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REVIEW ARTICLE

Immunity to Campylobacter: its role in risk assessment and epidemiology

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Abstract

Acquired immunity is an important factor in the epidemiology of campylobacteriosis in the developing world, apparently limiting symptomatic infection to children of less than two years. However, also in developed countries the highest incidence is observed in children under five years and the majority of Campylobacter infections are asymptomatic, which may be related to the effects of immunity and/or the ingested doses. Not accounting for immunity in epidemiological studies may lead to biased results due to the misclassification of Campylobacter-exposed but apparently healthy persons as unexposed. In risk assessment studies, health risks may be overestimated when immunity is neglected.

Introduction

Campylobacter jejuni, and its close relative *Campylobacter coli*, are well recognized causes of acute bacterial human enteritis. In the European Community, for the first time in 2005, the reported incidence of campylobacteriosis exceeded that of salmonellosis (Anonymous 2007). Although campylobacteriosis is largely perceived as a foodborne infection, with poultry meat being a common source, there is increasing evidence for other routes of transmission including direct animal contact, the consumption of raw food, and environmental sources (Kapperud et al. 2003; Evers et al. 2008; Studahl and Andersson 2000; Saeed, Harris, and DiGiacomo 1993; Adak et al. 1995; Potter, Kaneene, and Hall 2003; Neimann et al. 2003; Talsma et al. 1999). Human-to-human spread is also observed, albeit at low frequencies (Musher and Musher 2004). Nevertheless, due the ubiquitous presence of Campylobacter in the environment the exact contribution of different exposure routes to the incidence of illness remains difficult to quantify.

The clinical symptoms of campylobacteriosis are variable and may include watery diarrhea, bloody diarrhea, fever, abdominal pain and vomiting. The severity of illness varies from mild malaise to dehydration sufficient to require hospitalization. Infrequently systemic infections occur, as well as chronic illness and sequelae such as polyarthropathies (in particular reactive arthritis, ReA (Hill Gaston and Lillicrap 2003; Pope et al. 2007; Doorduyn et al. 2007) or neuropathies (in particular Guillain-Barré syndrome, GBS (Hughes and Cornblath 2005)). Furthermore, acute Campylobacter-enteritis may be linked to the induction of irritable

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bowel syndrome (Cumberland et al. 2003; Marshall et al. 2006; Gomez-Escudero, Schmulson-Wasserman, and Valdovinos-Diaz 2003) and, possibly, inflammatory bowel disease (Newman and Lambert 1980; Wassenaar, Kist, and de Jong 2007; Karlinger et al. 2000). The basis of the diversity in the manifestation of campylobacteriosis is not known but may be caused by differences in the pathogenicity of Campylobacter strains (which may or may not be dose dependent) and/or differences in the host susceptibility (innate or acquired) to the infection.

There is considerable evidence for diversity among Campylobacter strains and this may engender differences in disease presentation. For example, the presence of *C. jejuni* strains carrying a sialyltransferase gene (cst-II) appears to be associated with the development of GBS (Yuki and Koga 2006). However, evidence for strain-specific differences in the presentation of gastroenteritis are less obvious although this may reflect the lack of appropriate animal models and the absence of clear indicators of strain virulence. Thus far, the complete genome sequences have been determined for eight *C. jejuni* strains isolated from different sources. These data indicate considerable genetic variation, in particular in regions encoding surface exposed antigens such as the capsule, lipooligosaccharide (LOS) and flagellin (Parkhill et al. 2000). However, no specific genetic markers of virulence have been identified. The population genetics of *C. jejuni* is complex but a study using multilocus sequence typing (MLST) of a large set of patient isolates from the UK, (McCarthy et al. 2007) concluded that "there is no evidence for substantial virulence differences between the major *C. jejuni* clonal complexes." However, MLST is undertaken on house-keeping genes, which tend to be conserved and, therefore, their clonal spread may not coincide with that of virulence genes. Given the significant genomic and phenotypic variation in Campylobacter strains, and the well-recorded differences in putative virulence traits (Bacon et al. 2000), and in vitro invasive behavior (Fearnley et al. 2008), it seems likely that Campylobacter diversity influences disease presentation or symptoms.

The role of host susceptibility in the manifestation of campylobacteriosis is also poorly understood. The establishment of intestinal illness is likely to be determined by a chain of conditional events. Probable key factors are the local microbiota in the intestine, the status of protective processes like the physical barriers at the gastrointestinal mucosal surfaces, and the non-specific host defense. When such inherent protective processes are overcome, specific acquired host immunity becomes critically important for the outcome of infection. This immunity may limit the duration of the disease and may provide protection against subsequent infection and/or disease. The presence of high or variable numbers of immune individuals in a population clearly has the potential to impact on the epidemiology and risk assessment of campylobacteriosis. Variable immunity in the population may influence surveillance data, lead to misinterpretation of trends and to biased predictions of risks, which often form the basis of novel prevention and control strategies.

The aim of this review is to present an overview of the current knowledge of the epidemiology and risk assessment of Campylobacter and to discuss in particular the possible effects of immunity in the community on the outcome of such analyses. A short overview of the immune response against Campylobacter at the molecular, cellular and individual patient level will first be presented. In the remaining, major part of this review the consequences of acquired immunity for the response to Campylobacter at the population level will be discussed and gaps in knowledge that limit the interpretation of epidemiological and risk assessment studies will be identified.

Immunity to Campylobacter at the molecular and cellular level

Immunity against Campylobacter can theoretically be achieved via non-specific host defense mechanisms (innate immunity) as well as via a pathogen-specific immune response (adaptive immunity). Although the innate immune response is increasingly recognized as being an important director of any adaptive response, the interplay between both arms of the immune system has rarely been investigated for Campylobacter. Therefore, both responses will be discussed separately.

Innate immunity

The immediate and initial responses to bacterial enteric pathogens involve recognition of conserved microbial motifs by innate immune receptors present on mucosal epithelial and immune cells. Most studied are the Toll-like receptors (TLR) and Nod-like receptors. Activation of these receptors results in the production of an array of antimicrobial peptides, proinflammatory cytokines and chemokines. In vitro evidence indicates that *C. jejuni* stimulates the production of beta-defensins that have a direct bactericidal effect through disruption of the cell wall integrity (Zilbauer et al. 2005). Campylobacter also upregulates those chemokines and cytokines essential for proinflammatory responses and/or phagocyte recruitment (Hu and Hickey 2005; MacCallum et al. 2006; Hu et al. 2006; Borrmann et al. 2007; Watson and Galan 2005; Johanesen and Dwinell 2006; Bakhiet et al. 2004; Al-Salloom et al. 2003; Hickey et al. 2000; Chen et al. 2006b). Campylobacter is ingested and killed by professional phagocytes (Walan et al. 1992) to a variable extent (Wassenaar et al. 1997; Kiehlbauch et al. 1985).

For example, the capacity for bacterial killing can vary between cells derived from different hosts (Wassenaar et al. 1997) and certain *C. jejuni* strains appear to rapidly replicate within human monocytes (Hickey, Majam, and Guerry 2005). Nevertheless, in general, it is believed that the uptake and killing of Campylobacter by phagocytes limits the duration and systemic spread of the infection.

The bacterial factors that induce the innate responses include LOS (Hu et al. 2006), cytolethal distending toxin (CDT) (Hickey et al. 2000) and bacterial DNA (Dalpke et al. 2006). In contrast to most other enteric pathogens, Campylobacter flagellin is unable to activate TLR5 (Watson and Galan 2005; Johanesen and Dwinell 2006; Andersen-Nissen et al. 2005). Whether this aids the bacterium in delaying or evading the innate host response awaits further investigation. Furthermore, the existence of possible differences between Campylobacter isolates in their ability to activate or subvert the innate immune response has not been thoroughly investigated. Considering the variable expression of bacterial factors like LOS and CDT among strains, it seems likely that such differences exist and influence the host-pathogen interaction and thus the outcome of disease. Similarly, it can be expected that variations in the architecture of the innate immune systems between individuals will influence the efficacy of the innate response and thus impact on risk assessment. The role of genetic factors in the susceptibility to Campylobacter has recently been reviewed (Janssen et al. 2008).

