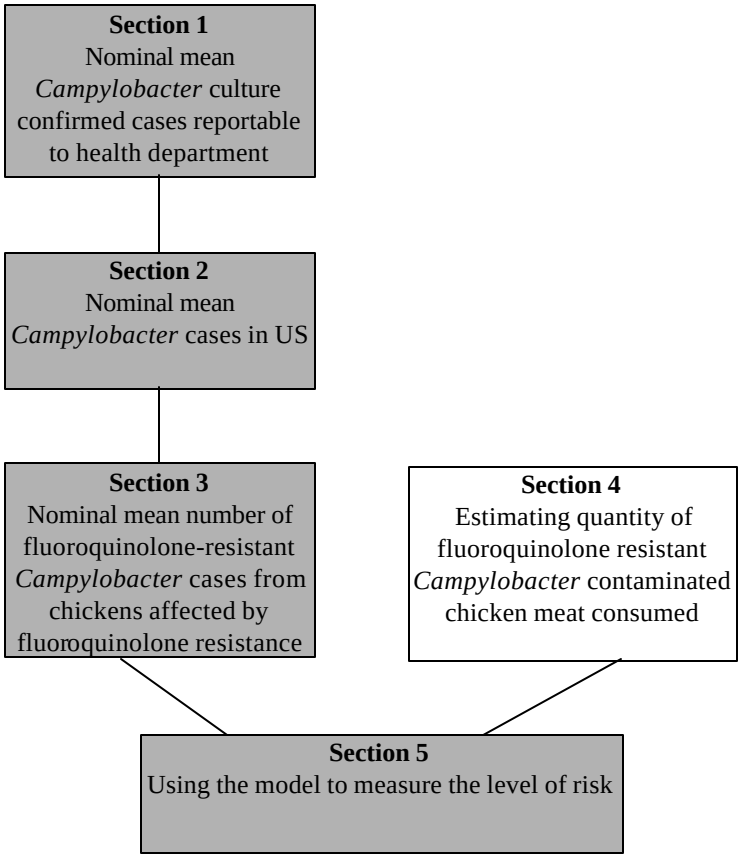
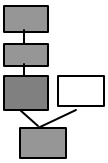
Section 4

Estimating quantity of fluoroquinolone resistant *Campylobacter* contaminated chicken meat consumed





Symbol	Description	Formula
p_c	Total prevalence of *Campylobacter* among broiler carcasses	Beta distribution based on data
p_{rc}	Prevalence of fluoroquinolone resistant *Campylobacter* among *Campylobacter* contaminated broiler carcasses	Beta distribution based on data
p_p	Estimated prevalence of fluoroquinolone-resistant *Campylobacter* in broiler carcasses	$= p_c * p_{rc}$
c	Consumption of boneless domestically reared chickens in U.S. per capita (lbs)	Data
V_c	Total consumption of boneless domestically reared chicken in U.S. (lbs)	$= c * n_{US}$
$\mathbf{V_i}$	Total consumption of boneless, domestically reared chicken contaminated with fluoroquinolone resistant *Campylobacter* in U.S. (lbs)	$\mathbf{=V_c * p_p}$

Overview for Section 4

This section estimates the burden of fluoroquinolone resistant *Campylobacter* on chicken carcasses by multiplying the carcass *Campylobacter* prevalence by the level of resistance in isolates from chickens. An estimate of the proportion of domestically reared chicken with fluoroquinolone resistant *Campylobacter* using food disappearance data, less imports, was calculated to account for changes in chicken consumption from year to year.

Parameters modeled include:

- Total prevalence of *Campylobacter* among broiler carcasses
- Prevalence of fluoroquinolone resistant *Campylobacter* among *Campylobacter* contaminated broiler carcasses

Output: Estimated prevalence of fluoroquinolone-resistant *Campylobacter* contaminated broiler carcasses

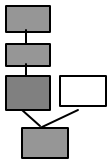
- Consumption of boneless domestically reared chickens in U.S. per capita (lbs)
- Total consumption of boneless domestically reared chicken in U.S. (lbs)

Output: Total consumption of boneless, domestically reared chicken contaminated with fluoroquinolone resistant *Campylobacter* in U.S. (lbs)

