Appendix B

List of Assumptions and Limitations of Data Used in the Risk Assessment

Below is a summary list of assumptions and a section reference for each assumption. The assumptions are listed in order of importance to the modeled risk.

Limitations in quantifying human health impact using attributable risk:
ASSUMPTION 1: The level of risk as calculated does not account for cases originating from chicken and contaminating other foods or the spread from chicken to other animal hosts and resulting in human exposure. (refer to Section 3.1)

DISCUSSION 1: The definition of the attributable risk included all cases of disease which may be attributed to a specific risk factor (122, 83). One limitation of epidemiologic tools used to determine the attributable risk or etiologic fraction is that those cases that were exposed to the risk factor of interest, even though the exposure may not have been the cause of the disease, would be included in the calculated level of risk, thereby potentially overestimating the level of actual risk. Conversely, another limitation of the epidemiologic tools used to determine the risk from the specific exposure of interest is that spread from the primary source of the pathogen, in this case chickens, is not included in the calculation of the level of risk. The magnitude of the bias introduced by false associations with chicken exposures (false positive associations) is likely to be much smaller than the lack of inclusion of the undeterminable cases from spread of the chicken associated resistant *Campylobacter* to other sources of human exposure. In addition, the risk assessment does not take into account the spread of the pathogen from chicken to other food sources. This can occur from cross contamination of other foods (29) or spread from chicken sources more proximate to the farm. For example, spread of Campylobacter can occur via many different pathways: from exposure of birds, insects and run-off surface water to chicken litter; from the use of chicken litter in aquaculture to fertilize fish ponds and from the use of litter in cattle feeds to increase the non-protein nitrogen content. Therefore, the risk assessment is likely to underestimate the overall risk of acquiring a resistant *Campylobacter* infection from exposure to chicken due to the secondary spread of *Campylobacter* from chickens to other sources of human exposure to the pathogen.

ASSUMPTION 2: The current level of risk of contracting campylobacteriosis from consumption of chicken is contained within the range of risk ascertained from studies conducted in the 1980's (refer to Section 3.1)

LIMITATIONS of studies used to determine the proportion of chicken associated cases:
Limitations of study 1 include: the demographic characteristics of the population, the frequency of chicken consumption, the proportion of the population consuming chicken and many other factors may have changed since this study. For example, the amount of chicken consumed has increased since 1982, and in 1998 people consumed 54.4% (72.60 lbs/47.02 lbs) more chicken, calculated in ready to cook pounds consumed per capita (80).

Limitations of study 2 include the lack of representativeness of the study population and the absence of some exposures, such as travel and raw milk that are frequently associated with risk in the population at large. In addition, the study was limited to enteric illnesses because more invasive infections were not eligible for inclusion in the study, although these usually comprise less than 1% of cases. These differences result in difficulty in generalizing the findings to the United States population but may represent the level of risk in some subgroups of the population.

DISCUSSION 2: In the two case control studies there was an increased risk of illness associated with consumption of chicken especially consumption of undercooked chicken. One study indicated a risk associated with raw milk consumption although the proportion of attributable risk was much less than that attributed to chicken. The proportions of disease attributable to consumption of chicken were 48.5% and 66.7%. The higher estimate of attributable risk from study 2 of 66.7% in the university student population indicates that in some subgroups of the population exposures are likely to differ and risk attributable to

consumption of chicken will vary accordingly. These estimates of the etiologic fraction represent a range of risk that is likely to reflect the level of risk in the early 1980's. More recent data do not exist for United States populations. Data analysis of a case control study, conducted by the CDC and participating State Health Departments (CA, CT, GA, MD, MN, OR), in 1998 within FoodNet sites is currently underway and will be published in the near future. The data from this study will provide updated risk factor information from which etiologic fractions associated with identified risk factors may be determined.

Limitations in data on resistant isolates:
ASSUMPTION 3: The fluoroquinolone resistance observed in persons ill from campylobacteriosis, (after removal of travelers, those who took a fluoroquinolone prior to culture and those for whom the time of taking the fluoroquinolone was unknown) is largely attributed to chickens (refer to Section 3.2).

