Risk Analysis, Vol. 28, No. 3, 2008 DOI: 10.1111/j.1539-6924.2008.01054.x

A Stochastic Assessment of the Public Health Risks of the Use of Macrolide Antibiotics in Food Animals

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Campylobacteriosis is an important food-borne illness with more than a million U.S. cases annually. Antibiotic treatment is usually not required. However, erythromycin, a macrolide antibiotic, is recommended for the treatment of severe cases. Therefore, it is considered a critically important antibiotic and given special attention as to the risk that food animal use will lead to resistant infections and compromised human treatment. To assess this risk, we used a retrospective approach; estimating the number of campylobacteriosis cases caused by specific meat consumption utilizing the preventable fraction. We then determined the number of cases with macrolide resistance *Campylobacter* spp. based on a linear model relating the resistance fraction to on-farm macrolide use. In this article, we considered the uncertainties in the parameter estimates, utilized a more elaborate model of resistance development and separated *C. coli* and *C. jejuni*. There are no published data for the probability of compromised treatment outcome due to macrolide resistance. Therefore, our estimates of compromised treatment outcome were based on data for fluoroquinolone-resistant infections. The conservative results show the human health risks are extremely low. For example, the predicted risk of suboptimal human treatment of infection with *C. coli* from swine is only 1 in 82 million; with a 95% chance it could be as high as 1 in 49 million. Risks from *C. jejuni* in poultry or beef are even less. Reduced antibiotic use can adversely impact animal health. These low human risks should be weighed against the alternative risks.

KEY WORDS: Antibiotic resistance; *Campylobacter*; macrolide

1. INTRODUCTION

Campylobacter spp. are important food-borne pathogens causing more than a million human cases annually. The two most common species are *C. coli* and *C. jejuni*. *C. jejuni* is responsible for the majority (95%) of reported human illness.[1] Campylobacteriosis typically results in self-limited gastroenteritis characterized by a few days of diarrhea and fever. The clinical symptoms are generally mild and antibiotic treatment is not usually required. Erythromycin, a macrolide antibiotic, is recommended for the treatment of severe campylobacteriosis, and is therefore considered a critically important antibiotic by Food and Drug Administration (FDA) and the World Health Organization.[2] Critically important antibiotics are given special attention as to the risk that on-farm use will lead to resistant *Campylobacter* spp. and suboptimal human treatment.

Macrolide antibiotics such as Tylosin and Tilmicosin are beneficial in food animal disease prevention, control, treatment, and growth promotion (performance enhancement). They can enhance overall animal health. Considering these benefits, it is

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important to develop useful models that may aid in making effective risk management decisions. In this assessment, we estimate the potential human health risk due to food-borne *Campylobacter* spp. infections derived from on-farm macrolide use.

Traditional food safety risk assessments frequently employ a farm to fork approach where all the events in the casual chain leading to the risk are explicitly modeled. This approach is data intensive (requiring data such as dose-response relationships) and is more appropriate for analyzing the relative benefits of different risk management options rather than accurately estimating an upper bound to the risk. For instance, Nauta *et al.*[3] found that the farm-to-fork assessment overestimated the risk compared to that based on epidemiological data by more than 10 times. Recently, Bartholomew *et al.*[4] proposed a retrospective approach of back calculating the number of infections caused by specific meat consumption (such as eating chicken) from the total number of human infections utilizing the associative population attributable fraction (PAF) from epidemiological studies. However, a measure that is more relevant for risk assessment is the preventable fraction, defined as the fraction of human cases that would be prevented if a specific risk factor were removed.[5] Preventable fraction is more useful for policy making as it conveys some sense of how much of the problem might go away if the putative causal factor is removed.[5] While it is difficult to determine the preventable fraction exactly, an upper bound can be estimated from methods such as epidemiological and subtyping studies, assuming the observed associations as causal.

Although conservative, estimating the preventable fraction from epidemiological and subtyping methods is still useful in obtaining a concrete upper bound on the risk due to resistant development.

Utilizing the above retrospective linear approach, Hurd *et al.*[6] showed a minimal risk of less than 1 in 10 million for compromised treatment outcomes due to macrolide use in food animals. This model was a deterministic risk assessment that obtained a worst-case bound. In this article, we aim to improve upon the early deterministic assessment by considering the uncertainties in parameter estimates, utilizing a more elaborate model of resistance development, combining various approaches to estimate the preventable fractions and separating *Campylobacter coli* and *Campylobacter jejuni*.

A unique feature of our assessment is the linear model relating on-farm macrolide use to the macrolide-resistant fraction of the *Campylobacter* population from a food animal. Previous risk assessments such as Bartholomew *et al.*[4] attributed all the observed resistance to on-farm antibiotic use, potentially overestimating the risk. Our model enables us to specifically determine the resistance fraction attributable to antibiotic use based on the resistance data in conventional compared to antibiotic-free farms, as well as the national resistance surveillance data. Conventional farms, in this article, are defined as those that use antibiotics for any purpose, such as the treatment of ill animals, prevention of disease, or performance enhancement.

The epidemiology and the resistance development in *Campylobacter* spp. are very active areas of research and a significant amount of new data are available. These data enabled us to conduct separate assessments for C. *coli* and *C. jejuni*. This is imperative as there is a substantial difference in the background resistance levels and the infectiousness of these bacteria. Specifically, C. *coli* is frequently resistant to erythromycin (40% in antibiotic-free farms) while it causes less than 4% of the human campylobacter infections in the United States.[1] Note that the background resistance is not necessarily due to antibiotic use but the genetic makeup of the organism.

2. MODELING APPROACH OVERVIEW

Similar to Cox and Popken,[7] we follow a retrospective modeling approach where epidemiological data rather than dose-response studies are used for exposure assessment. The FDA adopted this approach as a basis for banning fluoroquinolone in chicken.[8] Shore[9] recommends the use of epidemiological data for exposure assessment, if high-quality data are available. However, the FDA's risk assessment assumed that on-farm antibiotic use caused all *Campylobacter* spp. resistance. No attempt was made to link antibiotic use to the resistance levels on farm. We utilized a linear model that relates the overall population mean resistance fraction (r_t) to the overall fraction of the population exposed to macrolide (α).

We divided the risk assessment model into the release, exposure, and consequence assessment sections as suggested by FDA Guidance Document 152.[10] In the release assessment section, we calculated the fraction of *Campylobacter* from animals resistant due to macrolide use. Next, in the exposure assessment section, we calculated the number of culture-confirmed human infections resistant due to on-farm macrolide use. Finally, in the consequence assessment section, we estimated the human health consequences.

Consequence is defined as a compromised treatment outcome due to macrolide resistance. These outcomes could be persistence of symptoms, additional days of diarrhea, invasive illness, septicemia, or unlikely mortality. Note there are no published data suggesting that macrolide-resistant *Campylobacter* infections are likely to have any compromised treatment outcomes. Therefore, our estimates of compromised outcome probability were based on some data for fluoroquinolone resistance.

