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Immune response-eliciting exposure to *Campylobacter* vastly exceeds the incidence of clinically overt campylobacteriosis but is associated with similar risk factors: A nationwide serosurvey in the Netherlands

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SUMMARY

Background: We aimed to estimate population-level exposure to *Campylobacter* and associated risk factors, using three approaches for serological data analysis.
Methods: Nationwide, population-based serosurvey in the Netherlands (Feb 2006–Jun 2007). Anti-*Campylobacter* IgG, IgM and IgA were measured using ELISA, and analysed via: a) seroincidence estimation, using reference values of antibody peak levels and decay rates over-time after *Campylobacter* exposure; b) two normal distributions of true positives/negatives fitted to the IgG distribution to derive seroprevalence and individual probability of being positive/negative; and c) IgG levels. Risk factors were analysed using multiple linear regressions.
Results: From 1559 respondents, seroincidence was estimated at 1.61 infections/person-year (95%CI:1.58–1.64) and seroprevalence at 68.1% (65.4–70.9). The three approaches identified similar risk factors, although seroincidence had higher power and results were interpretable as risk: seroincidence was higher in females [exp(b) = 1.07(1.04–1.11)], older ages [vs. 15–34 years; for < 5, 5–14, 35–54 and 55–70 years: 0.60(0.58–0.63), 0.74(0.71–0.78), 1.08(1.03–1.13) and 1.08(1.01–1.16), respectively], non-Dutch background [Moroccan/Turkish: 1.25(1.14–1.37); Caribbean: 1.14(1.03–1.25)], low socioeconomic status [1.05(1.01–1.10)], traveling outside Europe [1.05(1.01–1.09)], and eating undercooked meat [1.04(1.01–1.08)].
Conclusion: *Campylobacter* exposure is much higher than clinical infection rates, but risk factors are similar to those previously described.Seroincidence is a powerful measure to study *Campylobacter* epidemiology, and is preferred over other methods.

Introduction

Campylobacter is a major causative agent of bacterial gastroenteritis worldwide.[1] In the Netherlands, the annual incidence of *Campylobacter* gastroenteritis was estimated at circa 5.6 cases/1000 inhabitants in 2009, and is the foodborne bacterium most frequently causing hospitalization.[2] Guillain–Barré syndrome, reactive arthritis, irritable bowel syndrome, and inflammatory bowel disease, are among the possible sequelae of *Campylobacter* clinical infection. Altogether, *Campylobacter* is the foodborne pathogen with the highest human disease burden and the second highest economic costs in the Netherlands.[3]

However, most *Campylobacter* infections are either asymptomatic or result in self-limiting episodes of gastrointestinal illness that do not usually prompt clinical consultation or laboratory investigation. Therefore, symptomatic cases are likely to represent only the tip of the iceberg and may not represent the full picture of associated risk factors, limiting our understanding of *Campylobacter* epidemiology.[4,5] The detection of a serological response to *Campylobacter* in seroepidemiological studies can identify exposures to the pathogen even in the absence of clinical manifestations, and has the potential to capture the full spectrum of infection and provide a more comprehensive overview of risk factors for exposure.

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Analyzing serological data for *Campylobacter* poses several challenges, mainly because of the lack of clear cut-off values to classify seropositivity, but also for the bias inherent in classification methods due to false negative and false positive results.[6,7] Several innovative methods have been proposed to analyze serological data from cross-sectional surveys. All have the common feature of avoiding classification of individuals into absolute positive/negative states by using probabilistic approaches.[8–10] These methods maximize the use of available information accounting for the individual variability in the serological response to a *Campylobacter* infection.

In this study, we analyze data from a large population-based serosurvey in the Netherlands to estimate exposure to *Campylobacter* in the resident population and to identify the associated risk factors, comparing different innovative approaches to modeling serology data.

Methods

Study design

A cross-sectional population-based serological survey was performed in the Netherlands between February 2006 and June 2007. Information on its design and the resulting serum bank has been published.[11] Briefly, a two-stage clustered design sampled eight municipalities, with probability proportional to population size, within each of five study-defined geographical regions of approximately equal size (total of 40 municipalities). Within these, an age-stratified sample (< 1, 1–4, 5–9, …, 75–79 years) was randomly drawn from municipal population registers.

Participants provided a blood sample and completed an epidemiological questionnaire on demographic characteristics, medical history, and different activities and behaviors putatively related to infectious diseases transmission. All participants gave informed consent. Information on socioeconomic status (SES) and urbanization degree per postcode area was obtained from Statistics Netherlands (www.cbs.nl). SES was classified using country-wise tertiles. For the present study, we randomly selected a sub-sample from individuals aged 0–70 years within the serum-bank.

