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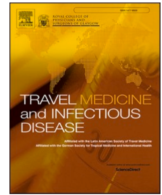

Measles seroprevalence among Dutch travelling families

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ABSTRACT

Background: 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 and 1975, compared to those born before 1965 and after 1975.
Methods: 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).
Results: In 188 (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 and 1975 were protected in 89%.
Conclusions: 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 and 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.

1. Introduction

Globally, measles cases are on the rise [1,2]. Measles, being a vaccine-preventable virus infection, is an important cause of childhood mortality and can induce neurological complications and long-lasting immune suppression [3]. Incidence rates are increasing as a consequence of declining vaccination coverage rates – worsened by the COVID-19 pandemic – driven by factors such as health care access and vaccine hesitancy [2]. 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 [4]. As measles outbreaks are currently happening in countries where 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 [1], unprotected travellers are at increased risk with respect to

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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 [5]. 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 [6]. We have to be careful to prevent a global measles outbreak being the next public health emergency of international concern [7].

Since its availability in 1960s, live-attenuated measles vaccines have been incorporated in national immunisation programmes (NIPs) worldwide [8]. 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 [9,10]. 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 [10]. If the immune status is not clear, serology can be performed, or direct measles vaccination can be considered [9]. Different serological assays are available to determine the level of antibodies. It is important to note that an individual is considered protected from measles if the concentration of antibodies that neutralize measles virus is higher than 120 mIU/ml [11]. However, subjects without detectable neutralizing antibodies may still be protected based on cellular immunity.

Since 1987, Dutch infants get vaccinated with two doses of MMR vaccine: one at the age of 14 months (MMR-1) and another one at nine years (MMR-2) [12]. In 2019, the reported vaccination coverage for MMR-1 was 92.9% among two-year olds in the Netherlands [13]. 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 to 2007) [14].

At this time of measles resurgence, travel clinics have to pay special attention to measles protection [15]. 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 [16]. 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.

2. Material and methods

2.1. 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 [16].

2.2. 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, share a copy of their vaccination records if available and donate blood by a finger prick. The questionnaires contained questions regarding demographics, medical history, participation in the national immunisation programme and travel vaccination history and travel characteristics. Vaccination records could enclose the national immunisation programme and/or a separate yellow booklet for travel vaccinations. 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 2 h, 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° Celsius until tested.

2.3. Elution of DBS samples

Filter paper cards were thawed and dried blood spots (DBS) were punched from these cards with a 3 mm diameter paper-hole punch. This spot size was considered to contain 1.5 μL (μl) of serum [17,18]. 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 1 h at 37° Celsius. If the remaining DBS was not sufficient, the 1:6 eluates from the previous study on this cohort [16] 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° 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.

2.4. Laboratory testing

2.4.1. Enzyme linked immunoassay

Previous studies have measured measles IgG concentrations in DBS samples using the enzyme immunoassay (EIA) Dade Behring Enzygnost [19]. 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.

2.4.2. Virus neutralization assay

Due to the relatively low sensitivity of measles ELISAs [20–22] we decided to retest all negative and equivocal samples with a focus reduction neutralization test (FRNT). The FRNT is a simplified neutralization test based on the gold standard PRNT [23]. 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 [24], 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

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

	Measles seronegative[a] 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 (Partly) other nationality than Dutch	2 (4.0)	16 (8.5)	18 (7.5)	0.426
NIP				0.588
Yes	48 (96.0)	178 (96.8)	227 (96.6)	
Education level (child)[a]				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)[a]				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.
[a] Including equivocal results.

serum (Sigma-Aldrich, USA), further referred to as D10F. Twofold serial dilutions were made by serial transfer of 24 μl. 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 2 h at 37° for neutralization. Subsequently, the virus-serum dilutions were transferred to Vero-humanCD150 [25] monolayers - that were seeded four days prior to the start of the assay - and incubated for another 4 h. Thereafter, virus-serum dilutions were replaced by 50 μl of D10F supplemented with 200 μM fusion inhibitory peptide (FIP: Zd-Phe-L-Phe-Gly-OH, Bachem, Heidelberg, Germany) to prevent cell-to-cell spread of the virus. After 48 h 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 min 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.

2.5. 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.

2.6. 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.

3. Results

Of the 246 travellers that were recruited between September 2016 and December 2018, 30% were a child (12–18 years old), 55% were female and 8% had a (partly) other nationality than Dutch. In this study population, 97% indicated being vaccinated following the NIP. Individuals were divided into 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). From 9 participants the year of birth was missing.

3.1. 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.