2.1. Release Assessment

The objective of this section is to estimate r_m the fraction of *Campylobacter* from an animal (swine, poultry, and cattle) population that has resistance due to on-farm macrolide use. In this article, *Campylobacter* refers to either *C. coli* or *C. jejuni*, which are evaluated separately, not an undefined conglomerate of either species as is reported by some government data as *Campylobacter* spp. (USDA Food Safety Inspection Service (FSIS)).[11,12]

Even in the absence of prior exposure, bacteria have naturally varying degrees of antibiotic resistance. For example, *C. coli* has higher resistance (13.6%) levels to most antibiotics compared to *C. jejuni* (1%) even in countries where antibiotics have been banned for several years.[13] Attributing all the resistance to on-farm antibiotic use can lead to a substantial overestimation of risk. This fact is recognized in GD 152, which suggests that we estimate the resistance "above background."

As a first step, we utilized a linear model relating the overall fraction of animals exposed to macrolide (α) and the steady-state resistance fraction (r_t), assuming several cycles of animal production. By resistant fraction, we mean the fraction of total *Campylobacter* population that is resistant to the antibiotic. The constant of proportionality (p) in our linear model is a key parameter representing the propensity of the bacteria to develop resistance upon exposure to antibiotic. The p can be different depending on whether exposure is for treatment or nontreatment (performance enhancing or preventive) purposes. Given p, the fraction of *Campylobacter* resistant due to macrolide use (r_m) can be calculated from the macrolide exposure (usage) data. In the following, we will derive the conditions under which the linear model is appropriate and discuss methods to calculate the constant p.

Consider a conventional farm that utilizes antibiotics in which fraction (x) of the animals is exposed to macrolide for any purpose. Let r_b be the background resistant fraction that exists without exposure to macrolide. The fraction of susceptible bacteria $(1 - r_b)$ acquiring resistance upon exposure to macrolide is some function $f(x)$. Therefore, we can relate the resistant fraction in this conventional farm after several cycles of macrolide use (r_t) to the fraction (x) as given by Equation (1).

$$r_t = r_b + (1 - r_b) f(x). \tag{1}$$

In practice, there are both the conventional farms that utilize antibiotics and the antibiotic free farms (ABF) that do not. Comparing the resistance fractions between these farm types can be useful in estimating (p). Let the national fraction of conventional farms be y. Then, the national average resistance fraction over all farm types $E(r_t)$ can be calculated as the sum of the average resistant fraction in ABF farms, $E(r_b)$, and the increase in resistant fraction due to macrolide use $yE(1 - r_b)f(x)_c$ as given by Equation (2). The subscript c refers to the average over conventional farms. In utilizing Equation (2), we assume that conventional and ABF farms have similar average herd size, *Campylobacter* prevalence, and microbial loads on carcass.

$$E(r_t) = E(r_b) + yE((1 - r_b) f(x))_c. \tag{2}$$

Multiplying and dividing $yE((1 - r_b) f(x))_c$ in Equation (2) by $E(x) E((1 - r_b)$, where $E(x)$ is the average fraction of animals exposed to macrolide in a conventional farm, gives Equation (3). Equation (3) is more readily estimable from available industry data on $\alpha = yE(x)$ compared to Equation (2), which requires data on the fraction of conventional farms (y).

$$E(r_t) = E(r_b) + yE(x)E(1 - r_b)\frac{E((1 - r_b) f(x))_c}{E(1 - r_b)\ E(x)}. \tag{3}$$

From Equation (1), the increase in the resistant fraction due to macrolide exposure $(1 - r_b)f(x)$ is equal to $(r_t - r_b)$. Substituting this term into Equation (3), gives Equation (4).

$$E(r_t) = E(r_b) + yE(x)\ E((1 - r_b)\ \frac{E(r_t - r_b)_c}{E((1 - r_b)\ E(x)}. \tag{4}$$

The overall fraction of the food animal population exposed to macrolide (α) is given by $yE(x)$. Therefore, we can define the constant p according to Equation (5).

$$p = \frac{E(r_t - r_b)_c}{E(1 - r_b)E(x)}. \quad (5)$$

Substituting Equation (5) into Equation (4), we have

$$E(r_t) = E(r_b) + \alpha E((1 - r_b)p. \quad (6)$$

Equation (6) expresses the average resistance fraction across all the farms as the sum of the background resistance fraction $E(r_b)$ and the fraction resistant due to macrolide use r_m. The fraction, r_m, in turn is given by Equation (7).

$$r_m = \alpha E((1 - r_b)p. \quad (7)$$

The constant p can be calculated from either Equation (5) or Equation (6) depending on the available data. Equation (5) is estimable if there are data on the resistant fractions comparing a few conventional and ABF farms. We utilized Equation (5) to calculate p for the case of C. *coli* in swine. Equation (6) is appropriate when the source of specific isolates (ABF or conventional farms) is unknown, but the average fraction of animals exposed to macrolide α can be estimated. We utilized Equation (6) to calculate p for the case of chicken and cattle where national surveillance data were available without specifying the farm type (*C. jejuni*). The constant p can be different for treatment, control, prevention, and performance enhancement uses. While it is possible to consider different ps for these various purposes, we combined them as a generic exposure for simplicity. This simplification seems reasonable as we estimated the p based on performance enhancement and prevention purposes, accounting for the majority of macrolide exposures. Additionally, our assessment is conservative in that performance enhancement exposures, which we based our estimation on, may lead to higher resistance fraction and a greater p relative to other uses, such as treatment.[14,15] Given p, we calculated r_m utilizing Equation (7) for all applications.

The linear relationship between the national average resistant fraction $E(r_t)$ and fraction of animals exposed (α) is valid if (1) changes in α are only caused by changes in the fraction of conventional farms (y) and if (2) there is no spread of resistance between the individual farms. These assumptions are more appropriate for prevention and performance enhancement uses, where typically either none or all the animals in a farm are exposed to antibiotics. Conversely, if some animals from conventional farms are mixed with those from ABF farms or vice versa, there could be additional spread of resistance. The linear model is still useful as a first-order approximation when the above assumptions are not satisfied.

The central assumption in this model is that macrolide use has no impact on the overall prevalence or quantity of *Campylobacter* in animals, carcasses, or products. Therefore, by making this assumption we neglect any beneficial impact of antibiotic-induced animal health that might reduce carcass contamination. Singer *et al.*[16] developed a risk-risk assessment model that shows that antibiotic use could result in reduced number of human infections if it can reduce the rate of animal illnesses and lesions leading to decreased carcass contamination.

