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Research Paper

Quantitative Risk Assessment of Antimicrobial-Resistant Foodborne Infections in Humans Due to Recombinant Bovine Somatotropin Usage in Dairy Cows

RANDALL S. SINGER,[1,2]* PAMELA L. RUEGG,[3] AND DALE E. BAUMAN[4]

[1]*University of Minnesota, St. Paul, Minnesota 55108;* [2]*Mindwalk Consulting Group, LLC, Falcon Heights, Minnesota 55113;* [3]*University of Wisconsin, Madison, Wisconsin 53706; and* [4]*Cornell University, Ithaca, New York 14853, USA*

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ABSTRACT

Recombinant bovine somatotropin (rbST) is a production-enhancing technology that allows the dairy industry to produce milk more efficiently. Concern has been raised that cows supplemented with rbST are at an increased risk of developing clinical mastitis, which would potentially increase the use of antimicrobial agents and increase human illnesses associated with antimicrobial-resistant bacterial pathogens delivered through the dairy beef supply. The purpose of this study was to conduct a quantitative risk assessment to estimate the potential increased risk of human infection with antimicrobial-resistant bacteria and subsequent adverse health outcomes as a result of rbST usage in dairy cattle. The quantitative risk assessment included the following steps: (i) release of antimicrobial-resistant organisms from the farm, (ii) exposure of humans via consumption of contaminated beef products, and (iii) consequence of the antimicrobial-resistant infection. The model focused on ceftiofur (parenteral and intramammary) and oxytetracycline (parenteral) treatment of clinical mastitis in dairy cattle and tracked the bacteria *Campylobacter* spp., *Salmonella enterica* subsp. *enterica,* and *Escherichia coli* in the gastrointestinal tract of the cow. Parameter estimates were developed to be maximum risk to overestimate the risk to humans. The excess number of cows in the U.S. dairy herd that were predicted to carry resistant bacteria at slaughter due to rbST administration was negligible. The total number of excess human illnesses caused by resistant bacteria due to rbST administration was also predicted to be negligible with all risks considerably less than one event per 1 billion people at risk per year for all bacteria. The results indicate a high probability that the use of rbST according to label instructions presents a negligible risk for increasing the number of human illnesses and subsequent adverse outcomes associated with antimicrobial-resistant *Campylobacter, Salmonella,* or *E. coli.*

Key words: Antimicrobial resistance; Clinical mastitis; Quantitative risk assessment; Recombinant bovine somatotropin

Recombinant bovine somatotropin (rbST) is a production-enhancing technology that allows the dairy industry to produce milk more efficiently. As one of the first proteins produced through the application of biotechnology, rbST has been marketed in the United States since 1994 under the trade name Posilac *(4)*. Although the milk composition is unaltered, cows receiving rbST require less feed nutrients, produce less animal waste, and have a reduced carbon footprint per unit of milk produced *(9)*. Over the first 20 years of use, over 35 million U.S. dairy cows have received rbST *(60)*.

Throughout the extensive use of rbST in the United States and many other countries, no evidence has been found that illness in rbST-supplemented cows has increased, including the incidence of clinical mastitis *(36, 60)*. In two recent reviews, a systematic review by the 78th meeting of the Joint Food and Agriculture Organization–World Health Organization Expert Committee on Food Additives *(36)* of clinical and epidemiological studies with dairy animals conducted from 1998 to 2013 and a comprehensive meta-analysis by St-Pierre et al. *(60)* of all peer-reviewed studies with dairy cows in which the rbST formulation approved by the U.S. Food and Drug Administration (FDA) was used in accordance with the approved label, use of rbST did not increase the risk of clinical mastitis. Although the difference between rbST-supplemented and nonsupplemented cows was not statistically significant, the point estimate of this difference was not zero *(60)*. A recent report by the European Food Safety Authority *(25)* included the following statement concerning antimicrobial resistance (AMR) in dairy cattle: "Assuming that treatment with rbST can lead to an increased incidence of mastitis in dairy cattle, that cases of mastitis are usually treated with antimicrobials, that the use of antimicrobials can lead to the development of AMR in dairy cattle (and in dairy cattle farms), and that AMR in humans may derive from both the exposure to AMR bacteria/genes of cattle origin and residues of antimicrobials, it is concluded that an increase of AMR in humans following to [sic] the use of rbST in dairy cattle is plausible" (p. 3).

