for Pertussis vaccination. Procedia Vaccinology. 2010;2(1):104-6.
128. Wilson R, Scronias D, Zaytseva A, Ferry MA, Chamboredon P, Dubé E, et al. Seasonal influenza self-vaccination behaviours and attitudes among nurses in Southeastern France. Hum Vaccines Immunother. 2019;15(10):2423-33.
129. Wilson R, Zaytseva A, Bocquier A, Nokri A, Fressard L, Chamboredon P, et al. Vaccine hesitancy and self-vaccination behaviors among nurses in southeastern France. Vaccine. 2020;38(5):1144-51.
130. Zhang J, While AE, Norman IJ. Nurses' knowledge and risk perception towards seasonal influenza and vaccination and their vaccination behaviours: A cross-sectional survey. Int J Nurs Stud. 2011;48(10):1281-9.
131. Zhang J, While AE, Norman IJ. Seasonal influenza vaccination knowledge, risk perception, health beliefs and vaccination behaviours of nurses. Epidemiol Infect. 2012;140(9):1569-77.
132. Akivis Y, Leong J, Rosenstein I, Myrie A, Moy M, Markell M. Vaccination rates and relation to health beliefs in inner-city kidney transplant recipients (KTRS). Am J Transplant. 2019;19:390.
133. Ahmad Hamidi A, Gelmez Taş B, Gündüz A, Nur Çelebi S, Esen ES, Toprak D, et al. Immunization rates of pneumococcal, influenza and tetanus vaccines and knowledge and attitudes of adult patients who receive inpatient treatment at hospital: Point prevalence study. Hum Vaccines Immunother. 2018;14(11):2649-53.
134. Teich N, Klugmann T, Tiedemann A, Holler B, Mössner J, Liebetrau A, et al. Vaccination coverage in immunosuppressed patients: results of a regional health services research study. Dtsch Arztebl Int. 2011;108(7):105-11.
135. Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and Gender Differences in the Outcomes of Vaccination over the Life Course. Annu Rev Cell Dev Biol. 2017;33:577-99.
136. Verger P, Fressard L, Collange F, Gautier A, Jestin C, Launay O, et al. Vaccine Hesitancy Among General Practitioners and Its Determinants During Controversies: A National Cross-sectional Survey in France. EBioMedicine. 2015;2(8):891-7.
137. ECDC. Vaccine hesitancy among healthcare workers and their patients in Europe - A qualitative study. Stockholm: 2015.
138. van Beek J, Veenhoven RH, Bruin JP, van Boxtel RAJ, de Lange MMA, Meijer A, et al. Influenza-like Illness Incidence Is Not Reduced by Influenza Vaccination in a Cohort of Older Adults, Despite Effectively Reducing Laboratory-Confirmed Influenza Virus Infections. The Journal of Infectious Diseases. 2017;216(4):415-24.
139. Hobson-West P. 'Trusting blindly can be the biggest risk of all': organised resistance to childhood vaccination in the UK. Sociol Health Illn. 2007;29(2):198-215.
140. Poltorak M, Leach M, Fairhead J, Cassell J. 'MMR talk' and vaccination choices: an ethnographic study in Brighton. Soc Sci Med. 2005;61(3):709-19.
141. Stahl JP, Cohen R, Denis F, Gaudelus J, Martinot A, Lery T, et al. The impact of the web and social networks on vaccination. New challenges and opportunities offered to fight against vaccine hesitancy. Médecine et Maladies Infectieuses. 2016;46(3):117-22.
142. Mollema L, Staal JM, van Steenbergen JE, Paulussen TGWM, de Melker HE. An exploratory

qualitative assessment of factors influencing childhood vaccine providers' intention to recommend immunization in the Netherlands. BMC Public Health. 2012;12(1):128.
143. Heinrich-Morrison K, McLellan S, McGinnes U, Carroll B, Watson K, Bass P, et al. An effective strategy for influenza vaccination of healthcare workers in Australia: experience at a large health service without a mandatory policy. BMC Infect Dis. 2015;15:42.
144. Jarrett C, Wilson R, O'Leary M, Eckersberger E, Larson HJ. Strategies for addressing vaccine hesitancy – A systematic review. Vaccine. 2015;33(34):4180-90.
145. Horne Z, Powell D, Hummel JE, Holyoak KJ. Countering antivaccination attitudes. Proceedings of the National Academy of Sciences. 2015;112(33):10321-4.
146. Riphagen-Dalhuisen J, Burgerhof JG, Frijstein G, van der Geest-Blankert AD, Danhof-Pont MB, de Jager HJ, et al. Hospital-based cluster randomised controlled trial to assess effects of a multi-faceted programme on influenza vaccine coverage among hospital healthcare workers and nosocomial influenza in the Netherlands, 2009 to 2011. Eurosurveillance. 2013;18(26):20512.
147. World Tourism Organization. UNTWO Tourism Highlights. Madrid: 2018.
148. World Tourism Organization. Global Report on Aviation: Responding to the needs of new tourism markets and destinations Madrid, Spain: 2012.
149. LCR. Protocollen. LCR-landenlijst2019.
150. ZorgverzekeringWijzer. Eén op de drie reizigers heeft geen vaccinatie: ZorgverzekeringWijzer; 2014 [updated 02-05-2018. Available from: https://www.zorgverzekeringwijzer.nl/zorgverzekering/en-op-de-drie-reizigers-heeft-geen-vaccinatie.
151. Eldering P. Vliegtoerist negeert advies. De Telegraaf. 2018.
152. Rowe K, Chaves N, Leder K. Challenges to providing pre-travel care for travellers visiting friends and relatives: an audit of a specialist travel medicine clinic. Journal of Travel Medicine. 2017;24(5):tax038-tax.
153. Herman JS, Hill DR. Advising the traveller. Medicine. 2014;42(2):107-11.
154. GGD Reisvaccinaties. Helft kinderen ongevaccineerd op reis: GGD; 2015 [updated 16-01-2015. Available from: https://www.ggdreisvaccinaties.nl/actueel/helft-kinderen-ongevaccineerd-op-reis.
155. Mollema L, de Melker HE, Hahne SJM, van Weert JWM, Berbers GAM, van der Klis FRM. PIENTER 2-project: second research project on the protection against infectious diseases offered by the national immunization programme in the Netherlands. Bilthoven: 2010 Contract No.: RIVM report 230421001/2009.
156. Beebeejaun K, Degala S, Balogun K, Simms I, Woodhall SC, Heinsbroek E, et al. Outbreak of hepatitis A associated with men who have sex with men (MSM), England, July 2016 to January 2017. Euro Surveill. 2017;22(5).
157. Porretta A, Quattrone F, Aquino F, Pieve G, Bruni B, Gemignani G, et al. A nosocomial measles outbreak in Italy, February-April 2017. Euro Surveill. 2017;22(33).
158. Markovinovic L, Kosanovic Licina ML, Tesic V, Vojvodic D, Vladusic Lucic I, Kniewald T, et al. An outbreak of tick-borne encephalitis associated with raw goat milk and cheese consumption, Croatia, 2015. Infection. 2016;44(5):661-5.
159. LCR. Hepatitis A. D70-1 ed2016.
160. Heywood AE, Nothdurft H, Tessier D, Moodley M, Rombo L, Marano C, et al. Pre-travel advice, attitudes and hepatitis A and B vaccination rates among travellers from seven

countries†. Journal of travel medicine. 2016;24(1):taw069.
161. Rijksoverheid. Leerplicht en kwalificatieplicht [Available from: https://www.rijksoverheid.nl/onderwerpen/leerplicht/leerplicht-en-kwalificatieplicht.
162. Gil A, Gonzalez A, Dal-Re R, Dominguez V, Astasio P, Aguilar L. Detection of antibodies against hepatitis A in blood spots dried on filter paper. Is this a reliable method for epidemiological studies? Epidemiology & Infection. 1997;118(2):189-91.
163. de Almeida LM, Azevedo RS, Guimaraes AA, Coutinho Eda S, Struchiner CJ, Massad E. Detection of antibodies against hepatitis A virus in eluates of blood spotted on filter-paper: a pilot study in Rio de Janeiro, Brazil. Trans R Soc Trop Med Hyg. 1999;93(4):401-4.
164. Melgaco JG, Pinto MA, Rocha AM, Freire M, Gaspar LP, Lima SM, et al. The use of dried blood spots for assessing antibody response to hepatitis A virus after natural infection and vaccination. J Med Virol. 2011;83(2):208-17.
165. Ott JJ, Wiersma ST. Single-dose administration of inactivated hepatitis A vaccination in the context of hepatitis A vaccine recommendations. International Journal of Infectious Diseases. 2013;17(11):e939-e44.
166. Melgaço JG, Morgado LN, Santiago MA, Oliveira JMd, Lewis-Ximenez LL, Hasselmann B, et al. A single dose of inactivated hepatitis A vaccine promotes HAV-specific memory cellular response similar to that induced by a natural infection. Vaccine. 2015;33(32):3813-20.
167. Van Herck K, Jacquet J-M, Van Damme P. Antibody persistence and immune memory in healthy adults following vaccination with a two-dose inactivated hepatitis A vaccine: Long-term follow-up at 15 years. J Med Virol. 2011;83(11):1885-91.
168. Schmidtke P, Habermehl P, Knuf M, Meyer CU, Sänger R, Zepp F. Cell mediated and antibody immune response to inactivated hepatitis A vaccine. Vaccine. 2005;23(44):5127-32.
169. Fujiyama S, Odoh K, Kuramoto I, Mizuno K, Tsurusaki R, Sato T. Current seroepidemiological status of hepatitis A with a comparison of antibody titers after infection and vaccination. Journal of Hepatology. 1994;21(4):641-5.
170. StatLine C. Bevolkingsdichtheid per gemeente: Volksgezondheidenzorg.info; 2015 [updated 2015. Available from: https://www.volksgezondheidenzorg.info/onderwerp/bevolking/regionaal-internationaal/bevolkingsomvang#node-bevolkingsdichtheid-gemeente.
171. CBS. Vo; leerlingen, onderwijssoort in detail, leerjaar: CBS, StatLine; 2019 [updated April 19, 2019. Available from: https://opendata.cbs.nl/statline/#/CBS/nl/dataset/80040ned/table?ts=1558955564791.
172. Nuffic. Education System The Netherlands. 2nd ed2018.
173. André FE, D'Hondt E, Delem A, Safary A. Clinical assessment of the safety and efficacy of an inactivated hepatitis A vaccine: rationale and summary of findings. Vaccine. 1992;10:S160-S8.
174. McMahon BJ, Williams J, Bulkow L, Snowball M, Wainwright R, Kennedy M, et al. Immunogenicity of an inactivated hepatitis A vaccine in Alaska Native children and Native and non-Native adults. J Infect Dis. 1995;171(3):676-9.
175. Nothdurft HD, Dahlgren AL, Gallagher EA, Kollaritsch H, Overbosch D, Rummukainen ML, et al. The Risk of Acquiring Hepatitis A and B Among Travelers in Selected Eastern and Southern Europe and Non-European Mediterranean Countries: Review and Consensus Statement on Hepatitis A and B Vaccination. Journal of Travel Medicine. 2007;14(3):181-7.
176. Boggild AK, Castelli F, Gautret P, Torresi J, von Sonnenburg F, Barnett ED, et al. Vaccine

