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Non-participation in a population-based seroprevalence study of vaccine-preventable diseases

H. E. DE MELKER[1]*, N. J. D. NAGELKERDE[2] AND M. A. E. CONYN-VAN SPAENDONCK[1]

[1]*Department of Infectious Diseases Epidemiology, National Institute of Public Health and the Environment, P.O. Box 1, 3720 BA Bilthoven, The Netherlands*
[2]*Computerization and Methodological Consultancy Unit, National Institute of Public Health and the Environment, Bilthoven, The Netherlands*

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SUMMARY

To estimate the immunity of the Dutch population against vaccine-preventable diseases, a population-based serum bank was established. Since a multi-tiered approach to enrol eligible individuals was used, both the overall non-response selection and the effect, on this selection, of including additional participants and of excluding a subgroup of non-participants (i.e. those without questionnaire data) could be studied. For some characteristics associated with non-participation, an association with seroprevalence of vaccine-preventable diseases is likely (e.g. age, gender). For other characteristics (e.g. marital status, receipt of reminder, degree of urbanization) the association with immune status is unclear but probably small. If the distribution in the population, or information on all participants and non-participants, of the characteristic is available, then the effect on the seroprevalence can be estimated. However, investigators have to be aware that studying only a subgroup of non-participants might lead to a biased insight into non-participation selection. Furthermore, merely including additional participants might not always reduce this bias.

INTRODUCTION

To estimate the immunity of the Dutch population against vaccine-preventable diseases, we established a serum bank of Dutch individuals in a population-based study [1]. Most previous serosurveys had used residual sera from blood banks, military recruits or specialist clinics which are not representative of the whole population. Despite an appropriate sampling frame, serum collection may still be biased due to selective non-participation [2–8]. To reduce this bias, one may incorporate information on characteristics of non-participants into sample estimates [9]. In our study, a multi-tiered approach to enrol eligible individuals was used. This approach made it possible to study, not only the overall selection bias due to non-response, but also to study the effect of including additional participants and of excluding a subgroup of non-participants on this selection [10–12].

* Author for correspondence.

MATERIALS AND METHODS

Sampling design

In each of five geographic regions in the Netherlands with similar population sizes, eight municipalities were sampled with a probability proportional to their population size. An age-stratified sample of approx. 380 individuals was randomly selected within each municipality from the municipal registers [1]. The age strata were 0, 1–4 and then by 5-year classes up to 75–79 years. Twenty individuals were sampled in each

Table 1. *Odds ratios (ORs) and 95% confidence intervals (95% CI) for characteristics ('Non-questionnaire' variables) comparing all non-participants with participants; Pienter Project, 1995–6, The Netherlands*

	Numbers in subgroup	Crude ORs	(95% CI)	Adjusted ORs	(95% CI)
Age group (years) by gender					
Male					
0–4	1636	1·34	(1·18–1·51)	1·00	(0·85–1·18)
5–14	820	0·48	(0·40–0·56)	0·36	(0·29–0·44)
15–29	1194	1·65	(1·44–1·89)	1·35	(1·14–1·59)
30–64*	2842	1·0		1·0	
65–79	1017	1·01	(0·88–1·17)	1·06	(0·92–1·23)
Female					
0–4	1551	2·06	(1·82–2·34)	1·62	(1·37–1·91)
5–14	785	0·78	(0·66–0·93)	0·58	(0·47–0·71)
15–29	1205	1·47	(1·28–1·68)	1·22	(1·03–1·43)
30–64*†	2756	1·0		1·0	
65–79	1383	2·02	(1·78–2·31)	1·88	(1·64–2·16)
Marital status					
Married*	6012	1·0		1·0	
Unmarried	7769	1·42	(1·33–1·52)	1·44	(1·27–1·63)
Widowed/divorced	1408	1·86	(1·66–2·09)	1·59	(1·40–1·80)
Country of nationality					
Netherlands*	14568	1·0		1·0	
Turkey	128	1·33	(0·94–1·89)	1·12	(0·78–1·61)
Morocco	128	0·80	(0·56–1·15)	0·65	(0·45–0·95)
Other	365	2·52	(2·03–3·15)	2·41	(1·91–3·03)
Reminder					
By telephone*	7348	1·0		1·0	
By mail	7386	1·38	(1·29–1·47)	1·31	(1·22–1·40)
Other	455	6·56	(5·16–8·35)	6·38	(4·99–8·15)
Degree of urbanization					
Very high	2280	2·03	(1·83–2·25)	1·92	(1·72–2·15)
High	1900	1·20	(1·07–1·34)	1·20	(1·06–1·35)
Moderate	3798	1·07	(0·98–1·17)	1·10	(1·00–1·22)
Low	3040	1·01	(0·92–1·11)	1·03	(0·92–1·14)
No*	4171	1·0		1·0	
Region					
Central	3040	0·98	(0·89–1·09)	0·93	(0·83–1·05)
Southeast	3040	0·90	(0·81–1·00)	0·84	(0·76–0·94)
Northwest	3038	1·06	(0·96–1·18)	1·00	(0·90–1·13)
Southwest	3033	1·10	(0·99–1·21)	0·95	(0·84–1·07)
Northeast*	3038	1·0		1·0	