The objective of the present study was to perform a quantitative risk assessment (QRA) to estimate the potential

* Author for correspondence. Tel: 612-625-6271; Fax: 612-625-5203; E-mail: rsinger@umn.edu.

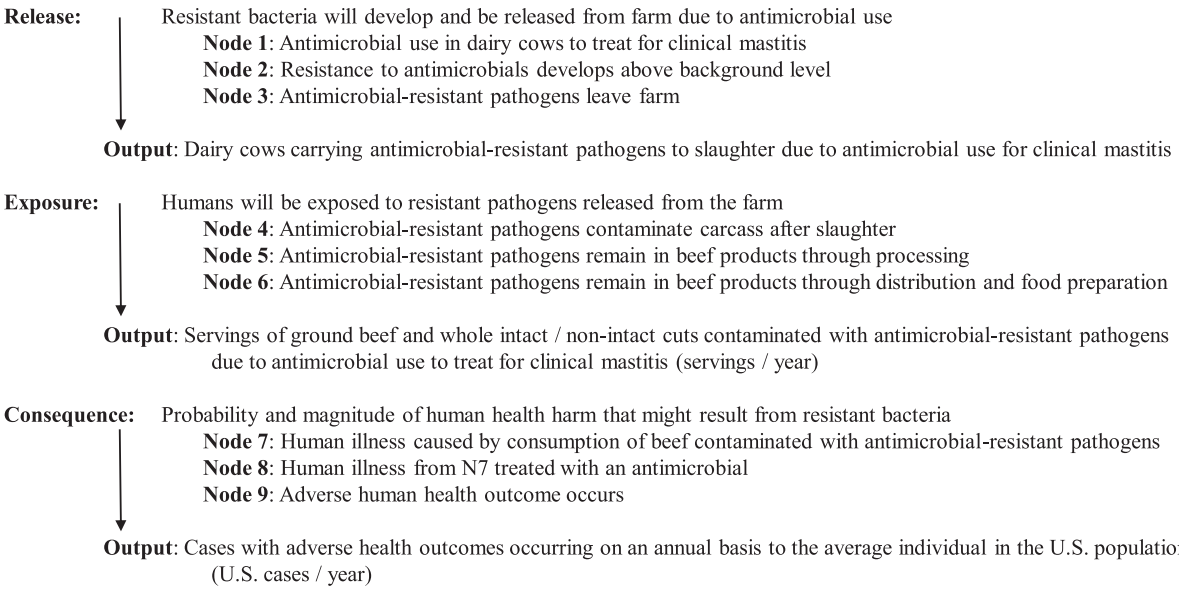

FIGURE 1. *Overview of model. Details concerning the inputs and outputs of the model are presented in Tables 1 through 10.*

increased risk in the United States of human infection with antimicrobial-resistant bacteria, namely *Campylobacter* spp., *Salmonella enterica* subsp. *enterica,* and *Escherichia coli,* derived from dairy beef products as a result of rbST usage in dairy cattle. Specifically, this QRA estimates the risk that use of rbST in lactating dairy cattle according to FDA-approved label instructions results in increased incidence of clinical mastitis and subsequent antimicrobial treatment, emergence and dissemination of antimicrobial-resistant bacteria through the dairy beef supply, and human illnesses with antimicrobial-resistant foodborne pathogens following consumption of these contaminated beef products. This pathway represents a more indirect route of potential risk compared with previous QRAs *(3, 31, 32)* because the potential risk pathway is initiated by rbST supplementation rather than an antimicrobial agent. The potential source of exposure to humans in this QRA is through consumption of beef products from slaughtered dairy cows; milk consumption was not considered in this model because almost all fluid milk for human consumption in developed countries is pasteurized, thereby minimizing any risk to human health *(24, 32, 73).*