preventable diseases in returned international travelers: Results from the GeoSentinel Surveillance Network. Vaccine. 2010;28(46):7389-95.
177. Warne B, Weld LH, Cramer JP, Field VK, Grobusch MP, Caumes E, et al. Travel-related infection in European travelers, EuroTravNet 2011. J Travel Med. 2014;21(4):248-54.
178. WHO. Special groups of travellers. International Travel and Health 2009. p. 177-9.
179. Van Damme P, Banatvala J, Fay O, Iwarson S, McMahon B, Van Herck K, et al. Hepatitis A booster vaccination: is there a need? The Lancet. 2003;362(9389):1065-71.
180. Verhoef L, Boot HJ, Koopmans M, Mollema L, Van Der Klis F, Reimerink J, et al. Changing risk profile of hepatitis A in The Netherlands: a comparison of seroprevalence in 1995-1996 and 2006-2007. Epidemiol Infect. 2011;139(8):1172-80.
181. Maltezou HC, Pavli A, Spilioti A, Patrinos S, Lymperi I, Theodoridou M. Preparedness of paediatric international travellers departing from Athens, Greece: an 18-month airport-based survey. Acta Paediatr. 2014;103(4):e161-4.
182. van Genderen PJ, van Thiel PP, Mulder PG, Overbosch D, Dutch Schiphol Airport Study G. Trends in knowledge, attitudes, and practices of travel risk groups toward prevention of hepatitis A: results from the Dutch Schiphol Airport survey 2002 to 2009. J Travel Med. 2012;19(1):35-43.
183. Decuyper I, Van Damme P, Soentjens P, Wojciechowski M. Low adherence with national travel medicine recommendations in Belgian expatriate children: A retrospective analysis. Travel Med Infect Dis. 2019.
184. Maltezou HC, Pavli A, Theodoridou K, Bournousouzis E, Minitsios A, Katerelos P, et al. Preparedness of adolescents departing from Athens International Airport to Africa or Asia: A five-year airport-based prospective study. Travel Medicine and Infectious Disease. 2018;21:69-73.
185. Yom-Tov E, Shembekar J, Barclay S, Muennig P. The effectiveness of public health advertisements to promote health: a randomized-controlled trial on 794,000 participants. npj Digital Medicine. 2018;1(1):24.
186. Omodior O, Luetke MC, Nelson EJ. Mosquito-borne infectious disease, risk-perceptions, and personal protective behavior among U.S. international travelers. Prev Med Rep. 2018;12:336-42.
187. Neave PE, Heywood AE, Gibney KB, Leder K. Imported infections: What information should be collected by surveillance systems to inform public health policy? Travel Medicine and Infectious Disease. 2016;14(4):350-9.
188. Schlagenhauf P, Weld L, Goorhuis A, Gautret P, Weber R, von Sonnenburg F, et al. Travel-associated infection presenting in Europe (2008-12): an analysis of EuroTravNet longitudinal, surveillance data, and evaluation of the effect of the pre-travel consultation. Lancet Infect Dis. 2015;15(1):55-64.
189. Van Herck K, Van Damme P, Castelli F, Zuckerman J, Nothdurft H, Dahlgren AL, et al. Knowledge, attitudes and practices in travel-related infectious diseases: the European airport survey. J Travel Med. 2004;11(1):3-8.
190. Cousins S. Measles: a global resurgence. Lancet Infect Dis. 2019;19(4):362-3.
191. Laksono BM, de Vries RD, Verburgh RJ, Visser EG, de Jong A, Fraaij PLA, et al. Studies into the mechanism of measles-associated immune suppression during a measles outbreak in the Netherlands. Nature Communications. 2018;9(1):4944.

192. Thornton J. Measles cases in Europe tripled from 2017 to 2018. Bmj. 2019;364:l634.
193. Dahl V, Wallensten A. Self-reported infections during international travel and notifiable infections among returning international travellers, Sweden, 2009-2013. PLOS ONE. 2017;12(7):e0181625.
194. MacIntyre CR, Karki S, Sheikh M, Zwar N, Heywood AE. The role of travel in measles outbreaks in Australia – An enhanced surveillance study. Vaccine. 2016;34(37):4386-91.
195. Durrheim DN, Crowcroft NS, Blumberg LH. Is the global measles resurgence a "public health emergency of international concern"? Int J Infect Dis. 2019;83:95-7.
196. Jan H, Stuart B. Measles Vaccination Before the Measles-Mumps-Rubella Vaccine. American Journal of Public Health. 2013;103(8):1393-401.
197. LCR. Bof, Mazelen, Rodehond (BMR). LCR; 2017. p. 1-6.
198. van den Hof S, Berbers GA, de Melker HE, Conyn-van Spaendonck MA. Sero-epidemiology of measles antibodies in the Netherlands, a cross-sectional study in a national sample and in communities with low vaccine coverage. Vaccine. 1999;18(9-10):931-40.
199. WHO. Manual for the Laboratory-based Surveillance of Measles, Rubella, and Congenital Rubella Syndrome. Immunization, Vaccines and Biologicals2020.
200. Blume S, Tump J. Evidence and policymaking: The introduction of MMR vaccine in the Netherlands. Social science & medicine (1982). 2010;71(6):1049-55.
201. van Lier EA, Oomen PJ, Giesbers H, van Vliet JA, Drijfhout IH, Zonnenberg-Hoff IF, et al. Vaccinatiegraad en jaarverslag Rijksvaccinatieprogramma Nederland 2018. Bilthoven: RIVM, 2019.
202. Mollema L, Smits GP, Berbers GA, Van Der Klis FR, Van Binnendijk RS, De Melker HE, et al. High risk of a large measles outbreak despite 30 years of measles vaccination in The Netherlands. Epidemiology and Infection. 2014;142(5):1100-8.
203. Heywood AE. Measles: a re-emerging problem in migrants and travellers. J Travel Med. 2018;25(1).
204. Doornekamp L, GeurtsvanKessel C, Slobbe L, Te Marvelde MR, Scherbeijn SMJ, van Genderen PJJ, et al. Adherence to hepatitis A travel health guidelines: A cross-sectional seroprevalence study in Dutch travelling families - The Dutch travel Vaccination Study (DiVeST). Travel Med Infect Dis. 2019:101511.
205. Mei JV, Alexander JR, Adam BW, Hannon WH. Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens. The Journal of Nutrition. 2001;131(5):1631S-6S.
206. Hall EM, Flores SR, De Jesus VR. Influence of Hematocrit and Total-Spot Volume on Performance Characteristics of Dried Blood Spots for Newborn Screening. Int J Neonatal Screen. 2015;1(2):69-78.
207. Riddell MA, Byrnes GB, Leydon JA, Kelly HA. Dried venous blood samples for the detection and quantification of measles IgG using a commercial enzyme immunoassay. Bull World Health Organ. 2003;81(10):701-7.
208. Cohen BJ, Doblas D, Andrews N. Comparison of plaque reduction neutralisation test (PRNT) and measles virus-specific IgG ELISA for assessing immunogenicity of measles vaccination. Vaccine. 2008;26(50):6392-7.
209. Dorigo-Zetsma JW, Leverstein-van Hall MA, Vreeswijk J, de Vries JJC, Vossen ACTM, ten Hulscher HI, et al. Immune status of health care workers to measles virus: evaluation of

