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Research paper

Impact of waning acquired immunity and asymptomatic infections on case-control studies for enteric pathogens

Arie H. Havelaar[a,b,*], Arno Swart[c]

[a] Emerging Pathogens Institute, Institute for Sustainable Food Systems, Animal Sciences Department, University of Florida, Gainesville, FL, USA
[b] Institute for Risk Assessment Sciences, Utrecht University, Utrecht, The Netherlands
[c] Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands

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ABSTRACT

Case-control studies of outbreaks and of sporadic cases of infectious diseases may provide a biased estimate of the infection rate ratio, due to selecting controls that are not at risk of disease. We use a dynamic mathematical model to explore biases introduced in results drawn from case-control studies of enteric pathogens by waning and boosting of immunity, and by asymptomatic infections, using *Campylobacter jejuni* as an example. Individuals in the population are either susceptible (at risk of infection and disease), fully protected (not at risk of either) or partially protected (at risk of infection but not of disease). The force of infection is a function of the exposure frequency and the exposure dose. We show that the observed disease odds ratios are indeed strongly biased towards the null, i.e. much lower than the infection rate ratio, and furthermore even not proportional to it. The bias could theoretically be controlled by sampling controls only from the reservoir of susceptible individuals. The population at risk is in a dynamic equilibrium, and cannot be identified as those who are not and have never experienced disease. Individual-level samples to measure protective immunity would be required, complicating the design, cost and execution of case-control studies.

1. Introduction

Foodborne pathogens such as *Campylobacter* spp., non-typhoidal *Salmonella* spp. and other enteric pathogens continue to be of public health importance in developed and developing countries alike (Havelaar et al., 2016). To inform a rational choice of intervention methods, source attribution models are increasingly applied (Pires et al., 2009). The ultimate goals of such studies are to identify the most important sources of exposure for intervention, and to monitor if interventions have been successful. Epidemiologic approaches to source attribution include case-control studies of outbreaks and of sporadic cases. The underlying assumption in case-control studies is that the observed exposure distributions in cases and controls are reflective of the infection incidence rate ratios. However, such relationships may be biased by acquired immunity or asymptomatic infections, due to selecting controls that are not at risk of disease (Havelaar et al., 2009). Swift and Hunter (2004) developed a mathematical model suggesting that given lifetime exposure to infectious disease agents at different intensities, risk ratios for high exposure are biased to the null by constant low exposures and that high exposure may even apparently become protective. The model assumes lifelong immunity, which may not be realistic for many enteric pathogens such as *Campylobacter* spp., to which protective immunity is of limited duration. As a consequence, an individual may not be at risk of disease at some point in time, but subsequently lose protective immunity and be at risk again. Hence, both the population of potential cases and of eligible controls varies over time and individuals may leave and enter the cohort repeatedly. It is suggested that the confounding effects of immunity may be controlled by the usual array of methods used in study design and data analysis (Rothman and Mahon, 2004). Such methods would, however, require information on the exposure history of the study population, which is rarely available.

Swart et al. (2012) have developed a simple mathematical model to quantify the impact of acquired immunity on the population dynamics of campylobacteriosis and concluded that due to the effects of waning and boosting of immunity, an increasing force of infection does not necessarily lead to an increase in the incidence of disease. Under certain conditions, a decrease of the force of infec-

* Corresponding author at: Emerging Pathogens Institute, Institute for Sustainable Food Systems, Animal Sciences Department, University of Florida, Gainesville, Florida, USA.
E-mail address: ariehavelaar@ufl.edu (A.H. Havelaar).

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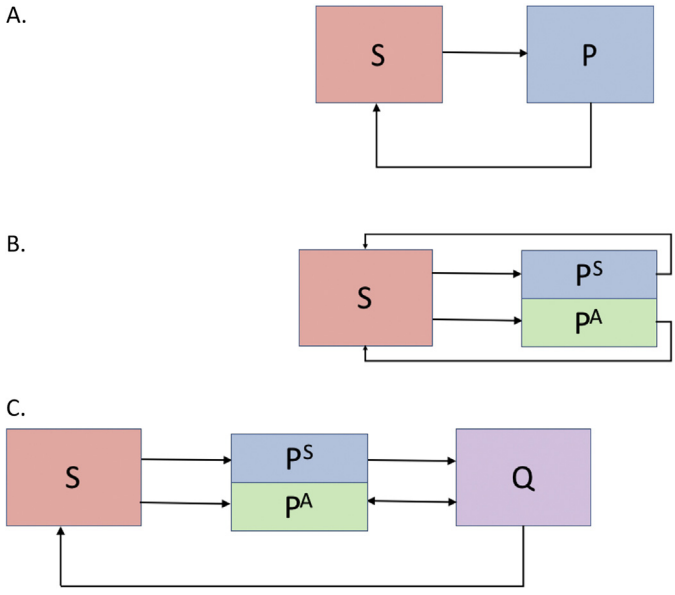

Fig. 1. Conceptual models for dynamics of enteric illness without (A) and with (B, C) asymptomatic infection and without (A, B) and with (C) waning acquired immunity.

tion may in fact lead to an increase of the incidence of disease. The model also includes the possibility of asymptomatic infection, leading to temporary protection without illness symptoms occurring. This model was subsequently used by Havelaar and Swart (2014) to explore the impact of acquired immunity in quantitative microbial risk assessment studies by explicitly including the frequency of exposure and the ingested dose into the estimation of the force of infection and the probability of (a)symptomatic infection. In this paper, we proceed to use this model as a basis to explore biases introduced in case-control studies of enteric pathogens by waning and boosting of immunity, and by asymptomatic infections, using *Campylobacter jejuni* as an example.