Parameter estimations

4.1 (p_c) - Total prevalence of *Campylobacter* among broiler carcasses

Approximately 200 broiler slaughter establishments were included in the sample, representing 87% of all broiler slaughter establishments under Federal inspection in 1994. The broilers slaughtered at these establishments accounted for more than 99.9% of all broilers slaughtered during the period. Sample size, to provide reasonable levels of precision for a national prevalence, was estimated at 1200 samples. To achieve this number of samples a random number of 1871 broiler carcass samples were requested during the 52-week sampling period. Some samples were not collected, some were collected but not analyzed and the total number of samples providing laboratory results for the prevalence estimate was 1297 samples (104). Sampling frame was based upon weekly identification of randomly selected establishments using probabilities for sample selection that were proportional to the slaughter volume of the selected establishments, therefore those establishments slaughtering a greater number of chickens were sampled more frequently than other establishments. Sample delivery constraints resulted in the restriction of



sampling to first shifts, Monday through Thursday. Carcasses were obtained from the drip line after the chill tank, the end point for slaughter and evisceration and prior to further handling and processing. Whole carcasses were randomly selected, and aseptically placed into a sterile bag that was securely closed, double bagged, packed with a gel pack and shipped to the laboratory via overnight delivery service. Only samples received at temperatures between 0 to 10 degrees C (inclusive) within one day of sample collection were analyzed. The analytical sample was obtained from rinse fluid recovered after shaking the broiler carcass in 400 ml of sterile Butterfield's Phosphate Diluent (104). Isolation was achieved using Hunt's Enrichment Broth, incubating the sample for 24 hours in a microaerophillic environment (5% O_2, 10% CO_2 and 85% N_2), followed by streaking onto Modified *Campylobacter* Charcoal Differential Agar for isolation of *Campylobacter* spp after incubation at 42 degrees C for 24 hours (81). Tests to identify *Campylobacter jejuni* and *coli* included wet mount examination, glucose fermentation, catalase, nalidixic acid, and oxidase tests. Nalidixic acid screening was performed to eliminate *Campylobacter* spp other than *jejuni* and *coli* from the prevalence estimate. Since fluoroquinolones were not licensed for use in poultry during the survey period, it was assumed that the level of nalidixic acid resistant isolates was low in 1994-5 because no selective pressure existed for chickens to develop fluoroquinolone resistance. Therefore, the prevalence estimate was unlikely to be affected by acquired resistance and potential misclassification of *Campylobacter* species.

ASSUMPTION: If a carcass was positive for *Campylobacter*, the predominant species isolated was *C. jejuni*.

The *Camplyobacter* prevalence estimate from the drip line was preferred because at this point carcasses were ready for further processing and had the least potential of human or other non-chicken sources of contamination. Post-chiller sampling of carcasses takes into account the cross-contamination from other chickens that occurs while in the chiller that leads to carriage of many diverse strains of *Campylobacter* on a single chicken product (92). The post-chiller location is a sampling point that is repeatable, practical, and provides isolates for susceptibility testing, closely linking these two parameters to provide a better estimate of the level of resistance. This would be more relevant for future surveys, when concurrent carcass prevalence and susceptibility testing could be conducted, as is currently underway in 1999.

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 parameter was thus modeled as:

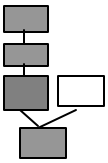
p_c = Beta(1144+1,1297-1144+1)

4.2 (p_{rc}) Prevalence of FQ resistant *Campylobacter* among *Campylobacter* broiler carcasses

Isolates were collected in a pilot survey by USDA-FSIS, from October to December 1998, from chicken carcass rinse samples (Section 4.1) and cultured as described previously (81). If growth was evident, a single colony was removed from the plate for susceptibility testing. A total of 128 *C. jejuni* isolates were collected from chicken carcasses for the period. The isolates were speciated using the biochemical hippurate assay and polymerase chain reaction (PCR) hippuricase primers to identify hippurase negative *C. jejuni* (74). The proportion of Fluoroquinolone resistant *C. jejuni* isolates was 9.4% (**12**/**128**), and *C.* "other" was 21.2% (14/66) (100). *C.* "other" were hippurate negative isolates, that were not *jejuni* as identified by PCR for the hippuricase gene and may include species such as *C. lari* that are intrinsically resistant to quinolones.