protective titers in four measles IgG EIAs. Journal of Clinical Virology. 2015;69:214-8.
210. Ratnam S, Gadag V, West R, Burris J, Oates E, Stead F, et al. Comparison of commercial enzyme immunoassay kits with plaque reduction neutralization test for detection of measles virus antibody. J Clin Microbiol. 1995;33(4):811-5.
211. Terletskaia-Ladwig E, Enders G, Meier S, Dietz K, Enders M. Development and evaluation of an automatable focus reduction neutralisation test for the detection of measles virus antibodies using imaging analysis. J Virol Methods. 2011;178(1-2):124-8.
212. de Swart RL, de Vries RD, Rennick LJ, van Amerongen G, McQuaid S, Verburgh RJ, et al. Needle-free delivery of measles virus vaccine to the lower respiratory tract of non-human primates elicits optimal immunity and protection. NPJ Vaccines. 2017;2:22.
213. Ono N, Tatsuo H, Hidaka Y, Aoki T, Minagawa H, Yanagi Y. Measles viruses on throat swabs from measles patients use signaling lymphocytic activation molecule (CDw150) but not CD46 as a cellular receptor. J Virol. 2001;75(9):4399-401.
214. MC E. Referentie-intervallen Afdeling Klinische Chemie. Rotterdam: AKC; 2020.
215. Di Pietrantonj C, Rivetti A, Marchione P, Debalini MG, Demicheli V. Vaccines for measles, mumps, rubella, and varicella in children. Cochrane Database of Systematic Reviews. 2020(4).
216. Rosario-Rosario G, Gareca M, Kincaid H, Knouse MC. Using Locally Derived Seroprevalence Data on Measles, Mumps, Rubella, and Varicella by Birth Cohort to Determine Risks for Vaccine-Preventable Diseases During International Travel. J Travel Med. 2015;22(6):396-402.
217. Turner DP, McGuinness SL, Cohen J, Waring LJ, Leder K. Use of pre-travel vaccine-preventable disease serology as a screening tool to identify patients in need of pre-travel vaccination: a retrospective audit. J Travel Med. 2017;24(3).
218. Basu S, Giri P, Adisesh A, Mc NR. Healthcare workers and measles-mumps-rubella (MMR) status: how worried should we be about further outbreaks? Epidemiol Infect. 2014;142(8):1688-94.
219. Anichini G, Gandolfo C, Fabrizi S, Miceli GB, Terrosi C, Gori Savellini G, et al. Seroprevalence to Measles Virus after Vaccination or Natural Infection in an Adult Population, in Italy. Vaccines. 2020;8(1):66.
220. Freund R, Krivine A, Prévost V, Cantin D, Aslangul E, Avril MF, et al. Measles immunity and measles vaccine acceptance among healthcare workers in Paris, France. Journal of Hospital Infection. 2013;84(1):38-43.
221. Poethko-Muller C, Mankertz A. Seroprevalence of measles-, mumps- and rubella-specific IgG antibodies in German children and adolescents and predictors for seronegativity. PLoS One. 2012;7(8):e42867.
222. RIVM. Vaccinatiegraad en jaarverslag Rijksvaccinatieprogramma Nederland 2017. Bilthoven: 2017.
223. Brinkman ID, de Wit J, Smits GP, ten Hulscher HI, Jongerius MC, Abreu TC, et al. Early Measles Vaccination During an Outbreak in the Netherlands: Short-Term and Long-Term Decreases in Antibody Responses Among Children Vaccinated Before 12 Months of Age. The Journal of Infectious Diseases. 2019;220(4):594-602.
224. Gellin B, Modlin JF, Crowe JE, Jr. Influence of Maternal Antibodies on Neonatal Immunization against Respiratory Viruses. Clinical Infectious Diseases. 2001;33(10):1720-7.
225. Woudenberg T, van Binnendijk R, Veldhuijzen I, Woonink F, Ruijs H, van der Klis F,

et al. Additional Evidence on Serological Correlates of Protection against Measles: An Observational Cohort Study among Once Vaccinated Children Exposed to Measles. Vaccines (Basel). 2019;7(4).
226. Xiong Y, Wang D, Lin W, Tang H, Chen S, Ni J. Age-related changes in serological susceptibility patterns to measles. Human Vaccines & Immunotherapeutics. 2014;10(4):1097-103.
227. Dine MS, Hutchins SS, Thomas A, Williams I, Bellini WJ, Redd SC. Persistence of vaccine-induced antibody to measles 26-33 years after vaccination. J Infect Dis. 2004;189 Suppl 1:S123-30.
228. Bolotin S, Hughes SL, Gul N, Khan S, Rota PA, Severini A, et al. What Is the Evidence to Support a Correlate of Protection for Measles? A Systematic Review. J Infect Dis. 2020;221(10):1576-83.
229. Hahné SJM, Nic Lochlainn LM, van Burgel ND, Kerkhof J, Sane J, Yap KB, et al. Measles Outbreak Among Previously Immunized Healthcare Workers, the Netherlands, 2014. The Journal of Infectious Diseases. 2016;214(12):1980-6.
230. Sundell N, Dotevall L, Sansone M, Andersson M, Lindh M, Wahlberg T, et al. Measles outbreak in Gothenburg urban area, Sweden, 2017 to 2018: low viral load in breakthrough infections. Eurosurveillance. 2019;24(17):1900114.
231. Haralambieva IH, Kennedy RB, Ovsyannikova IG, Schaid DJ, Poland GA. Current perspectives in assessing humoral immunity after measles vaccination. Expert Review of Vaccines. 2019;18(1):75-87.
232. CDC. Measles: CDC; 2020 [updated May 11, 2018. Available from: https://wwwnc.cdc.gov/travel/diseases/measles.
233. Koch D. Measles outbreaks: Plea for a regular check of the vaccination booklet. Swiss Med Wkly. 2019;149(23-24).
234. McAllister G, Shepherd S, Templeton K, Aitken C, Gunson R. Long term stability of HBsAg, anti-HBc and anti-HCV in dried blood spot samples and eluates. J Clin Virol. 2015;71:10-7.
235. van den Hof S, van Gageldonk-Lafeber AB, van Binnendijk RS, van Gageldonk PG, Berbers GA. Comparison of measles virus-specific antibody titres as measured by enzyme-linked immunosorbent assay and virus neutralisation assay. Vaccine. 2003;21(27-30):4210-4.
236. Lewis JD, Enfield KB, Sifri CD. Hepatitis B in healthcare workers: Transmission events and guidance for management. World J Hepatol. 2015;7(3):488-97.
237. Ryan KJ, Ray CG, Sherris JC. Sherris medical microbiology2010.
238. European Centre for Disease Prevention and Control. Monitoring the responses to hepatitis B and C epidemics in EU/EEA Member States, 2019. Stockholm: ECDC. 2020.
239. WHO. Hepatitis B 2020 [updated 27 July 2020. Available from: https://www.who.int/news-room/fact-sheets/detail/hepatitis-b.
240. European Centre for Disease Prevention and Control. Hepatitis B. ECDC Annual epidemiological report for 2017 Stockholm. 2019.
241. De Schryver A, Claesen B, Meheus A, van Sprundel M, François G. European survey of hepatitis B vaccination policies for healthcare workers. European Journal of Public Health. 2010;21(3):338-43.
242. Organization; WH. Hepatitis B vaccines: WHO position paper, July 2017 - Recommendations. Vaccine. 2019;37(2):223-5.
243. Immunity; ECGoHB. Are booster immunisations needed for lifelong hepatitis B immunity?

The Lancet. 2000;355(9203):561-5.
244. Rijksinstituut voor Volksgezondheid en Milieu. Richtlijn Uitvoering RVP 2020. 2020.
245. RIVM-LCI. Praktijkrichtlijn voor bedrijfsartsen: hepatitis B-vaccinatie van risicolopend personeel dat geen risicovormer is: RIV<; 2016 [Available from: https://lci.rivm.nl/sites/default/files/2019-06/Praktijkrichtlijn%20voor%20bedrijfsartsen%20hepatitis%20B-vaccinatie%20van%20risicolopend%20personeel.pdf.
246. Leuridan E, Van Damme P. Hepatitis B and the need for a booster dose. Clin Infect Dis. 2011;53(1):68-75.
247. Yang S, Tian G, Cui Y, Ding C, Deng M, Yu C, et al. Factors influencing immunologic response to hepatitis B vaccine in adults. Sci Rep. 2016;6:27251.
248. Are booster immunisations needed for lifelong hepatitis B immunity? European Consensus Group on Hepatitis B Immunity. Lancet. 2000;355(9203):561-5.
249. Authority HPR. Human vaccines [Available from: http://www.hpra.ie/homepage/medicines/medicines-information/vaccines.
250. Bini C, Grazzini M, Chellini M, Mucci N, Arcangeli G, Tiscione E, et al. Is hepatitis B vaccination performed at infant and adolescent age able to provide long-term immunological memory? An observational study on healthcare students and workers in Florence, Italy. Hum Vaccin Immunother. 2018;14(2):450-5.
251. Grosso G, Mistretta A, Marventano S, Ferranti R, Mauro L, Cunsolo R, et al. Long-term persistence of seroprotection by hepatitis B vaccination in healthcare workers of southern Italy. Hepat Mon. 2012;12(9):e6025.
252. Lamberti M, De Rosa A, Garzillo EM, Corvino AR, Sannolo N, De Pascalis S, et al. Vaccination against hepatitis b virus: are Italian medical students sufficiently protected after the public vaccination programme? J Occup Med Toxicol. 2015;10:41.
253. Verso MG, Costantino C, Vitale F, Amodio E. Immunization against Hepatitis B Surface Antigen (HBsAg) in a Cohort of Nursing Students Two Decades after Vaccination: Surprising Feedback. Vaccines (Basel). 2019;8(1).
254. Bagheri-Jamebozorgi M, Keshavarz J, Nemati M, Mohammadi-Hossainabad S, Rezayati MT, Nejad-Ghaderi M, et al. The persistence of anti-HBs antibody and anamnestic response 20 years after primary vaccination with recombinant hepatitis B vaccine at infancy. Hum Vaccin Immunother. 2014;10(12):3731-6.
255. Saffar H, Saffar MJ, Ajami A, Khalilian AR, Shams-Esfandabad K, Mirabi AM. Long-term T-cell-mediated immunologic memory to hepatitis B vaccine in young adults following neonatal vaccination. Hepat Mon. 2014;14(9):e22223.
256. Bauer T, Jilg W. Hepatitis B surface antigen-specific T and B cell memory in individuals who had lost protective antibodies after hepatitis B vaccination. Vaccine. 2006;24(5):572-7.
257. Wang RX, Boland GJ, van Hattum J, de Gast GC. Long-term persistence of T cell memory to HBsAg after hepatitis B vaccination. World J Gastroenterol. 2004;10(2):260-3.
258. Carollo M, Palazzo R, Bianco M, Pandolfi E, Chionne P, Fedele G, et al. Hepatitis B specific T cell immunity induced by primary vaccination persists independently of the protective serum antibody level. Vaccine. 2013;31(3):506-13.
259. CDC. Hepatitis B and the health care worker. In: CDC, editor.
260. ECDC. Hepatitis B. Stockholm: European Centre for Disease Prevention and Control, 2019.