2.2. Exposure Assessment

The objective of this section is to estimate C_m, the number of culture-confirmed human infections caused by *Campylobacter* that are macrolide resistant due to its use in the specific food animal (swine, poultry, cattle). This definition is consistent with FDA Guidance Document 152, which states "exposure assessment is intended to estimate the probability that humans will be exposed to the hazardous agent through consumption of animal derived food commodities."[17]

An important parameter in calculating C_m is the preventable fraction (η) defined as the fraction of human cases that would be prevented if specific food animal meat were removed (e.g., pork, poultry, and beef) or if the pathogen was eradicated from that meat, regardless of resistance. Given an estimate of η, C_m can be calculated as the product of η, C_t, and r_m; where C_t is the total number of culture-confirmed human infections per year and r_m as defined earlier is the fraction of *Campylobacter* from a specific food animal that has macrolide use derived resistance (Equation 8). This approach to calculate C_m assumes that susceptible and resistant *Campylobacter* are equally likely to survive and cause illness.[4,18]

$$C_m = C_t \eta_t r_m. \quad (8)$$

Equation (8) represents a retrospective approach to exposure assessment based on η. It is an alternative to traditional dose-response modeling. Bartholomew *et al.*[4] proposed that a variation of this approach be utilized to predict how changes in the number of contaminated servings affect the number of human infections. Cox,[19] discusses the limitations of this model,

such as disregarding the nonlinear nature of the empirical dose-response relationships, not considering microbial loads, and calculating risk when there is none. However, we do not attempt to predict how the number of contaminated servings impacts the number of human infections. Consequently, the preceding limitations are not as restrictive in our case. Instead, we focus on resistance development and how that affects the fraction of historically estimated human infections with on-farm macrolide use derived resistance. Another concern mentioned by Cox[19] relates to using the PAF from epidemiological studies as representative of the preventable fraction (η). In general, PAF that indicates association may not be equal to η that represents causation. For the PAF, confounding variables and their interactions may not have been modeled, and the study population, from which the PAF is derived, may not be representative of overall population.[20] Therefore, when estimating η, it is important to complement epidemiological studies with data from other approaches and to rule out alternate casual mechanisms. Hertz-Picciotto[21] and Shore[9] recommend the use of epidemiological data for exposure assessment if high-quality data are available. Other methods to estimate an upper bound on the η include subtyping, time series analysis, and meat contamination rates.

A central assumption of our exposure assessment is that only the culture-confirmed cases are treated with erythromycin and face a risk of compromised outcome due to resistance. The following explains why this assumption is reasonable. The chain of events that must occur for a patient infected with *Campylobacter* to be treated with erythromycin without a culture is: (1) patient seeks care, (2) the doctor prescribes an antibiotic without requesting a culture, (3) the antibiotic is erythromycin.

(1) Patient seeks care—Since Campylobacteriosis is usually mild and self-limited, most patients do not seek medical care.[22]
(2) Antibiotics are prescribed without a culture—Most campylobacteriosis cases are self-limiting; antibiotics are only indicated under specific circumstances such as severe cases, prolonged illness, and for immunocompromised states.[23–25] Moreover, antibiotics may worsen the risk of hemolytic uremic syndrome (HUS) if the infection is due to EHEC, which is a major cause of bloody diarrhea in the United States. Therefore, use of any antibiotics, especially without culture, is contraindicated.[23,25]
(3) The prescribed antibiotic is erythromycin-– Without a culture the prescribed antibiotic is unlikely to be erythromycin. Campylobacteriosis is clinically indistinguishable from gastroenteritis caused by other organisms such as salmonella and shigella that are generally susceptible to fluoroquinolones.[23,25] Salmonella is largely resistant to erythromycin. Therefore, fluoroquinolones are the drugs of choice for treatment of diarrhea without culture.[26,27] Alternately, Rifaximin is becoming an increasingly popular treatment of diarrhea as it has few side effects.[28] Other antibiotics such as gentamycin, imipenem, and cephalosporins are recommended for initial therapy of bacteremic and immunocompromised patients before the culture results are ready.[29] No clinical guidelines recommend erythromycin for nondiagnosed diarrhea. Therefore, our assumption that erythromycin is only prescribed for a culture-confirmed case of Campylobacteriosis seems reasonable. However, this assumption can be validated by starting with a different C_t that includes nonconfirmed cases and then estimating the probabilities of the patient seeking care and treatment with erythromycin without culture.

2.3. Consequence Assessment

The objective of this section is to calculate C_{ao}, the annual number of compromised treatment outcomes caused by specific food animal macrolide use derived resistance in *Campylobacter* infections.

By compromised outcome, we mean that the patient did not respond to treatment as expected, resulting in any of the following: persistence of symptoms, that is, additional days of diarrhea, invasive illness, septicemia, or mortality. We are not aware of any direct evidence that macrolide resistance causes compromised treatment outcome. However, from studies on the impact of fluoroquinolone resistance, the potential compromised outcome is most likely persistent, symptoms, that is, 1–3 extra days of diarrhea.[24,30]

We calculated C_{ao} according to Equation (9), where τ is the joint probability that a culture-confirmed infection is treated with an antibiotic and

the prescribed antibiotic is a macrolide. The probability of a compromised outcome due to macrolide resistance is ρ.

$$C_{ao} = C_m \rho \tau. \quad (9)$$

For macrolides, the parameter ρ is likely to be zero. There is no direct evidence that erythromycin resistance causes any harm or, conversely, that erythromycin treatment for campylobacteriosis has clinical benefit unless given early.[31–34] Note that such early treatment with erythromycin without a culture is not recommended and assumed to be unlikely.[30] However, to be conservative, as zero consequence computes to a zero risk, we assumed that erythromycin resistance and fluoroquinolone resistance have a similar probability of causing a compromised outcome, utilizing the data on fluoroquinolone-resistant infections, discussed later.

2.4. Simulation and Sensitivity Analysis Methods

Given the parameter estimates, discussed below, we utilized @Risk software (@Risk 4.5.5 Palisade Corporation, Ithaca, NY) with 20,000 iterations and Latin hypercube sampling for simulation. To calculate the overall risk of on-farm macrolide use in all food animals combined, we summed up the annual compromised outcome estimates from the individual models and simulated with @Risk. We performed sensitivity analysis to identify which parameters contribute the most toward the uncertainty in the risk estimate. To conduct the analyses, we chose the rank correlation option where Spearman rank correlation coefficients are calculated between the output values and each set of sampled input values. The results are displayed as a tornado chart.

3. ESTIMATION OF PARAMETERS FROM DATA

In this section, we describe the estimation of model parameters from the available data. We estimated the parameters to determine the risk due to macrolide use for (1) *C. jejuni* infection from chicken, (2) *C. jejuni* infection from cattle, (3) *C. coli* infection from swine, and (4) C. *coli* infection from chicken. We did not consider the risk due to *C. jejuni* from swine and *C. coli* from cattle as the prevalence of these bacteria in the corresponding animals is low, resulting in negligible risk.[35–38] Conservative or risk increasing assumptions were made wherever data were insufficient.

3.1. Release Assessment

The objective of the release assessment is to estimate the fraction of *Campylobacter* from an animal population that has resistance due to on-farm macrolide use (r_m) (Equation (7)).