MATERIALS AND METHODS

A probabilistic risk assessment was conducted and organized by (i) release of antimicrobial-resistant organisms from the dairy farm, (ii) exposure to humans via the foodborne route, and (iii) consequence of the antimicrobial-resistant infection *(13–15, 71).* The flow of the model is depicted in Figure 1. For the release assessment, the probability of emergence or selection of antimicrobial-resistant bacteria in lactating dairy cows treated with antimicrobial agents for clinical mastitis was estimated, and the potential excess mastitis cases due to rbST use was quantified. The model focused on the foodborne pathogens *Campylobacter, Salmonella,* and *E. coli* in the gastrointestinal tract of the cow. For the exposure assessment, the model evaluated the distribution of beef products harvested from cull dairy cows potentially contaminated with antimicrobial-resistant *Campylobacter, Salmonella,* or *E. coli* due to the use of antimicrobial agents for the treatment of clinical mastitis. The consequence assessment evaluated the probability of illness, antimicrobial treatment, and subsequent adverse health outcomes due to antimicrobial-resistant infections in human patients treated with an antimicrobial agent. The adverse health outcomes typically associated with antimicrobial-resistant *Campylobacter, Salmonella,* and *E. coli* include prolonged duration of clinical signs such as diarrhea *(15, 47).*

Modeling approach. The model was constructed as a quantitative event tree that begins with the use of an antimicrobial agent in lactating dairy cattle for the treatment of clinical mastitis. The structure of the model closely follows the approach taken by

TABLE 1. *Breakdown of intramammary (IMM) and parenteral treatment of clinical mastitis in U.S. dairy herds by severity of clinical mastitis (48, 49)*

Case type	% of cases
Clinical mastitis severity	
Mild mastitis case	50
Moderate mastitis case	35
Severe mastitis case	15
Clinical mastitis treatment based on severity	
Mild mastitis cases treated	90
Moderate mastitis cases treated	95
Severe mastitis cases treated	100
IMM treatment based on severity	
Mild cases treated IMM	100
Moderate mastitis cases treated IMM	100
Severe mastitis cases treated IMM	80
Parenteral treatment based on severity	
Mild cases treated parenteral	10
Moderate cases treated parenteral	30
Severe cases treated parenteral	80
Overall percentage of cases treated IMM or parenteral	
Cases treated IMM	90.25
Cases treated parenteral	26.475
Cases treated IMM and parenteral	93.25

TABLE 2. *Antimicrobial agent use for the treatment of clinical mastitis in dairy cows (node 1)*

Model node	Description	Parenteral	IMM[a]	Reference(s)
Input				
1a	No. of cows in lactation in U.S. dairy herd	9,317,000	9,317,000	*(65, 68)*
1b	% of lactating dairy cows with clinical mastitis when rbST is not used	24.1	24.1	*(70)*
1c	Excess % of lactating dairy cows with clinical mastitis when rbST is used	lognormal(1.249, 0.205) * 30 * 80 * 1b	lognormal(1.249, 0.205) * 30 * 80 * 1b	*(60)* and model assumptions
1d	% of clinical mastitis cases treated with an antimicrobial agent	26.475	93.25	Table 1
1e	% of cows shipped to slaughter following antimicrobial treatment for clinical mastitis	25	25	*(39, 63)*
1f	% of lactating dairy cows with recurring clinical mastitis in same lactation	20.4	20.4	*(48, 52)*
1g	% of cows shipped to slaughter following antimicrobial treatment for recurring clinical mastitis in same lactation	100	100	Model assumption
Output[b]				
1aa	Excess no. of lactating dairy cows with clinical mastitis in the U.S. dairy herd	125,298 (−30,951, 329,592)		Model calculation: (1a) × (1c)
1bb	Excess no. of lactating dairy cows with clinical mastitis that are shipped to slaughter following first treatment with an antimicrobial agent	8,293 (−2,049, 21,815)	29,209 (−7,213, 76,835)	Model calculation: (1aa) × (1d) × (1e)
1cc	Excess no. of lactating dairy cows with recurring clinical mastitis shipped to slaughter following two treatments with an antimicrobial agent	1,344 (−332, 3,535)	16,669 (−4,117, 43,849)	Model calculation: (1aa) × (1d) × (1 − e) × (1f) × (1g)
1dd	Excess no. of lactating dairy cows with clinical mastitis that stay in herd following treatment with an antimicrobial agent	445,307 (415,958, 483,682)	1,342,600 (1,254,119, 1,458,299)	Model calculation: [(1aa) × (1d)] − (1bb) − (1cc)