In 1999, collection of isolates was continuous throughout the year and a total of **481** *C. jejuni* isolates were obtained (101). Of these, **45** isolates were resistant so the proportion of isolates that were resistant was 9.4%.

The level of resistance to fluoroquinolone in *C. jejuni* from chickens was used in the risk assessment because the greater proportion of human disease, 92.7% in the *Campylobacter* Case Control Study, was due to *C. jejuni*. *C. coli* were not clearly distinguished from the group *C.* "other" which may have included *C.*



lari, a species intrinsically resistant to quinolones, therefore this precluded use of these isolates in the risk assessment.

DISCUSSION: Limitations in determination of the level of fluoroquinolone resistance in *Campylobacter* in 1998 included: the small number of isolates collected, the lack of seasonal representation. In addition, the presence of mixed colonies of organisms (*C. jejuni* and *C. coli*) when selecting a single colony makes species identification more complicated.

Unquantified Issues in the Assessment of the Prevalence of Resistance in Campylobacter isolates
Other problems were raised with the isolation and susceptibility testing of *Campylobacter*. Lack of agreement of MIC susceptibility test results occurs in up to 10% (2/20) of isolates subjected to repeat testing in one study (personal communication P. Fedorka-Cray). One explanation of the inconsistency is that the single colony may be composed of multiple isolates and that all isolates in the mixed colony may not have the same potential to survive storage, freezing, re-culture and testing. The effect of selecting a colony with multiple isolates decreases the reliability of susceptibility testing. The species of each reported isolate was confirmed by PCR of the *hipp-O* and *ceu* genes to identify *C. jejuni* and *C. coli* respectively.

In addition to the problem mentioned in the paragraph above, many varied *Campylobacter* colonies are present on a culture plate. The selection of a single colony from a plate of diverse colonies provides a "plate average," and the level reported will consistently underestimate the true carcass prevalence because a plate may carry resistant and susceptible isolates. A survey conducted in 1998-9 using a selective media for fluoroquinolone resistance indicated that of retail chicken products from which *Campylobacter* had been isolated, 24.5% (15/61) carried fluoroquinolone resistant isolates (82). Therefore, the actual prevalence of chicken carcasses carrying resistance may be much higher than the estimate obtained from testing a single isolate. Use of a quinolone-containing screening media would provide a better estimate of the true carcass prevalence and may give an indication of load of fluoroquinolone resistant *Campylobacter* on chicken. This may be a more accurate method to use to assess the impact of resistant pathogens.

The three issues described above; the lack of reliability of in identification of *Campylobacter* species using biochemical assay and 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 are issues that are currently not quantifiable. These issues need to be better characterized and methods developed to allow more meaningful assessments of their impact on both human and foodborne isolates. This risk assessment determined the measurable risk, limiting the model to those parameters for which data were relevant, valid and available.

The parameter was thus modelled as:

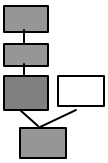
1998: p_{rc} = Beta(12+1,128-12+1)
1999: p_{rc} = Beta(45+1,481-45+1)

4.3 (p_p) - Estimated prevalence of fluoroquinolone-resistant *Campylobacter* in broiler carcasses

This parameter is calculated as:
$p_p = p_c * p_{rc}$

4.4 (c) - Consumption of boneless domestically reared chickens in U.S. per capita (lbs)
An annual value representing measurable human exposure to chicken in the United States less product sent for rendering, product diverted for pet food, exports, water added during processing and imports was the pounds of boneless broiler food disappearance, which in 1998 was 50.8 lbs per capita (102, 113).

1998 c = 50.8 lbs
1999 c = 54.3 lbs



4.5 (V_c) – Total consumption of boneless domestically reared chickens in U.S. per capita (lbs)

This parameter is calculated as:
$V_c = c * n_{US}$

4.6 (**V_i**) - Total consumption of boneless, domestically reared chicken contaminated with fluoroquinolone resistant *Campylobacter* in U.S. (lbs)

This parameter is calculated as:

$V_i = V_c * p_{rc}$

It represents the amount of boneless product contaminated with fluoroquinolone resistant *Campylobacter* consumed in the U.S. in the year. Figure 4.1 shows the uncertainty distribution for **V_i**.