2. Model

To evaluate the selection bias by acquired immunity and asymptomatic infections in case-control studies, we present several compartmental models. Panel A in Fig. 1 represents a basic model in which susceptible individuals (S) may become infected (defined as a state in which the pathogen has established itself and actively multiplies in the host, measurable by production of antibodies by the host), with all infected individuals becoming ill, who subsequently recover and become susceptible again. This model is similar to the standard SIS model (Anderson and May, 1991) but in our case the focus is on illness rather than infectivity, hence we use the symbol P (for protected) rather than I for this compartment and the symbol Q (sequential to P) for the partially protected compartment. This basic model also represents the assumptions in a standard analysis of case control studies: all individuals with clinical signs of illness are classified as cases and all asymptomatic cases are classified as controls. Note that typically, duration of protective immunity is longer than of clinical symptoms, hence even in this basic model, some asymptomatic individuals may be misclassified. We assume that this misclassification is countered by the usual practice to exclude persons with a recent history of gastrointestinal disease from the study, e.g., (MacDonald et al., 2015). Implicitly, this assumes that the average duration of protection in P is the same as the exclusion period in the epidemiological study.

A more detailed consideration of the nature of infectious diseases leads to the need to refine the simple SPS model:

a. Even upon first exposure, infection may not lead to illness, i.e. the probability of illness given an S → P transition is less than 1, depending on the ingested dose and other factors; see the panel B in Fig. 1. Asymptomatic, infected individuals (P^A) are protected from disease and hence potentially incorrectly classified as controls for estimating the infection rate ratio, while symptomatic individuals (P^S) are correctly classified as cases. We refer to this model as the SP^AS model.

b. After infection, individuals may be partially protected by acquired immunity, implying they may be re-infected but the reinfection does not lead to disease, see panel C in Fig. 1. Individuals in this state of partial protection (Q) are potentially incorrectly classified as controls for estimating the infection rate ratio as they are not at risk of disease. We refer to this model as the SP^AQS model.

As in Swart et al. (2012), we assume for the full SP^AQS model that:

- All individuals are born susceptible (S);
- Individuals may become (asymptomatically) infected with force of infection λ; incorporating both the intensity of exposure and the dose-response function;
- When infected, there is a probability π of developing symptomatic illness;
- an infected individual is immediately fully protected (P) against subsequent infection;
- Waning of immunity is represented by transitions from P to a state of partial protection (Q) with rate α and then back to S with rate γ;
- When a partially protected individual is re-exposed to the same pathogen, a transition to the fully protected state (P) takes place with rate λ given the same frequency and dose of exposure as for the S → P transition;

We first consider the distribution of individuals over the different compartments in a closed cohort. At the start of the study, we have unknown fractions of the population in S, P, Q denoted s_0, p_0, q_0, and $s_0 + p_0 + q_0 = 1$. The evolution of the numbers of individuals as a function of time in the compartments, in the absence of birth and death, is given by

$$\begin{pmatrix} s(t) \\ p(t) \\ q(t) \end{pmatrix} = \frac{1}{(\alpha+\lambda)(\gamma+\lambda)} \begin{pmatrix} \gamma\alpha \\ \lambda(\gamma+\lambda) \\ \lambda\alpha \end{pmatrix} +$$

$$\frac{\lambda - (\alpha+\lambda)p_0}{(\alpha-\gamma)(\alpha+\lambda)} \begin{pmatrix} -\gamma \\ \gamma-\alpha \\ \alpha \end{pmatrix} e^{-(\alpha+\lambda)t} +$$

$$\frac{\alpha\lambda s_0 - \alpha\gamma p_0 + \gamma(\lambda+\gamma-\alpha)q_0}{(\alpha-\gamma)(\gamma+\lambda)} \begin{pmatrix} 1 \\ 0 \\ -1 \end{pmatrix} e^{-(\gamma+\lambda)t} \quad (1)$$

Note that when working with age instead of time, and setting $(s_0, p_0, q_0) = (1, 0, 0)$, we retrieve the equations from Swart et al. (2012).

Individual components can be obtained from the above equation, e.g.

$$s(t) = \frac{\gamma\alpha}{(\alpha+\lambda)(\gamma+\lambda)} + \frac{\gamma}{(\alpha-\gamma)} \left\{ \left[p_0 - \frac{\lambda}{(\alpha+\lambda)} \right] e^{-(\alpha+\lambda)t} + \left[\frac{\alpha}{\gamma} s_0 + q_0 - \frac{\alpha}{(\gamma+\lambda)} \right] e^{-(\gamma+\lambda)t} \right\} \quad (2)$$

Table 1
Steady state population distributions for three different models.

Model	SPS	SP^AS	SP^AQS
s^*	$\frac{\alpha}{(\alpha+\lambda)}$	$\frac{\alpha}{(\alpha+\lambda)}$	$\frac{\alpha\gamma}{(\alpha+\lambda)(\gamma+\lambda)}$
p^{*A}	0	$\frac{(1-\pi)\lambda}{(\alpha+\lambda)}$	$\frac{(1-\pi)\gamma\lambda+\lambda^2}{(\alpha+\lambda)(\gamma+\lambda)}$
p^{*S}	$\frac{\lambda}{(\alpha+\lambda)}$	$\frac{\pi\lambda}{(\alpha+\lambda)}$	$\frac{\pi\lambda\gamma}{(\alpha+\lambda)(\gamma+\lambda)}$
q^*	0	0	$\frac{\alpha\lambda}{(\alpha+\lambda)(\gamma+\lambda)}$