3.2. 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

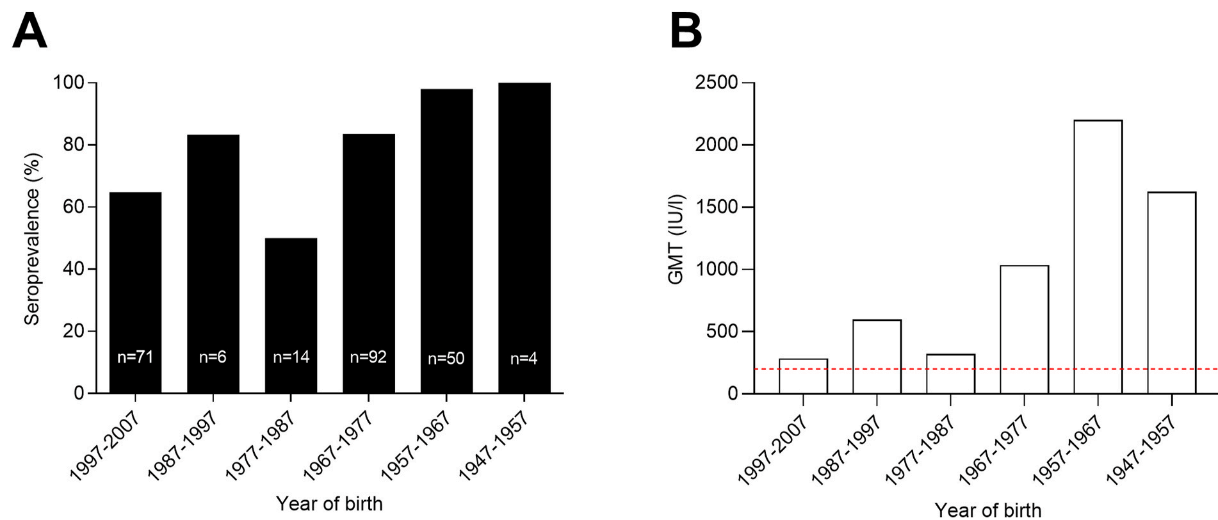

Fig. 1. Seroprevalence rates and geometric mean titres per age group.
The x-axis shows the different age groups. In panel A the y-axis shows the rate of travellers with a measles antibody titre >200 IU/l. In panel B the y-axis shows the geometric mean of the measles antibody titre (IU/l) with the cut-off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

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% (25 out of 25). In the children (all older than 12), we found a seroprevalence rate of only 67% (48 out of 72), compared to 84% (141 out of 167) 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 (Fig. 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%) were considered protected on basis of their antibody levels measured with ELISA. 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 [16]. 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 51 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 [26].

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 (Fig. 2). Of these 20 retested samples, 9 (45%) were children, which is comparable to the fraction of children in all 37 ELISA-negative samples (n = 18, 49%)

Out of the 14 samples that were found to be equivocal in the ELISA (150–200 IU/l), 5 were retested with the FRNT. All samples with equivocal results in the ELISA (titre range 173–191 IU/l) tested negative in 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. 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), 17 (85%, 95% CI 64–95) were negative in the FRNT as well. Two samples (10%) that were negative in the ELISA showed equivocal results in the FRNT (165 and 141 IU/l). One sample became positive (424 IU/l) (Fig. 2).

We assume, based on the most optimistic view on the combined ELISA and FRNT data in which 17 out of 20 ELISA negative samples

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

	Measles seronegative[a] n = 50	Measles seropositive n = 189	Total n = 239 (100%)	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)	

[a] Including equivocal results.