261. ECDC. Vaccine Scheduler: ECDC; 2019 [updated 2019. Available from: https://vaccine-schedule.ecdc.europa.eu/.
262. WHO. WHO Immunological Basis for Immunization Series. Geneva: WHO, 2017.
263. Kessels JA, Recuenco S, Navarro-Vela AM, Deray R, Vigilato M, Ertl H, et al. Pre-exposure rabies prophylaxis: a systematic review. Bull World Health Organ. 2017;95(3):210-9C.
264. WHO. WHO Expert Consultation on Rabies. Geneva: WHO, 2018.
265. LCI. Rabiës richtlijn: RIVM; 2016 [updated 13 december 2019. Available from: https://lci.rivm.nl/richtlijnen/rabies.
266. Smith JS, Yager PA, Baer GM. A rapid reproducible test for determining rabies neutralizing antibody. Bulletin of the World Health Organization. 1973;48(5):535-41.
267. Cliquet F, Aubert M, Sagne L. Development of a fluorescent antibody virus neutralisation test (FAVN test) for the quantitation of rabies-neutralising antibody. J Immunol Methods. 1998;212(1):79-87.
268. Dacheux L, Wacharapluesadee S, Hemachudha T, Meslin FX, Buchy P, Reynes JM, et al. More accurate insight into the incidence of human rabies in developing countries through validated laboratory techniques. PLoS Negl Trop Dis. 2010;4(11):e765.
269. Welch RJ, Anderson BL, Litwin CM. An evaluation of two commercially available ELISAs and one in-house reference laboratory ELISA for the determination of human anti-rabies virus antibodies. J Med Microbiol. 2009;58(Pt 6):806-10.
270. WHO. WHO Guidelines on hepatitis B and C testing. 2016.
271. Nichols BE, Girdwood SJ, Shibemba A, Sikota S, Gill CJ, Mwananyanda L, et al. Cost and Impact of Dried Blood Spot Versus Plasma Separation Card for Scale-up of Viral Load Testing in Resource-limited Settings. Clin Infect Dis. 2020;70(6):1014-20.
272. Wasniewski M, Labbe A, Tribout L, Rieder J, Labadie A, Schereffer JL, et al. Evaluation of a rabies ELISA as an alternative method to seroneutralisation tests in the context of international trade of domestic carnivores. Journal of Virological Methods. 2014;195:211-20.
273. Wasniewski M, Barrat J, Combes B, Guiot AL, Cliquet F. Use of filter paper blood samples for rabies antibody detection in foxes and raccoon dogs. Journal of Virological Methods. 2014;204:11-6.
274. Zero by 30: the global strategic plan to end human deaths from dog-mediated rabies by 2030 [press release]. Geneve: WHO, May 7, 2020 2018.
275. Grüner N, Stambouli O, Ross RS. Dried blood spots--preparing and processing for use in immunoassays and in molecular techniques. J Vis Exp. 2015(97).
276. De Swart RL, Nur Y, Abdallah A, Kruining H, El Mubarak HS, Ibrahim SA, et al. Combination of Reverse Transcriptase PCR Analysis and Immunoglobulin M Detection on Filter Paper Blood Samples Allows Diagnostic and Epidemiological Studies of Measles. Journal of Clinical Microbiology. 2001;39(1):270-3.
277. de Bruin E, Loeber JG, Meijer A, Castillo GM, Cepeda ML, Torres-Sepúlveda MR, et al. Evolution of an influenza pandemic in 13 countries from 5 continents monitored by protein microarray from neonatal screening bloodspots. J Clin Virol. 2014;61(1):74-80.
278. Banga N, Guss P, Banga A, Rosenman KD. Incidence and variables associated with inadequate antibody titers after pre-exposure rabies vaccination among veterinary medical students. Vaccine. 2014;32(8):979-83.

279. Bujang MA, Adnan TH. Requirements for Minimum Sample Size for Sensitivity and Specificity Analysis. Journal of Clinical and Diagnostic Research. 2016;10.
280. EURL. Rabies Serology Proficiency Test Report. ANSES; 2019 April 2019. Report No.
281. Mansfield KL, Andrews N, Goharriz H, Goddard T, McElhinney LM, Brown KE, et al. Rabies pre-exposure prophylaxis elicits long-lasting immunity in humans. Vaccine. 2016;34(48):5959-67.
282. Olugasa BO, Odeniyi AO, Adeogun AO, Adeola OA. Antibody levels against rabies among occupationally exposed individuals in a Nigerian University. Vet Ital. 2010;46(1):21-8.
283. Dietzschold B, Tollis M, Lafon M, Wunner WH, Koprowski H. Mechanisms of rabies virus neutralization by glycoprotein-specific monoclonal antibodies. Virology. 1987;161(1):29-36.
284. Lange B, Cohn J, Roberts T, Camp J, Chauffour J, Gummadi N, et al. Diagnostic accuracy of serological diagnosis of hepatitis C and B using dried blood spot samples (DBS): two systematic reviews and meta-analyses. BMC Infectious Diseases. 2017;17(1):700.
285. Colson KE, Potter A, Conde-Glez C, Hernandez B, Ríos-Zertuche D, Zúñiga-Brenes P, et al. Use of a commercial ELISA for the detection of measles-specific immunoglobulin G (IgG) in dried blood spots collected from children living in low-resource settings. Journal of Medical Virology. 2015;87(9):1491-9.
286. Ducrocq J, Proulx JF, Levesque B, De Serres G, Wood H, Lemire M. Assessment of naturally acquired neutralizing antibodies against rabies Lyssavirus in a subset of Nunavik's Inuit population considered most at risk of being exposed to rabid animals. Zoonoses Public Health. 2019.
287. Gold S, Donnelly CA, Nouvellet P, Woodroffe R. Rabies virus-neutralising antibodies in healthy, unvaccinated individuals: What do they mean for rabies epidemiology? PLOS Neglected Tropical Diseases. 2020;14(2):e0007933.
288. Cutts FT, Hanson M. Seroepidemiology: an underused tool for designing and monitoring vaccination programmes in low- and middle-income countries. Trop Med Int Health. 2016;21(9):1086-98.
289. Meidani M, Naeini AE, Rostami M, Sherkat R, Tayeri K. Immunocompromised patients: Review of the most common infections happened in 446 hospitalized patients. J Res Med Sci. 2014;19(Suppl 1):S71-3.
290. Thirumala R, Ramaswamy M, Chawla S. Diagnosis and Management of Infectious Complications in Critically Ill Patients with Cancer. Critical Care Clinics. 2010;26(1):59-91.
291. Murphey K, Weaver, C. Janeway's Immunobiology. 9th edition ed: Garland Science; 2017.
292. James EA, LaFond RE, Gates TJ, Mai DT, Malhotra U, Kwok WW. Yellow fever vaccination elicits broad functional CD4+ T cell responses that recognize structural and nonstructural proteins. J Virol. 2013;87(23):12794-804.
293. Croce E, Hatz C, Jonker EF, Visser LG, Jaeger VK, Buhler S. Safety of live vaccinations on immunosuppressive therapy in patients with immune-mediated inflammatory diseases, solid organ transplantation or after bone-marrow transplantation - A systematic review of randomized trials, observational studies and case reports. Vaccine. 2017;35(9):1216-26.
294. Lopez A, Mariette X, Bachelez H, Belot A, Bonnotte B, Hachulla E, et al. Vaccination recommendations for the adult immunosuppressed patient: A systematic review and comprehensive field synopsis. J Autoimmun. 2017;80:10-27.
295. Malhi G, Rumman A, Thanabalan R, Croitoru K, Silverberg MS, Hillary Steinhart A, et