Given homologous exposure (i.e. we consider only one antigenic variant of *C. jejuni*), the model reaches a dynamic equilibrium, which is characterized by the population distribution:

$$s^* = \frac{\alpha\gamma}{(\alpha+\lambda)(\gamma+\lambda)};\ p^* = \frac{\lambda}{(\alpha+\lambda)};\ q^* = \frac{\alpha\lambda}{(\alpha+\lambda)(\gamma+\lambda)} \tag{3}$$

When we split the compartment P into P^A and P^S, the equations for these compartments become:

$$\frac{dP^A}{dt} = (1-\pi)\lambda S - \alpha P^A - \lambda Q \text{ and } \frac{dP^S}{dt} = \pi\lambda S - \alpha P^S \tag{4}$$

Setting these to zero to describe the steady state yields

$$p^{*S} = \frac{\pi\lambda s^*}{\alpha} = \frac{\pi\lambda\gamma}{(\alpha+\lambda)(\gamma+\lambda)} \text{ and } p^{*A} = \frac{(1-\pi)\lambda s^* + \lambda q^*}{\alpha}$$
$$= \frac{(1-\pi)\gamma\lambda + \lambda^2}{(\alpha+\lambda)(\gamma+\lambda)} \tag{5}$$

One may verify that those compartments add up to p^*. Table 1 shows the steady state solutions for all three models, noting that the SP^AS model is a special case of the SP^AQS model with $\gamma \to \infty$, and that the SPS model is a special case of SP^AS model with $\pi = 1$.

3. Stratification of the cohort

Assume a cohort of size N is composed of two mutually exclusive groups (with population fractions ϕ_0 and ϕ_1; $\phi_0 + \phi_1 = 1$), each exposed to *C. jejuni* at a different force of infection $\lambda_1 > \lambda_0$ and different probability of illness $\pi_1 \geq \pi_0$. The exposure ratio for these two groups $E = \lambda_1/\lambda_0$. In the low exposure group, we denote by s_0^*, p_0^* and q_0^* the fractions of the individuals in the compartments S, P, Q in steady state. Similarly, we define s_1^*, p_1^* and q_1^* in the high exposure group.

The disease incidence I_j in each group is calculated on the basis of S → P transitions. For all models,

$$I_j = \lambda_j \pi_j s_j^* T_j \phi_j N \ \ (j = 0, 1) \tag{6}$$

new cases are observed in each group j during a study with observation time T_j. The incidence rate in each group equals the incidence divided by the person time in the at-risk population (PT), which differs between models. For both the SPS and the SPAS models (denoted by $SP^{(A)}S$):

$$PT_j^{SP^{(A)}S} = s_j^* T_j \phi_j N \tag{7}$$

Under steady-state conditions, this implies for the $SP^{(A)}S$ models:

$$IR_j^{SP^{(A)}S} = \frac{\lambda_j \pi_j s_j^* T_j \phi_j N}{s_j^* T_j \phi_j N} = \lambda_j \pi_j \tag{8}$$

At a given value of λ, the incidence increases proportional to π. When $\pi_0 = \pi_1$ which is a reasonable assumption, then $IRR = IR_1/IR_0 = \lambda_1/\lambda_0$, and the incidence rate ratio reflects the infection rate ratio.

For the SP^AQS model, the incidence is also calculated by Eq. (6), but numerically the results are lower because the proportion of susceptible individuals is lower in the SP^AQS model than in the $SP^{(A)}S$ model, particularly at higher values of λ as the increase of the force of infection is almost fully nullified by the decrease in the proportion of susceptible individuals. Without consideration of the immune status of individuals, the population time at risk would be identified as all who are not symptomatic (at any point in time as we assume steady state conditions):

$$PT_j^{SP^AQS} = \left(1 - p_j^{s*}\right) T_j \phi_j N \tag{9}$$

Hence

$$IR_j^{SP^AQS} = \lambda_j \pi_j s_j^* / (1 - p_j^{s*}) = \lambda_j \pi_j \frac{1}{\left(1 + \frac{\lambda_j}{\gamma}\right)\left(1 + \frac{\lambda_j}{\alpha}\right) - \frac{\lambda_j}{\alpha}\pi_j} \tag{10}$$

As the proportion of symptomatic individuals in the population is lower in the SP^AQS model than in the SP^AS model, the incidence rates are also lower. The observed incidence rate will in general not be equal to the exposure rate ratio, even when $\pi_0 = \pi_1$. An unbiased estimate of the incidence rate ratio could be obtained if the true susceptible individuals could be identified in the population and their number be used in the denominator of the incidence rate.

Table 2
Steady state population distributions for the SP^AS and SP^AQS models at different levels of the force of infection (λ) and probability of illness given infection $\pi = 0.1$.

Model	SP^AS		SP^AQS	
λ	1	10	1	10
s^*	0.929	0.565	0.486	0.056
p^{*A}	0.064	0.391	0.068	0.430
p^{*S}	0.007	0.044	0.004	0.004
q^*	0.000	0.000	0.442	0.510

4. Numerical example

Based on previous work (Swart et al., 2012), we assume $\alpha = 13\,yr^{-1}$ and $\gamma = 1.1\,yr^{-1}$. We compare two populations with different force of infection $\lambda \in \{1, 10\}$ and probability of symptomatic illness given infection varying between $\pi \in [0, 1]$. All computations were done in the statistical language R version 3.2.3 (R Core Team, 2015). Fig. 2 shows the distribution of the population over the different compartments, and Table 2 shows selected results. For the $SP^{(A)}S$ models, the proportion in P increases with λ, as expected. In the SP^AS model, the P compartment is split into two components P^A and P^S, proportional to π. Incorporating the possibility of acquired immunity in the SP^AQS model results in a considerable reduction of the proportion in S, compared to the $SP^{(A)}S$ models. The proportions in Q and P^S are relatively insensitive to changes in λ, with very low proportions in P^S compared to the $SP^{(A)}S$ models. in the SP^AQS model, the proportion in P^A increases strongly with λ.