Adaptive immunity

The bacterium-specific immune response is generally considered to contribute to the limitation of Campylobacter disease and to lead to the development of protective immunity. The importance of the adaptive immune system is illustrated by the reports of persistent and severe Campylobacter infections in agammaglobulinemic (Freeman and Holland 2007) and other immunocompromized individuals. In patients with HIV infection, serum antibody responses to *C. jejuni* are markedly impaired compared to immunocompetent controls (Perlman et al. 1988). Similarly, systemic disease, such as bacteremia, due to *Campylobacter* species is mainly seen in immunocompromized individuals (Pigrau et al. 1997; Schonheyder, Sogaard, and Frederiksen 1995; Melamed et al. 1988; Melamed et al. 1983). Certainly, antibodies directed against Campylobacter promote bacterial agglutination and complement activation (Jones, Eldridge, and Dale 1980; Jones et al. 1981), have bactericidal activity (Blaser, Smith, and Kohler 1985; Johnson et al. 1984; Pennie et al. 1986) and may display opsonic activity promoting uptake and killing by professional phagocytes (Walan et al. 1992; Bernatowska et al. 1989; Bar, Glenn-Calvo, and Krausse 1991). In contrast, the role of cell-mediated immunity in protection against campylobacteriosis is largely circumstantial. Serious and recurrent Campylobacter infections in patients with HIV infection or AIDS suggest that CD4+ T-cells are an important factor in recovery from infection. However, no comprehensive studies of T-cell-mediated responses during campylobacteriosis have yet been undertaken (Baqar et al. 1995, Baqar et al. 2001; Van Rhijn et al. 2003).

Campylobacter antigens

Analysis of the host immune status towards Campylobacter requires identification of the bacterial antigens that are recognized by the immune system. Early studies using rabbit polyclonal antisera demonstrated antigenic diversity between strains that was considered useable for typing purposes. Throughout the 1980s multiple typing schemes were developed but by 1990 two major schemes prevailed based on "heat-labile" (HL) and "heat-stable" (HS) antigens and termed the Lior and Penner schemes respectively. Derivatives of Penner scheme have also been subsequently developed. There has been considerable debate about the molecular basis of these schemes and the serotypes derived from them. Both schemes depend on complex and polyclonal reactions which are to a large extent dependant on the methodologies utilized. Serotyping has not yet been found reproducible by either monoclonal antibodies or genotyping methods so definitive conclusions of the responsible antigens are not possible.

Initially the HS antigen of the Penner scheme was thought to be based on the Campylobacter LPS. However, in 1996, Chart et al. (1996) using a modified Penner serotyping scheme concluded that the HS antigen was capsular in nature. Nevertheless, Moran and Penner (1999) in reviewing the literature have proposed that the serotyping antigen of the Penner scheme is based on a Campylobacter "glycolipid analogous to the LPS of enteric bacteria and the LOS of non-enteric mucosal pathogens, but with significant differences from each of them to be considered as a different, but related glycolipid."

The molecular basis of the Lior scheme is just as contentious. As early as 1985 the Campylobacter flagellar protein was considered an essential component of the HL typing scheme (Wenman et al. 1985). This was supported by evidence from monoclonal antibody studies (Newell 1986a; Newell 1986b) which clearly indicate that at least for some serotypes the flagellin is serotype specific but also that by immunogold staining, with these monoclonals, the serotypic antigens are not only confined to the flagella but may also be surface expressed. The surface expression of the HL antigens was further investigated by Alm et al. (1991), who demonstrated

using defined mutants that the product of the flaA gene is not the only serotype-specific HL antigen expressed on some C. jejuni strains. Thus current evidence indicates that HL serotyping is complex and is dependent on a number of surface exposed antigens of which flagellin is one.

Serological studies of patients and volunteers infected with Campylobacter have indicated an array of immunogenic bacterial components (Blaser, Hopkins, and Vasil 1984; Mills and Bradbury 1984; Panigrahi et al. 1992; Cordwell et al. 2008; Winsor, Mathewson, and DuPont 1986; Cawthraw et al. 2000; Cawthraw et al. 2002; Nachamkin and Hart 1985). Major antigens include flagellin (Martin et al. 1989; Wenman et al. 1985; Nachamkin and Yang 1989; Nachamkin and Yang 1992), the major outer membrane protein (MOMP) (Mills and Bradbury 1984; Nachamkin and Hart 1985), and glycoantigens (capsule, LOS) (Preston and Penner 1989; Nachamkin and Hart 1985; Prendergast et al. 2004). These studies show that often, elicited antibodies display little cross-reactivity between different Campylobacter isolates, indicating considerable antigenic variation. This phenomenon is frequently observed for many immunodominant antigens of mucosal pathogens and is often caused by variation in surface-exposed epitopes induced by selective pressures from the immune system. This epitopic variation has a major impact on both serodiagnostics and the development of cross-protective immunity. For example, epitope mapping with polyclonal sera and monoclonal antibodies indicates the presence of both variable and conserved flagellin epitopes. In silico epitope analysis suggests that the variable domains are surface exposed, while the cross-protective epitopes can be cryptic and thus may elicit poorly functional antibodies in the host. This is further complicated by the antigenic preparations used, which may comprise fully (as in western blots), partly (as in acid extracts) or un-folded (as in whole isolated flagella) protein. The repertoire of flagellin diversity may be even further enhanced by variable glycosylation of the protein (Logan and Trust 1983; McNally et al. 2006; Thibault et al. 2001; Logan 2006; Alm et al. 1992). The antigenic variation in flagellin has been demonstrated with multiple studies using polyclonal and monoclonal antibodies. Obviously, only antibodies that react with conserved surface-exposed regions of the flagellins may provide effective immunity against heterologous Campylobacter strains. The same holds true for antibodies directed against LOS. LOS is immunogenic *in vivo* but antigenically highly diverse (Prendergast et al. 2004; Mills et al. 1992; Karlyshev et al. 2005). The outer core of LOS of some strains resembles host cell gangliosides. It is not yet clear whether this antigen mimicry may limit a powerful immune response or lead to more invasive behavior, leading to immunopathology including the development of autoimmune sequelae, like GBS.

Antibodies against MOMP may be directed against both linear and conformational epitopes. During the natural infection antibodies appear primarily directed against the native porin (Huang, Sahin, and Zhang 2007). These epitopes are mostly conformational as determined by immunoprecipitation and ELISA, and, like the surface-exposed flagellin regions, also generally poorly conserved among strains. This implies that serological assays based on the detection of antibodies against the denatured regions of MOMP, such as standard Western blotting techniques, may indicate exposure but have no predictive value as to the immunity of an individual towards Campylobacter.

Apart from the major surface antigens described above, a number of additional bacterial components have been demonstrated to be immunogenic, including several so-called PEB antigens (Blaser, Hopkins, and Vasil 1984; Logan and Trust 1983; Dubreuil et al. 1990), the fibronectin binding protein CadF (Cordwell et al. 2008), CDT (Abuoun et al. 2005), OMP18 (Cordwell et al. 2008) and a 76-kDa iron-regulated protein (Schwartz et al. 1994). The PEB proteins are amino-acid transporters that are primarily attached to the inner membrane of the bacterium and thus may have only limited exposure at the bacterial cell surface. The conserved CadF protein is surface exposed and may be a valuable diagnostic target. However, to our knowledge, CadF-based protective immunity towards heterologous Campylobacter strains has thus far not been reported, which limits the use of this antigen in risk assessment studies.

The mechanisms by which Campylobacter-specific antibodies elicit a therapeutic and/or protective effect appear to involve complement-mediated killing of the bacteria (Johnson et al. 1984; Blaser, Smith, and Kohler 1985; Pennie et al. 1986; Bernatowska et al. 1989) as well as opsonization and subsequent killing of the Campylobacter by professional phagocytes (Walan et al. 1992; Bar, Glenn-Calvo, and Krausse 1991; Wassenaar et al. 1997; Kiehlbauch et al. 1985). In the intestine, Campylobacter-specific sIgA antibodies may also promote bacterial agglutination, which limits colonization capacity and enhances bacterial elimination and excretion. *In vitro* the sensitivity of Campylobacter to human serum appears to vary with the surface composition of the bacterial strain as capsule and sialylation of LOS may interfere with complement-mediated killing (Guerry et al. 2000; Bacon et al. 2001). Thus the functionality of anti-Campylobacter antibodies may vary significantly among different strains.

The strong antigenic diversity of Campylobacter and the immune-evasion strategies described above are likely to limit the generation of (cross-) protective immunity.

This antigen variation may explain why Campylobacter appears to elicit a humoral immune response that primarily affords protection against challenge with homologous but not, or only to a limited extent, with heterologous strains. The results of the above studies of the antigen repertoire of Campylobacter suggest that only repeated exposure to different strains may lead to sufficient immunity to provide at least partial protection against the huge diversity of strains that are present in the environment.