3.1.1. Release Assessment for C. coli *from Swine*

Resistant fractions in antibiotic-free farms (ABF) can be used to represent the background resistance (r_b). From a couple of recent studies on ABF farms in North Carolina, 38% of the 745 C. *coli* isolates were resistant.[37,39] The beta $(s + 1, n - s + 1)$ is the posterior uncertainty distribution for the probability of success with a uniform prior when s successes have been observed in n trials. Since $s = 283$ and $n = 745$, we utilized the beta (284, 463) distribution with a mean of 38% for r_b. Thakur and Gebreyes[39] found a resistant fraction of 77.1% based on 347 isolates in conventional farms where antibiotics were routinely used. Therefore, for r_t, we utilized the beta (268, 81) distribution with a mean of 77%.

From r_t and r_b, we calculated $p = 62\%$ for *C. coli* in swine (Equation (5)). Finally, from the industry surveys, the national fraction exposed α was estimated as 65%.[40] We calculated r_m utilizing Equation (7) as approximately 25%.

3.1.2. Release Assessment for C. jejuni *from Chicken*

The resistant fractions in *C. jejuni* from chicken have remained relatively low. A recent study found no resistance in conventional and organic broiler farms (not utilizing macrolides).[41] Therefore, we assumed r_b is zero. Data on the resistant fractions in chicken farms utilizing macrolide are scarce. From the NARMS USDA national surveillance data, the average-resistant fraction of *C. jejuni* in the years 1998–2003 was 0.89% from 2,245 isolates.[42] As these data were obtained from sampling over all farm types (regardless of conventional or ABF) the data represent the average resistance fraction $E(r_t)$. In addition, there was significant variation in the fraction exposed (α) during those years. To elaborate, α varied between 8.5% in 1999 and 0.07% in 2003 with an average of 4.3% (Rennier Associates 1999 and 2003 surveys[43]). We utilized the average α of 4.3% to calculate $p = 20\%$ assuming $r_b = 0$ (Equation (6)). This estimate of p is very conservative, as the data do not show any decrease in resistance fraction from 1999 to 2003

while fraction of chicken exposed to macrolide has decreased significantly. Finally, we calculated the current r_m utilizing the most recent estimate of α (07% in 2003) and Equation (7) ($0.07\% \times 1 \times 20\% = .0145\%$).

3.1.3. Release Assessment for C. coli *from Chicken*

For *C. coli* from chicken, the available data are not consistent with the hypothesis that macrolide use in chickens increases the resistant fractions. The resistant fraction was 15% in 1999 where α was 8.4% while it was 20% in 2003 when α was 0.07%. Similarly, studies comparing the resistance prevalence of *C. coli* in conventional and ABF chicken have found, conversely, a greater macrolide resistant fraction in ABF chicken.[41,44] According to these studies, the probability (p) is zero for *C. coli* from chicken. However, to be extremely conservative (risk increasing), we used the probability of resistance development for *C. coli* in swine (62%) for *C. coli* in chicken as well.

3.1.4. Release Assessment for C. jejuni *from Cattle*

The resistant fractions (r_t) of *C. jejuni* in cattle have remained low despite substantial use of macrolide. Inglis *et al.*[45] found no resistance in the 64 *C. jejuni* isolates from a macrolide treatment group. Sato *et al.*[46] found no resistance in 117 *C. jejuni* isolates from conventional dairy herds. Bae *et al.*[35] found a resistant fraction of 2.9% from 272 isolates from mixed (dairy, feedlot, etc.) farms. Englen *et al.*[36] found a resistance fraction of 0.8% from 381 *C. jejuni* isolates from feedlot cattle. The average resistance fraction (r_t) from the above data was estimated as 1.4%.

From a large industry survey, α for cattle is 45%.[40] Conservatively assuming that r_b is 0, and solving Equation (6), we have $p = 3.1\%$.

3.2. Exposure Assessment

The objective of the exposure assessment is to estimate the parameters required for calculating C_m according to Equation (8).

3.2.1. The Number of Culture-Confirmed Cases in United States (C_t)

A total of 5,665 culture confirmed *Campylobacter* spp. infections were observed in the CDC Foodnet catchment area in 2004.[47] We extrapolated these data to the U.S. population by multiplying by the ratio of the U.S. population of 298 million to the population of the Foodnet catchment area of 44.1 million (6.76). The gamma(X, 1) distribution has a theoretical basis for representing the uncertainty in the number of events given that X events have been observed in a period. We utilized a gamma (5,665, 1) $\times$ 6.76 with a mean of 38,315 cases for the overall number of culture-confirmed cases in the United States.[8]

Gupta *et al.*[1] reported that about 4.3% of all *Campylobacter* spp. clinical isolates were from *C. coli*. From this, we estimated that 1,665 of the *Campylobacter* spp. cases are *C. coli* while the remaining 36,650 cases were *C. jejuni*.

3.2.2. Preventable Fraction (η)

To estimate η for *C. jejuni* from chicken, we utilized a combination of studies employing different approaches such as epidemiologic studies, subtyping studies, and time-series analysis. Data on η for swine and beef are relatively scarce. For these cases, we estimated η from the meat contamination rates and risk increasing assumptions.

3.2.2.1. Preventable fraction of C. jejuni *from chicken (η)* For chicken, we utilized a combination of studies employing different approaches such as epidemiologic studies, subtyping studies, and time-series analysis to derive a general beta distribution for η. Details on the η estimates from various sources are summarized in Table I.

The PAF from epidemiological studies should be considered an upper bound to η because it is a measure of association and does not necessarily imply causation. Subtyping methods provide an upper bound on the preventable fraction based on classifying the *Campylobacter* isolates from various sources into subtypes. For example, if all the chicken and 20% of human isolates are subtype A, then the remaining 80% of human cases are not caused by chicken. The fraction, 20%, then is only an upper bound for η, as both chicken and humans could have been infected by some other source such as water.[48] From time-series analysis Vellinga and Van Loock[49] claimed that there was a 40% reduction in *Campylobacter* cases during the dioxin crisis when chicken was banned in Belgium. However, the 40% decrease was not unexpected or statistically significant based on the observed variability for the same time interval in other years.[50]

Giving equal weights for the η values from above data sources, the mean was set at 33%, the most likely (η) was set at 23% with a range of 20–61%. We utilized

No	Data Source	Country and Methodology	Preventable Fraction
1	Friedman *et al.*[69]	USA: Epidemiological	24%[a]
2	Nauta *et al.*[3]	Denmark: Epidemiological	23%[a]
3	Wingstrand *et al.*[70]	Denmark: Epidemiological	23%[a]
4	Nadeu *et al.*[71]	Canada: Subtyping	20%[a]
5	Safe Food[72]	UK: Subtyping	28.3%[a]
6	Champion *et al.*[73]	UK: Subtyping	44%[a]
7	Nielson[51]	Denmark: Subtyping	61%[a]
8	Vellinga and Loock[49]	Belgium: Time series analysis	40%[b]

Table I. Data Sources and Estimated Values for the Preventable Fraction of *C. jejuni* from Chicken

[a]The estimated value is an upper bound to the preventable fraction.
[b]The estimated value is not an upper bound. However, the study's conclusions have been debated.[50]

a general beta distribution, beta (1.11, 2.43, range: 0.2–0.61) with the above characteristics as the distribution for η in chicken.