Laboratory methods

Levels of IgG, IgM and IgA against *Campylobacter* were determined in the serum samples using an ELISA as described previously.[12] The antigen used for the ELISA was an acid glycine extract of *C. jejuni* strain SSDZ-01. Data for all isotypes were expressed as ratios. The ratio was calculated by dividing the mean optical density (OD) value of the serum, tested in duplicate, by the mean OD value of the reference sample that was included in triplicate on each ELISA plate. Specificity of the *Campylobacter* ELISA was investigated by pre-incubating serum samples from individuals with documented *Campylobacter* infection and healthy controls with known anti-*Campylobacter* IgG reactivity with bacterial suspensions of the following species: *C. jejuni, C. coli, C. upsaliensis, C. lari, C. fetus, C. hyointestinalis, Helicobacter pylori, Legionella pneumophila*. After pre-incubation the samples were centrifuged and further tested by ELISA.

Statistical analysis

Sampling weights were used to improve representativeness, with strata defined by urbanization degree, sex, age group, and ethnicity; weights were the inverse of the ratio between sample and population numbers per stratum. Standard errors were corrected for the clustering of municipalities within regions. Analyses were performed for the whole sample and separately for children below 15 years. Serology data were analysed in three different ways.

We first calculated seroincidence (i.e. number of infections per person-year) of *Campylobacter* as a *proxy* for risk of exposure or infection pressure. The method, proposed by Teunis et al.,[4,9] has been adopted as a standard by the European Centre for Disease Control (ECDC).[13] Briefly, we used reference values of peak levels and decay rates over time of IgG, IgM and IgA following *Campylobacter* infection to estimate time since last infection given any observed IgG, IgM and IgA. An estimate of seroincidence is obtained together with a 95% confidence interval; further analyses were weighted for the inverse of its width to down-weight estimations with low precision. We calculated seroincidence in the above-defined sample strata, and applied the sampling weights to obtain overall seroincidence estimates. To analyze risk factors associated with seroincidence, an individual estimate was obtained, log-transformed, and analyzed using multivariable generalized linear models (GLMs). Although log-transformation failed to fully satisfy normal residuals distribution assumption, it drew fully comparable results to the best transformation (i.e. inverse of square root), with the advantage of facilitating the interpretation of exponentiated coefficients as a relative change in risk of exposure.

Calculation of seroprevalence using mixture distributions was used as an alternative approach. A binary mixture of normal distributions (representing “true” positives and “true” negatives) was fitted to the log-transformed antibody values (i.e. ODs) by maximum likelihood, separately for IgG, IgM and IgA. In this method, means, variances and fraction of positive subjects – seroprevalence – follow no a priori assumption but are estimated from the data. Sensitivity and specificity resulting from the overlap of the distributions was assessed using a ROC curve. We estimated the parameters of the two mixture component distributions (μ_0, σ_0 and μ_1, σ_1) in the full data set and then, while keeping these parameters constant, the stratum-specific seroprevalence. Overall seroprevalence in the Netherlands and by sociodemographic variables was further estimated applying sampling weights. To analyze risk factors in a GLM model, we calculated the Z-standardized log-odds (i.e. the ratio between the probability of a value being positive and its probability of being negative) for all observed IgG values using the fitted mixture component distributions.

Finally, GLMs were used to directly model the logarithm of the observed antibody OD values and evaluate risk factors associated to higher antibody values.

For all multivariate analyses, the saturated model included variables with $p < 0.2$ in univariate analysis, plus age, sex and ethnicity, selected a priori. Variables were sequentially dropped until all had $p < 0.05$. Finally, dropped variables were reevaluated and retained if $p < 0.05$. Wald significance tests was used. Assumptions were verified by residuals diagnostics.

Multiple imputation using chained equations[14] was used to deal with missing data in covariates: incomplete binary and ordinal variables were imputed using logistic and ordered logistic regression models, respectively. Results from thirty imputed datasets were combined using Rubin’s rules.[15] We used R v.3.3.1 for the seroincidence calculations and the mixture models and STATA v.14.2 for other analyses.

Results

Description of the sample

Out of 6386 total participants in the serum-bank, 1559 were selected. Table 1 shows sample characteristics before and after imputing missing values and applying sampling weights. Overall, 43.9% were males, 29.0% under 15 years, 15.7% of non-Dutch ethnic background and 8.4% were migrants (born outside the Netherlands

Table 1
Characteristics of the study sample ($N = 1559$) before and after imputation of missing values and application of sampling weights. Mean IgG, IgM and IgA OD values (and 95% confidence intervals) by independent factors.