Immunity to Campylobacter at the individual level

Sero-diagnosis of uncomplicated Campylobacter infection

In addition to data based on culture-confirmed cases, serological data are frequently used as an indicator of exposure to Campylobacter. For example, sero-diagnostic tools are essential in studies on post-infectious complications (GBS, ReA), as isolation of *C. jejuni* after cessation of diarrhea is rarely successful (Svedhem and Kaijser 1980). Several different methods are being used to detect *C. jejuni*-specific antibodies, including complement-fixation assays (CFA), immunoblotting, and ELISAs. Compared to CFA, the sensitivity of ELISA-based assays is superior and therefore the use of CFA for determining a recent infection with Campylobacter should be discouraged. However, most of these serological assays have not been standardized with regard to antigens used and/or the criteria for defining cut-off values.

Most ELISAs for the detection of Campylobacter antibodies utilize crude antigen preparations derived from either acid-glycine extracts or whole cell sonicates. The assays are often validated using cohorts of patients with uncomplicated Campylobacter enteritis. Only very few assays are validated further with serum samples from patients infected with potentially cross-reacting micro-organisms such as Yersinia, Helicobacter and Legionella (Strid et al. 2001; Ang et al. 2007b). ELISA assays based on acid-glycine extracted antigens antigens have a sensitivity of 60–90%, depending on the age of the patient and on the time of sampling relative to the onset of symptoms. One major advantage is that antibodies of the IgA, IgM, and IgG isotypes, alone or in combination, can be detected in serum from patients with culture-confirmed Campylobacter infection (Ang et al. 2007a; Strid et al. 2001). Using proper cut-off values, it is possible to detect a recent Campylobacter-infection based on an elevated value of either IgA, IgM, or IgG. This is in contrast to widely reported criteria using sero-positivity in two or more immunoglobulin classes.

In order to obtain more detailed data on the host humoral immune responses against *C. jejuni*, ELISAs using immunodominant (conserved) antigens can be applied, such as those present in flagellin acid extracts (Newell and Nachamkin 1992). However, as mentioned above, the use of this antigen is hampered by the fact that the surface-exposed epitopes, as well as those on the flagellar hook protein FlgE2, are HL serotype-specific (Newell and Nachamkin 1992; Power, Alm, and Trust 1992). Alternative antigens with potentially better specificities and sensitivities include the PEB antigens and the putative peptidoglycan-associated protein Cj0113 (Pei, Ellison, and Blaser 1991; Cordwell et al. 2008). Recently, with the combined use of the recombinant *C. jejuni* antigens P18 (a homolog of the peptidoglycan-associated lipoprotein Pal of *E. coli*) and the ATP/GTP-binding protein P39 as antigens, a specificity and sensitivity of detecting IgA antibodies of respectively 99.0% and 91.9% was achieved for sera from patients with uncomplicated Campylobacter infections (Schmidt-Ott et al. 2005).

Sero-diagnosis in relation to post-infectious complications

Many serological studies have tried to assess the frequency of Campylobacter infections as triggers of post-infectious complications, such as reactive arthritis (ReA) and GBS. Unfortunately, results are often unreliable due to the use of ELISA systems that, at best, have only been validated on cohorts of campylobacteriosis patients without post-infectious sequelae. Only three studies used serum samples from Campylobacter culture-positive ReA or GBS patients (Strid et al. 2001; Koga et al. 1998; Ang et al. 2007a), and these studies indicated that the sensitivity of the ELISA for detecting a recent Campylobacter infection, in patients with immune-mediated disease, was 95–100% depending on the criteria for a recent infection.

Reports indicate that the proportion of Campylobacter culture-positive patients reporting joint symptoms (ReA) ranges from 2–30%. Data on the prevalence of Campylobacter as the trigger for arthropathies is lacking as very few serological studies have been performed in cohorts of patients with early arthritis. In two independent Scandinavian studies, between 24–63% of patients with ReA had a preceding Campylobacter infection (Mäki-Ikola et al. 1991; Soderlin et al. 2003). However, in a German study, using a different ELISA system to detect Campylobacter antibodies, none of the ReA patients tested showed evidence of a recent Campylobacter infection (Fendler et al. 2001).

Similar issues are evident in assessing the relationship between Campylobacter infection and GBS. Nevertheless, depending on the geographical region, 15–80% of GBS patients have a serologically documented recent infection with Campylobacter (Hao et al. 1998; Guarino, Casmiro, and D'Alessandro 1998; Nachamkin

et al. 2007; Ho et al. 1995; Van Koningsveld et al. 2001). Interestingly the application of improved ELISA formats, using recombinant Campylobacter antigens, indicated that a surprisingly high percentage of GBS patients (80%) had serological evidence of a preceding *C. jejuni* infection compared to healthy blood donors (3.5%) (Schmidt-Ott et al. 2006).

Kinetics and efficacy of the natural immune response

In human volunteers experimentally infected with *C. jejuni*, both mucosal and systemic (serum) humoral immune responses were detectable (Black et al. 1992). In naturally infected patients anti-Campylobacter-sIgA (Winsor, Mathewson, and DuPont 1986) is detectable in feces, while in serum, a rise in IgA and IgM antibodies is detectable by one week post-infection, and IgG antibodies peak a few weeks later (Mascart-Lemone et al. 1987; Rautelin and Kosunen 1987). IgA and IgM antibodies disappear after two to three months, while IgG antibody titers remain present for much longer. Notably, IgA antibodies directed against Campylobacter can also be detected in breast milk (Ruiz-Palacios et al. 1990; Torres and Cruz 1993; Renom et al. 1992; Nachamkin et al. 1994). These antibodies may contribute to the limited incidence of campylobacteriosis during the first 6 months of infancy (see below).

Obviously, the presence of antibodies in sera and mucosal secretions provides no clue as to the therapeutic efficacy of these antibodies or of their possible protective effectiveness against re-infection. Yet, several observations suggest that the humoral immune response contributes to resolving Campylobacter infection (Black et al. 1992; Cawthraw et al. 2002). There is a temporal association between the rise in antibody titers with the decline in clinical symptoms and, as previously indicated, Campylobacter infection may persist in immunocompromized individuals (Johnson et al. 1984; Perlman et al. 1988; Pigrau et al. 1997; Molina et al. 1995). Nevertheless, evidence of the impact of immunity on the outcome of infection is sparse and is largely based on the analysis of the limited experimental infections in human volunteers. Black et al. (1988) performed a series of experiments whereby American adults were exposed to different doses of two clinical isolates of *C. jejuni*. For one strain (A3249), the probability of infection increased with dose (8×10^2 to 1×10^8) and 15% of the individuals became ill. The other strain (81–176) was only given in doses higher than 10^6 cfu and in this case, all volunteers became colonized and approximately half of them fell ill with no apparent dose-dependent probability. A remarkable observation in this dataset is that, even at doses of approximately 10^6 cfu, only 80% of the volunteers developed positive stool cultures for strain A3249. This indicates that either the culture methods used did not allow isolation of the pathogen from the stools of these persons or, more likely, that some hosts were particularly resistant. It seems likely that this resistance was due to pre-existing immunity. In contrast to human volunteer studies for other pathogens, these individuals were not selected on the basis of absence of measurable antibody titers. Indeed, those volunteers, who did not become infected, had a higher pre-existing IgA level in their serum than those who did. Coincidentally during the study period the serum IgA antibody levels only increased in those infected volunteers who also became ill. Similar differences were not detected for serum IgM or IgG antibodies. Additional data from a later experiment, published only on the Internet (Tribble 1998), indicated that all 5 volunteers when pre-selected for sero-negative status, developed positive stool cultures after receiving a dose of 10^5 cfu of *C. jejuni* strain 81–176 of (the lowest dose tested). These results supported the assumption that protective immunity reduced the risk of infection and illness in the original experiment.

This assumption is also supported by the evidence that, upon rechallenge after 28 days with high doses of the homologous strains, none of those volunteers who had previously developed illness, became ill again; even though one of the two challenge strains (81–176) was able to colonize the majority of volunteers. In contrast, all previously unexposed controls were colonized by both strains, and 50% of those exposed to strain 81–176 developed illness. Interestingly, after clearance of the infection, IgG antibody titers remained at an elevated level for longer time periods in the rechallenged individuals.