3.2.2.2. Preventable fraction for C. jejuni *in cattle (η).* Given the limited data, we implemented a method of estimating an upper bound on η based on the meat contamination rates of beef relative to chicken. To calculate η, we conservatively assumed a maximum of 66% of all human *C. jejuni* cases are caused by beef and chicken combined, based on two subtyping studies.[24,51] We then allocated the total 66% into the fractions caused by either beef or chicken according to relative *C. jejuni* contaminated servings from beef and chicken.

The relative contaminated servings were estimated from carcass contamination rates and total volume of meat produced. The *Campylobacter* spp. carcass contamination rates at the slaughter and the retail sources varied considerably. Therefore, we calculated η separately from these two sources. For the uncertainty distribution of η, we utilized a uniform distribution between the η calculated from the slaughter data and the retail data. Table II summarizes the notation, data sources, and the estimated values of the parameters utilized in calculating η as explained below.

From slaughter, the Food Safety and Inspection Service (FSIS) provided *Campylobacter* spp. contamination rates but did not differentiate between C. *coli* and *C. jejuni*.[11,52] For beef, we assumed all isolates were *C. jejuni* with a 4% carcass contamination rate (Table II).

For chicken, we calculated the *C. jejuni* contamination rate (61.6%) as the product of *Campylobacter* spp. contamination rate (88%) from FSIS data and the fraction of *C. jejuni* (30%) among the *Campylobacter* spp. as reported by NARMS retail data.[53,54]

From the carcass contamination rates calculated above and the annual meat production quantities as shown in Table II, 4% $\times$ 11.1 billion = 450 million kilograms of beef servings and 34% $\times$ 16 billion = 9.8 billion kilograms of the chicken servings are contaminated with *C. jejuni* each year. From the above, 4.3% of the total (chicken and beef) contaminated servings are from beef. However, since chicken and beef together cause at most 66% of *C. jejuni*

Table II. Calculation of the Preventable Fraction (η) of *C. jejuni* for Beef from Carcass and Retail Meat Contamination Data

Parameter Details	Beef	Chicken
Annual kilograms of meat produced[74,75]	11.1 billion	16 billion
Campylobacter spp. carcass contamination rates from FSIS data (1994)[11,52]	4% beta (831,984)	88% beta (1,149, 190)
Fraction of *C. jejuni* among *Campylobacter* spp. isolates using NARMS retail data[53,54]	1 (assumption)	70% beta (527,231)
Carcass contamination rate of *C. jejuni* at slaughter	4%	61.6%
Annual kilograms of contaminated meat servings from slaughter	450 million	9.8 billion
Preventable fraction η from slaughter data	2.8%	63.2%
Contamination rate of *C. jejuni* on retail samples[53,54]	.12% beta (21,522)	34% beta (6,141,180)
Annual kilograms of contaminated meat servings from retail data	13 million	5.4 billion
Preventable fraction η from retail data	0.16%	65.84%
Distribution for preventable fraction η	Uniform (0.16%,2.8%)	

Note: The beta distributions were utilized to model the uncertainty in the parameter estimates. The η values for chicken were shown for completeness and were not utilized in the chicken risk assessments.

Table III. Calculation of the Preventable Fraction (η) of *C. coli* for Swine and Chicken from Campylobacter Carcass Contamination Data

Parameter Details	Chicken	Swine
Annual kilograms of meat produced[74,75]	16 billion	9.3 billion
Campylobacter spp. carcass contamination rate from FSIS data[11,12]	88% beta (1,149, 190)	32% beta (6,661,448)
Fraction of *C. coli* among *Campylobacter* spp. isolates using NARMS retail data[53,54]	30% beta (231,527)	1 (assumption)
C. coli carcass contamination rate at slaughter	27%	32%
Expected fraction of swine carcass processed into ground meat[6]	–	21%
Overall fraction *C. coli* contaminated servings from slaughter	27%	6.72%
Annual kilograms of *C. coli* contaminated servings	4.4 billion	0.620 billion
Preventable fractions η for *C. coli*	87.6%(86–89)%	12.4%(11–14)%

Note: The beta distributions were utilized to model the uncertainty in the parameter estimates.

cases, the corresponding η for beef is 66% $\times$ 4.3% = 2.8%.

Alternatively, using retail data from the NARMS, we calculated a η of 0.16% using a similar approach as for slaughter sources. Details for estimation of η from retail sources are provided in Table II. Finally, combining the retail and slaughter estimates, we utilized a uniform distribution between 0.16% and 2.8% with a mean of 1.4% for the preventable fraction (η).

3.2.2.3. Preventable fraction for C. coli *for swine or chicken* For *C. coli* from swine or chicken, we used a similar method as beef assuming all the *C. coli* infections (C_t) are caused by eating only pork or chicken and then allocating the cases according to relative carcass contamination rates. Table III provides the data sources and the estimated values of the parameters utilized in calculating η. We calculated the *C. coli* chicken carcass contamination rate from the FSIS data of 27% as the product of the *Campylobacter* spp. contamination rate from FSIS data and the fraction of *C. coli* among chicken isolates from the NARMS data.

To estimate the kilograms of contaminated pork, we assumed that all ground pork from a contaminated swine carcass is contaminated, while the nonground pork is not. This assumption seems reasonable as the prevalence of *C. coli* in nonground meat was extremely low (0.4%) from NARMS retail data. Roughly 21% of the swine carcass is processed into ground meat.[6] Hence we calculated that 21% $\times$ 32% = 6.72% of all servings of pork are contaminated with *C. coli*. For chicken, we assumed all servings from a contaminated carcass were contaminated. We calculated the preventable fraction (η) for swine as 12.4%, as shown in Table III.

3.3. Consequence Assessment (C_{ao})

The objective of the consequence assessment is to calculate the annual number of compromised treatment outcomes due to on-farm macrolide use, according to Equation (9).

3.3.1. Probability That a Culture-Confirmed Case of Campylobacteriosis Is Treated with Macrolide (τ)

We calculated the probability that a culture-confirmed case of campylobacteriosis is treated with a macrolide as the joint probability of being treated with an antibiotic and the prescribed antibiotic being a macrolide. Data on these parameters are available from epidemiological studies. In Minnesota, Smith *et al.*[55] found that 83% of 390 culture-confirmed patients were treated with antimicrobials and 25% (81) of those treated patients took macrolide. A large case series study in the United States found that 83% of the 740 culture-confirmed patients took some antibiotic, of which 497 patients knew which antibiotic they used.[56] Among them, 258 patients took only fluoroquinolone while 239 patients took some other antibiotic. We very conservatively assumed that the 239 (40%) of patients who had taken an antibiotic other than fluoroquinolone took erythromycin. Combining the data from the above studies, 938 of 1,130 (83%) patients were prescribed an antibiotic and at most, 320 of 819 (38.9%) patients could have taken macrolide.