Variable		Study sample		IgG mean	IgM mean	IgA mean	Imputed sample	Imputed & weighted sample
		n	(%)	(95%CI)	(95%CI)	(95%CI)	(%)	(%)
Gender	Man	685	(43.9)	3.5 (3.3–3.7)	0.49 (0.47–0.51)	0.43 (0.42–0.45)	43.9	51.0
	Woman	874	(56.1)	4.1 (3.9–4.3)	0.54 (0.52–0.55)	0.47 (0.45–0.49)	56.1	49.0
Age	<5 years	184	(11.8)	1.2 (1.0–1.37)	0.37 (0.36–0.39)	0.40 (0.37–0.43)	11.8	7.3
	5-14	267	(17.1)	2.2 (2.0–2.4)	0.47 (0.45–0.49)	0.39 (0.38–0.40)	17.1	15.0
	14-35	481	(30.9)	4.4 (4.2–4.7)	0.56 (0.53–0.58)	0.44 (0.43–0.46)	30.9	27.9
	35-54	487	(31.2)	4.8 (4.6–5.1)	0.55 (0.53–0.57)	0.51 (0.47–0.54)	31.2	35.6
	55-70	140	(9.0)	5.1 (4.6–5.6)	0.54 (0.49–0.59)	0.51 (0.47–0.56)	9.0	14.2
Educational level	None/Primary	266	(17.1)	3.8 (3.5–4.2)	0.50 (0.48–0.52)	0.44 (0.41–0.46)	17.3	20.5
	Secondary	611	(39.2)	3.7 (3.5–4.0)	0.51 (0.49–0.52)	0.44 (0.42–0.45)	39.6	38.0
	Post-secondary	665	(42.7)	4.0 (3.7–4.)	0.53 (0.51–0.55)	0.48 (0.45–0.50)	43.0	41.5
	Unknown	17	(1.1)	3.4 (2.3–4.4)	0.61 (0.49–0.73)	0.47 (0.38–0.56)	–	–
Net income	<1.150 €	173	(11.1)	4.0 (3.6–4.4)	0.54 (0.49–0.59)	0.43 (0.41–0.46)	14.6	15.2
	1.151-3.050 €	725	(46.5)	3.8 (3.6–4.0)	0.52 (0.50–0.54)	0.43 (0.42–0.45)	60.1	60.5
	>=3.051€	317	(20.3)	4.0 (3.7–4.4)	0.51 (0.48–0.54)	0.51 (0.46–0.56)	25.2	24.3
	Unknown	344	(22.1)	3.7 (3.4–4.0)	0.51 (0.48–0.53)	0.46 (0.43–0.49)	–	–
Ethnicity	Dutch	1315	(84.3)	3.8 (3.7–4.0)	0.51 (0.50–0.53)	0.46 (0.44–0.47)	84.3	82.1
	Other Western	107	(6.9)	4.2 (3.6–4.8)	0.54 (0.49–0.60)	0.45 (0.41–0.48)	6.9	7.3
	Moroccan/Turkish	44	(2.8)	3.6 (2.8–4.3)	0.54 (0.47–0.61)	0.42 (0.38–0.45)	2.8	3.5
		28	(1.8)	3.9 (2.9–4.9)	0.53 (0.44–0.61)	0.41 (0.37–0.45)	1.8	2.2
	Suriname/Aruba/Antilles							
	Other Non-Western	65	(4.2)	3.7 (3.1–4.4)	0.50 (0.47–0.54)	0.41 (0.39–0.44)	4.2	4.9
Origin	Netherlands	1426	(91.6)	3.8 (3.6-3.9)	0.51 (0.50–0.53)	0.45 (0.44–0.47)	91.6	90.0
	Other	130	(8.4)	4.5 (3.9–5.0)	0.55 (0.51–0.60)	0.46 (0.43–0.50)	8.4	10.0
	Unknown	3	(0.0)	4.2 (0.0–8.8)	0.72 (0.09–1.35)	0.80 (0.0–1.71)	–	–
Travelled abroad [a]	No	1090	(69.9)	3.5 (3.3–3.7)	0.50 (0.49–0.51)	0.44 (0.42–0.45)	69.9	68.2
	Yes	469	(30.1)	4.6 (4.4–4.9)	0.55 (0.53–0.58)	0.50 (0.46–0.53)	30.1	31.8
Persons living in the house	1–2	414	(26.8)	4.8 (4.5–5.1)	0.56 (0.52–0.59)	0.47 (0.45–0.49)	26.7	30.9
	3-4	789	(51.0)	3.6 (3.4–3.8)	0.50 (0.49–0.52)	0.46 (0.44–0.48)	51.0	47.9
	>=5	344	(22.2)	3.1 (2.8–3.4)	0.49 (0.47–0.51)	0.42 (0.41–0.44)	22.3	21.3
	Unknown	12	(0.8)	4.4 (2.8–6.0)	0.61 (0.46–0.76)	0.47 (0.35–0.59)	–	–
Garden	No	533	(34.2)	3.7 (3.4–3.9)	0.51 (0.49–0.54)	0.44 (0.42–0.46)	35.5	33.3
	Yes	987	(63.3)	4.0 (3.8–4.2)	0.52 (0.50–0.53)	0.46 (0.44–0.48)	64.5	66.7
	Unknown	39	(2.5)	2.7 (2.1–3.4)	0.50 (0.44–0.56)	0.42 (0.38–0.46)	–	–
House animals	No	508	(32.6)	3.9 (3.6–4.2)	0.52 (0.50–0.54)	0.47 (0.44–0.50)	32.6	35.3