The role of such immune responses in the protection against natural challenge particularly from heterologous strains is debatable. Certainly repeated infections may occur in some individuals. For example reinfection with *C. jejuni* of different serotypes occurs. Karmali et al. (1981) and Baqar et al. (2001) described the development of severe campylobacteriosis in a patient despite the presence of pre-existing humoral immunity and cellular recognition of *C. jejuni* antigens of the strain causing the illness as well as of the standard strain 81–176. Nevertheless, collectively, these results are consistent with the hypothesis that Campylobacter infection primarily elicits immunity against homologous but not heterologous strains.

Animal studies

Animal models have been extensively used to study immunity to enteric infections. Most laboratory animal species can be experimentally colonized with campylobacters but the consistent development of disease is largely confined to the higher primates and possibly to piglets.

Work with non-human primates is fraught with difficulties but recently, Jones *et al.* (2006) described the susceptibility of New World monkeys (*Aotus nancymae*) to Campylobacter. All animals were infected at challenge doses ranging from 8×10^8 to 5×10^{12} cfu, and severity of diarrhea increased with dose. Strong serum IgG and IgA and fecal IgA antibody responses, were observed and upon rechallenge to the homologous strain (56 days after initial exposure), a significantly reduced proportion of animals developed diarrhea, even though all animals were infected.

Rodent models are particularly important for the modeling of immune responses but only immune-deficient or artificial challenge routes generate disease symptoms. For example, IL-10-deficient mice develop intestinal pathological lesions similar to those seen in diseased humans (Mansfield et al. 2007) while BALB/c mice are reported to show high mortality rates, when exposed intra-nasally to Campylobacter in doses above 10^9 cfu. Such models can be used to investigate acquired immune protection and when previously exposed to sub-lethal doses, or when immunized with inactivated whole-cell vaccines, significant protection was observed in mice against an intranasal challenge dose of 4×10^9 cfu (Baqar et al. 1996).

Overall, the results from the few experimental animal studies available supports the human volunteer studies that the immune system can protect against clinical disease but not necessarily against colonization.

Immunity to Campylobacter at the population level

The epidemiology of campylobacteriosis is complicated but provides considerable circumstantial evidence of the development of protective immunity against campylobacteriosis. Epidemiological investigations of use in this context include comparison of disease in developing and industrialized countries, age-related studies, investigation of travelers/migrants, outbreak studies, rural compared with urban populations, occupationally exposed individuals, asymptomatic infection, serosurveillance, and conflicting results from epidemiological and risk assessment studies.

Comparison of the characteristics of the disease in developing and industrialized countries

Campylobacteriosis is an important disease in both industrialized and developing countries, though with different characteristics. In industrialized countries, the average number of Campylobacter infections is less than one per lifetime (Blaser 1997), and disease principally affects young adults. Widespread immunity, among adults in such regions of the world, is considered to be absent. The laboratory confirmed campylobacteriosis incidence in the population varies from 22/100,000 in the US to 400/100,000 in New-Zealand (Samuel et al. 2004; Baker et al. 2006). In contrast, in developing countries the annual incidence of campylobacteriosis in the first two years of life can be up to 2 episodes per child (Rao et al. 2001) but disease thereafter is rare (Calva et al. 1988).

In industrialized countries Campylobacter infections can result in severe disease with diarrhea, abdominal cramps, fever, and bloody stools (Blaser 1997). About 10% of laboratory-confirmed cases are hospitalized (Helms, Simonsen, and Molbak 2006). In contrast, in developing countries the clinical symptom associated with campylobacteriosis, is primarily watery diarrhea (Rao et al. 2001; Bhadra et al. 1992; Echeverria et al. 1989) and occurs predominantly in infants with symptom-producing infections inversely related to age. For example, the illness-to-infection ratio, which in infants younger than 6 months is about 1:2, is negatively associated with age (Rao et al. 2001; Calva et al. 1988).

These studies provide evidence of protection against clinical disease in older groups from such non-industrialized regions. This finding is confirmed by several case-control studies on the causes of diarrheal illness in children. Hasan et al. (2006) found that in children of less than 5 months of age, there was a higher Campylobacter isolation rate in diarrheic stools (9.7% vs. 3.9% in controls). However, in the cohort groups of up to 2 and 5 years of age there was no statistical difference in the isolation rates between cases and controls. There may even have been an overestimation of the Campylobacter contribution to the cases as, in up to 50% of cases, co-infection with other enteric pathogenesis likely (Georges-Courbot et al. 1990).

The conclusions of epidemiological studies in the developing world are not always consistent. One population-based survey on children in Guinea-Bissau reported that *Campylobacter* spp. is more prevalent in stools from controls than from cases of diarrhea (Molbak et al. 1994). On the other hand Jain et al. (2005) isolated Campylobacter from only 0.6% of healthy controls in a community-based study of diarrheal illness in rural northern India compared with 13.5% of cases.

A further interesting difference between the characteristics of campylobacteriosis in geographical regions is that individuals in the developing world tend to be infected with multiple strains. This is not due to strain differences, because individuals traveling from industrialized to developing regions experience the same symptoms of campylobacteriosis abroad as they do at home (Walz et al. 2001). There have been few such comparisons but Sjögren, Ruiz-Palacios, and Kaijser (1989) compared patients from Sweden and Mexico. Unlike Swedish patients, Mexican patients, with or without

diarrhea, were generally infected with multiple strains and frequently re-infected without symptoms with new strains, of different HS and HL serotypes.

In summary the observed differences in the epidemiology and presentation of campylobacteriosis between developing and industrialized countries is explainable by a frequent and multiple exposures at a very young age to the wide range of Campylobacter strains present in the endemic environment of developing countries. This exposure generates cross-protective host immunity, which protects from subsequent infection and illness later in life. This immunity is associated with raised specific serum antibodies, but does not protect against asymptomatic re-infection (Taylor et al. 1988). Evidence to be presented indicates that similar mechanisms occur in the developed world, but at a generally lower level of Campylobacter exposure and leading to an extension of the age-range during which Campylobacter infection causes symptoms.

Age-related studies

If protective immunity is acquired over time with repeated exposure, then resistance to infection should correlate directly with increasing age. Indeed, a peak in campylobacteriosis in infancy is consistently observed in both developed and developing countries and even in infant travelers returning from the tropics (Ekdahl and Andersson 2004). This is unsurprising given, as Newburg (2005) has beautifully described, "the infant is born with an immature and naïve acquired immune system, a gut devoid of micro-flora, a stomach less able to exclude pathogens, a mode of locomotion that results in intimate contact with the dirtiest and most contaminated part of our environment and a propensity to explore the environment orally."

Susceptibility in early infancy may be reduced by the acquisition of passive immunity from milk and/or placentally transferred immunity from immune mothers. Certainly, breastfeeding, in countries like Mexico where exposure to Campylobacter is common, can reduce infection rates significantly (Nachamkin et al. 1994; Ruiz-Palacios et al. 1990). Breastfeeding also protected children from HIV-infected women in Botswana where mortality is associated with a range of pathogens including *C. jejuni* (Shapiro et al. 2007). Whether this protection is dependent on specific passive immunity is unclear as the presence of non-specific, innate protective factors directed against bacterial agents is well recognized in milk (Newburg 2005). In addition, some milk glycans have a specific receptor activity against certain *C. jejuni* surface lectins (Morrow et al. 2005). However, the importance of this to campylobacteriosis may be confounded by the protective activity of IgA antibodies to *C. jejuni* flagellin in breast milk (Nachamkin et al. 1994).

Illness rates quickly fall with age from >1 year to 5 years, to achieve either a baseline, or insignificant levels, in developed and developing countries, respectively. This is consistent with the acquisition of immune protection following repeated exposure. However, the analysis of prevalence of infection against age in the developed world also indicates a secondary peak at 15–25 years, (Van Pelt et al. 2003). One explanation of this peak of infection in young adults is increasing exposure as a result of poor hygiene practices when moving out of the family home or when associated with childcare. Certainly there is some evidence that young adults engage in more risky eating behaviors (Byrd-Bredbenner et al. 2008). An alternative explanation would be that young adults are exposed to new Campylobacter types because they move to new environments. Regional differences may also be observable within countries. For example, Manaseki, Hawker, and Ali (2004) demonstrated that in the Asian population in Birmingham, UK, the age-pattern follows that commonly observed in developing countries, such that compared to the non-Asian population, a higher incidence rate was observed in children under 10 years of age, but lower rates were observed at higher ages. Also, the secondary peak expected of early adulthood in a developed country was absent in these Asians.

