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Campylobacter seroconversion rates in selected countries in the European Union

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SUMMARY

As a major foodborne pathogen, *Campylobacter* is frequently isolated from food sources of animal origin. In contrast, human *Campylobacter* illness is relatively rare, but has a considerable health burden due to acute enteric illness as well as severe sequelae. To study silent transmission, serum antibodies can be used as biomarkers to estimate seroconversion rates, as a proxy for infection pressure. This novel approach to serology shows that infections are much more common than disease, possibly because most infections remain asymptomatic. This study used antibody titres measured in serum samples collected from healthy subjects selected randomly in the general population from several countries in the European Union (EU). Estimates of seroconversion rates to *Campylobacter* were calculated for seven countries: Romania, Poland, Italy, France, Finland, Denmark and The Netherlands. Results indicate high infection pressures in all these countries, slightly increasing in Eastern EU countries. Of these countries, the differences in rates of notified illnesses are much greater, with low numbers in France and Poland, possibly indicating lower probability of detection due to differences in the notification systems, but in the latter case it cannot be excluded that more frequent exposure confers better protection due to acquired immunity.

Key words: *Campylobacter*, epidemiology, incidence, serology, statistics.

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INTRODUCTION

Among bacterial causes of gastroenteritis, *Campylobacter jejuni* and *C. coli* are important because they are such common pathogens [1, 2] with a considerable health burden due to acute enteric disease [3]. In the European Union (EU) notification rates vary from 0·5 to 70/100 000 (1/year) [4]. In addition, *Campylobacter* is associated with potentially severe sequelae. Infection with *C. jejuni* is a strong risk factor for Guillain–Barré syndrome: an immune-mediated disease of the peripheral nerves [5–7]. Studies of the occurrence of *Campylobacter* in foods (poultry, pork, raw milk) and the environment (untreated water) indicate that human exposure may be a frequent event [8, 9], perhaps much more frequent than reported cases of *Campylobacter* enteritis would suggest [10–13]. An important cause for the gap between risk estimates of *Campylobacter* infection and epidemiological estimates of the incidence of campylobacteriosis is the difference in modality of these two measures. Notification rates are based on reported cases of illness, occasionally corrected for under-ascertainment. Risk estimates can be obtained by translating exposure estimates, based on microbiological surveillance of foods and water into estimates of the probability of infection, including asymptomatic cases, using a dose–response model for infection [14]. Estimation of the fraction of infections that are symptomatic is difficult, because it may depend on covert properties of the infected host or the infecting microorganism [14].

Here we present a new method based on simple assumptions that allows analysis of measured antibody levels (as optical densities in an immunosorbent assay) quantitatively, instead of first categorizing them (into 'positives' and 'negatives'). The distribution of antibody titres at the time of sampling is related to the infection rate: the higher the infection pressure the more frequently seroconversion occurs and, as a consequence, antibody titres are shifted towards higher values. When the serum antibody response to infection is known, the incidence of infection (seroconversion) can thus be estimated from the distribution of antibody titres in a cross-sectional serum sample.

MATERIALS AND METHODS

Serological data

To describe the serum antibody response to infection, peak levels and decay rates were estimated from a published longitudinal study of anti-*Campylobacter* IgA, IgM, or IgG antibodies in symptomatic cases. Longitudinal sera were obtained from a Danish study (1996–1997) of 210 culture-confirmed cases of campylobacteriosis (*C. jejuni* or *C. coli*) with blood samples taken about 3 weeks, 3 months, 6 months and 2 years after infection. Ages of patients ranged from 10 to 76 years (median age 33·5 years). Details of the study design can be found in Strid *et al.* [15]. Serological testing was performed as reported previously [16]: IgA, IgM and IgG antibodies were measured as a ratio against a reference sample [17]. The ELISA measured antibodies against *C. jejuni* and *C. coli* but not against non-thermophilic *Campylobacter* spp.

Cross-sectional samples of sera were obtained from existing serum banks in Denmark, Finland, France, Italy, Poland, Sweden and The Netherlands. Sera from Finland, Sweden and The Netherlands were subsamples of national studies representative of the general population. Three sets of samples from The Netherlands were included, for successive periods (1995–1996, 1998–2002, 2006–2007). Sera from Denmark were also obtained from an existing collection, sampled in Copenhagen and its peri-urban region. Sera from Poland and Italy were obtained by sampling persons consulting health services for reasons unrelated to gastrointestinal problems. In Romania blood samples were collected prospectively, from people attending district medical services with non-gastrointestinal problems, in the course of the present study (September 2007). As a consequence, the sera from Italy, Romania and Poland may not be representative samples of the general population, because they selected for health problems, and excluded gastroenteric illnesses. Further details on the representativeness of samples have been published [18]. The sampling period and sample sizes are summarized in Table 2.