[a] IMM includes both the intramammary route and the parenteral routes of administration, following the breakdown in Table 1.
[b] Model output values are the median (95% prediction interval).

Hurd et al. *(32)* for the assessment of AMR risks associated with the use of antimicrobial agents in animal agriculture and compares the likelihood of adverse human health outcomes *(15)* when rbST is not used with the likelihood of these outcomes when rbST is used in the U.S. dairy herd. For the present study, it was assumed that rbST was used in 30% of the U.S. dairy herd, even though the National Animal Health Monitoring System 2014 survey *(70)* revealed that rbST was used in 14.7% of all cows in the United States; other researchers have estimated rbST usage to be somewhat higher *(6)*.

The model is divided into nine nodes (Fig. 1), where the output of each node is the probability of the event occurring in that node *(31, 32)*. Data from the peer-reviewed literature, governmental and nongovernmental organization reports, and expert opinion were used to estimate model parameters and probabilities of each event. Parameters were modeled as either deterministic or stochastic, depending on data reliability and availability. For each model simulation (set of parameters), a Monte Carlo simulation of 50,000 iterations was performed in Excel 2013 (Microsoft Corp., Redmond, WA) and @Risk 7.5 (Palisade Corp., Ithaca, NY).

A sensitivity analysis was performed on the influence of stochastic input parameters on model output values. For each simulation, a global sensitivity analysis was performed, with the importance of individual parameters on the outcome variable (excess number of human cases with persistent symptoms) assessed with Spearman rank correlation coefficients. For those parameters deemed most important based on the global sensitivity analysis, a targeted sensitivity analysis was performed. This analysis assesses the influence on the outcome variable of each individual parameter over its range. Spider plots were then created to depict the effect on the output variable of each input parameter over its range.

The most commonly used antimicrobial agents for treatment of clinical mastitis in the United States were considered for inclusion in the model *(70)*: ceftiofur, oxytetracycline, and ampicillin via the parenteral route of administration and ceftiofur, pirlimycin, and cephapirin via the intramammary (IMM) route. Because of the overlap of some of these antimicrobial classes, we decided to focus on the most medically important antimicrobial agents in this list: ceftiofur (parenteral and IMM) and oxytetracycline (parenteral) *(71, 75)*. For the simulations in which ceftiofur was used on the farm, we modeled the treatment of *Salmonella* or *E. coli* infections in humans using a human analog third- or fourth-generation cephalosporin; ceftriaxone is the most common class drug for this group of cephalosporins. *Campylobacter* was not included in these simulations because it is considered to be intrinsically resistant to ceftiofur *(76)*. For the simulations in which oxytetracycline was used on the farm, we used a different approach. *Salmonella* and *E. coli* infections in humans would not normally be treated with a tetracycline and thus would appear to have no relationship to the use of oxytetracycline on the farm. However, the assumption was made that the use of oxytetracycline would also select for resistance to ceftriaxone via co-resistance (genetic linkage) *(15)*, and this antimicrobial agent is often used in