DISCUSSION 3: It is difficult to know what proportion of resistance in human campylobacteriosis may be attributable to a source when human exposures are multiple and varied and when the data are limited. A single source of resistant bacteria may be disseminated from its origins into the environment or maintained in secondary hosts further spreading resistant *Campylobacter* to additional sources of human exposure further complicating the ability to measure the impact.

Fluoroquinolone use has been associated with the development of fluoroquinolone resistance in *Campylobacter* in clinical trials in poultry production units (58) in poultry production in the Netherlands (36) and in the United States (92) after the introduction of veterinary fluoroquinolones. In countries where fluoroquinolones have been approved for human and companion animal use but are not allowed in food animals the level of fluoroquinolone resistance in food animals and human clinical cases is low (8, 54).

An Extra Label Use Prohibition of fluoroquinolone use in food-producing animals was published in 1997 (21CFR530.41), limiting food animal drug use to species listed on the product label. Approvals of fluoroquinolone drugs for use in animals include feline and canine oral and canine injectable products (available in 1989), poultry water soluble and in-ovo injectable products (available in 1995) and feedlot cattle injectable products (available in October, 1998). There are no fluoroquinolones currently approved for use in swine.

Campylobacteriosis is primarily an animal derived foodborne disease, with the predominant source of human infections attributed to poultry (22, 31, 36 64). There is little surveillance data available to describe the level of fluoroquinolone resistance in *Campylobacter* isolated from animal derived food in the United States, either before or after the approval of these drugs for food animal use. Chicken *Campylobacter* isolates collected in 1998 indicated an overall level of 13.4% resistance to Ciprofloxacin (see Section 4.1). Because there was no food animal fluoroquinolone use other than use in poultry until late 1998, and only rare, sporadic and isolated resistance was observed prior to 1992 in human cases[1] it is unlikely that the increase in domestically acquired fluoroquinolone resistance observed in people since 1996[2] can be

[1] In two surveys encompassing 474 human isolates from 1982 to 1992 in the United States, only a single Ciprofloxacin resistant isolate was identified and subsequently speciated as *C. lari* (70).
[2] After removal of persons who had traveled within 7 days of illness onset and removal of those taking fluoroquinolones prior to culture, quinolone resistance in Minnesota was observed in 0.8% of isolates in 1996 and had increased to 3.0% in 1998 (chi square for linear trend, 9.8; $p \leq 0.002$) (71). In Minnesota quinolone resistance, screened by nalidixic acid disc diffusion was highly correlated with resistance to ciprofloxacin using the E-Test, (sensitivity 99.6%, specificity 98.4%) (71). A survey of *Campylobacter* isolated from 88% of 91 chicken products resulted in *C. jejuni* from 67(74%) and *C. coli* from 19 (21%) of samples and six samples were the source of both pathogens. Products carrying resistant isolates were purchased from 11 stores representing 8 franchises and originated in seven processing plants in five states (70, 71) indicating widespread resistance in chicken campylobacter isolates. Molecular subtyping was performed using PCR restriction endonuclease length polymorphism typing of the flagellin gene in the C. jejuni human and chicken product isolates. 12 subtypes were identified from 13 *C. jejuni* positive chicken products. Six of seven resistant subtypes in the chicken products were also identified in the quinolone resistant human isolates. For people acquiring infections during 1997, excluding cases that had taken fluoroquinolones prior to culture, persons with non-traveler resistant infections were more likely to have C.

attributed to a consistently distributed source of resistant *Campylobacter* exposures. Distribution of resistance from foodborne sources is more likely to be associated with specific exposures and limited predominantly to poultry.