al. Vaccination in inflammatory bowel disease patients: attitudes, knowledge, and uptake. J Crohns Colitis. 2015;9(6):439-44.
296. Martino C, Gallone MS, Quarto M, Germinario C, Tafuri S. Immunization coverage among splenectomized patients: Results of an ad hoc survey in Puglia Region (South of Italy). Hum Vaccin Immunother. 2016;12(5):1277-9.
297. Yeung JH, Goodman KJ, Fedorak RN. Inadequate knowledge of immunization guidelines: a missed opportunity for preventing infection in immunocompromised IBD patients. Inflamm Bowel Dis. 2012;18(1):34-40.
298. Balvert F, van den Bemd G. Amazing [Online magazine]. Erasmus MC; 2015 [updated 28-06-2018. Available from: https://www.erasmusmc.nl/overerasmusmc/publicaties/amazing/.
299. O'Cathain A, Murphy E, Nicholl J. The quality of mixed methods studies in health services research. Journal of health services research & policy. 2008;13(2):92-8.
300. Coignard-Biehler H, Lanternier F, Hot A, Salmon D, Berger A, de Montalembert M, et al. Adherence to preventive measures after splenectomy in the hospital setting and in the community. J Infect Public Health. 2011;4(4):187-94.
301. Patel RR, Liang SY, Koolwal P, Kuhlmann FM. Travel advice for the immunocompromised traveler: prophylaxis, vaccination, and other preventive measures. Ther Clin Risk Manag. 2015;11:217-28.
302. Bialy C, Horne K, Dendle C, Kanellis J, Littlejohn G, Ratnam I, et al. International travel in the immunocompromised patient: a cross-sectional survey of travel advice in 254 consecutive patients. Intern Med J. 2015;45(6):618-23.
303. Stine JG, Khokhar OS, Charalambopoulos J, Shanmugam VK, Lewis JH. Rheumatologists' awareness of and screening practices for hepatitis B virus infection prior to initiating immunomodulatory therapy. Arthritis Care Res (Hoboken). 2010;62(5):704-11.
304. Boeije H. Analyseren in kwalitatief onderzoek: Boom Lemma Uitgevers; 2005.
305. GmbH L. LimeSurvey: An Open Source survey tool Hamburg, Germany.
306. IBM SPSS Statistics for Windows. 24.0 ed. Armonk, NY2016.
307. GraphPad Prism 5. 5 ed: GraphPad Software, inc.; 2007.
308. Sorgdrager W. Wet medisch-wetenschappelijk onderzoek met mensen 1998 [Available from: http://wetten.overheid.nl/BWBR0009408/2017-03-01.
309. The Netherlands Code of Conduct for Academic Practice, (2014).
310. Jung YS, Park JH, Kim HJ, Cho YK, Sohn CI, Jeon WK, et al. Insufficient knowledge of korean gastroenterologists regarding the vaccination of patients with inflammatory bowel disease. Gut Liver. 2014;8(3):242-7.
311. Kumar D, Ferreira VH, Blumberg E, Silveira F, Cordero E, Perez-Romero P, et al. A Five-year Prospective Multi-center Evaluation of Influenza Infection in Transplant Recipients. Clinical Infectious Diseases. 2018.
312. El-Alfy MS, El-Sayed MH. Overwhelming postsplenectomy infection: is quality of patient knowledge enough for prevention? The hematology journal : the official journal of the European Haematology Association / EHA. 2004;5(1):77-80.
313. Loubet P, Verger P, Abitbol V, Peyrin-Biroulet L, Launay O. Pneumococcal and influenza vaccine uptake in adults with inflammatory bowel disease in France: Results from a web-based study. Dig Liver Dis. 2018.
314. Nguyen M, Lindegaard H, Hendricks O, Friis-Moller N. Factors associated with influenza

and pneumococcal vaccine uptake among rheumatoid arthritis patients in Denmark invited to participate in a pneumococcal vaccine trial (Immunovax_RA). Scand J Rheumatol. 2017;46(6):446-53.

315. Moro JA, Almenar L, Martnez-Dolz L, Aguero J, Sanchez-Lazaro I, Iglesias P, et al. Support program for heart transplant patients: initial experience. Transplant Proc. 2008;40(9):3039-40.
316. Evans EW, Redmond EC. An assessment of food safety information provision for UK chemotherapy patients to reduce the risk of foodborne infection. Public Health. 2017;153:25-35.
317. Cutrona SL, Golden JG, Goff SL, Ogarek J, Barton B, Fisher L, et al. Improving Rates of Outpatient Influenza Vaccination Through EHR Portal Messages and Interactive Automated Calls: A Randomized Controlled Trial. J Gen Intern Med. 2018;33(5):659-67.
318. Sheth H, Moreland L, Peterson H, Aggarwal R. Improvement in herpes zoster vaccination in patients with rheumatoid arthritis: A quality improvement project. J Rheumatol. 2017;44(1):11-7.
319. Isenor JE, Edwards NT, Alia TA, Slayter KL, MacDougall DM, McNeil SA, et al. Impact of pharmacists as immunizers on vaccination rates: A systematic review and meta-analysis. Vaccine. 2016;34(47):5708-23.
320. Posadzki P, Mastellos N, Ryan R, Gunn LH, Felix LM, Pappas Y, et al. Automated telephone communication systems for preventive healthcare and management of long-term conditions. Cochrane Database Syst Rev. 2016;12:CD009921.
321. Wasilczuk K, Korzeniewski K. Immunocompromised travellers. Int Marit Health. 2017;68(4):229-37.
322. Mikati T, Griffin K, Lane D, Matasar M, Shah MK. International travel patterns and travel risks for stem cell transplant recipients. J Travel Med. 2015;22(1):39-47.
323. Melmed GY, Ippoliti AF, Papadakis KA, Tran TT, Birt JL, Lee SK, et al. Patients with inflammatory bowel disease are at risk for vaccine-preventable illnesses. Am J Gastroenterol. 2006;101(8):1834-40.
324. WHO. Up to 650 000 people die of respiratory diseases linked to seasonal flu each year.: World Health Organization; 2017 [Available from: https://www.who.int/news-room/detail/14-12-2017-up-to-650-000-people-die-of-respiratory-diseases-linked-to-seasonal-flu-each-year.
325. Tinsley A, Navabi S, Williams ED, Liu G, Kong L, Coates MD, et al. Increased Risk of Influenza and Influenza-Related Complications Among 140,480 Patients With Inflammatory Bowel Disease. Inflamm Bowel Dis. 2018.
326. Beck CR, McKenzie BC, Hashim AB, Harris RC, Zanuzdana A, Agboado G, et al. Influenza vaccination for immunocompromised patients: summary of a systematic review and meta-analysis. Influenza Other Respir Viruses. 2013;7 Suppl 2:72-5.
327. Andrisani G, Frasca D, Romero M, Armuzzi A, Felice C, Marzo M, et al. Immune response to influenza A/H1N1 vaccine in inflammatory bowel disease patients treated with anti TNF-alpha agents: effects of combined therapy with immunosuppressants. J Crohns Colitis. 2013;7(4):301-7.
328. Hagihara Y, Ohfuji S, Watanabe K, Yamagami H, Fukushima W, Maeda K, et al. Infliximab and/or immunomodulators inhibit immune responses to trivalent influenza vaccination in

adults with inflammatory bowel disease. J Crohns Colitis. 2014;8(3):223-33.
329. Shirai S, Hara M, Sakata Y, Tsuruoka N, Yamamoto K, Shimoda R, et al. Immunogenicity of Quadrivalent Influenza Vaccine for Patients with Inflammatory Bowel Disease Undergoing Immunosuppressive Therapy. Inflamm Bowel Dis. 2018;24(5):1082-91.
330. Marin AC, Gisbert JP, Chaparro M. Immunogenicity and mechanisms impairing the response to vaccines in inflammatory bowel disease. World J Gastroenterol. 2015;21(40):11273-81.
331. Feagan BG, Sandborn WJ, Gasink C, Jacobstein D, Lang Y, Friedman JR, et al. Ustekinumab as Induction and Maintenance Therapy for Crohn's Disease. N Engl J Med. 2016;375(20):1946-60.
332. Mamula P, Markowitz JE, Piccoli DA, Klimov A, Cohen L, Baldassano RN. Immune response to influenza vaccine in pediatric patients with inflammatory bowel disease. Clin Gastroenterol Hepatol. 2007;5(7):851-6.
333. Launay O, Abitbol V, Krivine A, Slama LB, Bourreille A, Dupas JL, et al. Immunogenicity and Safety of Influenza Vaccine in Inflammatory Bowel Disease Patients Treated or not with Immunomodulators and/or Biologics: A Two-year Prospective Study. J Crohns Colitis. 2015;9(12):1096-107.
334. Cullen G, Bader C, Korzenik JR, Sands BE. Serological response to the 2009 H1N1 influenza vaccination in patients with inflammatory bowel disease. Gut. 2012;61(3):385-91.
335. deBruyn J, Fonseca K, Ghosh S, Panaccione R, Gasia MF, Ueno A, et al. Immunogenicity of Influenza Vaccine for Patients with Inflammatory Bowel Disease on Maintenance Infliximab Therapy: A Randomized Trial. Inflamm Bowel Dis. 2016;22(3):638-47.
336. Davignon JL, Rauwel B, Degboe Y, Constantin A, Boyer JF, Kruglov A, et al. Modulation of T-cell responses by anti-tumor necrosis factor treatments in rheumatoid arthritis: a review. Arthritis Res Ther. 2018;20(1):229.
337. Aversa G, Punnonen J, de Vries JE. The 26-kD transmembrane form of tumor necrosis factor alpha on activated CD4+ T cell clones provides a costimulatory signal for human B cell activation. J Exp Med. 1993;177(6):1575-85.
338. Sands BE, Sandborn WJ, Panaccione R, O'Brien CD, Zhang H, Johanns J, et al. Ustekinumab as Induction and Maintenance Therapy for Ulcerative Colitis. N Engl J Med. 2019;381(13):1201-14.
339. Biemans VBC, van der Meulen-de Jong AE, van der Woude CJ, Lowenberg M, Dijkstra G, Oldenburg B, et al. Ustekinumab for Crohn's disease: results of the ICC Registry, a nationwide prospective observational cohort study. J Crohns Colitis. 2019.
340. Liefferinckx C, Verstockt B, Gils A, Noman M, Van Kemseke C, Macken E, et al. Long-term Clinical Effectiveness of Ustekinumab in Patients with Crohn's Disease Who Failed Biologic Therapies: A National Cohort Study. J Crohns Colitis. 2019;13(11):1401-9.
341. Rahier JF, Magro F, Abreu C, Armuzzi A, Ben-Horin S, Chowers Y, et al. Second European evidence-based consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. J Crohns Colitis. 2014;8(6):443-68.
342. Teng MW, Bowman EP, McElwee JJ, Smyth MJ, Casanova JL, Cooper AM, et al. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. Nat Med. 2015;21(7):719-29.
343. Schmitt N, Bustamante J, Bourdery L, Bentebibel SE, Boisson-Dupuis S, Hamlin F, et al. IL-12 receptor beta1 deficiency alters in vivo T follicular helper cell response in humans.