TABLE 3. *Resistance to ceftiofur and oxytetracycline develops above background (node 2)*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference(s)
	A. Resistance to ceftiofur				
Input					
2a	Prevalence of pathogens in treated herds (% of animals positive); data from references were used generate a beta distribution ($s + 1$, $n - s + 1$)		6.3	100	*(7, 26, 33, 64, 69, 74)*
	s		2,756		
	n		43,664		
	Beta distribution		(27.57, 409.07)		
2b	Background sensitivity of pathogen to antimicrobial agent in untreated animals (% of positive animals carrying sensitive isolates); data from references were used generate a beta distribution ($s + 1$, $n - s + 1$)		95.8	87.1	*(21, 53, 57, 69)*
	s		1,798	3,394	
	n		1,876	3,896	
	Beta distribution		(179.8, 7.8)	(339.5, 50.1)	
2c	Probability that antimicrobial-sensitive pathogens develop resistance due to antimicrobial use (%)[c]		0.5	0.5	Model assumption
	Beta distribution		(10, 2,000)	(10, 2,000)	
2d	No. of dairy cows contacted by each dairy cow treated for mastitis		4	4	Model assumption
Output					
Parenteral					
2aa	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that are culled and shipped to slaughter after first antimicrobial treatment		2 (−1, 7)	33 (−8, 104)	Model calculation: (1bb) × (2a) × (2b) × (2c)
2bb	Excess no. of dairy cows with recurring clinical mastitis and carrying resistant pathogens that are culled and shipped to slaughter following second treatment with an antimicrobial agent		0 (0, 1)	5 (−1, 17)	Model calculation: (1cc) × (2a) × (2b) × (2c)
2cc	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that stay in herd following treatment with an antimicrobial agent		6 (−2, 21)	94 (−24, 294)	Model calculation: (1dd) × (2a) × (2b) × (2c)
2dd	Excess no. of dairy cows carrying resistant pathogens due to commingling with cows treated for mastitis		3 (−1, 9)	38 (−10, 120)	Model calculation: (2cc) × (2d)
Parenteral and IMM					
2aa	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that are culled and shipped to slaughter after first antimicrobial treatment		8 (−2, 26)	116 (−29, 365)	Model calculation: (1bb) × (2a) × (2b) × (2c)
2bb	Excess no. of dairy cows with recurring clinical mastitis and carrying resistant pathogens that are culled and shipped to slaughter following second treatment with an antimicrobial agent		4 (−1, 15)	66 (−17, 209)	Model calculation: (1cc) × (2a) × (2b) × (2c)
2cc	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that stay in herd following treatment with an antimicrobial agent		19 (−5, 63)	282 (−71, 888)	Model calculation: (1dd) × (2a) × (2b) × (2c)
2dd	Excess no. of dairy cows carrying resistant pathogens due to commingling with cows treated for mastitis		12 (−3, 41)	182 (−46, 574)	Model calculation: (2cc) × (2d)

TABLE 3. *Continued*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference(s)
	B. Resistance to oxytetracycline				
Input					
2a	Prevalence of pathogens in treated herds (% of animals positive); data from references were used generate a beta distribution ($s + 1$, $n - s + 1$)	26.6	6.3	100	*(2, 7, 23, 26, 29, 33, 55, 56, 64, 69, 74)*
	s	2,088	2,756		
	n	7,847	43,664		
	Beta distribution	(20.89, 57.58)	(27.57, 409.07)		
2b	Background sensitivity of pathogens to antimicrobial agent in untreated animals (% of positive animals carrying sensitive isolates); data from references were used generate a beta distribution ($s + 1$, $n - s + 1$)	37.6	89.9	7.6	*(53, 57, 69)*
	s	208	1,687	16	
	n	553	1,876	223	
	Beta distribution	(20.8, 34.5)	(168.7, 18.9)	(1.7, 20.6)	
2c	Probability that antimicrobial-sensitive pathogens develop resistance due to antimicrobial use (%)[c]	0.5	0.5	0.5	Model assumption
	Beta distribution	(10, 2,000)	(10, 2,000)	(10, 2,000)	
2d	No. of dairy cows contacted by each dairy cow treated for mastitis	4	4	4	Model assumption
Output, parenteral					
2aa	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that are culled and shipped to slaughter after first antimicrobial treatment	4 (−1, 13)	2 (−1, 7)	2 (−1, 12)	Model calculation: (1bb) × (2a) × (2b) × (2c)
2bb	Excess no. of dairy cows with recurring clinical mastitis and carrying resistant pathogens that are culled and shipped to slaughter following second treatment with an antimicrobial agent	1 (0, 2)	0 (0, 1)	0 (0, 2)	Model calculation: (1cc) × (2a) × (2b) × (2c)
2cc	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that stay in herd following treatment with an antimicrobial agent	10 (−3, 36)	6 (−2, 20)	6 (−2, 34)	Model calculation: (1dd) × (2a) × (2b) × (2c)
2dd	Excess no. of dairy cows carrying resistant pathogens due to commingling with cows treated for mastitis	4 (−1, 15)	2 (−1, 8)	2 (−1, 14)	Model calculation: (2cc) × (2d)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.