5. Inference from a case control study

We now assume a case-control study is done in this cohort. We assume that a fraction ϕ_0 is exposed to a low force of infection and a low probability of illness and a fraction $\phi_1 = 1 - \phi_0$ of the population is exposed to a high force of infection, and a high probability of illness. According to a density sampling design, each time a case is observed, one control is selected from the asymptomatic population (excluding recently diseased cases, see above). The exposure rate ratio in the two groups is $ERR = \lambda_2/\lambda_1$ and the disease incidence rate ratio is $IRR = \lambda_1\ \pi_1/\lambda_0\ \pi_0$.

The proportion of cases and controls exposed to risk factors resulting from a theoretical case-control study is shown in Table 3. The Table shows the results for the SP^AQS model. For the SP^AS

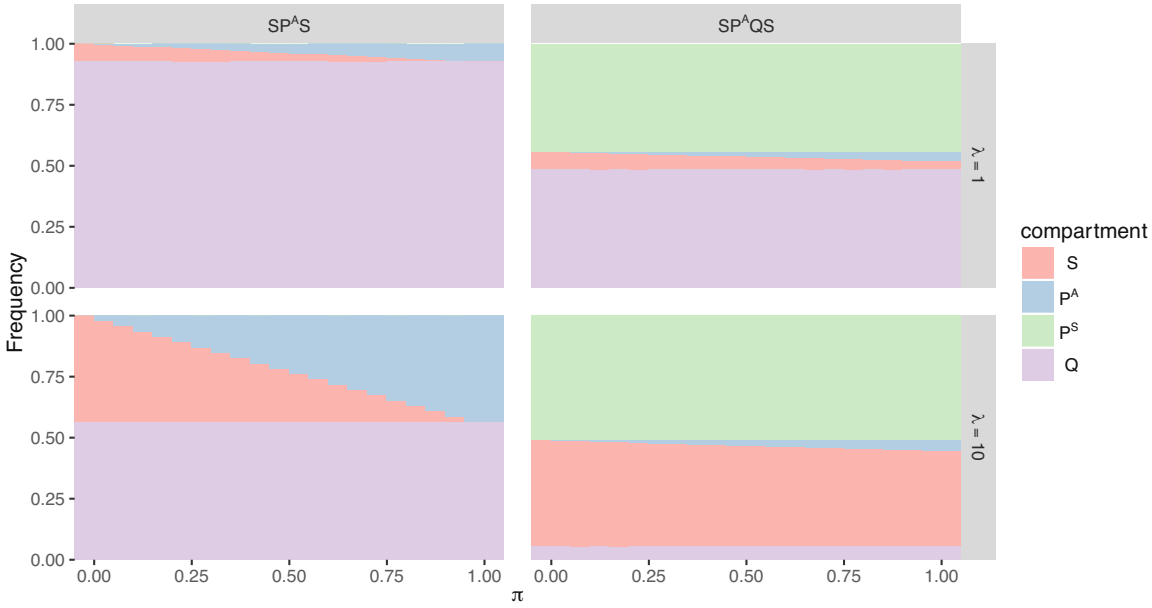

Fig. 2. Steady state distribution of symptomatic and asymptomatic cases in a cohort, according to different models for infectious disease dynamics as a function of the probability of illness given infection (π) and for two levels of the force of infection (λ). For an explanation of symbols, see text. Note that the SPS model is a limiting case of the SP^{A}S model with $\pi = 1$.

Table 3
Proportions (a) and numbers (b) of observed cases and controls, without (Havelaar et al., 2016) or with (Pires et al., 2009) consideration of immune status, in each of two exposure groups, according to the SP^{A}QS model.

(a1)

	High exposure	Low exposure
Cases	$p_1^{s*}\phi_1$	$p_0^{s*}\phi_0$
Controls	$\left(s_1^* + p_1^{a*} + q_1^*\right)\phi_1 = \left(1 - p_1^{s*}\right)\phi_1$	$\left(s_0^* + p_0^{a*} + q_0^*\right)\phi_0 = \left(1 - p_0^{s*}\right)\phi_0$
Sum	ϕ_1	ϕ_0

(a2)

	High exposure	Low exposure
Cases	$p_1^{s*}\phi_1$	$p_0^{s*}\phi_0$
Controls	$s_1^*\phi_1$	$s_0^*\phi_0$
Non-eligible	$\left(p_1^{a*} + q_1^*\right)\phi_1$	$\left(p_0^{a*} + q_0^*\right)\phi_0$
Sum	ϕ_1	ϕ_0

(b1)

	High exposure	Low exposure	Sum
Cases	$\frac{p_1^{s*}\phi_1}{p_1^{s*}\phi_1 + p_0^{s*}\phi_0}A$	$\frac{p_0^{s*}\phi_0}{p_1^{s*}\phi_1 + p_0^{s*}\phi_0}A$	A
Controls	$\frac{\left(1-p_1^{s*}\right)\phi_1}{\left(1-p_0^{s*}\right)\phi_0 + \left(1-p_1^{s*}\right)\phi_1}A$	$\frac{\left(1-p_0^{s*}\right)\phi_0}{\left(1-p_0^{s*}\right)\phi_0 + \left(1-p_1^{s*}\right)\phi_1}A$	A

(b2)

	High exposure	Low exposure	Sum
Cases	$\frac{p_1^{s*}\phi_1}{p_1^{s*}\phi_1 + p_0^{s*}\phi_0}A$	$\frac{p_0^{s*}\phi_0}{p_1^{s*}\phi_1 + p_0^{s*}\phi_0}A$	A
Controls	$\frac{s_1^*\phi_1}{s_0^*\phi_0 + s_1^*\phi_1}A$	$\frac{s_0^*\phi_0}{s_0^*\phi_0 + s_1^*\phi_1}A$	A

model, set $q_j^* = 0$ and for the SPS model, additionally set $p_j^{A*} = 0$, and $p_j^{s*} = p_j^*$.