Age-related differences are also apparent at the other end of the spectrum. Recent studies using the Scottish surveillance data indicate that a lower prevalence of infection in adults (aged 20–60) is associated with a reduced occurrence of the more common HS serotypes (Miller et al. 2005). Such observations are consistent with the development of protective immunity against the more prevalent serotypes as a consequence of repeated exposures to multiple strains over time.

Investigation of travelers/migrants

It is generally considered that travel from industrialized countries to foreign, especially underdeveloped, countries is associated with a significant risk of traveler's diarrhea. This is due to a higher environmental burden, poorer standards of food preparation hygiene and possibly the presence of "new" Campylobacter types. The number of travelers suffering enteritis on return home, varies with both the countries of origin and of travel, but Campylobacter is a frequent cause (Mattila et al. 1992; Ekdahl and Giesecke 2004), particularly in Southeast Asia (Riddle et al. 2006; Walz et al. 2001). As mentioned previously, the symptoms of campylobacteriosis experienced have the same spectrum regardless of whether the infection was acquired abroad or at home suggesting that strain virulence properties are not geographically distinct.

Susceptibility to infection is related to the duration of stay in a foreign land. A comparison the incidence

of diarrhea in Nepal, in expatriate residents or tourists, indicated that protection from infection linearly correlated with length of duration of residence (Hoge et al. 1996). Similarly, among U.S. expatriates living in Thailand, Campylobacter was isolated more frequently from diarrheic stools if the patients had lived there less than 1 year (Gaudio et al. 1996). Moreover, Walz et al. (2001) found that those U.S. military volunteers, who had low pre-travel anti-*C. jejuni* titers, before deployment to Thailand, were more likely to have had diarrhea during their tour of duty compared with those with high pre-travel titers.

Outbreak studies

Most cases of campylobacteriosis are sporadic. However, outbreaks, sometimes with many hundreds of cases, can occur. Unpasteurized or inadequately pasteurized milk, is a frequent source of such outbreaks and is presumed, and sometimes proven to be, fecally contaminated by cattle (Fahey et al. 1995; Evans et al. 1996; Peterson 2003; Schildt, Savolainen, and Hanninen 2006; Teunis et al. 2005). A consistent finding is that people exposed to the same outbreak strain have variable symptoms from asymptomatic to severe enteritis (Porter and Reid 1980). This is supported by serological studies that demonstrate increased levels of anti-Campylobacter antibodies, suggestive of asymptomatic infections and thus higher attack rates than based on the occurrence of symptomatic infection. Furthermore a high prevalence of antibodies and a low attack rate of illness correlated with the habitual consumption of raw milk (Jones, Robinson, and Eldridge 1981). Thus Campylobacter-related raw milk outbreaks offer an opportunity to study both dose-response effects, and protective immunity as indicated by serology. In an outbreak among 31 students, Blaser, Sazie, and Williams (1987) found that the quantity of raw milk consumed was directly related to the occurrence and severity of illness. Acutely infected and ill students, but not unexposed persons, showed significant rises in *C. jejuni*-specific immunoglobulins. In contrast, those persons with a chronic exposure to raw milk and who did not develop symptoms, showed elevated antibody levels to *C. jejuni* in serum samples. These findings indicate that elevated levels of *C. jejuni*-specific serum antibodies are related to immunity against symptomatic infection. In a raw milk outbreak of campylobacteriosis involving 100 young children (9–12 years of age) in the Netherlands, a clear relationship was found between the amount of milk ingested and the occurrence, severity and duration of disease (Teunis et al. 2005). Independent from the dose, an added effect of age was also shown. The youngest children had more severe symptoms, were ill for a longer time and more often visited their doctor. In a comparable outbreak in the UK (Evans et al. 1996) the dose correlated with the occurrence of illness but not with its duration and severity.

Small but continuous outbreaks can also provide valuable information. (Schildt, Savolainen, and Hanninen 2006) reported an outbreak of illness of 5 months duration in a farming family with recurrent infections and periods of diarrhea in some of the cases. The same strain of Campylobacter was found in the cases as well as in the unpasteurized cows' milk of which large quantities were consumed before and during the outbreak by all of the patients. This study raises some interesting questions. Unfortunately, serological data were not available but, it seems likely that, in this family, with the repeated consumption of potentially contaminated milk at least some degree of protective immunity should have developed to prevent disease symptoms. One explanation of this outbreak would be that such protection is never complete and can be overcome by larger challenge doses.

Rural compared with urban populations

In many countries, clear differences are observed between the incidence of campylobacteriosis in rural and urban areas, but the patterns are complicated and sometimes conflicting. Such observations date back to the 1980s when, in Northern and Central Ontario, Canada, a higher incidence of disease was observed among farm residents than among rural, non-farm, or urban residents (Thompson, Cahoon, and Hodge 1986). At that time raw milk consumption was common among farm residents in Canada but similar observations were also reported in Scotland where the incidence in the predominantly urban greater Glasgow area of Scotland was consistently lower than in the Lothian area which, although it includes the city of Edinburgh, also encompasses large amounts of farmland (Miller et al. 2004). Interestingly, the summer peak was also more pronounced in the Lothian compared with the Glasgow area. These reports suggest that rural living raises the risk of Campylobacter infection. Similarly, Ethelberg et al. (2005) have reported that in Denmark, living in the types of houses found in rural areas, and living in areas with a low population density, were both associated with an increased risk of campylobacteriosis. This relationship was particularly clear among children under 15 years of age. In the province of Manitoba, Canada, the incidence of campylobacteriosis was higher in rural than in urban areas for all age classes, but particularly for children under 5 years of age (Green, Krause, and Wylie 2006).

Raised levels of environmental contamination with Campylobacters seem a likely reason for this rural/urban difference. In Sweden, there was a significant association between incidence of campylobacteriosis and livestock density (Nygard et al. 2004). However, in one region of the Netherlands, an increasing incidence

among routine laboratory-confirmed cases has been observed with increasing index of urbanization; although there was no data on cities larger than 100,000 inhabitants (Van Pelt et al. 2003). The same trend was observed for *Salmonella* Enteritidis but a reverse trend was observed for *Salmonella* Typhimurium (Doorduyn et al. 2006).

Overall, there appears to be a higher incidence in rural areas than in urban areas, which is particularly pronounced in young children. This may be consistent with a higher exposure in rural areas due to increased contact with livestock or their excreta, combined with the development of protective immunity at older ages. This hypothesis is supported by serological data from Wisconsin, where 59% of children in a rural area were sero-positive (≥ 2 immunoglobulin classes of *C. jejuni* antibodies) (Belongia et al. 2003). In this serological study increasing age and farm residence were independently and positively associated with sero-positivity. Moreover, the incidence of diarrheal illness was lower if children resided on a farm. The incidence of diarrhea was slightly, but not significantly, lower in sero-positive children (Belongia et al. 2003). The higher incidence among adults in rural areas suggests that protection by acquired immunity is incomplete. Under such circumstances antigen-related strain differences or exposure to very high doses may overcome any immunity developed. In addition, behavioral factors leading to increased exposure or differences in health-care seeking have also been offered as possible explanations for higher numbers of rural laboratory-confirmed cases (Van den Brandhof et al. 2006; Ethelberg et al. 2005).

Occupationally exposed individuals

Anecdotal evidence suggests that poultry workers in abattoirs, veterinary students, and sewage workers all acquire symptomatic intestinal disease within the first few days of starting work and thereafter are usually free of symptoms. Certainly the environment within a poultry abattoir is heavily laden with Campylobacter, especially in the aerosols around the plucking and evisceration machinery (Posch et al. 2006; Johnsen, Kruse, and Hofshagen 2007). Early studies demonstrated that abattoir workers have a higher prevalence of Campylobacter antibodies than the normal population (Jones and Robinson 1981). In an outbreak of campylobacteriosis in a Swedish poultry abattoir (Christenson et al. 1983), the attack rate was higher among inexperienced seasonal workers than in the regular staff (71 vs. 29%). In addition among the regular workers, the highest rates were seen in younger individuals and asymptomatic carriage was noted, but only among experienced workers. Serological studies (Cawthraw et al. 2000) have demonstrated significantly raised anti-Campylobacter antibodies in poultry slaughterhouse workers and that these antibody levels increased with length of employment. In this study excretion of Campylobacter was only accompanied by clinical symptoms in one new worker; while seven long-term employees were symptomless excreters. Similarly, experienced poultry workers in the Delmarva Peninsula (USA) did not excrete Campylobacter, nor did they suffer from more gastrointestinal symptoms than a control group in the general population (Price et al. 2007). These poultry workers had higher IgG, but not IgA, anti-Campylobacter serum antibody titers. However, in contrast, a prospective study in rural Michigan (Potter, Kaneene, and Hall 2003) indicated that poultry husbandry workers (but not professional farmers) had a higher risk of campylobacteriosis despite the likelihood of routine exposure to chickens carrying campylobacters and in Canada, 7 out of 9 casual farm workers, but also 4 permanent workers, developed Campylobacter-associated diarrheal illness when catching and transporting 13,000 turkey poults (Ellis et al. 1995).