Blood. 2013;121(17):3375-85.
344. Murphy K, Weaver C. Janeway's immunobiology. 9th edition ed. New York: Ww Norton & Co; 2017. 374, 466 p.
345. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol. 2005;19 Suppl A:5A-36A.
346. Hirst GK. The Quantitative Determination of Influenza Virus and Antibodies by Means of Red Cell Agglutination. J Exp Med. 1942;75(1):49-64.
347. Fonville JM, Wilks SH, James SL, Fox A, Ventresca M, Aban M, et al. Antibody landscapes after influenza virus infection or vaccination. Science. 2014;346(6212):996-1000.
348. Soesman NM, Rimmelzwaan GF, Nieuwkoop NJ, Beyer WE, Tilanus HW, Kemmeren MH, et al. Efficacy of influenza vaccination in adult liver transplant recipients. J Med Virol. 2000;61(1):85-93.
349. Trombetta CM, Montomoli E. Influenza immunology evaluation and correlates of protection: a focus on vaccines. Expert Rev Vaccines. 2016;15(8):967-76.
350. Beyer WE, Palache AM, Luchters G, Nauta J, Osterhaus AD. Seroprotection rate, mean fold increase, seroconversion rate: which parameter adequately expresses seroresponse to influenza vaccination? Virus Res. 2004;103(1-2):125-32.
351. Brodmerkel C, Wadman E, Langley RG, Papp KA, Bourcier M, Poulin Y, et al. Immune response to pneumococcus and tetanus toxoid in patients with moderate-to-severe psoriasis following long-term ustekinumab use. J Drugs Dermatol. 2013;12(10):1122-9.
352. Haykir Solay A, Eser F. High dose hepatitis B vaccine is not effective in patients using immunomodulatory drugs: a pilot study. Hum Vaccin Immunother. 2019;15(5):1177-82.
353. Brodmerkel C, Zhu Y, Jiao Q, Cornacoff J, Treacy G, Mascelli MA, et al. Effects of ustekinumab administration on primate/human antigen-recall and humoral immune response functions. J Drugs Dermatol. 2010;9(6):677-83.
354. Ma CS, Avery DT, Chan A, Batten M, Bustamante J, Boisson-Dupuis S, et al. Functional STAT3 deficiency compromises the generation of human T follicular helper cells. Blood. 2012;119(17):3997-4008.
355. Odegard JM, Marks BR, DiPlacido LD, Poholek AC, Kono DH, Dong C, et al. ICOS-dependent extrafollicular helper T cells elicit IgG production via IL-21 in systemic autoimmunity. J Exp Med. 2008;205(12):2873-86.
356. Luytjes W, Enouf V, Schipper M, Gijzen K, Liu WM, van der Lubben M, et al. HI responses induced by seasonal influenza vaccination are associated with clinical protection and with seroprotection against non-homologous strains. Vaccine. 2012;30(35):5262-9.
357. Hoft DF, Lottenbach KR, Blazevic A, Turan A, Blevins TP, Pacatte TP, et al. Comparisons of the Humoral and Cellular Immune Responses Induced by Live Attenuated Influenza Vaccine and Inactivated Influenza Vaccine in Adults. Clin Vaccine Immunol. 2017;24(1).
358. Schleker T, Jacobsen EM, Mayer B, Strauss G, Debatin KM, Posovszky C. Preserved in vitro immunoreactivity in children receiving long-term immunosuppressive therapy due to inflammatory bowel disease or autoimmune hepatitis. Mol Cell Pediatr. 2018;5(1):1.
359. Colombel JF, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med.

2010;362(15):1383-95.
360. Singh S, Facciorusso A, Dulai PS, Jairath V, Sandborn WJ. Comparative Risk of Serious Infections With Biologic and/or Immunosuppressive Therapy in Patients With Inflammatory Bowel Diseases: A Systematic Review and Meta-Analysis. Clin Gastroenterol Hepatol. 2019.
361. Matsumoto H, Ohfuji S, Watanabe K, Yamagami H, Fukushima W, Maeda K, et al. Booster influenza vaccination does not improve immune response in adult inflammatory bowel disease patients treated with immunosuppressives: a randomized controlled trial. J Gastroenterol. 2015;50(8):876-86.
362. Caldera F, Hillman L, Saha S, Wald A, Grimes I, Zhang Y, et al. Immunogenicity of High Dose Influenza Vaccine for Patients with Inflammatory Bowel Disease on Anti-TNF Monotherapy: A Randomized Clinical Trial. Inflamm Bowel Dis. 2019.
363. Farraye FA, Melmed GY, Lichtenstein GR, Kane SV. ACG Clinical Guideline: Preventive Care in Inflammatory Bowel Disease. Am J Gastroenterol. 2017;112(2):241-58.
364. Wyant T, Leach T, Sankoh S, Wang Y, Paolino J, Pasetti MF, et al. Vedolizumab affects antibody responses to immunisation selectively in the gastrointestinal tract: randomised controlled trial results. Gut. 2015;64(1):77-83.
365. Coenen S, Weyts E, Jorissen C, De Munter P, Noman M, Ballet V, et al. Effects of Education and Information on Vaccination Behavior in Patients with Inflammatory Bowel Disease. Inflammatory Bowel Dis. 2017;23(2):318-24.
366. Kuster SP, Shah PS, Coleman BL, Lam PP, Tong A, Wormsbecker A, et al. Incidence of influenza in healthy adults and healthcare workers: a systematic review and meta-analysis. PLoS One. 2011;6(10):e26239.
367. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. Nature. 2008;451(7181):990-3.
368. Cleaveland S, Laurenson MK, Taylor LH. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Philos T Roy Soc B. 2001;356(1411):991-9.
369. Woolhouse MEJ. Population biology of emerging and re-emerging pathogens. Trends Microbiol. 2002;10(10):3-7.
370. Funk S, Gilad E, Watkins C, Jansen Vaa. The spread of awareness and its impact on epidemic outbreaks. P Natl Acad Sci USA. 2009;106(16):6872-7.
371. Higgins C, Lavin T, Metcalfe O. Health Impacts of Education a review: Institute of Public Health in Ireland; 2008. 25- p.
372. Valente TW, Paredes P, Poppe PR. Matching the message to the process: the relative ordering of knowledge, attitudes, and practices in behavior change research. Hum Commun Res. 1998;24(3):366-85.
373. Schrader P, Lawless KA. The knowledge, attitudes, & behaviors approach how to evaluate performance and learning in complex environments. Performance Improvement. 2004;43(9):8-15.
374. Visser PS, Krosnick JA. Development of attitude strength over the life cycle: surge and decline. J Pers Soc Psychol. 1998;75(6):1389-410.
375. Langford R, Bonell C, Jones H, Pouliou T, Murphy S, Waters E, et al. The World Health Organization's Health Promoting Schools framework: a Cochrane systematic review and meta-analysis. BMC Public Health. 2015;15:130.