[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.

[c] Assumed 1 in 200 cows would develop resistance following antimicrobial agent administration.

human medicine to treat *Salmonella* and *E. coli* infections. *Campylobacter* infections in humans also would not normally be treated with a tetracycline and thus would appear to have no relationship to the use of oxytetracycline on the farm. However, the assumption was made that the use of oxytetracycline would also select for resistance to macrolides via co-resistance (genetic linkage) *(15),* and macrolides are often used in human medicine to treat *Campylobacter* infections.

Model assumptions. Throughout the model, we have made a focused effort to use estimates for each parameter that would be considered maximum risk and have tried to err on the side of overestimating the risk to humans associated with the use of rbST in dairy cattle. Some of the explicit and implicit assumptions that we considered to be maximum risk are listed below.

(i) The total number of dairy cows in lactation in the United States was kept fixed, even though the increased milk yield associated with the use of rbST would require fewer cows to produce the same amount of milk *(8, 9)*. Fixing the total number of cows in lactation and not adjusting the risk estimates by the amount of milk produced results in a relative overestimate of excess mastitis incidence when comparing the rbST-supplementation model to the no-rbST model.

(ii) Proportionally more cases of mastitis (about 30%) occur during the first 60 days postpartum *(37, 48)* than during the final 245 days of the lactation cycle (the period when rbST would be used). However, the model used the assumption that the risk of mastitis was constant during the entire lactation cycle (305 days), and thus 80% of mastitis cases would occur in the final 245 days when rbST would be administered.

(iii) For *E. coli,* the model focused on diarrheagenic *E. coli* and enterotoxigenic *E. coli*; the model does not include Shiga toxin–producing *E. coli*. The model uses the assumption that 100% of cows are carrying diarrheagenic *E. coli* and enterotoxigenic *E. coli*.

(iv) Even though animals that return to the healthy herd after antimicrobial treatment for clinical mastitis may not be sent to slaughter for months or years, the probability of carrying bacteria to slaughter that are resistant to the antimicrobial agent because of the clinical mastitis treatment was assumed to be high (mean, 58%).

(v) Ceftiofur is administered by both the parenteral and IMM routes. There is no evidence that the IMM use affects bacteria in the gut of the cow. However, the model used the assumption that all ceftiofur use (parenteral and IMM) affects gut bacteria equally.

(vi) The model used the assumption that each animal treated for clinical mastitis that stays in the herd following treatment can commingle and transmit any antimicrobial-resistant bacteria that developed following treatment to four additional cows and that these commingled cows then carry the resistant bacteria to slaughter with the same probability as the treated cohort. Even though the gut microflora of the commingled cows are under no selection pressure, the untreated cows are still assumed to carry the AMR for extended periods. This notion is similar to the concept of R_0, the basic reproduction number *(1),* which is defined as the number of secondary infections that would result if a single infected individual were introduced into a completely susceptible population. In studies of *E. coli* and *Salmonella* in cattle, this value has been estimated as 1.5 or less *(12, 40),* although under extreme assumptions, the estimated R_0 value was slightly greater than 4 *(40)*. We used the maximum risk parameter estimate of $R_0 = 4$ secondary infections for this model, i.e., each treated cow will share antimicrobial-resistant bacteria with four untreated cows.