If a total number of A cases is observed, and the number of controls equal by design, relative proportions are shown in Table 3a and numbers of cases and controls in Table 3b.

From the cross-tabulation of Table 3b, the odds ratio can be calculated:

$$\Omega^{Obs} = \frac{p_1^{s*}\left(1 - p_0^{s*}\right)}{p_0^{s*}\left(1 - p_1^{s*}\right)} \tag{11}$$

Table 4
Exposure scenarios for C. jejuni in subgroups of the hypothetical population, see text for explanation of symbols. f, d = low exposure frequency or dose, F, D = high exposure frequency or dose.

Exposure scenario(j) Frequency/Dose	ϕ_j	E_j (year^{-1})	D_j (cfu)	λ_j (year^{-1})	π_j
f/d	0.901	1	5	5.9×10^{-2}	4.3×10^{-4}
f/D	0.029	1	100	2.7×10^{-1}	8.3×10^{-3}
F/d	0.012	100	5	5.9×10^{0}	4.3×10^{-3}
F/D	0.058	100	100	2.7×10^{1}	8.3×10^{-3}

Under different models, this will yield differing results, from Table 1 we find:

$$\Omega^{Obs,SPS} = \frac{\lambda_1}{\lambda_0} \tag{12}$$

$$\Omega^{Obs,SP^AS} = \frac{\pi_1\lambda_1(\alpha+(1-\pi_0)\lambda_0)}{\pi_0\lambda_0(\alpha+(1-\pi_1)\lambda_1)} \tag{13}$$

$$\Omega^{Obs,SP^AQS} = \frac{\pi_1\lambda_1\left((\alpha+\lambda_0)(\gamma+\lambda_0)-\pi_0\lambda_0\gamma\right)}{\pi_0\lambda_0\left((\alpha+\lambda_1)(\gamma+\lambda_1)-\pi_1\lambda_1\gamma\right)} \tag{14}$$

Hence, under the SP^A(Q)S model assumptions, the incidence rate ratio cannot be reconstructed on the basis of the observed odds ratio, even when all other parameters are known.

Moreover, the odds ratio should be calculated on the basis of susceptible controls (in the S compartment) only. Assuming this is possible, this translates to removing the terms corresponding to the P and Q compartments in Table 3a, and re-calculating the odds ratio adjusted for protective immunity:

$$\Omega^{Adj} = \frac{p_1^{s*}s_0^*}{p_0^{s*}s_1^*} \tag{15}$$

Depending on model choice we obtain:

$$\Omega^{Adj,SPS} = \frac{\lambda_1}{\lambda_0} \tag{16}$$

$$\Omega^{Adj,SP^AS} = \frac{\pi_1\lambda_1}{\pi_0\lambda_0} \tag{17}$$

$$\Omega^{Adj,SP^AQS} = \frac{\pi_1\lambda_1}{\pi_0\lambda_0} \tag{18}$$

Hence, we can reconstruct the incidence rate ratio in each model. The bias in the odds ratio is due to selection bias in the denominators of the odds of exposure in both cases and controls: $\left(1-p_j^{s*}\right) \neq s_j^*$ in the SP^{A}S and SP^{A}QS models.

6. Numerical example

We set $\lambda_0 = 1$; $\pi_0 = 0.01$ and $\lambda_1 = 10$; $\pi_1 = 0.1$, hence $ERR = 10$ and $IRR = 100$. The observed odds ratio for the SP^{A}QS model $\Omega^{obs} = 11.6$, strongly biased to the null compared to the unbiased $IRR = 100$.

0. In the SP^{A}S model, $\Omega^{obs} = 63.6$. Apparently, asymptomatic infections do affect the observed odds ratio but to a lesser extent than acquired immunity. In the SPS model, $\Omega^{obs} = 100$, as expected. An unbiased estimate of the odds ratio would only sample controls from the S compartment. Then, in all models $\Omega^{Adj} = 100$, equal to the unbiased disease incidence rate ratio.

Fig. 3 shows the results for the observed and adjusted odds ratio for the SP^{A}QS model for all combinations of $0 \leq (\lambda_0, \lambda_1) \leq 10$ and $\pi_1 = 0.1$; $\pi_0 = 0.01$. The adjusted odds ratio is a linear function of λ_1 for all values of λ_0. The observed odds-ratio is considerably lower for all values of λ_1 and λ_0, and shows non-linear behavior with a maximum at $\lambda_1 = \sqrt{\alpha\gamma}$ for all values of λ_0.

7. Consideration of exposure frequency and dose

For pathogens from inanimate sources such as food or the environment, the force of infection λ and probability of illness π depend on the exposure frequency E and the ingested dose D per exposure event.