	Yes	1051	(67.4)	3.8 (3.6–4.0)	0.51 (0.50–0.53)	0.45 (0.43–0.46)	67.4	64.7
Pets (multiple possible)	Dog	429	(27.5)	3.8 (3.5–4.1)	0.52 (0.49–0.55)	0.45 (0.43–0.47)	27.5	27.2
	Cat	428	(27.5)	3.9 (3.6–4.2)	0.52 (0.50–0.54)	0.44 (0.42–0.45)	27.5	25.9
	Bird	182	(11.7)	3.9 (3.5–4.3)	0.49 (0.47–051)	0.46 (0.42–0.51)	11.7	11.8
	Mouse	55	(3.5)	3.7 (3.0–4.3)	0.52 (0.40–0.64)	0.48 (0.35–0.62)	3.5	3.5
	Fish	300	(19.2)	3.5 (3.1–3.8)	0.49 (0.46–0.51)	0.43 (0.41–0.45)	19.2	18.6
	Rabbit	398	(25.5)	3.6 (3.3–3.8)	0.50 (0.48–0.53)	0.44 (0.42–0.47)	25.5	24.4
Farm animals	No	1459	(93.6)	3.8 (3.7–4.0)	0.52 (0.50–0.53)	0.45 (0.44–0.47)	93.6	93.9
	Yes	100	(6.4)	4.0 (3.3–4.6)	0.51 (0.45–0.58)	0.46 (0.40–0.52)	6.4	6.1
Farm animals (multiple possible)	Pigs	6	(0.4)	2.6 (0.3–4.9)	0.41 (0.34–0.49)	0.48 (0.39–0.57)	0.4	0.3
	Cows	27	(1.7)	4.3 (3.0–5.6)	0.51 (0.45–0.58)	0.46 (0.40–0.51)	1.7	1.6
	Sheep	24	(1.5)	3.4 (2.3–4.4)	0.58 (0.33–0.83)	0.43 (0.37–0.48)	1.5	1.5
	Goat	19	(1.2)	3.3 (2.2–4.4)	0.48 (0.41–0.46)	0.39 (0.35–0.43)	1.2	1.2
	Poultry	59	(3.8)	4.0 (3.2–4.9)	0.54 (0.44–0.65)	0.48 (0.38–0.58)	3.8	3.7
Bitten by a tick	No	1207	(77.4)	3.9 (3.7–4.0)	0.51 (0.50–0.53)	0.45 (0.44–0.47)	85.5	85.7
	Yes	205	(13.2)	3.8 (3.4–4.2)	0.50 (0.48–0.53)	0.46 (0.42–0.51)	14.5	14.3
	Unknown	147	(9.4)	3.8 (3.4–4.2)	0.54 (0.50–0.59)	0.46 (0.43–0.48)	–	–
Vegetarian	No	1515	(97.2)	3.8 (3.7–4.0)	0.51 (0.50–0.53)	0.46 (0.44–0.47)	98.2	98.7
	Yes	28	(1.8)	3.5 (2.3–4.8)	0.53 (0.44–0.62)	0.39 (0.35–0.42)	1.8	1.3
	Unknown	16	(1.0)	3.3 (1.9–4.7)	0.54 (0.42–0.66)	0.46 (0.37–0.55)	–	–
Eaten under-cooked meat	No	618	(39.6)	3.1 (2.9–3.3)	0. 49 (0.47–0.51)	0.43 (0.41–0.45)	39.6	38.5
	Yes	941	(60.4)	4.3 (4.1–4.5)	0.53 (0.51–0.55)	0.47 (0.45–0.49)	60.4	61.5
Type of meat (multiple possible)	Beef	917	(58.8)	4.3 (4.1–4.5)	0.53 (0.51–0.55)	0.47 (0.45–0.48)	58.8	59.9
	Pork	305	(19.6)	4.7 (4.3–5.0)	0.55 (0.51–0.58)	0.48 (0.45–0.51)	19.6	21.2
	Poultry	73	(4.7)	4.9 (4.3–5.6)	0.60 (0.48–0.73)	0.46 (0.51–0.51)	4.7	5.2
Eaten unwashed raw vegetables	No	1.051	(67.4)	3.7 (3.5–3.9)	0.51 (0.49–0.52)	0.46 (0.44–0.47)	69.0	69.8
	Yes	475	(30.5)	4.1 (3.8–4.3)	0.53 (0.50–0.56)	0.45 (0.43–0.47)	31.0	30.2
	Unknown	33	(2.1)	4.2 (3.3–5.1)	0.59 (0.51–0.68)	0.46 (0.40–0.51)	–	–
Socio-economic status	High	437	(28.1)	3.8 (3.5–4.1)	0.52 (0.49–0.54)	0.45 (0.43–0.47)	28.0	29.1
	Middle	644	(41.3)	3.7 (3.5–4.0)	0.51 (0.49–0.53)	0.45 (0.43–0.47)	41.4	40.5
	Low	477	(30.6)	4.0 (3.7–4.3)	0.52 (0.50–0.55)	0.47 (0.44–0.50)	30.6	30.4
	Unknown	1	(0.0)	10.5 (–)	0.58 (–)	0.42 (–)	–	–
Urbanization degree	Highest	267	(17.1)	4.3 (3.9–4.6)	0.55 (0.52–0.59)	0.46 (0.43–0.49)	17.1	19.9
	Medium	1099	(70.5)	3.8 (3.69–4.0)	0.51 (0.49–0.52)	0.45 (0.44–0.47)	70.5	68.9
	Rural	193	(12.4)	3.6 (3.2–4.0)	0.51 (0.48–0.55)	0.45 (0.42–0.49)	12.4	11.1