Thus although some of the evidence from studies related to occupational exposure indicates that persistent exposure induces protective immunity to disease, anomalies again suggest that exposure to relatively high doses and/or to an unusual antigenic type could overcome this protection.

Asymptomatic infections

It has been widely reported that asymptomatic Campylobacter infections occur rarely, if at all, in industrialized countries. A longitudinal community-based cohort study on infectious intestinal disease (IID) in four regions in the Netherlands was undertaken from March–July 1991 and over 2200 persons were followed (Hoogenboom-Verdegaal et al. 1993). All respondents completed a weekly questionnaire including information on symptoms of gastro-enteritis and 562 respondents also submitted a weekly stool sample for Campylobacter culture. Seventeen persons excreted Campylobacter during the study period; two of whom experienced two excretion periods, separated by time intervals of 6 and 9 weeks respectively. Overall, excretion lasted between 1 and 10 weeks, with an average of 3 weeks. Only 3 of the 17 persons were symptomatic during these excretion periods. One person was symptomatic 3 weeks before excretion and the other two 3 and 7 weeks after excretion. (De Wit et al. 1996). The incidence of Campylobacter excretion in the community was 105 per 1000 person years, which corresponds with a prevalence of 0.6%, similar to that typically found in case-control studies.

According to Wheeler et al. (1999), the incidence rate of a community-based IID study in England was 19.4/100 person years. With an average duration of an episode of gastroenteritis of 3.9 days (Food Standards

Agency 2000), this implies an average prevalence of 0.2% of symptomatic IID at any one time point.

In the nested case-control study (Tompkins et al. 1999), *Campylobacter* spp. were isolated from 32/761 cases (4.2%). Hence, the prevalence of symptomatic IID patients excreting *Campylobacter* spp. is 0.009%. The isolation rate from healthy controls (i.e., the prevalence of asymptomatic infection with *Campylobacter* spp.) was 4/555 (0.7%). This implies that healthy carriers outnumbered diseased persons by a factor of approximately 80, or in other words, only 1.2% of all excreters of Campylobacter are actually experiencing an episode of illness. Similar calculations for the Netherlands (De Wit et al. 2001b) suggest the ratio of asymptomatic to symptomatic shedders is 120:1 and in developing countries, asymptomatic excretion of Campylobacter is even more common, as discussed previously.

These data suggest that Campylobacter excretion is frequently asymptomatic, and the hypothesis would be that in these infected individuals immunity has protected against illness but not against colonization.

Serosurveillance

Further support for the hypothesis of the occurrence of frequent asymptomatic infections is provided by sero-epidemiology. In a recent study, Ang et al. (2007b) using ELISA assays based on acid glycine extracted antigens, demonstrated an increasing incidence of sero-prevalence for anti-Campylobacter IgG antibodies in a representative sample of the Dutch population (the Pienter cohort, De Melker and Conyn-van Spaendonck 1998). There was a linear increase of seroprevalence to virtually 100% in young adults (20–29 years of age), which was maintained in higher age groups. This increase in sero-prevalence with increasing age was also observed in a study using sera from the United States. Belongia et al. (2003) determined anti-Campylobacter IgA, IgM, and IgG antibodies in sera from children living in rural Wisconsin. In this case the criterion for sero-positivity was defined as an optical density (OD) greater than 3 standard deviations above the mean of an "unexposed pediatric reference population." Despite these stringent criteria, the percentage of sero-positive farm-resident children increased from 40% in 1–4 year olds to almost 90% in 15–18 year olds. Surprisingly, using the same method, children in Colorado and Maryland only showed a very slight increase in IgG anti-Campylobacter antibody levels in the age groups from one year to 2–4 years old. With further increasing age groups, IgG levels remained constant at low level (Blaser et al. 1985).

In developing countries, most studies document an increase in sero-prevalence with advancing age. Blaser et al. found a strong IgG anti-Campylobacter antibody response in Bangladeshi and Thai children. Infants under one year of age had the lowest IgG antibody levels, albeit higher than their U.S. counterparts. With rising age (1–4 years old), levels of anti-Campylobacter antibodies increased with a peak at one year of age in Thai children and 2–4 years of age in Bangladeshi children, followed by a decrease during puberty (Blaser, Taylor, and Echeverria 1986; Blaser et al. 1985). In the Central African Republic, Martin et al. (1989) studied the anti-Campylobacter antibody response in children from birth to two years of age. They found a linear increase in antibody levels during the first two years of life. In Chilean infants, an identical pattern was observed (Figueroa et al. 1989).

In summary, the data from sero-surveillance studies indicate that both in developing and developed countries, frequent exposure to Campylobacter occurs, leading to an detectable antibody response. In some regions, there is an increase of serum antibody levels with advancing age. However, comparison of sero-prevalence rates between countries or regions is hampered by the widely divergent serological methodologies used.

Antibody decay, although highly variable between individuals, was demonstrated in longitudinal studies (Strid et al. 2001; Cawthraw et al. 2002; Taylor et al. 2004). Hence, even though anti-Campylobacter IgG antibodies may persist for several years, their high prevalence indicates repeated (symptomless) infection by Campylobacter during life. These studies again emphasize that the presence of specific serum antibodies indicates a previous infection but does not allow any conclusions to be drawn regarding the possible protection provided by these antibodies.

Conflicting results from epidemiological and risk assessment studies

There are reported conflicts between estimates of infection with Campylobacter from theoretical and empirical studies. For example, Evers et al. (2008) estimated the average exposure of the Dutch population to Campylobacter by risk assessment methods. For this purpose, the quantitative estimates of the occurrence of the bacteria from different sources (including food, water and animals) were combined with data on the frequency of contact (e.g. food and water consumption, transmission during direct animal contact) and survival of the organism during food preparation. The average exposure was then introduced into a dose-response model (Teunis and Havelaar 2000) to give an estimate of more than 9 million infections and approximately 3 million clinical cases of campylobacteriosis per year in the Netherlands (population 16 million). In contrast, a population-based study (De Wit et al. 2001b) from the same country indicated that in 1999 the incidence of acute Campylobacter-associated gastro-enteritis was only 80,000 cases (Mangen et al. 2005). Thus, there is an

approximately 40-fold difference between the risk-based and the epidemiological estimates of disease incidence. Such a difference is of concern even when taking the uncertainties in both estimates into account. Partly, the difference can be explained by clustering of exposure in certain groups, which can reduce the risk estimate but this alone cannot explain the observation.

An alternative explanation is that, as a consequence of the impact of protective immunity, not every individual in the population is fully susceptible to illness from a Campylobacter infection. A high level of exposure would frequently result in asymptomatic infections (including infections that are associated with a mild illness that does not fulfill the case-definition of gastro-enteritis as used in the population-based study). Such infections may still result in a boosting of the immune-response, thus maintaining a high level of protection to more severe illness. These findings are in accord with the high prevalence of antibodies detected by sero-surveillance as discussed above.

Impact of immunity on epidemiological studies

The epidemiological investigation of campylobacteriosis is an essential component for identifying the importance of this infection to public health; in prioritizing the disease in terms of economic and social cost; and finally in identifying sources and routes of transmission so that effective control strategies can be developed.