DATA GAP 3: Quantification of the proportion of human disease attributable to various sources and the determination of the level of resistance carriage within the specific exposures would more precisely allow the determination of the relative contributions of the various exposures to fluoroquinolone resistant human disease. This ability to determine the relative contributions of various sources of infection to the level of resistance in human cases becomes increasingly important once fluoroquinolones are available for use in more than one food animal species. A model intended to determine the human health impact of the level of resistance in *Campylobacter* attributable to fluoroquinolone use in food animals will need to distribute the burden of resistant human disease amongst many different food animal species and attribute levels of resistance to sources of human infection.

Limitations in microbiological methods:
LIMITATION 4: The lack of accuracy in the determination of the level of resistance using a single isolate leading to an underestimation of the level of resistance in chicken carcasses is currently not quantifiable. The limitation in the accuracy of reported carcass prevalence and the lack of reliability in the results needs further characterization and methods need to be developed to provide more accurate and reliable data which would improve the ability to measure the impact on human health. This risk assessment determined the measurable risk, limiting the model to those parameters for which data were relevant, valid and available.

ASSUMPTION 5: If a carcass was positive for *Campylobacter*, the predominant species isolated was *C. jejuni.* (refer to Section 4.1)

DISCUSSION: The prevalence of *Campylobacter* in chickens was estimated from a 1994-95 survey of **1,297** broiler carcass rinse samples at 88.2% of carcasses, indicating that **1,144** carcasses tested positive (104). The isolates were speciated using the biochemical hippurate assay and *C. jejuni* and *coli* were included in the carcass prevalence estimate.

Assumptions relating to the use of surrogate data on diarrheal illness for seeking care, submitting stools and rate of prescription of antimicrobials for campylobacteriosis:
ASSUMPTION(s) 6: The rate at which people reporting bloody stools seek care is similar to the rate at which people with campylobacteriosis reporting bloody stools seek care. The rate at which people with non-bloody stools seek care for diarrheal illness is similar to the rate at which people with campylobacteriosis reporting non-bloody stools seek care. (refer to Section 2.1 for discussion)

DISCUSSION 6: These estimates are for diarrheal illness, and not campylobacteriosis specifically. Data describing care seeking behavior for campylobacteriosis was not available. Bacterial foodborne disease is typically more severe than viral foodborne disease (42) and rates of seeking care may differ by pathogen.

In the population survey, factors that were most important in influencing the decision to seek care were fever, vomiting, "how sick they felt," stomach cramps, reporting blood in stool and duration of diarrhea (26). Some of these factors were evaluated for diarrheal illness in the telephone survey and compared with the same characteristics in individuals who had culture-confirmed *Campylobacter* infections or diarrheal disease (Table 2.1). Comparing the groups, a greater proportion of people with culture-confirmed *Campylobacter* cases were affected by fever and blood in the stool than the people seeking care for diarrheal illness. Therefore, the actual rate of seeking care for campylobacteriosis may be underestimated by the 20.5% for persons with non-bloody and 33.2% for persons with bloody stools. However, because a greater proportion of people with fever and bloody stools would be cultured and enrolled in the case control study, such comparisons are difficult.

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jejuni subtype also found in the quinolone resistant C. jejuni from chicken products (odds ratio 15.0, 5^{th} and 95^{th} percentile 1.9 to 321.8) (70).

DATA GAP 6: Additional studies to define the rate at which people with campylobacteriosis seek care would be helpful and would provide a more accurate estimate. These data would require very large community-based surveys that are likely to require considerable resources to conduct.

ASSUMPTION 7: The probability that a stool specimen was requested among people with diarrheal illness reporting bloody stools is similar to the probability that a stool specimen was requested among people with campylobacteriosis reporting bloody stools. The probability that a stool specimen was requested among people with diarrheal illness reporting non-bloody stools is similar to the probability that a stool specimen was requested among people with campylobacteriosis reporting non-bloody stools. (refer to Section 2.2)

ASSUMPTION 8: The population survey proportion of cases of all acute diarrheal illness seeking care, not submitting a stool sample and receiving an antibiotic (38.1%) is similar to that for persons ill with campylobacteriosis. (refer to Section 3.5)

DISCUSSION 8: Severity of illness is one of many factors that lead physicians to prescribe antibiotics to patients with a diarrheal illness.