(*continued on next page*)

Table 1
(*continued*)

Variable		Study sample		IgG mean	IgM mean	IgA mean	Imputed sample	Imputed & weighted sample
		n	(%)	(95%CI)	(95%CI)	(95%CI)	(%)	(%)
Variables only collected in the children < 15 sub-sample (*N* = 451)								
Played in sandpit	No	207	(45.9)	2.1 (1.8–2.4)	0.44 (0.42–0.47)	0.40 (0.37-0.43)	46.7	40.7
	Yes	239	(53.0)	1.5 (1.3–1.6)	0.42 (0.40–0.43)	0.38 (0.37–0.39)	53.3	59.2
	Unknown	5	(1.1)	2.5 (0.1–4.9)	0.55 (0.32–0.77)	0.40 (0.34–0.45)	–	–
Ate the sand	No	55	(12.2)	1.5 (1.1–.9)	0.43 (0.39–0.48)	0.40 (0.37–0.43)	20.9	17.3
	Yes	183	(40.6)	1.5 (1.3–1.6)	0.41 (0.40–0.43)	0.38 (0.37–0.39)	79.1	82.7
	Unknown	213	(47.2)	2.1 (1.8–2.4)	0.45 (0.42–0.47)	0.40 (0.37–0.43)	–	–

[a] In Asia, Africa or South America.

Table 2
Estimates of seroincidence (incidence rate per person-year) and seroprevalence by sampling strata, and 95% Confidence Interval.

		Seroincidence Cases/person-year (95%CI)	Seroprevalence % (95%CI)
Gender	Man	1.51 (1.47–1.56)	65.0 (61.2–68.8)
	Woman	1.71 (1.66–1.75)	71.3 (67.5–75.2)
Age	<5 years	0.97 (0.94–0.99)	7.7 (2.8–12.7)
	5–14	1.17 (1.15–1.19)	25.4 (21.0–29.7)
	15–34	1.75 (1.71–1.79)	77.1 (73.7–80.5)
	35–54	1.91 (1.86–1.96)	84.1 (80.2–88.1)
	55–70	1.91 (1.84–1.97)	96.5 (79.9–93.2)
Ethnicity	Dutch	1.61 (1.57–1.64)	67.7 (64.7–70.6)
	Other Western	1.71 (1.59–1.83)	73.6 (64.9–82.3)
	Moroccan/Turkish	1.59 (1.44–1.73)	79.8 (68.1–91.5)
	Suriname/Aruba/Antilles	1.54 (1.32–1.76)	61.7 (37.0–86.4)
	Other Non-Western	1.58 (1.33–1.83)	67.9 (51.3–84.5)
Urbanization degree	Highest	1.78 (1.70–1.86)	76.3 (70.9–81.7)
	Medium	1.60 (1.56–1.63)	68.2 (65.3–71.2)
	Rural	1.53 (1.41–1.66)	60.8 (46.8–74.9)
Overall		1.61 (1.58–1.64)	68.1 (65.4–70.9)

or of non-Dutch nationality). 42.7% had post-secondary education (self or parents' if respondent < 15 years), 12.4% lived in rural settings and 30.6% in municipalities of low SES. 67.0% had pets in the last 5 years, mainly dogs, cats and rabbits, while 6.4% had contact with farm animals, mainly poultry. 60.4% reported eating undercooked meat in the last 12 months, mainly beef, and 30.5% had eaten unwashed raw vegetables, while only 1.8% were vegetarian. Results of OD for IgG, IgM and IgA by potential risk factors are shown in Table 1.