Multiplying the appropriate conditional probabilities of being treated with an antibiotic and the prescribed antibiotic being a macrolide, we estimated the distribution of τ as beta (321, 500) $\times$ beta (939, 193) with a resulting mean of 32% for τ.

3.3.2. Probability of Suboptimal Treatment Response for Patients with Macrolide-Resistant Infections (ρ)

We are not aware of any epidemiological studies on the compromised treatment outcomes of campylobacteriosis due to macrolide resistance. In addition, numerous clinical trials found no clinical benefit of erythromycin treatment of macrolide susceptible campylobacteriosis.[31–34] Hence, for erythromycin, ρ is likely zero. However, to be conservative, we estimated this parameter from two studies on the efficacy of fluoroquinolone treatment for U.S. troops stationed in Thailand. The first study found that 5 of 16 (31%) patients infected with fluoroquinolone resistant *Campylobacter* experienced a compromised outcome (such as persistence or relapse of diarrhea).[56] The second study found a compromised treatment response among 13(7.7%) ciprofloxacin-resistant infections.[57] Here, adverse outcome or compromised treatment was defined as either continuance of symptoms or a relapse of diarrhea and did not include septicemia or death. From these two studies, we have that 6 out of 29 infections with fluoroquinolone resistance experienced a compromised outcome. Therefore, we utilized a beta (7, 24) distribution with a resulting mean of 22% for the probability of a compromised outcome (ρ).

4. RESULTS

The parameter estimates, their distributions, and the results for the risk assessment are provided in Table IV. The results show that the risk of a compromised treatment outcome due to macrolide-induced resistance in *Campylobacter* spp. is relatively low. Recall, this estimate is based on probability of a

Table IV. Parameter Estimates and Risk of Adverse Treatment Outcomes to *Campylobacter* Infections that Have Food Animal Macrolide Use Derived Resistance

Parameter	Swine *C. coli*	Chicken *C. coli*	Chicken *C. jejuni*	Cattle *C. jejuni*
Release Assessment				
Fraction of animals exposed to macrolide (α)	65%	0.07%	0.07%	45%
Fraction resistant in absence of macrolide use (r_b)	38% beta (284,463)	(equal swine)	0% (assumed)	0% (assumed)
Resistant fraction in farms utilizing macrolide (r_t)	77%) beta (26,8 81)	(equal swine)	0.89% beta (202,227)	1.4% beta (12,823)
Proportionality constant (p)	62%	(equal swine)	20%	3.1%
Fraction resistant due to macrolide use (r_m)	25.119%	0.0437%	0.0145%	1.4%
Exposure Assessment				
Annual number of culture-confirmed *Campylobacter* spp. cases		38315 gamma (5,665,1) × 6.76		
Fraction of *C. coli* among *Campylobacter* spp. cases		4.3% beta (641,409)		
Annual number of *C. coli* or *C. jejuni* cases (C_t)	1,665	1,665	36,650	36,650
Preventable fraction (η)	12.4%	87.6%	33% beta (1.1, 2.4)	1.48% uniform (0.16%, 2.8%)
Number of human cases resistant due to macrolide use in food animals/year (C_m)	206	0.638	1.746	7.96
Consequence Assessment				
Probability that a culture-confirmed infection is treated with a macrolide (τ)		32% beta (321,500) × beta (939,193)		
Probability of a compromised outcome given macrolide resistance (ρ)		22% beta (7,24)		
Results Estimated				
Annual number of adverse outcomes (C_{ao}) (median)*	3.62 (1.8–6.3)	0.04 (.022–.076)	.11 (.05–.24)	0.49 (.08–1.4)
Annual risk of a compromised outcome due to macrolide use for a person in the United States (median)	1 in 82 (49–165) million	1 in 6.2 (3.6–12.5) billion	1 in 2.4 (1.2–5.6) billion	1 in 608 (212–3,700) million

*Based on fluoroquinolone-resistant infection data.

Confidence intervals provided are two sided at 90%. The human health consequences are given as medians. Annual risk was calculated as the ratio of the U.S. population of 298 million/C_{ao}.

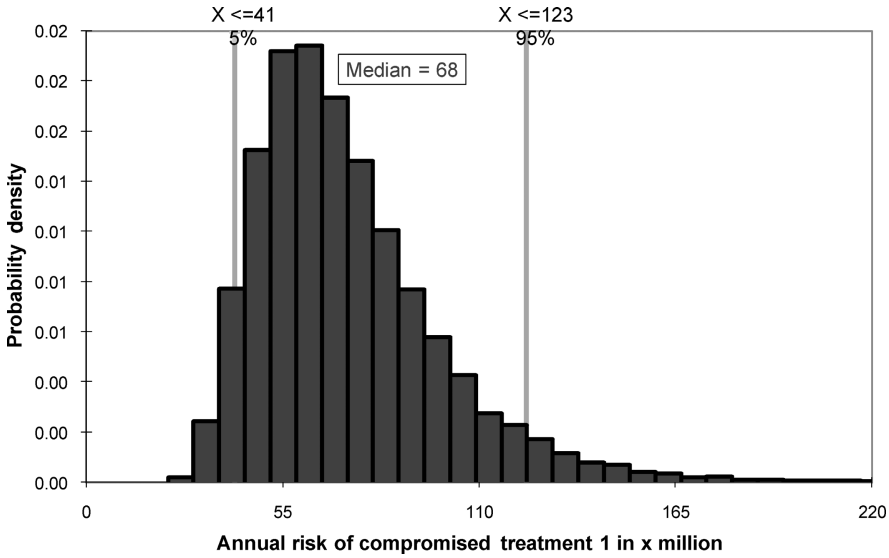

Fig. 1. Annual risk of a compromised outcome for a person in the United States due to macrolide use induced resistance in *Campylobacter* spp. from cattle, chicken, and swine combined.

compromised outcome (ρ) derived from data on fluoroquinolone resistance. The median risk of an adverse outcome is less than 1 in 82 million for *C. coli* from swine, 1 in 2.4 billion for *C. jejuni* from chicken, 1 in 6.2 billion for *C. coli* from chicken, and 1 in 608 million for *C. jejuni* from cattle. The distribution for the overall macrolide use derived risk from all food animals is shown in Fig. 1. The median risk due to macrolide use in all the three animals combined is less than 1 in 68 million. The risk is less than 1 in 41 million with 95% confidence. We can observe that the distribution has a long right tail, implying that there is some small chance that the risk is below negligible (1 in 123 million). Conversely, the probability of higher risk (greater than 1 in 25 million) is extremely low.