These sera, sampled from the adult population (aged 18–60 years) in the contributing countries, were analysed with the same ELISA as used for the longitudinal sample, to ensure identical units [17].

In Denmark, Finland, France, Poland, Sweden, and The Netherlands serum banks had been approved by the appropriate ethics committee. Sera from Italy and Romania were left over from blood samples taken for diagnostics, where patients had consented to their use for research purposes. For this reason formal ethics committee approval was deemed unnecessary

Table 1. *Longitudinal characteristics of response to Campylobacter infection*

	Geometric mean	Median	$Q_{0\cdot05}$	$Q_{0\cdot95}$
IgG				
A (U/ml)	10·04	10·42	2·50	34·25
$T_{½}$ (days)	498·52	472·36	60·39	$4\cdot64 \times 10^3$
IgM				
A (U/ml)	2·10	1·85	0·59	12·54
$T_{½}$ (days)	215·28	243·08	24·97	$1\cdot25 \times 10^3$
IgA				
A (U/ml)	1·35	1·31	0·37	5·78
$T_{½}$ (days)	114·11	112·23	40·56	$3\cdot34 \times 10^2$

A, peak titre; $T_{½}$, decay halftime.
$Q_{0\cdot05}$ and $Q_{0\cdot95}$ indicate 0·05 and 0·95 quantiles, respectively.

by the responsible public health institutes. All serum samples were anonymized.

Statistical methods

The serum antibody response to infection consists of a short-term increase (seroconversion) to peak levels, followed by a slow decrease towards baseline [19, 20]. Antibody responses may vary substantially between individual subjects [21, 22], therefore peak levels and decay rates are specified as distributions, representing the variation between individuals in the population. For use in the marginal cross-sectional model, peak titre and decay rate were characterized by parametric distributions: heterogeneity in peak titre as a gamma distribution, heterogeneity in decay rate as an inverse gamma distribution. Correlation between peak level (A) and halftime ($T_{½}$) is weak (0·10, 0·34, −0·003 for IgG, IgM, IgA, respectively), therefore we conveniently assumed A and $T_{½}$ (and A and k) to be independent.

Seroconversions (infections) were assumed to occur at random with a given rate, the incidence of infection. Given the response to infection, the distribution of antibody levels in a cross-sectional (snapshot) sample can be expressed as a function of the incidence: when infection is infrequent, low titres are likely; when infection occurs frequently the probability of finding high antibody levels increases. A parametric model assuming that incident infections occur as a homogeneous time Poisson process, with gamma-distributed antibody peak levels and inverse gamma-distributed antibody decay rates was used to obtain likelihood-based estimates of the seroconversion rate [22].

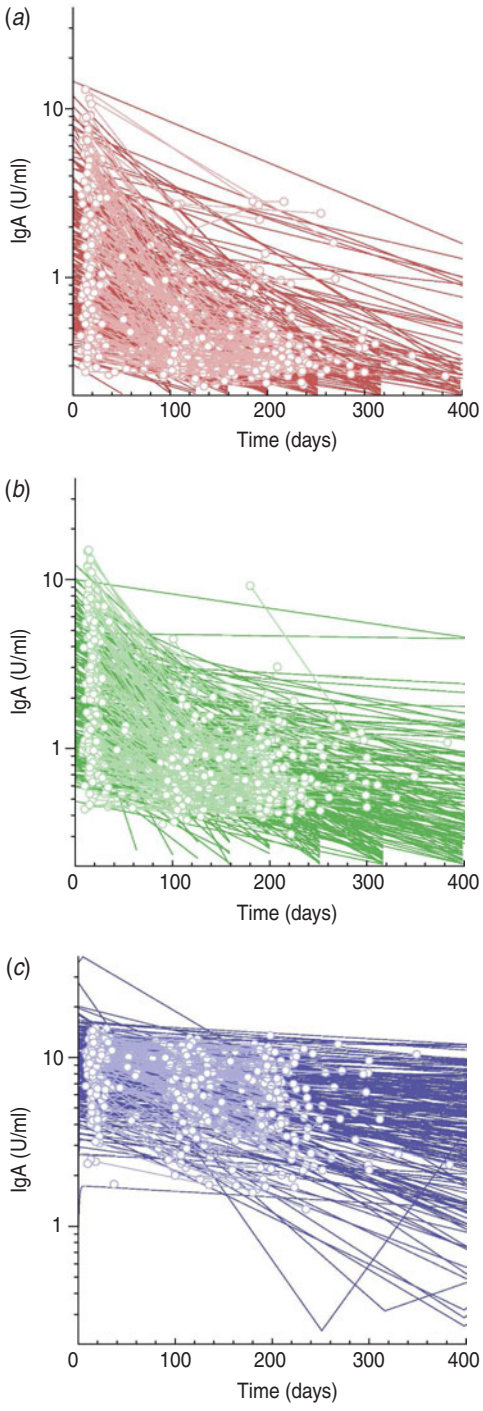

Fig. 1. [*colour online*]. Longitudinal model output: serum antibody responses of (*a*) IgA, (*b*) IgM and (*c*) IgG to *Campylobacter* infection. Observed data (circles) and model responses, for each individual person in the study.