* Reference group.

† Women aged 30–64 years versus men aged 30–64 year crude OR 0·64 (95% CI 0·58–0·72) and adjusted OR 0·62 (95% CI 0·56–0·70).

stratum except the youngest two, where 40 individuals were sampled, as a lower response rate was observed in these age groups in a pilot study [13]. Data were collected between October 1995 and December 1996. Subjects were contacted by mail and requested to fill out a questionnaire and to visit a clinic for blood sampling on one of two dates, during daytime or evening hours. Before these dates, individuals received a reminder by telephone, if possible, or in writing. Individuals who were unable to attend on either of the

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Table 2. *Odds ratios (ORs) and 95% confidence intervals (95% CI) for characteristics ('Non-questionnaire' variables) comparing additional participants (AP;* n = *455), non-participants with a full questionnaire (OQ;* n = *1618), non-participants with a non-response questionnaire (NQ;* n = *1053) and absolute non-participants (NP;* n = *4159) with initial participants (IP); Pienter Project, 1995–6, The Netherlands*

	Adjusted ORs AP	Adjusted ORs OQ	Adjusted ORs NQ	Adjusted ORs NP
Age group (years) by gender				
Male				
0–4	0·63 (0·39–1·01)	3·54 (2·62–4·78)	1·11 (0·81–1·52)	0·51 (0·42–0·62)
5–14	0·54 (0·32–0·91)	0·74 (0·50–1·09)	0·45 (0·30–0·68)	0·26 (0·20–0·33)
15–29	1·29 (0·83–2·02)	1·72 (1·23–2·41)	1·49 (1·08–2·06)	1·29 (1·07–1·56)
30–64*	1·0	1·0	1·0	1·0
65–79	0·46 (0·27–0·78)	1·04 (0·76–1·41)	0·87 (0·63–1·20)	1·07 (0·91–1·26)
Female				
0–4	1·11 (0·69–1·78)	4·07 (3·01–5·49)	1·67 (1·21–2·32)	0·94 (0·77–1·16)
5–14	0·86 (0·51–1·46)	1·37 (0·97–1·95)	0·61 (0·40–0·93)	0·38 (0·29–0·49)
15–29	0·92 (0·58–1·48)	1·46 (1·07–2·01)	1·47 (1·07–2·02)	1·06 (0·88–1·29)
30–64*†	1·0	1·0	1·0	1·0
65–79	0·51 (0·30–0·88)	1·83 (1·42–2·37)	1·30 (0·96–1·77)	1·97 (1·68–2·31)
Marital status				
Married*	1·0	1·0	1·0	1·0
Unmarried	1·33 (0·93–1·89)	1·33 (1·03–1·71)	1·55 (1·21–1·99)	1·46 (1·26–1·68)
Widowed/divorced	1·26 (0·84–1·88)	1·33 (1·04–1·71)	1·70 (1·31–2·20)	1·63 (1·41–1·88)
Country of nationality				
Netherlands*	1·0	1·0	1·0	1·0
Turkey	0·19 (0·03–1·41)	0·33 (0·13–0·83)	0·21 (0·06–0·67)	1·73 (1·18–2·53)
Morocco	1·32 (0·62–2·83)	0·34 (0·14–0·79)	0·54 (0·26–1·11)	0·83 (0·54–1·27)
Other	1·29 (0·66–2·51)	1·09 (0·68–1·76)	1·46 (0·93–2·29)	3·26 (2·54–4·19)
Reminder				
By telephone*	1·0	1·0	1·0	1·0
By mail	1·49 (1·22–1·82)	0·62 (0·55–0·70)	2·29 (1·99–2·64)	1·60 (1·47–1·73)
Other	2·58 (1·26–5·30)	4·97 (3·61–6·85)	5·19 (3·36–8·02)	8·11 (6·17–10·7)
Degree of urbanization				
Very high	2·26 (1·67–3·06)	1·31 (1·07–1·59)	2·76 (2·23–3·43)	2·21 (1·94–2·52)
High	1·42 (1·02–1·98)	1·04 (0·85–1·27)	1·95 (1·54–2·47)	1·17 (1·01–1·35)
Moderate	1·43 (1·07–1·91)	1·03 (0·87–1·22)	1·75 (1·42–2·16)	1·07 (0·95–1·21)
Low	1·01 (0·73–1·40)	0·99 (0·83–1·19)	1·12 (0·89–1·40)	1·02 (0·90–1·15)
No*	1·0	1·0	1·0	1·0
Region				
Central	0·62 (0·44–0·89)	1·03 (0·85–1·25)	0·71 (0·57–0·90)	0·92 (0·80–1·06)
Southeast	1·08 (0·79–1·48)	1·01 (0·84–1·22)	0·63 (0·50–0·79)	0·85 (0·75–0·97)
Northwest	1·13 (0·81–1·57)	1·11 (0·91–1·34)	0·80 (0·63–1·00)	1·01 (0·88–1·15)
Southwest	0·89 (0·63–1·25)	1·13 (0·93–1·37)	0·80 (0·64–1·01)	0·89 (0·78–1·03)
Northeast*	1·0	1·0	1·0	1·0