Estimates of exposure to Campylobacter

Seroincidence of *Campylobacter* infection in the Netherlands was estimated at 1.61 infections per person-year (95%CI: 1.58–1.64). Seroincidence was slightly higher in women, increased rapidly with age, and was higher in highly urbanized areas compared to intermediate or rural areas (Table 2). However, it was homogeneous across ethnic backgrounds.

Seroprevalence was calculated only based on the mixture distributions for IgG, as for IgM and IgA, there was considerable overlap between the mixture distributions and the ROC curve showed poor discrimination capacity (Supplementary Fig. 1). Seroprevalence of *Campylobacter* infection in the Netherlands was estimated at 68.1% (95%CI: 65.4–70.9). Differences by sociodemographic variables had similar direction but were more marked than those observed for seroincidence (Table 2).

Risk factors for exposure

Factors associated with *Campylobacter* exposure were analyzed based on modeled seroincidence, odds of infection (based on IgG), and observed IgG OD levels. Supplementary Tables 1 and 2 show the univariate analysis results for the full sample and the subset of children < 15 years. For the multivariate analyses, only a general category of undercooked meat was included, due to collinearity and low number of individuals consuming undercooked pork or poultry.

In the full sample, the three approaches selected the same independent risk factors (Fig. 1A). Seroincidence models had lower magnitude of effects and narrower confidence intervals. However, since they model different outcomes, differences in magnitude of effects were expected. For example, compared to being Dutch, people of Moroccan or Turkish ethnic background had 25% higher seroincidence [exp(b):1.25 (95%CI: 1.14–1.37)], while IgG OD levels were 72% higher [exp(b):1.72 (1.38–2.14)], and odds of infection was 84% standard deviations higher [exp(b):1.84(1.43–2.37)]. Other risk factors were female gender, increasing age, Caribbean and other non-Western ethnicity, low SES, having ever travelled to Asia, Africa or South America, and having eaten undercooked meat in the last 12 months. Urbanization degree was not a risk factor in any of the approaches but was on the limit of statistical significance. For example, compared to municipalities with the highest urbanization degree, IgG levels were 8% lower [exp(b):0.92 (0.83–1.01; $p = 0.066$)] in intermediately urbanized and 10% lower [exp(b):0.90 (0.79–1.02);$p = 0.104$] in rural areas.

In individuals < 15 years, models using seroincidence identified more risk factors than the other approaches (Fig. 1B). Models using IgG or the odds of infection identified as risk factors: older age, non-Western ethnicities and eating undercooked meat. Models of seroincidence additionally detected higher risk for females and people in contact with pigs. Additionally, some other exposures were of borderline significance, such as being vegetarian [exp(b): 0.95 (0.91–1.00); $p = 0.055$], or having owned pet birds in the last 5 years [exp(b): 1.03 (1.00–1.06); $p = 0.085$].