RESULTS

Longitudinal serum antibody responses to *Campylobacter* infection are illustrated in Figure 1. Important characteristics of the serological response to infection are summarized in Table 1.

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Table 2. *Estimated yearly seroconversion rates of* Campylobacter [*maximum likelihood value* $\hat{\gamma}$ *and likelihood-based 95% confidence interval* (*CI*)] *from joint* (*IgG, IgM, IgA*) *antibodies* (*also shown: notification rates as reported by the European Food Safety Agency and ratio of seroconversion and notification rates*)

Country	Sampling period	*N*	$\hat{\gamma}$ (1/year)	95% CI (1/year)	Notification rate $\times 100\,000$ (1/year)	Ratio
The Netherlands	1995–1996	456	0·67	0·60–0·75		
The Netherlands	1998–2002	1108	0·75	0·69–0·81		
The Netherlands	2006–2007	1566	0·71	0·67–0·76	38·6	1839
Finland	2000–2001	499	0·87	0·77–0·98	77·8	1118
Denmark	2006–2007	1801	0·80	0·75–0·85	71·0	1127
France	2003–2004	1010	0·77	0·71–0·84	4·8	16 042
Italy	2003–2004	529	0·96	0·85–1·09	1·1	87 273
Poland	2004	500	0·84	0·74–0·94	0·5	167 000
Romania	2007	510	0·85	0·75–0·95		

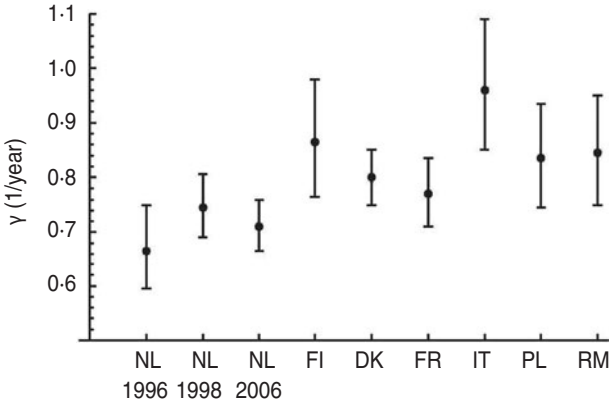

Fig. 2. Seroconversion rates based on combined antibodies (joint IgG, IgM, IgA).

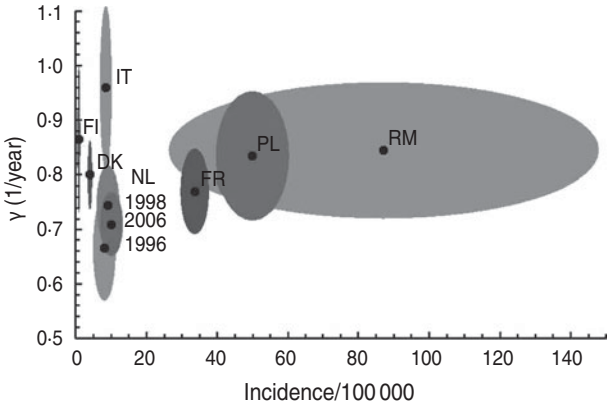

Fig. 3. Comparing seroconversion rates with incidences estimated in returning Swedish travellers (1997–2003) for Finland (FI), Denmark (DK), The Netherlands (NL 1996, 1998, 2006), Italy (IT), France (FR), Poland (PL) and Romania (RM) using data from Ekdahl & Giesecke [27]. Ellipsoids indicate 95% confidence intervals for either estimate.

Given the serum antibody response, cross-sectional (1-point) serum samples from the surveyed countries were translated into seroconversion rates, shown in Table 2.

Incidence rates (maximum likelihood estimates $\hat{\gamma}$) are given in Table 2 as estimated numbers of seroconversions per person per year. Seroconversion rates estimated from IgA, IgM and IgG data were consistent (judged by the likelihood ratio test): estimates in Table 2 are therefore based on joint IgA, IgM and IgG data. Table 2 also gives notification rates reported to the EU in 2007 (no figure reported for Romania).

Figure 2 shows seroconversion rates for all cross-sectional samples. Error bars indicate (95%) predictive intervals, predominantly reflecting differences in sample size between countries. Differences between countries were small with error bars overlapping.