We consider a population of size N, composed of j mutually exclusive groups (with population fractions $\phi_j \sum_j \phi_j = 1$), each exposed to *Campylobacter* spp. at different exposure frequencies and doses. We use a conditional dose-response model based on (Teunis and Havelaar, 2000; Teunis et al., 1999) to estimate the force of infection and the probability of illness given infection. This model assumes that illness results from a series of events: exposure → infection → illness. The probability of a susceptible individual to get infected (equivalent to an S → P transition) from a single exposure to dose D_{ij} (the i^{th} dose in group j) is characterized by the hypergeometric model:

$$p_{inf}(\mathrm{D_{ij}}|\alpha,\beta) = 1 - {}_1\mathrm{F}_1(\alpha, \alpha+\beta, -\mathrm{D_{ij}}) \tag{19}$$

with a and b parameters of the dose-response model.

If E exposure events occur in a fixed time period (e.g. a year), then the average force of infection is

$$\lambda_j = \sum_{i=1}^{E} p_{inf}\left(D_{ij}\right) \tag{17}$$

We assume in the following that individuals are exposed to the average dose D_j at each exposure event. The conditional probability of illness given infection π_j is also assumed to depend in the ingested dose and quantified by

$$\pi_j = p_{ill|inf}(D_j|\rho,\eta) = 1-(1+\eta D_j)^{-\rho} \tag{18}$$

with ρ and η parameters of the dose-response model.

The illness incidence can then be calculated according to Eq. (6).

For each group j, the referent incidence (i.e. the average incidence in all other exposure groups) can be calculated as

$$\tilde{I}_j = \sum_{k\neq j} I_k \Big/ \sum_{k\neq j} \phi_k s_k^* N \tag{19}$$

and the incidence rate ratio as $IRR_j = I_j/\tilde{I}_j$ (20)

8. Numerical example

We use scenarios based on (Havelaar and Swart, 2014) to explore the impact of differences in exposure frequency and dose on the bias in odds rations induced by acquired immunity, see Table 4. In contrast to the earlier work, we have implemented a symmetrical design, i.e. low or high exposure frequency is defined as 1 or 100 yr^{-1}, and low or high dose is defined as 5 or 100 cfu, respectively. We indicate exposure groups as e.g. f/D for low exposure frequency and high dose. The Table also shows the computed values of λ_j, which ranges between 6.0×10^{-23} yr^{-1} for f/d and 2.7×10^{1} yr^{-1} for F/D, and for π_j, which is 4.2×10^{-4} yr^{-1} for low doses and 8.3×10^{-3} yr^{-1} for high doses. For these computations, we used the following dose-response parameters: $\alpha = 0.145$,

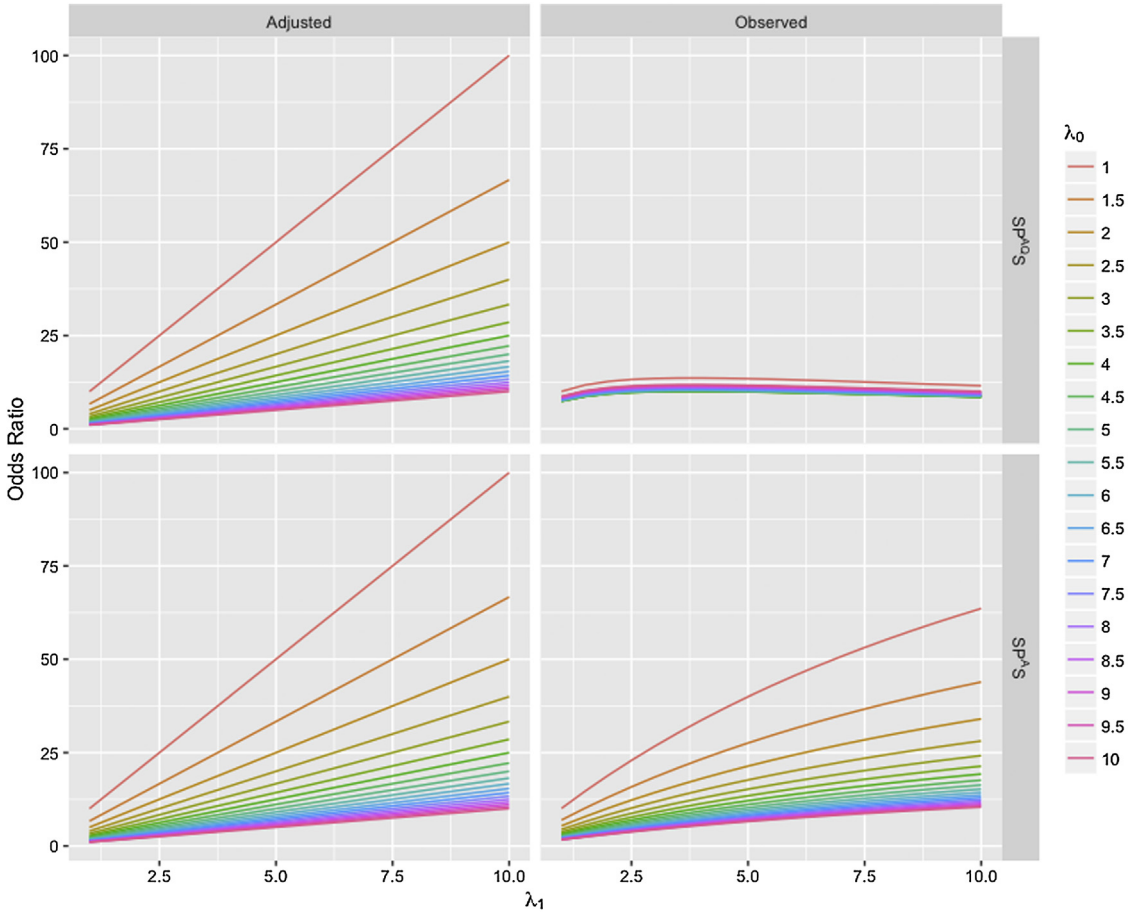

Fig. 3. Observed and adjusted odds ratio for the SP^AQS model as a function of the force of infection in two exposed groups.