4.1. Sensitivity Results

Figs. 2, 3, and 4 show the tornado plots for the risk of a compromised outcome due to macrolide-induced resistance in *C. coli* from swine, *C. jejuni* from chicken, and *C. jejuni* from cattle, respectively. We did not perform sensitivity analysis for *C. coli* from chicken as the risk was too small. The probability of a compromised outcome (ρ) is contributing the most toward uncertainty in all the three cases. This is because (ρ) was

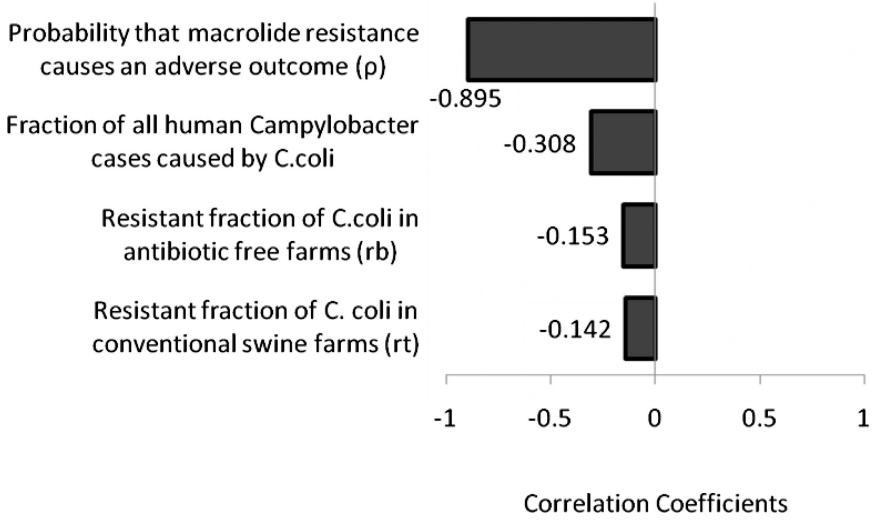

Fig. 2. Tornado plot for annual risk of a compromised treatment outcome due to macrolide induced resistance in *C. coli* from swine.

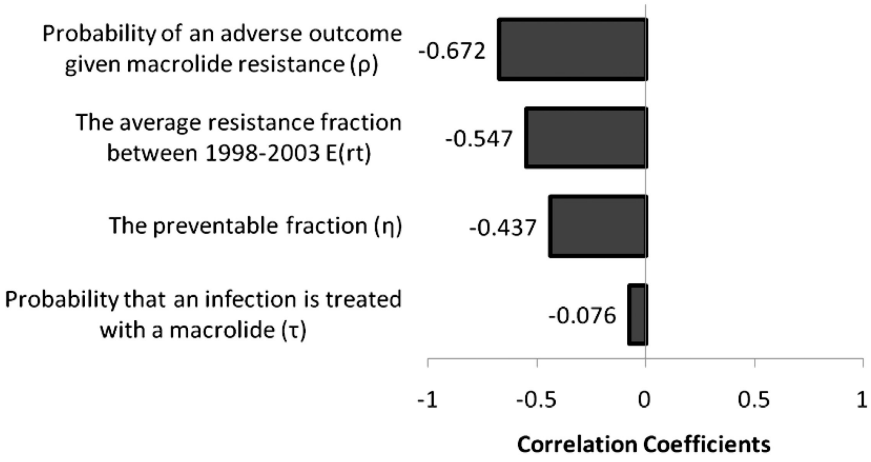

Fig. 3. Tornado plot for annual risk of a compromised treatment outcome due to macrolide induced resistance in *C. jejuni* from chicken.

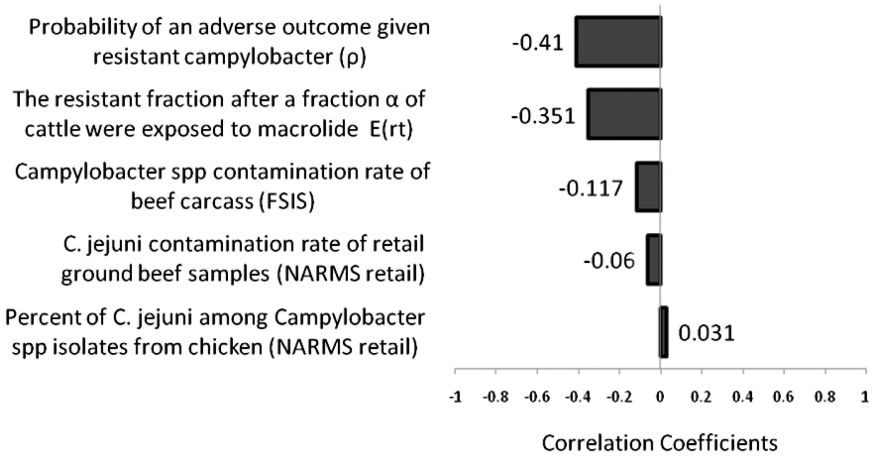

Fig. 4. Tornado plot for annual risk of a compromised treatment outcome due to macrolide induced resistance in *C. jejuni* from cattle.

estimated from two small fluoroquinolone studies in Thailand with only 29 cases overall.

Additionally, for *C. coli* from swine (Fig. 2), the risk is quite sensitive to the fraction of human infections caused by *C. coli* compared to *C. jejuni*. As there is a large number of campylobacteriosis cases, any variation in this parameter would cause a relatively large change in the total number of *C. coli* cases (C_t).

For *C. jejuni* from chicken (Fig. 3), the preventable fraction (η) and $E(r_t)$ the average resistance fraction in the years 1998–2003 (NARMS USDA) is leading to significant uncertainty in risk. The preventable fraction for chicken is a widely debated parameter with varying values estimated by different studies. Therefore, the estimate we used was given a large uncertainty distribution. Although the data utilized to estimate $E(r_t)$ had a large sample size, the mean was quite low (.84%), resulting in a relatively high coefficient of variation (.22). The high coefficient of variation is leading to a wider uncertainty since the risk is proportional to $E(r_t)$ given our assumption of $r_b = 0$ when calculating p.

For the risk due to *C. jejuni* from cattle, the average resistance fraction among all the farms $E(r_t)$ and the *C. jejuni* contamination rate in NARMS retail data are leading to substantial uncertainty (Fig. 4). Data utilized for estimating $E(r_t)$ for beef had a smaller sample size compared to chicken, leading to a greater uncertainty. Similarly, the ground beef contamination rate at retail from NARMS data had a greater uncertainty also owing to the smaller sample size.

5. DISCUSSION

In this stochastic risk assessment, we estimated the annual average risk of adverse health outcomes due to macrolide-induced resistance in *C. coli* and *C. jejuni* from cattle, chicken, and swine assuming macrolide resistant infections cause adverse

outcomes. We also estimated the uncertainty in the risk by utilizing data-centered distributions for the parameters. Overall, our results show that the human health risk due to macrolide-induced resistance in *C. coli* and *C. jejuni* from cattle, chicken, and swine is very low, especially if the uncertainties in the parameter estimates are considered. Furthermore, these risks are based on adverse treatment probability (ρ) of 22%. There is no published evidence that ρ is nearly this high for erythromycin. If erythromycin treatment is not very beneficial for recovery, then the actual risk of resistant infection and on-farm macrolide uses could be virtually zero.