Age profiles of *Campylobacter* seroconversion rates were flat (not shown), showing no marked differences across the range tested (18–60 years). Gender differences could not be detected at any age (likelihood ratio test for incidence estimates in males and females).

Comparing the illness rates in Swedish travellers to the seroconversion rates for the same countries did not show strong correlation (Fig. 3). Countries with high illness rates (Poland, Romania) also had high seroconversion rates, but countries with low illness rates did not have low seroconversion rates. This is especially true for Italy and Finland, with high estimated seroconversion rates and very low reported

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numbers of campylobacteriosis in returning Swedish travellers.

DISCUSSION

A recent estimate of the incidence of culture-confirmed cases of *Campylobacter* infections in The Netherlands (2000–2004) was 36/100 000 (1/year) [23]. A report by the European Food Safety Agency (EFSA) on foodborne illnesses in the EU [4] provides estimated rates of notified cases of campylobacteriosis for several EU countries (see Table 2). These estimates of symptomatic illness rates range from 40 to 70/100 000 (1/year) down to 0·5–5/100 000 (1/year) with the lowest reported numbers in Poland. It should be noted that the reported illness rates for France are based on a sentinel surveillance for *Campylobacter*, where only a small proportion of laboratories submit data and strains, leading to low reported rates of notified illnesses.

The estimated seroconversion rates are several orders of magnitude higher than the notification rates. This discrepancy reflects not only the detection deficit in the surveillance of foodborne diseases [24], but also the often unnoticed fact that enteric infection may remain completely asymptomatic. For *Campylobacter*, this was known from a human challenge study [25] where human volunteers were given different doses of a *C. jejuni* isolate; however, other epidemiological studies have also noted considerable asymptomatic carriage of various enteric bacterial pathogens [9, 26].

Recent prior exposure to specific *Campylobacter* strains may lead to protective immunity as illustrated by illness data collected from Swedish travellers who were exposed abroad to strains they were less likely to have been exposed to at home [27]. Estimated seroconversion rates for *Salmonella* were strongly correlated with illness rates in Swedish travellers [18]. The same is not true for *Campylobacter* (Fig. 3). *Campylobacter* strains in Finland may not differ much from those in Sweden, so that most infections in the Swedish travellers visiting Finland remain asymptomatic, but it remains unclear why so few Swedish travellers return from Italy with campylobacteriosis. In contrast, illness incidence does seem to correlate with prevalence of *Campylobacter* in broiler flocks [28].

Asymptomatic infections may add to the public health burden, as complications and/or sequelae may also result from infections that do not result in acute enteric illness. Most asymptomatic infections would, however, not result in any health effects. Seroincidence therefore is not a good indicator of health burden. Because seroconversion is closely related to exposure (more so than symptomatic illness) seroincidence is a good indicator of infection pressure. The rate of ingesting a dose of *Campylobacter* high enough to cause infection informs about the presence of these pathogens in the food and other environmental compartments.

Whether a seroconverting subject moves on to symptomatic illness may depend on prior experience: an episode of campylobacteriosis may lead to transient protective immunity, possibly not preventing seroconversion, but protecting against symptomatic illness [14]. In immunodeficient subjects this protective response may be impaired. Protection against illness in recently infected subjects may lead to an interesting relationship between infection pressure and illness incidence [29].

The cross-sectional serum samples collected in some of the surveyed countries may not be completely representative of the general population in those countries. Consideration might also be given to data collected in different time-frames (different years, or even different seasons) representing different exposures. The small differences between estimated incidence does not show any indication that such differences in exposure may be present.

A mismatch between longitudinal and cross-sectional units would cause a bias in the seroconversion rate estimates. If the cross-sectional data are measured in smaller units than the longitudinal data (so that the same number indicates a smaller 'true' antibody titre) the incidence is overestimated. If the cross-sectional data are measured in larger units the incidence is underestimated.

Longitudinal data have been collected for symptomatic cases, because only then is symptom onset known. In contrast the cross-sectional data were collected in the general population, from asymptomatic individuals. While we cannot know whether the serum antibody response to symptomatic infection is identical to the response to asymptomatic infection, it is unlikely that they are very different. If the magnitude of the longitudinal (symptomatic) response were higher than that of the cross-sectional (asymptomatic) data, such a difference would be interpreted as a lower incidence. This means that then the estimated incidence would be an underestimate, which is unlikely given the high incidence estimates. The

converse: higher levels in the cross-sectional (asymptomatic) data than in the (symptomatic) longitudinal data would mean that the estimated incidence is biased upwards, but high titres in the cross-sectional sample would then exceed the highest peaks in the longitudinal response patterns (based on the longitudinal observations they would not be expected to ever occur) and the model would simply fail. Since that does not happen it is concluded that there is no reason for concern that asymptomatic responses are very different from the observed symptomatic responses.

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DECLARATION OF INTEREST

None.

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