* Reference group.

† Women aged 30–64 years versus men aged 30–64 years: additional participants adjusted OR 0·68 (95% CI 0·50–0·91); nonparticipants with a full questionnaire adjusted OR 0·86 (95% CI 0·68–1·08); non-participants with a nonresponse questionnaire adjusted OR 0·64 (95% CI 0·51–0·81); absolute non-participants adjusted OR 0·56 (95% CI 0·49–0·63).

two dates could visit an extra clinic. Those who refused to participate were asked to fill out the full, self-administered, questionnaire or, failing that, a short 'non-response' questionnaire. Five groups of participants and non-participants were distinguished: (1) 7904 (52·0%) 'initial participants' gave blood at the regular clinic and completed the questionnaire; (2) 455 (3·0%) 'additional participants' gave blood at the extra clinic and completed the questionnaire; (3) 1618 (10·7%) 'non-participants' completed the full ques-

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Table 3. *Odds ratios (ORs) and 95% confidence intervals (95% CI) for characteristics ('Non-questionnaire' variables) comparing non-participants with questionnaire data with participants; Pienter Project, 1995–6, The Netherlands*

	Numbers in subgroup	Crude ORs	(95% CI)	Adjusted ORs	(95% CI)
Age group (years) by gender					
Male					
0–4	1293	3·01	(2·57–3·54)	2·51	(2·00–3·16)
5–14	689	0·78	(0·61–0·99)	0·65	(0·49–0·86)
15–29	719	1·94	(1·60–2·37)	1·60	(1·26–2·05)
30–64*	1892	1·0		1·0	
65–79	670	0·99	(0·78–1·23)	0·94	(0·74–1·18)
Female					
0–4	1219	3·70	(3·13–4·36)	3·18	(2·52–4·00)
5–14	681	1·31	(1·04–1·64)	1·06	(0·81–1·41)
15–29	883	1·75	(1·44–2·12)	1·52	(1·20–1·92)
30–64*†	2110	1·0		1·0	
65–79	874	1·80	(1·48–2·19)	1·58	(1·29–1·94)
Marital status					
Married*	4366	1·0		1·0	
Unmarried	5809	2·23	(2·02–2·46)	1·37	(1·14–1·64)
Widowed/divorced	855	1·73	(1·45–2·06)	1·49	(1·24–1·80)
Country of nationality					
Netherlands*	10697	1·0		1·0	
Turkey	70	0·40	(0·19–0·84)	0·29	(0·13–0·61)
Morocco	93	0·60	(0·34–1·04)	0·42	(0·24–0·75)
Other	170	1·26	(0·90–1·76)	1·14	(0·80–1·63)
Reminder					
By telephone*	5738	1·0		1·0	
By mail	5066	1·05	(0·96–1·15)	1·03	(0·94–1·14)
Other	226	5·55	(4·21–7·32)	4·82	(3·61–6·44)
Degree of urbanization					
Very high	1377	1·66	(1·44–1·91)	1·67	(1·43–1·95)
High	1384	1·21	(1·05–1·41)	1·30	(1·11–1·53)
Moderate	2857	1·13	(1·00–1·27)	1·22	(1·06–1·40)
Low	2275	1·00	(0·88–1·14)	1·03	(0·89–1·20)
No*	3137	1·0		1·0	