376. Fonner VA, Armstrong KS, Kennedy CE, O'Reilly KR, Sweat MD. School based sex education and HIV prevention in low- and middle-income countries: a systematic review and meta-analysis. PLoS One. 2014;9(3):e89692-e.
377. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2197-223.
378. WHO. Dengue and severe dengue 2015 [28 December, 2015]. Available from: http://www.who.int/mediacentre/factsheets/fs117/en/.
379. Przewlocka T. Evaluation of the hepatitis B prevention education programme in Poland. Vaccine. 2000;18 Suppl 1:S46-8.
380. Acemoglu H, Palanci Y, Set T, Vancelik S, Isik M, Polat H. An intervention study for viral hepatitis. Peer-led health education among high school students. Saudi medical journal. 2011;32(2):183-7.
381. Kwan TT, Tam KF, Lee PW, Chan KK, Ngan HY. The effect of school-based cervical cancer education on perceptions towards human papillomavirus vaccination among Hong Kong Chinese adolescent girls. Patient education and counseling. 2011;84(1):118-22.
382. Ibrahim N, Abalkhail B, Rady M, Al Bar H. An educational programme on dengue fever prevention and control for females in Jeddah high schools. East Mediterr Health J. 2009;15(5):1058-67.
383. Mangklakeeree N, Pinitsoontorn S, Srisaenpang S. Effects of an influenza prevention program using non-pharmaceutical prevention measures to improve the knowledge, attitudes and practices of elementary school students in Nakhon Phanom province, Thailand. Southeast Asian J Trop Med Public Health. 2013;44(4):630-5.
384. The World Bank. Countries and Economies [Available from: http://data.worldbank.org/country.
385. Technasium. Stichting Technasium [Available from: https://www.technasium.nl/content/stichting-technasium.
386. Nuffic. The Surinamese education system described and compared with the Dutch system 2015 [Available from: https://www.epnuffic.nl/documentatie/publicaties/onderwijssysteem-suriname.pdf.
387. Nuffic. The Indonesian education system described and compared with the Dutch system 2015 [3rd Edition:[Available from: https://www.epnuffic.nl/documentatie/publicaties/onderwijssysteem-indonesie.pdf.
388. Akpinar M, del Campo C, Eryarsoy E. Learning effects of an international group competition project. Innovations in Education and Teaching International. 2015;52(2):160-71.
389. Michael J. Where's the evidence that active learning works? Adv Physiol Educ. 2006;30(4):159-67.
390. Gardner J, Belland BR. A Conceptual Framework for Organizing Active Learning Experiences in Biology Instruction. Journal of Science Education and Technology. 2012;21(4):465-75.
391. Barab SA. Doing science at the elbows of experts: Issues related to the science apprenticeship camp. Journal of research in science teaching. 2001;38(1):70-102.
392. Linton DL, Farmer JK, Peterson E. Is Peer Interaction Necessary for Optimal Active Learning? CBE-Life Sciences Education. 2014;13(2):243-52.
393. Viruskenner. Viruskenner - Knowledge is the antidote 2015 [Available from: http://

viruskenner.nl/?lang=en.
394. Viruskenner. YouTube channel 2015 [Available from: https://www.youtube.com/channel/UCHrYwQA5KghNdfDAkKot5yw.
395. Viruskenner. Viruskenner Facebook Page. 2015.
396. Field A. Discovering statistics with SPSS. 2nd ed ed: London: Sage; 2005.
397. Abdi H. Factor rotations in factor analysis. Encyclopedia of Social Sciences Research Methods. Thousand Oaks (CA): Sage; 2003.
398. Kaiser HF. An index of factorial simplicity. Psychometrika. 1974;39(1):31-6.
399. Schönrock-Adema J, Heijne-Penninga M, van Hell EA, Cohen-Schotanus J. Necessary steps in factor analysis: Enhancing validation studies of educational instruments. The PHEEM applied to clerks as an example. Medical Teacher. 2009;31(6):e226-e32.
400. Kaiser HF. The application of electronic computers to factor analysis. Educational and Psychological Measurement. 1960;20:141-51.
401. Kline P. The handbook of psychological testing. Londen: Routledge; 1999.
402. Hojat M, Xu G. A visitor's guide to effect sizes: statistical significance versus practical (clinical) importance of research findings. Advances in health sciences education : theory and practice. 2004;9(3):241-9.
403. Prince M. Does active learning work? A review of the research. J Eng Educ. 2004;93:223-32.
404. Johnson TP, Van de Vijver FJ. Social desirability in cross-cultural research. Cross-cultural survey methods. 3252003. p. 195-204.
405. Al-Iryani B, Basaleem H, Al-Sakkaf K, Crutzen R, Kok G, van den Borne B. Evaluation of a school-based HIV prevention intervention among Yemeni adolescents. BMC Public Health. 2011;11:279.
406. Barss P, Grivna M, Ganczak M, Bernsen R, Al-Maskari F, El Agab H, et al. Effects of a rapid peer-based HIV/AIDS educational intervention on knowledge and attitudes of high school students in a high-income Arab country. J Acquir Immune Defic Syndr. 2009;52(1):86-98.
407. Michielsen K, Beauclair R, Delva W, Roelens K, Van Rossem R, Temmerman M. Effectiveness of a peer-led HIV prevention intervention in secondary schools in Rwanda: results from a non-randomized controlled trial. BMC Public Health. 2012;12:729.
408. Cole BP, Nelson TD, Steele RG. An evaluation of a peer-based HIV/AIDS education program as implemented in a suburban high school setting. J HIV AIDS Prev Child Youth. 2008;9(1):84-96.
409. Gao X, Wu Y, Zhang Y, Zhang N, Tang J, Qiu J, et al. Effectiveness of School-based Education on HIV/AIDS Knowledge, Attitude, and Behavior among Secondary School Students in Wuhan, China. PLoS ONE. 2012;7(9).
410. Poobalan AS, Pitchforth E, Imamura M, Tucker JS, Philip K, Spratt J, et al. Characteristics of effective interventions in improving young people's sexual health: a review of reviews. Sex Education. 2009;9(3):319-36.
411. Espada JP, Orgiles M, Morales A, Ballester R, Huedo-Medina TB. Effectiveness of a school HIV/AIDS prevention program for Spanish adolescents. AIDS education and prevention : official publication of the International Society for AIDS Education. 2012;24(6):500-13.
412. van der Maas F, Otte WM. Evaluation of HIV/AIDS secondary school peer education in rural Nigeria. Health education research. 2009;24(4):547-57.

413. Givaudan M, Leenen I, Van de Vijver FJ, Poortinga YH, Pick S. Longitudinal study of a school based HIV/AIDS early prevention program for Mexican adolescents. Psychology, health & medicine. 2008;13(1):98-110.
414. Cartagena RG, Veugelers PJ, Kipp W, Magigav K, Laing LM. Effectiveness of an HIV Prevention Program for Secondary School Students in Mongolia. Journal of Adolescent Health. 2006;39(6):925.e9-.e16.
415. Ali B, Esmaiili S, Leila R, Alinaghi SAS, Foroughi M. The effects of a social-cognitive method based education on knowledge and attitudes intentions with respect to HIV transmition among school learners in Maragheh, Iran. Asian Pacific Journal of Tropical Disease. 2014;4(2):166-8.
416. Huszti HC, Clopton JR, Mason PJ. Acquired immunodeficiency syndrome educational program: Effects on adolescents' knowledge and attitudes. Pediatrics. 1989;84(6):986-94.
417. Paul-Ebhohimhen VA, Poobalan A, van Teijlingen ER. A systematic review of school-based sexual health interventions to prevent STI/HIV in sub-Saharan Africa. BMC Public Health. 2008;8:4.
418. Thato R, Jenkins RA, Dusitsin N. Effects of the culturally-sensitive comprehensive sex education programme among Thai secondary school students. J Adv Nurs. 2008;62(4):457-69.
419. Doyle AM, Ross DA, Maganja K, Baisley K, Masesa C, Andreasen A, et al. Long-term biological and behavioural impact of an adolescent sexual health intervention in Tanzania: follow-up survey of the community-based MEMA kwa Vijana Trial. PLoS Med. 2010;7(6):e1000287.
420. Cowan FM, Pascoe SJ, Langhaug LF, Mavhu W, Chidiya S, Jaffar S, et al. The Regai Dzive Shiri project: results of a randomized trial of an HIV prevention intervention for youth. AIDS (London, England). 2010;24(16):2541-52.
421. Langford R, Bonell CP, Jones HE, Pouliou T, Murphy SM, Waters E, et al. The WHO Health Promoting School framework for improving the health and well-being of students and their academic achievement. Cochrane Database Syst Rev. 2014(4):Cd008958.
422. Gortmaker SL, Peterson K, Wiecha J, Sobol AM, Dixit S, Fox MK, et al. Reducing obesity via a school-based interdisciplinary intervention among youth: Planet Health. Arch Pediatr Adolesc Med. 1999;153(4):409-18.
423. Khan MA, Bass DM. Viral infections: new and emerging. Curr Opin Gastroenterol. 2010;26(1):26-30.
424. Tregoning JS, Schwarze J. Respiratory Viral Infections in Infants: Causes, Clinical Symptoms, Virology, and Immunology. Clinical Microbiology Reviews. 2010;23(1):74-98.
425. Billington J, Deschamps I, Erck SC, Gerberding JL, Hanon E, Ivol S, et al. Developing Vaccines for SARS-CoV-2 and Future Epidemics and Pandemics: Applying Lessons from Past Outbreaks. Health Secur. 2020;18(3):241-9.
426. Funk S, Gilad E, Watkins C, Jansen VAA. The spread of awareness and its impact on epidemic outbreaks. Proceedings of the National Academy of Sciences. 2009;106(16):6872-7.
427. Doornekamp L, Stegers-Jager KM, Vlek OM, Klop T, Goeijenbier M, van Gorp ECM. Experience with a Multinational, Secondary School Education Module with a Focus on Prevention of Virus Infections. The American Journal of Tropical Medicine and Hygiene. 2017;97(1):97-108.