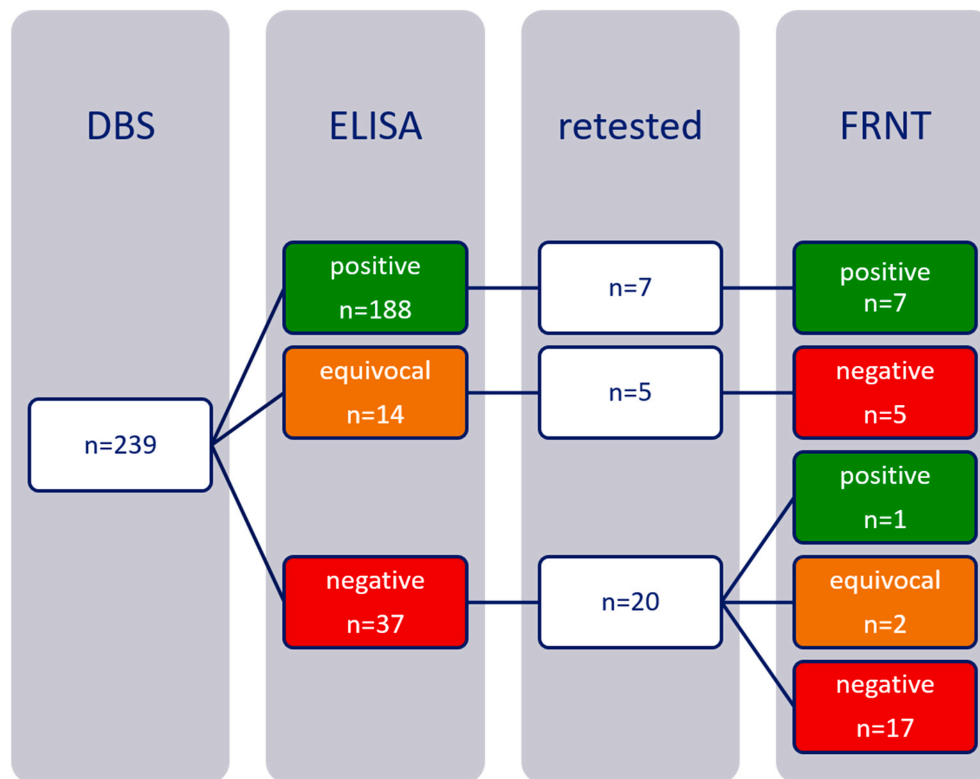

Fig. 2. Diagnostic flowchart.
This flowchart shows which serological tests were performed on what part of the samples. ELISA = enzyme linked immunoassay. FRNT = focus reduction neutralization test.

were FRNT negative as well, that 85% of the seronegative individuals measured with the ELISA are truly seronegative. This means that there would have been 43 negative DBS instead of the measured 51. 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).

4. 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 [9] 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% [27] 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 [14]. 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) [21]. However, when they retested these samples with the PRNT, the rate increased to 99% [21]. This raise in seroprevalence after retesting is in accordance with the increase we found and shows higher sensitivity of a neutralization assay compared to an ELISA. However, the difference in our study was restricted.

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%) [28]. Specifically in travellers, a large retrospective study in Australia reported lacking serological evidence of protection against measles in 8% of the 683 travellers [29]. They also noticed higher rates of seronegative results in those born after 1982 (15%) [29]. In addition, low measles seroprevalence rates in young people were reported by other European studies [30–32]. 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) [30]. A French study reported that the seroprotection rate (CAPTIA anti-measles IgG >90 IU/l) among HCWs younger than 30 years old was 87% compared to 96% among HCW older than 30 [32]. In an Italian study, seroprevalence rates between 73 and 79% were found in people aged 19–36 years, while seroprevalence rates ranged from 82 to 99% in those older than 37 years (measured with LIAISON XL) [31]. On the other hand, in a German pediatric population, only 9% of the 14–17 years old had a negative measles titre (Siemens Enzygnost anti-measles IgG titre <150 IU/l) [33]. 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 [21].

The increasing vaccine 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) [34]. 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 [35], 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 [36]. 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 [27,37]. 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 only 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 [27].

A more probable explanation for the low seroprevalence rate in younger people might be waning vaccine-induced immunity [38]. 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. [39] have shown that in 92% protective titres persisted 26–33 years after vaccination. Also, younger age groups had a lesser chance of getting a natural booster by exposure.

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 [11,37,40]. 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 [41], defined as breakthrough infections [42]. 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 [42]. 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 [38], 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 [27]. Therefore, it is important to aim for the highest possible compliance to the two-doses MMR in national immunisation programmes and perform check-ups to find out if every willing individual has been properly vaccinated [43]. Even more so since herd immunity against measles is only reached if the seroprevalence rate is at least 95%.

The CDC stated that in the United States, the majority of measles cases are seen in international travellers [44]. 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 [45].

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° Celsius we expect this effect to be marginal [46]. 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 [47]. 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) [11]. Although earlier studies reported good agreement between the FRNT and PRNT [23], 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.

CRediT authorship contribution statement

Laura Doornekamp: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **Anouskha D. Comvalius:** Methodology, Validation, Investigation. **Corine H. GeurtsvanKessel:**

Resources, Writing – review & editing. **Lennert Slobbe:** Writing – review & editing. **Sandra M.J. Scherbeijn:** Methodology, Validation. **Perry J.J. van Genderen:** Writing – review & editing. **Rob S. van Binnendijk:** Writing – review & editing. **Eric C.M. van Gorp:** Supervision, Project administration, Funding acquisition. **Rik L. de Swart:** Methodology, Validation, Supervision, Writing – review & editing. **Marco Goeijenbier:** Conceptualization, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

All authors declare no conflicts of interest.

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