Region					
Central	2215	0·97	(0·84–1·11)	0·91	(0·78–1·07)
Southeast	2253	0·89	(0·77–1·02)	0·82	(0·70–0·95)
Northwest	2174	1·05	(0·92–1·21)	0·96	(0·82–1·12)
Southwest	2181	1·13	(0·99–1·30)	0·98	(0·83–1·14)
Northeast*	2210	1·0		1·0	

* Reference group.

† Women aged 30–64 years versus men aged 30–64 years: crude OR 0·78 (95% CI 0·67–0·94), adjusted OR 0·78 (95% CI 0·66–0·93).

tionnaire; (4) 1053 (6·9%) 'non-participants' completed the non-response questionnaire; (5) 4159 (27·4%) 'absolute non-participants' neither gave blood nor completed any questionnaire.

For individuals in all groups, data were available on age, gender, marital status (with the exception of one municipality), nationality (with the exception of two municipalities) from the municipal database. Mu-

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nicipality and degree of urbanization of the municipality were assigned based on the place of residence of the individuals ('non-questionnaire' variables). Missing data on nationality and/or marital status were imputed with data from other municipalities. Information on educational level, self-perception of health, religion and opinion of the importance of immunization were collected by both questionnaires ('questionnaire' variables).

Definition of variables and statistical analysis

The 17 age strata were grouped into five classes: 0–4, 5–14, 15–29, 30–64 and 65–79 years, which corresponded well with variations in participation rates. Marital status was classified as 'married', 'unmarried' or 'widowed or divorced'. Nationality was classified as 'Dutch', 'Turkish', 'Moroccan' or 'other'. Although the numbers of Turks and Moroccans were small, they were analysed separately as a special effort was made to improve their response. Self-perception of health was classified as '(very) good' and 'less than good' and need for reminder as 'by telephone', 'by mail' or 'other' (i.e. no reminder or unknown). Degree of urbanization was categorized as 'very high' ($>$ 2500 addresses/km^2), 'high' (1500–2500), 'moderate' (1000–1500), 'low' (500–1000) and 'none' ($<$ 500). The geographical regions were based on the provinces in the Netherlands: 'Central' (Utrecht and Gelderland), 'Southeast' (Brabant and Limburg), 'Northwest' (Noord-Holland and Flevoland), 'Southwest' (Zeeland and Zuid-Holland) and 'Northeast' (Groningen, Drente, Overijssel and Friesland).