428. Nutbeam D. Health literacy as a public health goal: a challenge for contemporary health education and communication strategies into the 21st century. Health Promotion International. 2000;15(3):259-67.
429. Marks DF. Health Psychology in Context. J Health Psychol. 1996;1(1):7-21.
430. Marmot MG, Shipley MJ, Rose G. Inequalities in death - Specific explanations of a general pattern? The Lancet. 1984;323(8384):1003-6.
431. Marmot MG, Stansfeld S, Patel C, North F, Head J, White I, et al. Health inequalities among British civil servants: the Whitehall II study. The Lancet. 1991;337(8754):1387-93.
432. Super S, Wagemakers MAE, Picavet HSJ, Verkooijen KT, Koelen MA. Strengthening sense of coherence: opportunities for theory building in health promotion. Health Promotion International. 2015;31(4):869-78.
433. Bandura A. Health promotion from the perspective of social cognitive theory. Psychology and health. 1998;13(4):623-49.
434. WHO. Health promotion: a discussion document on the concept and principles Summary report of the Working Group on Concept and Principles of Health Promotion. Copenhagen: WHO Regional Office for Europe; 1984.
435. Perkins DD, Zimmerman MA. Empowerment theory, research, and application. American Journal of Community Psychology. 1995;23(5):569-79.
436. Zimmerman MA. Psychological empowerment: Issues and illustrations. . American Journal of Community Psychology. 1995;23(5):581-99.
437. Van Regenmortel T. Empowerment als uitdagend kader voor sociale inclusie. Journal of Social Intervention: Theory and Practice. 2009;18(4):22-42.
438. Boumans J. Naar het hart van empowerment deel 1. Een onderzoek naar de grondslagen van empowerment van kwetsbare groepen. Movisie, 2012.
439. Wallerstein N, Bernstein E. Empowerment Education: Freire's Ideas Adapted to Health Education. Health Education Quarterly. 1988;15(4):379-94.
440. Freire P. Pedagogy of the oppressed. New York: Continuum; 1971.
441. Beeker C, Guenther-Grey C, Raj A. Community empowerment paradigm drift and the primary prevention of HIV/AIDS. Social Science & Medicine. 1998;46(7):831-42.
442. Jacobs G. Participatie en empowerment in de gezondheidsbevordering: Professionals in de knel tussen ideaal en praktijk? Journal of Social Intervention: Theory and Practice. 2005;14:29-39.
443. Wallerstein N. Powerlessness, empowerment, and health: implications for health promotion programs. Am J Health Promot. 1992;6(3):197-205.
444. Sleeboom I, Hermanns J, Hermanns V. Meedoen leer je door mee te doen: Een inventarisatie van de wijze waarop in zorg voor kinderen van 0 tot 12 jaar met beperkingen aandacht besteed wordt aan (toekomstige) maatschappelijke participatie. Den Haag/Nieuwegein: ZonMw/CrossOver, 2010.
445. Laverack G, Labonte R. A planning framework for community empowerment goals within health promotion. Health Policy and Planning. 2000;15(3):255-62.
446. Matthews C. Critical pedagogy in health education. Health Education Journal. 2014;73(5):600-9.
447. Jacobs G, Braakman, M., & Houweling, J. Op eigen kracht naar gezond leven: empowerment in de gezondheidsbevordering; concepten, werkwijzen en onderzoeksmethoden. Utrecht:

Universiteit voor de Humanistiek; 2005.
448. Mohajer M, Earnest, J. Youth empowerment for the most vulnerable: A model based on the pedagogy of Freire and experiences in the field. Health Education. 2009;109(5):424-38.
449. Leurs MTW, Bessems K, Schaalma HP, de Vries H. Focus points for school health promotion improvements in Dutch primary schools. Health education research. 2006;22(1):58-69.
450. Hsieh H-F, Shannon SE. Three Approaches to Qualitative Content Analysis. Qualitative Health Research. 2005;15(9):1277-88.
451. Leech NL, Onwuegbuzie AJ. An array of qualitative data analysis tools: A call for data analysis triangulation. School Psychology Quarterly. 2007;22(4):557-84.
452. Boeije H. Analysis in qualitative research. Los Angeles: Sage; 2010.
453. Holmberg C, Larsson C, Korp P, Lindgren E-C, Jonsson L, Fröberg A, et al. Empowering aspects for healthy food and physical activity habits: adolescents' experiences of a school-based intervention in a disadvantaged urban community. International Journal of Qualitative Studies on Health and Well-being. 2018;13(sup1):1487759.
454. Mann K, Gordon J, MacLeod A. Reflection and reflective practice in health professions education: a systematic review. Advances in Health Sciences Education. 2007;14(4):595.
455. Frymier AB, Shulman GM. "What's in it for me?": Increasing content relevance to enhance students' motivation. Communication Education. 1995;44(1):40-50.
456. Langford R, Bonell CP, Jones HE, Pouliou T, Murphy SM, Waters E, et al. The WHO Health Promoting School framework for improving the health and well-being of students and their academic achievement. Cochrane Database of Systematic Reviews. 2014(4).
457. Kerrigan D, Mbwambo J, Likindikoki S, Davis W, Mantsios A, Beckham SW, et al. Project Shikamana: Community Empowerment-Based Combination HIV Prevention Significantly Impacts HIV Incidence and Care Continuum Outcomes Among Female Sex Workers in Iringa, Tanzania. J Acquir Immune Defic Syndr. 2019;82(2):141-8.
458. Trilling B, Fadel C. 21st century skills: Learning for life in our times. San Francisco: John Wiley & Sons; 2009.
459. Dahl KKB. 'From worse to better': how Kenyan student-teachers can use participatory action research in health education. Educational Action Research. 2014;22(2):159-77.
460. Cornelius-White J. Learner-Centered Teacher-Student Relationships Are Effective: A Meta-Analysis. Review of Educational Research. 2007;77(1):113-43.
461. Wicker S, Maltezou HC. Vaccine-preventable diseases in Europe: where do we stand? Expert Rev Vaccines. 2014;13(8):979-87.
462. Jacobson RM, Ovsyannikova IG, Vierkant RA, Pankratz VS, Poland GA. Independence of measles-specific humoral and cellular immune responses to vaccination. Hum Immunol. 2012;73(5):474-9.
463. de Vries RD, de Swart RL. Evaluating measles vaccines: can we assess cellular immunity? Expert Review of Vaccines. 2012;11(7):779-82.
464. Verso MG, Costantino C, Vitale F, Amodio E. Immunization against Hepatitis B Surface Antigen (HBsAg) in a Cohort of Nursing Students Two Decades after Vaccination: Surprising Feedback. Vaccines. 2019;8(1):1.
465. Rupprecht CE, Salahuddin N. Current status of human rabies prevention: remaining barriers to global biologics accessibility and disease elimination. Expert Review of Vaccines. 2019;18(6):629-40.

466. Doherty M, Schmidt-Ott R, Santos JI, Stanberry LR, Hofstetter AM, Rosenthal SL, et al. Vaccination of special populations: Protecting the vulnerable. Vaccine. 2016;34(52):6681-90.
467. Doornekamp L, van Gorp ECM, Koopmans MPG. Combatting Ebola anno 2019: the role of available vaccines and therapies. Nederlands Tijdschrift voor Medische Microbiologie. 2019;27:218-26.
468. Doornekamp L, van Gorp ECM, Koopmans MPG. The fight against Ebola – lessons learned from the epidemic in Western Africa. Tijdschrift voor Infectieziekten. 2019;14:11-7.
469. Yaqub O, Castle-Clarke S, Sevdalis N, Chataway J. Attitudes to vaccination: A critical review. Social Science & Medicine. 2014;112:1-11.
470. Halliley JL, Kyu S, Kobie JJ, Walsh EE, Falsey AR, Randall TD, et al. Peak frequencies of circulating human influenza-specific antibody secreting cells correlate with serum antibody response after immunization. Vaccine. 2010;28(20):3582-7.
471. Vinck P, Pham PN, Bindu KK, Bedford J, Nilles EJ. Institutional trust and misinformation in the response to the 2018–19 Ebola outbreak in North Kivu, DR Congo: a population-based survey. The Lancet Infectious Diseases. 2019;19(5):529-36.
472. Gefenaite G, Smit M, Nijman HW, Tami A, Drijfhout IH, Pascal A, et al. Comparatively low attendance during Human Papillomavirus catch-up vaccination among teenage girls in the Netherlands: Insights from a behavioral survey among parents. BMC Public Health. 2012;12(1):498.
473. Krawczyk A, Knäuper B, Gilca V, Dubé E, Perez S, Joyal-Desmarais K, et al. Parents' decision-making about the human papillomavirus vaccine for their daughters: I. Quantitative results. Hum Vaccin Immunother. 2015;11(2):322-9.
474. Dodd RH, Cvejic E, Bonner C, Pickles K, McCaffery KJ, Ayre J, et al. Willingness to vaccinate against COVID-19 in Australia. The Lancet Infectious Diseases. 2020.
475. Group C. A future vaccination campaign against COVID-19 at risk of vaccine hesitancy and politicisation. Lancet Infect Dis. 2020;20(7):769-70.
476. Giles-Vernick T, Kutalek R, Napier D, Kaawa-Mafigiri D, Dückers M, Paget J, et al. A new social sciences network for infectious threats. Lancet Infect Dis. 2019;19(5):461-3.
477. Price A, Schroter S, Snow R, Hicks M, Harmston R, Staniszewska S, et al. Frequency of reporting on patient and public involvement (PPI) in research studies published in a general medical journal: a descriptive study. BMJ Open. 2018;8(3):e020452.
478. Eitzel MV, Cappadonna JL, Santos-Lang C, Duerr RE, Virapongse A, West SE, et al. Citizen science terminology matters: exploring key terms. Citizen Science: Theory and Practice. 2017;2(1).
479. Wiggins A, Wilbanks J. The Rise of Citizen Science in Health and Biomedical Research. The American Journal of Bioethics. 2019;19(8):3-14.
480. Larson H, de Figueiredo A, Karafllakis E, Rawal M. State of vaccine confidence in the EU 2018. LSHTM, Commission E; 2018.
481. de Figueiredo A, Simas C, Karafillakis E, Paterson P, Larson HJ. Mapping global trends in vaccine confidence and investigating barriers to vaccine uptake: a large-scale retrospective temporal modelling study. Lancet. 2020;396(10255):898-908.

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