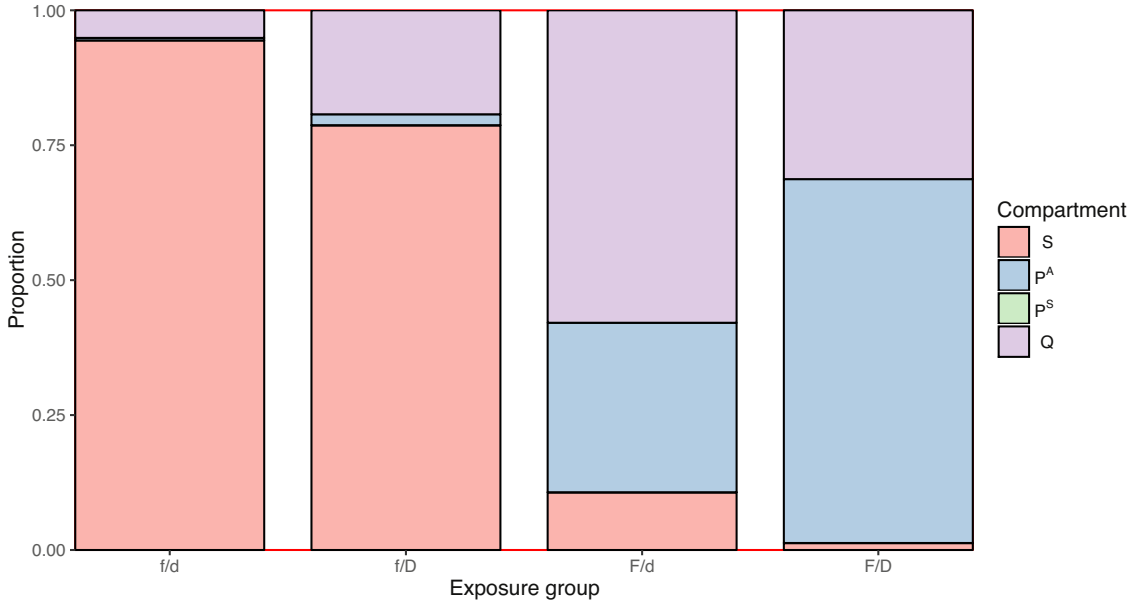

Fig. 4. Population distribution according to infection and protection status for four different exposure scenarios. For definition of exposure groups, see Table 4.

$\beta = 8.007$, $\eta = 0.000514$, $\rho = 0.167$ (Teunis and Havelaar, 2000; Teunis et al., 1999). Fig. 4 shows the distribution over the different compartments of the SP^AQS model for the different exposure groups. The proportion in P^S is very small in all groups (ranging between 2×10^{-6} and 2×10^{-4}). In the L/L group, 94% of the population is in S, and this proportion decreases in other scenarios to a low value of 1.2% in the F/D group. The proportion in Q is largest (58%) in the F/d group, whereas the proportion in P^A is largest (67%) in the F/D group.

Table 5
Incidence rate ratios (IRR) in the hypothetical population in comparison with the observed (Ω^{Obs}) and adjusted (Ω^{Adj}) odds ratios, according to the SP^AQS model.

Exposure scenario	*IRR*	Ω^{Obs}	Ω^{Adj}
f/d	0.00029	0.011	0.00029
f/D	10	8.9	10
F/d	9.3	1.1	9.3
F/D	2600	35	2600

The results of a case control study in this population are shown in Table 5 for the SP^AQS model. The results show that all observed odds ratios are biased to the null, with the bias stronger for frequent exposures. Sampling controls from the susceptible compartment only provides an unbiased estimate of the incidence rate ratio. The biased and unbiased odds ratio for the f/d group are below 1, which would be interpreted as a protective effect. Each individual is a member of one of the groups (f/d, f/D, F/d, F/D). When studying the f/d risk-factor, the referent group is f/D + F/d + F/D. Compared to this group, being in f/dis associated with considerably lower risks, resulting in odds ratios below 1, even though exposure at these low levels does constitute a risk of infection and illness. Note that inclusion of a proper non-exposed group is not possible: the force of infection of zero would lead to infinite odds ratios.

9. Discussion

We demonstrate that in infectious diseases research, standard assumptions to estimate the infection rate ratio from case-control studies can be violated by the effects of boosting and waning of acquired immunity, and asymptomatic infections. In a cohort, the population at risk of disease is decreased by symptomatic or asymptomatic infection. Only symptomatically infected persons will be recognized as such, whereas asymptomatically infected persons will not be recognized and will be eligible as controls if a case-control study is carried out in this cohort. When individuals have cleared the infection, they may enter a state of partial protection, in which they can be re-infected, but such re-infection does not lead to disease. Partially protected individuals would also be eligible as controls. At a low force of infection, waning of partially protective immunity may cause partially protected individuals to become susceptible to infection and disease again, thus adding to the population at risk. The population at risk is in a dynamic equilibrium, and cannot be identified as those who are not and have never been diseased.