The highest educational level achieved by the individual, for those aged 17 years or older, and by one of the parents, for those younger than 17 years, were classified as 'low' (primary school, lower vocational or lower general secondary education), 'intermediate' (ntermediate vocational or intermediate general secondary and higher general secondary education), and 'high' (higher vocational secondary education and university education). Three religious groups were distinguished: 'Orthodox Reformed', who are known to be opposed to vaccination, 'Reformed Bond' of whom about a quarter reject vaccination, and 'other or no religion' [14]. Views on 'importance of immunization' were distinguished as: (1) Diphtheria (D), pertussis (P), tetanus (T), poliomyelitis (IPV), *Haemophilus influenzae* type b (Hib), mumps (M), measles (Me) and rubella (R) immunization were considered necessary; (2) 7 of the 8 immunizations just mentioned were considered necessary; (3) all immunization was considered unnecessary; (4) D, T, P, IPV and Hib were thought necessary and M, Me and R unnecessary; (5) M, Me, R were thought necessary and D, T, P, IPV and Hib 'otherwise' (less than four necessary); (6) D, T, P, IPV and Hib were considered necessary and M, Me, R 'otherwise' (less than two necessary); (7) The remaining group had some other opinion.

Although the number of subjects in the categories 'D, T, P, IPV, Hib, M, Me and R unnecessary' and 'Orthodox Reformed' was small, these were considered separately because these groups decline immunization often, which might affect evaluation of the national immunization programme.

All participants were compared to all non-participants by dichotomous logistic regression and 'non-questionnaire' variables that were associated with (non)participation were identified. Variables remained in the multivariate model if either the likelihood ratio test was significant ($P < 0{\cdot}05$) or the estimates of the beta coefficients for other variables in the model changed by at least 10%. These variables were included in the polytomous logistic regression model.

The second dichotomous logistic regression analysis was restricted to (non-participant groups with questionnaire data. 'Non-questionnaire' variables from the first dichotomous logistic model were included. 'Questionnaire' variables were included in the dichotomous and polytomous models according to the same criteria as before. Participants were used as the reference group in the dichotomous logistic models; initial participants, in the polytomous logistic models.

RESULTS

Univariate and multivariate analyses showed that several subgroups were less likely to participate (Tables 1 and 2). The results from the multivariate polytomous logistic regression analysis (Table 3), and from the univariate analysis (not presented) were similar. The odds ratios for certain substrata (by age and sex) of the variously defined non-participant groups (above) compared with the initial participants were different. This was most obvious in the youngest age groups: for both boys and girls the odds ratio for non-participants completing a full questionnaire compared with initial participants was $>$ 3, while it

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was < 1 for absolute non-participants compared with initial participants. Nationality, kind of reminder, and degree of urbanization affected (non)participation differently (Table 2).

Looking at questionnaire variables and excluding absolute non-participants, in the univariate analysis, intermediate and higher levels of education, orthodox reformed religion and viewing all or some of the vaccines as unnecessary, were associated with non-participation. In multivariate analysis only opinion on immunization was associated with non-participation. Polytomous logistic regression showed that opinions about the importance of immunization were different in the different non-participating groups. Individuals who considered none of the immunizations necessary, or M, Me and R necessary, but the other vaccinations otherwise, were most likely to be in the non-participant with questionnaire group.

The exclusion of absolute non-participants affects the association with non-participation for some 'non-questionnaire' variables considerably (Table 1 versus Table 3).

The effect of considering additional participants as non-participants would only slightly affect the association with non-participation as most of the 95% confidence intervals include unity. For degree of urbanization and kind of reminder, the difference between participants and non-participants is slightly less when additional participants are included, while the difference increases slightly for nationality.

DISCUSSION

The only way to eliminate bias from selection is to take a random sample and to achieve complete response, which is impossible in practice [15]. The participation rate of 55% in our study was higher than expected, but differences found between participants and non-participants imply that our serum collection might not be representative of the general population [9, 16].

When these differences are also associated with the subjects' immune status to the disease in question they could lead to incorrect estimates of seroprevalence. Since the serum collection will be used for many seroprevalence studies, mainly of vaccine-preventable diseases, this association should be studied separately for each seroprevalence study. Below we summarize the different characteristics of the (non)participation groups and whether any associations with immune status might be expected.

The likelihood of participation was lower for men than women for those aged 15–64 years. Although some studies have found that the participation rate was not related to gender, others reported that men were more difficult to recruit [3, 5, 10, 17–20]. The participation rate of the men was lowest among 15–29-year-olds, while the participation rate of the women was lowest among the oldest and youngest age group. Frequently, but not always, it has been reported that non-participants were older than participants [4–7, 20–24]. The high participation rate of 5–14-year-olds, an age group recently vaccinated, might be explained by the perceived importance of the topic of the study [25]. The low participation rate of the younger age group is probably related to parents' fear of possible ill effects of blood sampling on their young children.