In recent years, many case-control studies have been carried out in different countries to identify risk factors for sporadic cases of campylobacteriosis. Overviews can be found elsewhere (Havelaar 2002; Neimann et al. 2003). Studies involving large numbers of cases and controls have shown that a multitude of risk factors, transmission routes and vehicles can be associated with an increased risk of campylobacteriosis. Surprisingly all these factors together can only explain a minority of the cases. Furthermore studying of the identified risk factors show conflicting results. For example, considering that poultry is a major reservoir for Campylobacter and that the monitoring of poultry products at retail shows high levels of contamination in most developed countries, it would be expected to find chicken consumption as a major risk factor. Indeed it is often found as a significant risk factor but less important than expected, sometimes not statistically significant and sometimes even protective. Eating undercooked, or almost raw, poultry meat is always a significant risk factor, but several studies have shown chicken prepared and eaten at home to be a protective factor whereas the (presumably occasional) eating of chicken in a restaurant is a risk factor (Adak et al. 1995; Eberhart-Phillips et al. 1997; Effler et al. 2001; Friedman et al. 2000). Several explanations for obvious risk factors that appear as protective in the statistical analyses of case-control studies have been suggested. In addition to a real protective effect these include: information bias, recall bias, competitive exclusion of risk foods, statistical coincidence, and proxy measures for differences in behavior and food preferences (Neimann et al. 2003). Protective immunity may also be suggested as an explanation. Indeed, a recent report from the UK found that consumption of chicken from a commercial establishment was a major risk factor. However, the degree of risk was higher for those who did not habitually eat chicken (Tam et al. 2007). How any of these hypotheses could explain the confusing results in the same study of chicken being protective in one setting and a risk factor in another, is not immediately obvious. The regular exposure to low doses of common Campylobacter types resulting in asymptomatic infection and allowing the development of protective immunity, as opposed to an occasional exposure to high doses or non-common Campylobacter types with a symptomatic outcome, might be a key explanation.

Protective immunity would result in the introduction of misclassification bias, because a part of the controls are classified as not exposed when in fact they were but did not develop illness because of protective immunity. This would bias the odds-ratio towards the null, but might even result in generating the apparently protective effects associated with consistent high-risk exposures. A simple model to evaluate the impact of protective immunity on analytical epidemiological studies has been recently proposed by Swift and Hunter (2004). These authors showed that, at relatively high levels of exposure, which are consistent over many years, a relative risk less than 1 can be found in case-control studies. The model assumes lifelong immunity, which may not be realistic for enteric pathogens such as Campylobacter. 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 require information on the exposure history of the study population.

Impact of immunity on risk assessment studies

Increasingly, risk assessment is applied as a complementary tool to understand the complex dynamics of the potential transmission chains of Campylobacter and to predict the effects of interventions. Risk assessment consists of four stages: hazard identification, exposure assessment, hazard characterization and risk characterization (Anonymous 2000). For the purposes of this review, the last two stages are of particular relevance. Hazard characterization is defined as the qualitative or quantitative description of the severity and duration

of adverse health effects. A dose-response assessment should be presented if the data are obtainable. Risk characterization is the qualitative and/or quantitative assessment of the probability of occurrence and severity of known or potential adverse health effects in a given population based on the three previous stages.

Dose-response models as commonly applied in microbiological risk assessment typically consider the infectious disease process as a conditional chain of events: exposure may lead to infection, which may lead to illness. This chain of events is represented by the probability of infection given exposure P(inf|exp) and the (conditional) probability of illness given infection P(ill|inf).

Modeling the risk of infection

The models for infection, given exposure to a certain dose of pathogenic microorganisms, are based on three fundamental assumptions (Zwietering and Havelaar 2006):

a. *Single hit*.
The single hit assumption states that, no matter how low the dose, there is always a non-zero probability of illness and infection, however low this probability might be. This is in contrast to the frequently used (minimal) infectious dose assumption, in which a certain threshold (MID) exists for infection. Experimental evidence supporting the single-hit hypothesis was published as early as 1957 by Meynell and Stocker (1957) and later by Moxon and Murphy (1978).

b. *Independent action*.
The hypothesis of independent action states that the mean probability per inoculated pathogen to cause disease or illness is independent of the number of pathogens inoculated. Experimental evidence to support this hypothesis has been summarized by Rubin (1987). This hypothesis implies that the probability of infection by any number of organisms is a simple binomial function.

c. *Random distribution*.
In the mathematical derivation of dose-response models, it is typically assumed that micro-organisms are randomly distributed in the inoculum, which can be described by a Poisson distribution. However, dose-response models can also be derived for other distributions (Haas 2002).

The basic form is the exponential model:

$$P(\text{inf}|\text{exp}) = 1 - e^{-rD} \approx rD.$$

In this model, D is the mean ingested dose and r is the dose-response parameter, indicating the probability that a single "unit" of the organism will survive all barriers and cause infection. This model requires the assumption that the bacteria have a Poisson distribution (see above), and that all cells have an equal probability of causing infection in any host. For low doses this equation can be approximated by a linear relationship as indicated (as long as $rD < 0.1$ less than 5% error is made).

The assumption that every one of the bacterial cells consumed by each host has an equal probability of causing infection may not be realistic and can be replaced by the assumption that this probability follows a beta-distribution with parameters α and β. The resulting dose-response model is the so-called hypergeometric model (Teunis and Havelaar 2000):

$$P(\text{inf}|\text{exp}) = 1 - {}_1F_1(\alpha, \alpha+\beta, -D) \approx \alpha/(\alpha/\beta)D$$

in which ${}_1F_1(\,)$ is the Kummer confluent hypergeometric function.

This model contains one more parameter than the exponential model. In the lower dose range the model can also be approximated by a linear relationship.

For cases where $\alpha << \beta$ and $\beta >> 1$, the hypergeometric model can be approximated by the Beta-Poisson model:

$$P(\text{inf}|\text{exp}) = 1 - [1 + D/\beta]^{-\alpha} \approx (\alpha/\beta)D$$

which also behaves linearly in the low dose range.

For infection with *C. jejuni*, the most widely used dose response for modeling the risk of infection is based on the volunteer experiments by Black et al. (1988), using two laboratory-adapted strains as described earlier. While the data for strain A3249 cannot be fitted with an exponential model, a hypergeometric model (with parameters $\alpha = 0.145$ and $\beta = 8.007$) provided a significant fit to the data confirming heterogeneity in the host-pathogen interaction, possibly related to protective immunity in some volunteers exposed to high doses (Teunis and Havelaar 2000). The low dose approximation is $\alpha/(\alpha+\beta) = 0.018$. This can be interpreted as the average probability of one cfu to cause infection being approximately 2%. It is difficult to generalize from this limited set of observations to general exposure scenarios due to the impact of immunity, and to the use of laboratory adapted strains. Chen et al. (2006a) have recently demonstrated the different colonization potential of *C. jejuni* strains in a one-day chicken model. The results indicated that *C. jejuni* is highly infectious in chickens. Fresh isolates showed higher colonization potential (mean $ID_{50} = 29$ cfu) than laboratory adapted isolates (mean $ID_{50} = 340$ cfu). In particular, it was noted that the (geno)type strain 11168 was a poor colonizer of chickens and transcript arrays studies by Gaynor et al. (2004) have shown that this is a consequence of laboratory adaptation.

Modeling illness given infection

Little work has been done on modeling the probability of illness given infection. A key question, that remains to be answered is, "Does this probability also depend on dose?" Defining P(ill|inf) as the conditional probability of illness given infection leads to the following formula for the unconditional probability of illness due to a certain dose:

$$P(ill|exp) = P(inf|exp) \times P(ill|inf)$$

Three possibilities can be suggested:

a. P(ill|inf) has a constant value. In this case, the probability of illness given dose has the same shape as the probability of infection given dose, but is scaled to a maximum of P(ill|inf). Note that several published dose-response models have directly estimated the parameters from dose-illness data, silently assuming that P(ill|inf) has a constant value. As most models reach a maximum probability of illness of 1, it is also assumed that P(ill|inf) is 1.
b. P(ill|inf) increases with dose. In this case, the probability of illness given dose increases more steeply than the probability of infection given dose, but can only reach values near 1 if both conditional probabilities are near 1.
c. P(ill|inf) decreases with dose. In this case, the probability of illness shows a maximum at an intermediate dose value, which will usually be considerably less than 1.

Teunis, Nagelkerke, and Haas (1999) have developed a hazard model assuming that the probability of illness given infection is related to the duration of the infection. Analysis of available datasets showed that P(ill|inf) was constant for *Cryptosporidium parvum*, was increasing for *Salmonella* spp., and decreasing for *C. jejuni*. The latter result (for strain A3249) many have been due to resistant volunteers who were assigned by chance to the higher dose groups. In the data presented by Tribble (1998), in which all participants were screened for seropositivity prior to challenge, approximately 80% of the infected (sero-negative) volunteers exposed to strain 81–176 developed clinical illness with the highest proportion at a dose of 10^9 cfu.