The bias introduced in epidemiologic studies by acquired immunity could theoretically be addressed by the usual array of methods used in study design and data analysis (Rothman and Mahon, 2004). Such methods would, however, require information on the exposure history of the study population, which is rarely available. Tam et al. (2009), in a case-control study in England, acquired information on frequency of chicken consumption and showed that eating chicken in the last 5 days was a highly significant risk factor (OR = 5.0, 95% CI 2.1–11.9) for those who did not regularly eat chicken, but was not a significant risk factor (OR = 0.8, 95% CI 0.6–1.0) for those who did. Ideally, controls should only be selected from individuals in the S compartment. This is not possible on the basis of questionnaire-based approaches, but would require obtaining body fluids (blood, saliva) from selected individuals to measure the presence of antibodies against the pathogen of interest. This would greatly complicate the design, ethical clearance, execution and costs of epidemiologic studies. Furthermore, measurements of protective immunity are not available for many enteric pathogens, including Campylobacter. Although an elevated antibody titer is indicative of past infection, it is not at all clear that the numerical value of a titer is proportional to some level of protection. In other words, the model parameters α and γ, describing waning and boosting of immunity, are likely not simple functions of measured antibody levels. Standardized serological assays have recently been applied to study the sero-epidemiology of Campylobacter and Salmonella in Europe, using hierarchical dynamic Bayesian models to interpret the data at population level (Teunis et al., 2012; Molbak et al., 2014). These measurements indicate that Campylobacter infection is a frequent event, occurring approximately once every year in any adult person, in the Netherlands. This supports the conclusion that only a small fraction of infections does lead to symptoms severe enough for notification or even unreported illness. In our exposure scenarios, the force of infection varies between 0.003 and 13 yr^{-1}, bracketing the observed force of infection from the sero-surveillance studies. Havelaar et al. (2012) estimated that in 2009, there were 92,000 (95% uncertainty interval 13,000–251, 000) symptomatic cases per year in the Netherlands (1.7×10^7 inhabitants); the average duration is 0.02 years. Hence, the expected prevalence of symptomatic cases is approximately 10^{-4} (10^{-5}–3×10^{-4}). In comparison to these numbers, the proportion of symptomatic individuals in Fig. 2 is only realistic for small values of π. In our exposure scenarios, the prevalence of symptomatic cases varies between 10^{-7} and 10^{-5} (data not shown), so one or more orders of magnitude lower than the observed value. This suggests that either the values for π estimated by the infection-illness dose-response model are unrealistically low, or that the predicted prevalence is too low because strain variability is ignored in our model.

We therefore study the impact of acquired immunity from a theoretical perspective and present a simple model to explore the biases involved.

Our simple model has several limitations, that need to be addressed in further work. It only covers two risk factors and assumes constant force of infection, but can be extended to cover more risk factors and variable exposures. The force of infection is a single parameter, and needs to be linked to exposure frequency and dose by dose-response models as suggested by Havelaar and Swart (2014). As in previous studies, one of the main limitations is that only one study is available for parameter estimation regarding immunity, and no data are available to account for heterogeneity in bacterial populations and cross-protection that may result from exposure to antigenically related strains. We have currently analyzed for steady state conditions, and need to extend the modeling approach to include age-related effects and variable force of infection.

Despite these limitations, our work impacts several key aspects of foodborne diseases epidemiology and risk assessment. A case-control study in the Netherlands suggests that the fraction of cases of human campylobacteriosis that can be attributed to exposure by broiler meat is 28% (Doorduyn et al., 2010; Mughini Gras et al., 2012). This attributable fraction is also used in evaluation of the cost-effectiveness of setting microbiological criteria for broiler meat after processing (Swart et al., 2013). Interestingly, the fraction of human cases of campylobacteriosis associated with the poultry reservoir, as based on comparing MLST types of isolates from humans and animal reservoirs is 66% (Mughini Gras et al., 2012). The discrepancy between these two attribution estimates has been interpreted to imply that other pathways than broiler meat, such as environmental transmission from broiler and laying hen reservoirs might be important in the epidemiology of human campylobacteriosis as was also suggested by Friesema et al. (2012). Our findings suggest that at least part of the discrepancy could be due to bias in the attributable fraction as estimated in the case-control study, but it is not currently possible to estimate the degree of underestimation.

Our results also have an impact on the interpretation of case-control studies for etiology of diarrheal diseases. The Sensor study

was a prospective population-based cohort study with a nested case-control study to estimate the incidence of gastroenteritis and the associated pathogens in the general Dutch population, carried out in 1998–1999. Stool samples of 1.3% of gastroenteritis cases were culture-positive for Campylobacter, as was 0.6% of samples from matched controls (De Wit et al., 2001). When calculating the proportion of gastroenteritis attributable to Campylobacter, Havelaar et al. (2012) decided not to correct for the isolation of Campylobacter from controls. This was considered one of the major uncertainties in the model for the burden of foodborne disease in the Netherlands (Bouwknegt et al., 2014) and is in contrast with the interpretation of results from large cohort studies in low-income countries, such as the GEMS (Blackwelder et al., 2012) and MAL-ED studies (The MAL-ED Network Investigators, 2014), who use an estimation method based on the population attributable fraction (PAF). Our results suggest that the PAF may underestimate the true proportion of cases of enteric disease that is attributed to a particular pathogen, as was also suggested by Lopman et al. (2014) for norovirus. More work is needed to develop methods for estimating etiological proportions taking infection dynamics into account.

While immunity may protect against disease, the public health balance of high exposure may still be negative due to triggering of immune-mediated diseases such as Guillain-Barre syndrome and reactive arthritis. Also, in low-income countries, a high force of infection is typically associated with a high number of childhood deaths and environmental enteropathy resulting from both symptomatic and asymptomatic infections (Korpe and Petri, 2012).

Conflict of interest

The authors declare that they have no conflict of interest.

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