Age and gender are likely to be associated with immune status for vaccine-preventable diseases. Seroprevalence will depend on age as a result of age-specific differences in chance of exposure to the pathogen, time since exposure, chance of vaccination and time since last vaccination [1]. For example those born before the introduction of mass vaccination in 1952 with DTP-IPV were more likely to have been exposed to diphtheria and poliomyelitis but are less likely to have received vaccination. Gender differences in seroprevalence can be expected due, for example, to vaccinations given in military service. In our recent study on tetanus antitoxin antibodies, men with military service history were more likely to have antibodies [26].

The special efforts to enhance the response rate of the Turks and Moroccans (the largest groups of non-Dutch nationals) seemed successful. In contrast to our pilot-study, Turks had a participation rate similar to the Dutch. Moroccans had an even higher rate than the Dutch [13]. Relatively fewer individuals of other nationalities participated. Due to differences in force of infection and immunication programmes in country of origin, immune status is likely to depend on nationality. For example the likelihood of protective tetanus antitoxin levels for Turkish and Moroccan individuals was lower than for Dutch individuals and individuals of other nationalities [26]. Since non-Dutch nationals account for only a small part of the population any impact on the overall estimate is likely to be small.

Like others, we found that fewer unmarried individuals than married individuals participated [3, 4, 6, 27]. The finding that a lower degree of

urbanization was associated with higher participation, is consistent with other reports [18, 24]. Active refusers accounted for the largest part of the group contacted by 'other reminder', which caused the high odds ratio for non-participation in this group. Consistent with other studies, individuals who could be reached and reminded by telephone participated more frequently than those who were reminded by mail [11, 16, 28]. Although the relationship of these three characteristics to immune status is unclear, we expect it to be small. This has been supported by the absence of associations with tetanus immunity [26].

Although including additional participants probably affected the non-response bias in our study very little, our results show that any assumption that additional (late) participants resemble non-participants more than initial participants, might be incorrect [9, 11, 19, 29, 30]. Identifying non-response bias in our study on the basis of completed questionnaires would overestimate the non-response bias for 0–4-year-olds and for Turks and Moroccans, and underestimate it for individuals of another non-Dutch nationality and for those reminded by mail.

Thus results from the analysis restricted to those with questionnaire information are difficult to interpret. In this analysis, opinions about the importance of immunization turned out to be an independent predictor for non-participation. Individuals who considered none of the immunizations necessary, or M, Me and R necessary and D, T, P, IPV and Hib otherwise, were most likely to be in the non-participant (with questionnaire) group. The likelihood in the univariate analysis of being a non-participant who filled in a questionnaire was also higher for individuals with an intermediate or high educational level and for those who were Orthodox Reformed, and slightly lower for individuals who considered their health less than good. These findings are inconsistent with those in other studies of educational level and self-perception of health, whereas there are no known studies on the effects of opinion concerning importance of immunization and of religion [3–6, 10, 20–23]. Vaccination history and hence, as a result of refusing vaccination, orthodox reformed religion might be expected to be associated with immune status to vaccine-preventable diseases. Since the vaccine coverage in the Netherlands is high (97% for DTP-IPV, Hib; 94% for M, Me and R) and the members of orthodox reformed groups are few (about 300000) the impact on seroprevalence estimates is probably limited. Again these expectations were supported by the seroprevalence study on tetanus [26].

In this population-based study addressing vaccine-preventable diseases differences were found between participants and non-participants as they were in other studies.

Correction for differential participation can be made by taking the distribution of the relevant characteristic in the Dutch population into account in the seroprevalence estimates. Furthermore, when information for all participants and non-participants is known the effect on the seroprevalence can be estimated by weighting the seroprevalence estimate by differential response rates. However, our results show that when information is available only for a subgroup of non-participants it might lead to a biased insight into the reasons for non-participation. If such a characteristic is associated with the immune status of the individual to the disease in question, one can not be certain on the exact impact on the overall seroprevalence estimate. Furthermore, investigators have to be aware that merely including additional participants might not always reduce this bias.

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