At least two large, well-documented raw milk outbreaks have shown a clear positive relationship with the ingested dose of not only the occurrence, but also the severity and duration, of the disease. The first outbreak involved 21 college students in the USA with low levels of *C. jejuni*-specific immunoglobulins (Blaser, Sazie, and Williams 1987). The second outbreak comprised 100 children, between 9–12 years of age, in the Netherlands (Teunis et al. 2005). In these outbreaks, no information was available on the numbers of Campylobacter in the milk, but inference could be made by combining the observed responses with the dose-response relation from the volunteer study. The best fitting parameter estimates of the unconditional model for illness given dose were $\alpha = 0.024$ and $\beta = 0.011$, with linear approximation $\alpha/(\alpha + \beta) = 0.69$. In other words, 69% of subjects exposed to a single cfu would become ill. It must be noted that the second outbreak occurred among urban schoolchildren during a visit to a dairy farm. One might speculate that protective immunity among such children is relatively low, resulting in a high susceptibility to infection and illness.

Risk characterization

In microbial risk assessment, there is frequently a need to account for repeated exposures, e.g., for individuals who consume a possibly contaminated product more than once within a period of time (albeit weeks or years). The current method is to estimate the risk of each exposure event separately and then to estimate the combined risk by assuming the events to be independent. If, for example, the risk per event is p and the number of events is n, then the combined risk follows from a binomial distribution:

$$p_n = 1 - (1 - p)^n \approx p \cdot n$$

If in the case of development of protective immunity, the assumption of independence between subsequent exposures is no longer valid, and the risk characterization must take this into account. As the level of immunity may be age-dependent, more attention must also be paid to the age distribution of the exposed population.

Dynamic models to analyze (partial and temporary) protective immunity

Classical dynamical infectious disease models (Anderson and May 1991) divide the population into several categories with respect to their disease status. Thus, the SIR class of models recognizes individuals who are susceptible to infection and illness (S), those who are currently infected and ill (I) and those who have recovered from the illness (R). Waning immunity can be modeled by allowing the resistant individuals to become susceptible again (SIRS-models). Mathematically, such models are implemented as a set of coupled differential equations that quantify the rate at which transitions occur from one state to another. These models are widely used for diseases that are directly transmissible between humans or animals, where the "force of infection" is directly related to the prevalence of infected individuals. Such diseases include childhood infections, such as measles,

Table 1. The surveillance pyramid of campylobacteriosis in the Netherlands (based on Helms et al. 2003; Van Pelt et al. 2003; De Wit et al. 2001a; De Wit et al. 2001b; Ang et al. 2007b; Evers et al. 2008)

Cases	Incidence (per 100,000 person years)	Background information
Fatal	0.15–0.30	Extrapolation from Danish registry-based study
Hospitalized	3.5–4.0	Laboratory surveillance
Reported	35–45	Laboratory surveillance
Consulting general practitioner	90–150	GP-based study (NIVEL)
Non-consulting	400–600	Population-based study (Sensor)
Asymptomatic, sero-conversion	10,000–20,000	Sero-surveillance
Asymptomatic, infected	40,000–60,000	Risk assessment model

sexually transmitted diseases, such as HIV/AIDS and respiratory infections, such as influenza. In contrast, the risk of campylobacteriosis and many other enterically transmitted diseases does not directly depend on the prevalence of infected individuals. The main reservoirs are warm-blooded animals (including humans) and transmission to humans may occur by an intricate maze of pathways. The risk assessment approach is typically used to model such pathways by combining exposure models with dose-response models to estimate the risk of infection or illness to an individual. A combination of risk assessment models and dynamic infectious disease models has been proposed to account for the effect of secondary (human-human) transmission after initial exposure to an environmental source, such as rotavirus in drinking water (Eisenberg et al. 1996). This combination of models is also capable of accounting for the impact of immunity on the dynamics of enteric infections (McBride and French 2006).

Dynamical models do not usually distinguish between asymptomatic and symptomatic infections, either in terms of health outcomes after first exposure or in terms of the resulting protection against future exposures. However, Aguas, Goncalves, and Gomes (2006) have proposed a model for *Bordetella pertussis* that differentiates those primary infections in fully susceptible individuals leading to serious illness, from those infections in previously infected or vaccinated individuals, which lead to mild symptoms only. Such an approach could also be applicable for campylobacteriosis and is in agreement with the observation that in developing countries, Campylobacter infections above the age of 5 are asymptomatic or lead to mild diarrheal symptoms only. A key outcome of this model is that there is a non-monotonous relationship between the incidence of severe illness and the force of infection. Initially, the incidence of severe illness increases with exposure, but at higher exposures, the incidence of severe illness decreases and most cases are mild or asymptomatic. Due to this non-linear behavior, reducing the force of infection may paradoxically result in increasing the rate of severe illness. This might be an explanation for the results of Oberhelman et al. (2006) who studied the effect of corralling free-ranging chickens in Peruvian peri-urban shantytown households. Unexpectedly, rates of Campylobacter-related diarrhea in local children were significantly higher when associated with the corraled chickens than in controls. Conversely, rates of asymptomatic carriage with Campylobacter were similar.

A key aspect in Campylobacter epidemiology is the heterogeneity in the bacterial population, implying that exposure to one particular strain may not be protective towards subsequent challenge with another strain. A possible approach to model cross protective-immunity has been proposed by Gog and Swinton (2002). These authors assumed that exposure to a strain in one homogeneous group (say group 1) would lead to complete protection towards homologous strains, partial protection towards strains in related groups but no protection towards unrelated strains. The partial protection towards related strains was modeled by assuming that among those individuals infected with strains from group 1, only a certain proportion would develop immunity to related strains. For Campylobacter, it appears necessary to differentiate protection from infection and protection from disease. Factors involved in colonization appear to be less conserved than factors involved in disease, implying that cross-immunity to disease is more common than cross-immunity to colonization.

Conclusions

The evidence presented above suggests that acquired immunity exerts a strong influence on the epidemiology of campylobacteriosis. This has been widely recognized for developing countries, but has previously been assumed to be of less importance in industrialized countries.

The experimental evidence suggests that in naïve humans every primary exposure can generate an immune response as indicated by circulating anti-Campylobacter antibodies and secondary exposures can enhance and contribute to that response. Even low or moderate dose

levels especially with organisms, which may be environmentally stressed and therefore have compromised infectivity could further boost this acquired immunity. Individuals with such detectable immune responses appear less likely to develop symptoms on further exposure and are thus, to some degree, protected from campylobacteriosis, but not apparently from colonization i.e. they can be asymptomatically infected. One confounding aspect is that the degree of protection is apparently finite, has a restricted cross-reactivity and may wane over time. As such it may be overcome by excessive challenge doses or perhaps by strains not previously experienced. All these characteristics of acquired immunity need to be taken into account when investigating the potential effects on Campylobacter epidemiology.

Table 1 presents the surveillance pyramid of campylobacteriosis in the Netherlands, based on current evidence. The risk assessment models indicate that each person can expect an exposure leading to asymptomatic infection on average every 2 years. However, only 1 out of 100 of such infections leads to symptoms of campylobacteriosis. It seems likely that the high incidence of asymptomatic infections is, at least in part, related to the effects of protective immunity, even in developed countries.

A series of mathematical models have been discussed for investigating the role of acquired immunity in the epidemiology of campylobacteriosis. These models suggest that the relationship between the force of infection and the risk of illness at the population level may be non-linear and non-monotonous. Thus reducing exposure may lead to either an increase or a decrease in the risk of symptomatic illness, depending on the baseline level of exposure. Given the importance of current public health targets worldwide to reduce campylobacteriosis, quantifying this relationship is critical to the evaluation of instigated risk mitigation strategies.

This study also demonstrates that knowledge of the protective immune status of individuals is necessary to reduce misclassification bias in future epidemiological studies and to prevent the overestimation of health risks in risk assessment studies. Such knowledge requires the development and implementation of accurate and appropriate assays of protective immunity. This in turn requires greater knowledge and understanding of the host immune responses to Campylobacter infections.

In conclusion, it is clear that a the dynamics of protective immunity against Campylobacter at the individual and population levels is a key factor in the interpretation of epidemiological and risk assessment studies and particularly to enable the correct interpretation of interventions at the farm and during food processing. This requires the urgent multidisciplinary collaboration between microbiologists, immunologists, epidemiologists, risk assessors, and mathematical modelers.

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