We have endeavored to be conservative (risk increasing), particularly for parameters for which data are insufficient. For example, to estimate p (probability of resistance development), wherever the data comparing antibiotic-free farms and conventional farms are not available, we assumed that all of the observed resistance is due to macrolide use ($r_b = 0$). This assumption is clearly incorrect as NARMS USDA data do not show any decrease in *Campylobacter* resistance from chicken during 1999–2003 although the usage reduced drastically (8.5% to .07%). This observation suggests $r_b > 0$. In the exposure assessment (*C. jejuni* from chicken), we included data from subtyping studies that are actually upper bound (worst-case) estimates of the proportion of illness due to chicken. Similarly, for *C. coli*, we assumed all human infections were caused by eating either chicken or pork, ignoring all other possible sources. Finally, as noted, we assumed that erythromycin-resistant human infections lead to the same consequences as fluoroquinolone-resistant infections (ρ), although clinical trials of erythromycin actually show no effect on diarrhea symptoms.

It is interesting that the health risks from each food animal group are low, in this model, for different reasons. In chicken, there has been a drastic reduction in the use of macrolide since 1999. Only a negligible fraction is currently exposed (0.07%). For beef, the carcass and the retail meat prevalence of *C. jejuni* is very low (0.1–4%). For swine, *C. coli* causes only 4% of human infections, and the preventable fraction for *C. coli* in swine is low (12.5%). These differences in the factors affecting the risk demonstrate the importance of performing a separate assessment for each combination of pathogen and animal.

Models such as these are important to identify and prioritize data needs. The uncertainty analyses (Figs. 2–4) identify key parameters whose ambiguity affects the risk most. It is noteworthy that these parameters have a significant place in the current public policy debate about antibiotic use in food animals; the probability of adverse outcome (ρ), the amount of resistant bacteria in the food animal population (r_t), and the preventable fraction (η). The discussions in FDA 152, and the WHO around "critically important" drugs are focused on the magnitude of ρ. The assumption seems to be that a macrolide resistant *Campylobacter* infection will have a high likelihood of adverse outcome because erythromycin is listed as the drug of choice in clinical guidelines such as the Sanford Guide.[58] However, based on available published data, the value of ρ seems low and the uncertainty is high. Several clinical trials did not find any difference in the duration of diarrhea between the erythromycin treatment and placebo groups.[31–34] Given the lack of data for erythromycin resistance, we assumed that erythromycin resistance leads to a similar rate of adverse outcomes as fluoroquinolone resistance. Hence, there may be a need for more studies on the impact of erythromycin resistance.

The amount of resistant bacteria in chicken (r_t) was based on relatively few recovered resistant *Campylobacter* isolates (mean 0.89% for 1998–2003) (NARMS USDA.[42]) A large number of susceptible isolates were recovered, but very few resistant. Some people's entire basis of concern is based on this small foundation of data. Statistically, based on the beta distribution, the coefficient of variation (0.22) is fairly large when the mean is small, producing a wide range of uncertainty in the final risk estimate. Hence, it is advisable for resistance surveillance programs such as NARMS to maintain large and representative sampling frames. Additionally, for pork, relatively few *Campylobacter* (12 from NARMS retail 2002–2004[53,54]) (susceptible or resistant) isolates were recovered from chops. Perhaps another pork sample type should be considered.[59] A potential improvement that can greatly benefit risk assessments is to classify the retail samples according to conventional and antibiotic-free sources. These data will allow better estimates of the background (r_b) and antibiotic usage (r_t) resistance levels.

As noted, the preventable fraction (η) is difficult to determine, contributing to much uncertainty for this parameter. We used many different studies and methods to estimate this parameter. In an effort to include all these studies, the resulting uncertainty distribution for chicken was quite large (20% to 61%). Insufficient data on the preventable fraction necessitated the use of very conservative assumptions, resulting in inflated risk estimates. Better estimation

of η will remove cases that are not caused by meat consumption, and therefore not responsive to antibiotic reduction efforts. Fortunately, research efforts are beginning that may better address this parameter (Med-Vet-Net[60] and WHO: FERG[61]). It is likely that many of the campylobacteriosis cases will eventually be attributed to nonfood sources and the preventable fraction will be reduced.[62–64] As a result, so will the estimated risk.

The linear release assessment model proposed in this article is most appropriate when changes in the fraction of animals exposed to macrolide are caused only by the number of farms using antibiotics, not the number of animals exposed within the farms. This assumption applies best to prevention and growth promotion uses where the whole barn is typically exposed. Hence, our model can be utilized to predict the effects of an increase in the fraction of farms utilizing macrolide. In other cases (treatment uses), our model is less accurate, but still useful as an approximation.

Although we did not explicitly model cross-contamination of other foods and meats, our model incorporates cross-contamination implicitly for the following reasons. For cattle or chicken, the preventable fraction includes subtyping and time-series studies that are independent of the route of contamination. In the case of swine, we allocated all reported cases to contaminated meat, including those caused by cross-contamination. In all cases the model "starts" with culture-confirmed cases, from all sources, similar to RRRT framework proposed by Cox *et al*.[65]

The cross-transfer of resistance from *C. coli* to *C. jejuni* has been observed for tetracycline and kanamycin resistance. However, we are not aware of any reports showing such transfer for erythromycin resistance. Moreover, erythromycin resistance in *C. jejuni* in chicken has remained low despite the coexistence with a significant proportion of *C. coli* in poultry. Further study is required to determine whether such cross-transfer is possible.

Our model is focused on the impact of on-farm derived resistance. It does not model any changes to the total number of human infections that might change due to antibiotic reduction policies that might affect animal health (i.e., risk-risk tradeoffs). Some studies have shown higher *Campylobacter* prevalence in organic (antibiotic-free) farms compared to conventional.[39,41,66] Additionally, Singer *et al*.[16] and Cox[18] suggest that an antibiotic ban in poultry could increase the fraction of ill animals, leading to greater *Campylobacter* loads and increased human illness with nonresistant *Campylobacter*. Preliminary work by Hurd *et al.*[67] suggests that reductions in swine health results in increased carcass contamination with *Campylobacter* and fecal indicator organisms. When assessing the impact of a risk management decision such as an antibiotic use restriction these secondary consequences should be considered. Additional secondary consequences include increased use of treatment antibiotics to treat those diseases prevented by so-called performance enhancing uses.[68]

Overall, our results show that the human health risks due to macrolide-induced resistance in *Campylobacter* are extremely low with 95% confidence. Hence, considering the low risk, it may be prudent to investigate any human health benefits of antibiotic use in animals before restricting usage.

ACKNOWLEDGMENTS

At the time of publication, Dr. Hurd was serving as Deputy Undersecretary for Food Safety at the U.S. Department of Agriculure. The opinions expressed in this article do not necessarily reflect those of the USDA.

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