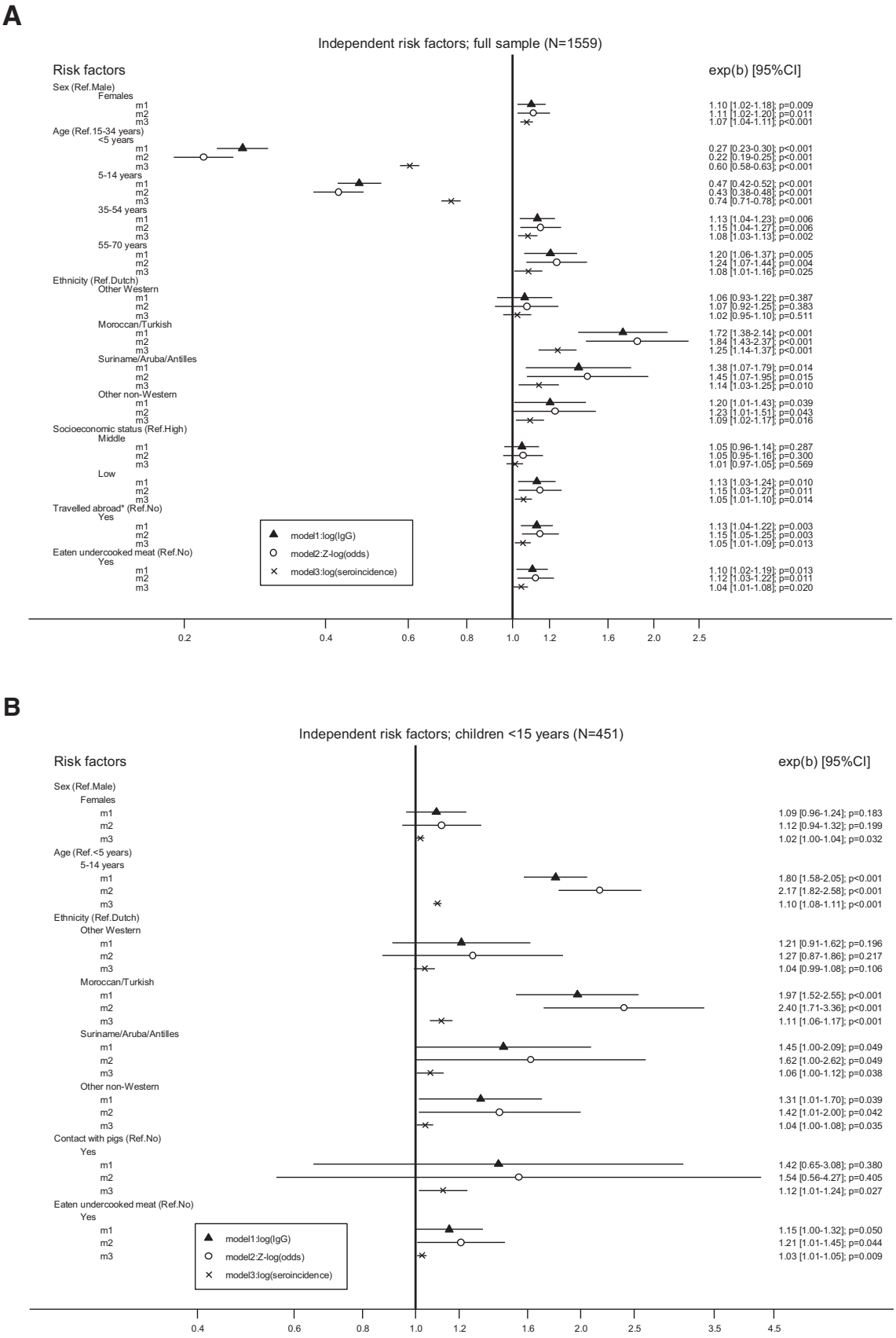

Fig. 1. Independent risk factors for Campylobacter: Results for multivariate models using three different approaches. Results are shown A) for the full sample ($N = 1{,}559$) and B) for the subset of children < 15 years of age ($N = 451$).* In Asia, Africa or South America. Three different approaches: Model 1: log(IgG), shows the association of independent variables with IgG Optical Density (log transformed) values; Model 2: Z-log(odds), shows the association of independent variables with normalized z-distribution of the log transformed Odds for infection by *Campylobacter*; and Model 3: log(seroincidence), shows the association of independent variables with the individual seroincidence (log transformed).

Discussion

Our results show that exposure to *Campylobacter* is high in the Netherlands, with every person being exposed, on average, around one and a half times a year and a prevalence of around two thirds of the population. These estimates are approximately two fold higher than the ones reported in a previous study using a similar methodology[4] due to a correction in the calculations.[13] Accounting for this correction, our estimates from 2006 to 2007 can be considered similar to those found one decade earlier (1996-1997) in another Dutch study.[9] This indicates that, in contrast to other enteropathogens such as *Salmonella* or *Yersinia enterocolitica*, the incidence of *Campylobacter* does not seem to be decreasing in the last decades.[16] Updated estimations of the seroincidence will allow to further monitor population-level exposure to Campylobacter throughout time.

The estimated seroincidence of 1600 infections per 1000 person-years is higher than the incidence of clinical campylobacteriosis by magnitude factor of approximately 285, as clinical campylobacteriosis has been estimated to occur at a rate of around 5.6 per 1000 persons-year in 2009.[2] This illustrates that *Campylobacter* cases captured in the clinical setting represent only the tip of the iceberg of the total *Campylobacter* infections occurring at the population level.[4,5,9,17,18] Moreover, the age pattern for clinical cases is reversed, peaking in toddlers (1–4 years) and younger adults (15–24 years),[19] whereas both seroincidence and IgG-seroprevalence increase progressively throughout life, with seroprevalence reaching ~100% from 55 years onwards. However, a limitation of the method is that, since there are no longitudinal data of the immunological response to *Campylobacter* in children, seroincidence estimates assume that the seroresponse is similar to that observed in adults.