Year	Model output	5th percentile	Mean	95th percentile
1998	V_I	7.34E+08	1.21E+09	1.77E+09
1999	V_I	9.68E+08	1.24E+09	1.54E+09

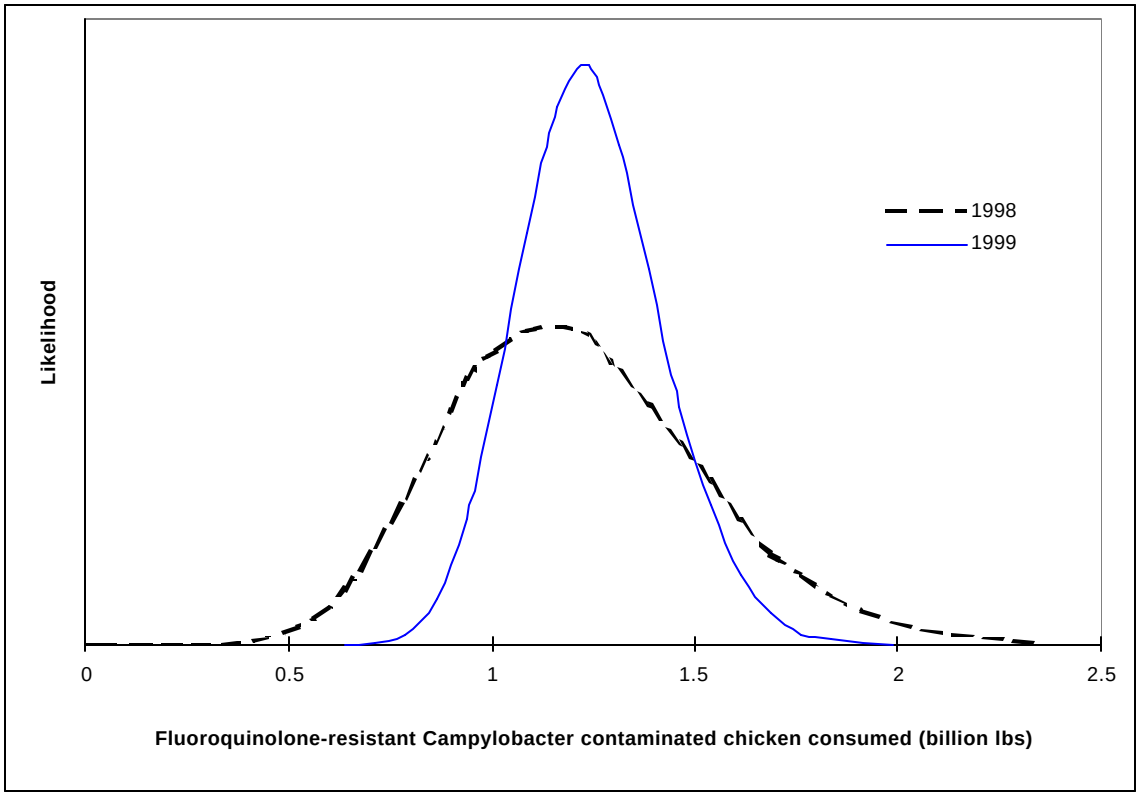
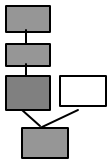


Figure 4.1. Uncertainty distribution for **V_i**.



Section 4 Summary

The mean estimated value for pounds of boneless chicken carrying fluoroquinolone resistant *Campylobacter* consumed in 1998 is 1,210,000,000 pounds. The 5th percentile estimate is 734,000,000 and the 95th percentile estimate is 1,770,000,000 pounds. In 1999, the mean estimated value was 1,240,000,000 pounds with a 5th percentile of 968,000,000 and a 95th percentile of 1,540,000,000. Relative contributions of the various components of the model to the model uncertainty will be presented in Section 5, Sensitivity Analysis.