Assumptions relating to bloody vs. non-bloody diarrhea:
ASSUMPTION 9: Patients with campylobacteriosis who have sought care and been requested to submit stool cultures and have submitted stool cultures are prescribed antibiotics at a rate that is the same whether they had bloody or non-bloody diarrhea. Conversely, if patients have sought care but have not been requested to submit stool cultures, they are prescribed at another rate that is the same whether they had bloody or non-bloody diarrhea.(refer to Section 3.5)

FoodNet to US extrapolation:
ASSUMPTION 10: The incidence rates for culture-confirmed *Campylobacter* infections in the FoodNet catchment are representative of incidence rates for culture-confirmed *Campylobacter* infections in the U. S. (refer to Section 1.2)

DISCUSSION 10: Although the incidence rates varied by site, from 10.2/100,000 in Maryland to 37.7/100,000 in California in 1998 (21), the overall rate of *Campylobacter* isolation is likely to reflect isolation rates in the U.S. population. Comparisons of demographic characteristics between the FoodNet sites and the U.S. population show similar distributions of sex, age, race and rural/urban distributions (Table 1.1).

In addition to demonstrating similarity in population composition, an evaluation of potential exposure is important. In a 1994-5 United States Department of Agriculture, Food Safety Inspection Service, survey, 88% of chicken carcasses were reported to carry *Campylobacter* at slaughter (Table 1.2)(104). Another estimate, of Campylobacter carriage on retail chicken products was demonstrated at a level of 88% in a Minnesota survey of chicken products in 1997 (92).

Another factor affecting incidence rates may be the sensitivity of stool culture methods as the methods for culturing stools are extremely diverse. Specimen handling is another factor that can greatly decrease the sensitivity of stool culture methods. In a review of non-typhoidal salmonellosis, an assumed estimate of the sensitivity of culture was 70% and was used to estimate the burden of salmonellosis in the United States (2). This estimate was adopted for determining the burden of campylobacteriosis in a recent review of foodborne disease (9).

DATA GAP 10: Incomplete knowledge of the sensitivity and specificity of culturing specimens for *Campylobacter* exists.

Invasive disease assumptions:
ASSUMPTION 11: All invasive campylobacteriosis cases seek care, have a specimen collected that yields *Campylobacter*, and is ascertained by FoodNet. (refer to Section 1.3)

DISCUSSION 11: It is not known precisely what proportion of persons with invasive *Campylobacter* infections seek care, but because persons with invasive *Campylobacter* infections will be moderately to severely ill, it is likely that most of these patients will seek care.

There is little knowledge of the completeness of ascertainment of invasive campylobacteriosis; the frequency with which laboratories are requested to test blood, CSF or other sterile specimens for *Campylobacter* and the sensitivity and specificity of the diagnostic tests used for isolation from blood and other sterile sites. Blood cultures usually represent more than 99% of all invasive isolations and most currently used blood culture systems are good for isolating *Campylobacter*, when it is present. The lack of information on the frequency of diagnostic requests and sensitivity may result in an underestimate of actual invasive disease rates. However, because the currently ascertained proportion of invasive cases is very small, approximately 1.0% of all confirmed cases, and most cases are likely to seek care, an increase in isolation of specimens classified as invasive is unlikely to have much impact on the overall number of cases of campylobacteriosis in the U.S.

DATA GAP 11: Data describing rates or cases of invasive disease seeking care, requests for diagnostic tests and the sensitivity of diagnostic procedures, such as blood culture, are not available.

ASSUMPTION 12: The proportion of fluoroquinolone prescriptions of total antibiotic prescriptions is the same for patients with invasive campylobacteriosis treated by their health care providers as it is for patients with enteric campylobacteriosis treated by their health care providers. (refer to Section 3.6)

ASSUMPTION 13: Because of the severity of illness upon presentation, all cases with invasive disease are presumed to seek care and are presumed to take antibiotics for their illness. (refer to Section 3.5)