The relationship between seroincidence and disease incidence is not completely understood and probably reflects several simultaneous factors. Human challenge studies have convincingly shown how pathogen shedding, as an indicator of infection and seroconversion, are consistent markers for infection in *Campylobacter*[20–24] and several other pathogens. The biggest discrepancy between asymptomatic exposures and clinical cases is likely attributable to the development of immunity following repeated contacts with *Campylobacter*.[5] Correlates of protection of this immunity are unclear, but would probably protect to a certain degree against clinical disease, but not against colonization or asymptomatic infection in successive exposures to *Campylobacter*.[5,24] Therefore, they contribute to serological estimates of infection, but not to disease incidence, which is concentrated in the less immune individuals: younger ages[19] and travellers that may encounter different *Campylobacter* strains.[25]

Illness/infection ratio, severity of symptoms and dose-response relationship will also depend on the strain[16] and host characteristics.[5] While *C. jejuni* requires a relatively low dose for the development of clinical campylobacteriosis in susceptible persons,[26] other species are less pathogenic, contributing to prevalence of antibodies not correlated with clinical disease.[5,16] Moreover, the great majority of exposures are probably occurring at very low doses, being therefore insufficient to cause illness.[27] Finally, severity and duration of symptoms, health-seeking behavior, clinical practice, surveillance and reporting systems in a given setting will influence significantly the proportion and type of patients identified in clinical studies.[28]

Data from clinical or laboratory-confirmed cases can be useful to estimate the burden of disease, but these are a minority and represent a non-random selection of all *Campylobacter* infections, not providing enough information on the underlying epidemiology and factors driving exposure to *Campylobacter* in the population.[5] Indeed, it has been demonstrated that case-control studies may suffer from substantial bias in case of frequent exposures that persist overtime and generate immunity.[29,30] For example, handling and/or consumption of chicken is one of the most recognized risk factors for human campylobacteriosis,[16,31,32] but its effect can sometimes be surprisingly moderate or even appear to be protective, supporting the notion of acquired immunity in people regularly exposed to (low doses) of *Campylobacter* bacteria via chicken.[5,30,33] Seroepidemiological studies can address this limitation in allowing the study of risk factors for exposure, regardless of clinical correlate. However, with very frequent exposures such as the scenario described above, the large proportion of the population with high antibody levels may limit the discriminatory capacity to identify risk factors.

In this study, we have compared three different approaches to analyse risk factors for *Campylobacter* exposure. All of them have the common goal of avoiding classification of individuals into prespecified positive and negative categories. All produced comparable results, however, models based on seroincidence resulted in estimates with narrower confidence intervals and identified additional risk factors, particularly in children. Moreover, results are directly interpretable as the relative change in risk of *Campylobacter* infection, making it our preferred method. Also, thanks to the seroincidence R package available through ECDC[13] it is now fairly straightforward to implement.

Using these methods, some previously known risk factors were again identified, such as eating undercooked meat or travelling outside Europe, but the magnitude of the effect was smaller than expected. Of all the animal contacts elicited, we observed an increased risk only in children in contact with pigs and borderline for those with pet birds. The important role of contact with animals in campylobacteriosis in children is concordant with previous reports,[34] but it was surprising that our results showed this only for pigs, as poultry and cattle are the main attributable sources of human campylobacteriosis in the Netherlands.[31] We cannot rule out that contact with pigs is acting as a *proxy* for a risk linked to farming activities as a whole rather than transmission from pigs per se. Surprisingly, although most studies show an increased incidence of *Campylobacter* infection in rural compared to urban areas,[5] we found a lower seroincidence in rural areas, which has been previouly described in the Netherlands.[18,35] This may depend on definitions and living conditions in rural settings,[18] although there are indications that chicken-associated *Campylobacter* infections may be more relevant in urban than rural settings.[16,31]

Finally, several sociodemographic variables were identified as risk factors for exposure to *Campylobacter*, such as age, female gender, non-Western ethnic background or low SES. Probably these variables act as *proxies* for hitherto unmeasured exposures related to lifestyle and habits. Indeed, although *Campylobacter* is largely perceived as a foodborne infection, there is growing evidence for other routes of transmission, including environmental exposure (likely to occur mainly at low doses).[5,16,25,31,33,34,36–38] Unfortunately, since this survey was originally designed for vaccine-preventable diseases, most environmental sources were not included in the epidemiological questionnaire. Other previously described risk factors were also not measured, notably factors related to exposure to recreational and drinking water, to people with gastroenteritis symptoms, to preferential consumption of meat products at restaurants vs. at home, or treatment with gastric antacids. These factors should need to be included in further studies to fully evaluate exposure routes to *Campylobacter*, which may also be different between societies with varying living habits. Finally, we were unable to estimate the risk associated with raw chicken consumption due to low numbers and collinearity with consumption of other undercooked meats.

In conclusion, the exposure to *Campylobacter* of the population in the Netherlands is much higher than the incidence of clinical cases, but factors driving such exposure were not found to differ substantially. Seroincidence is a powerful measure to study *Campylobacter* epidemiology, and was preferred over other methods to analyse *Campylobacter* serological data. This can potentially be used to assess the impact of interventions aimed at reducing exposure to *Campylobacter* in the population.

Conflict of interest

None declared.

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Ethical approval

Medical Ethics Committee, Almere, ISRCTN 20164309.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2018.04.016.

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