(vii) For a carcass testing positive for any of the modeled bacteria, the model used the assumption that all of the ground beef from that carcass is contaminated (100% probability), and 50% of the whole intact or nonintact cuts from this carcass is contaminated.

(viii) The model used the assumption of a linear relationship between the amount of beef consumed annually that contains antimicrobial-resistant pathogens and the number of illnesses in the U.S. population annually from these pathogens. This modeling approach has been used previously *(3, 32)* and is a risk-increasing assumption that provides an upper bound on adverse human health outcome estimates *(18)*.

(ix) The model used the assumption that all of the *Campylobacter* found in cattle are capable of causing human disease, even though *C. coli* is frequently isolated but not normally considered a human pathogen.

(x) The model used the assumption that 100% of the tetracycline-resistant *Salmonella* and *E. coli* isolates were resistant to third-generation cephalosporins (ceftriaxone) due to co-resistance and that 100% of the tetracycline-resistant *Campylobacter* isolates were resistant to macrolides due to co-resistance.

(xi) Even though there is a low probability that antimicrobial-resistant bacterial infections treated with an antimicrobial agent result in persistent symptoms compared with their antimicrobial-susceptible counterparts *(50),* the model used the assumption that 100% of human infections with a resistant bacterial strain will result in an adverse health outcome. An adverse outcome associated with the modeled pathogens would potentially include diarrhea of prolonged duration *(15, 47)*.

Other model assumptions are described below along with the detailed information provided for the appropriate model node.

Release assessment. The release assessment describes the probability that resistant bacteria will develop and be released from the dairy operation due to use of an antimicrobial agent for treatment of clinical mastitis in lactating dairy cows. The model compares this probability for the U.S. dairy herd when rbST is not used versus situations in which rbST is used in 30% of lactating dairy cows in the U.S. dairy herd. The release assessment estimates the annual number of lactating dairy cows developing clinical mastitis, the number being treated for mastitis with antimicrobial agents, the number in which key foodborne bacteria develop AMR because of use of antimicrobial agents, and the number carrying resistant organisms to slaughter.

Perhaps the most contentious parameter of the entire model is the change in percentage of dairy cows experiencing clinical mastitis when given rbST. Without an increased risk of mastitis due to rbST there would be no increase in farm usage of antimicrobial agents and consequently no increase in AMR nor increased risk to human health. In their meta-analysis, St-Pierre et al. *(60)* identified and extracted data from 14 articles concerning rbST use according to the FDA-approved label; these authors estimated the odds ratio of clinical mastitis between rbST-supplemented and nonsupplemented (control) cows to be 1.249. The 95% confidence interval of this odds ratio was 0.942 to 1.655 ($P = 0.122$), indicating a nonsignificant difference in the odds of mastitis between supplemented and nonsupplemented cows. For the present risk assessment, the actual point estimate of the odds ratio and the 95% confidence interval were used to generate a probability distribution for the difference in mastitis risk between rbST-supplemented and nonsupplemented cows. We generated a lognormal(1.249, 0.205) probability distribution, which has a mean of 24.9% excess mastitis cases and a 90% confidence interval between 5.7% reduction in mastitis and 61.2% excess. The random value obtained from this probability distribution is multiplied by the baseline incidence per lactation of clinical mastitis in dairy cows (24.1%), the market share of rbST in the U.S. dairy herd (30%), and the percentage of clinical mastitis cases that occur during the period in which rbST is administered (80%).

Node 1 estimates the percentage of clinical mastitis cases that will receive antimicrobial treatment. Using a mastitis severity scoring system (Table 1), the percentage of mastitis cases that would be considered mild, moderate, or severe was estimated *(49)*. Treatment with an antimicrobial agent is more common as the severity of the mastitis increases. Finally, parenteral administration of antimicrobial agents is typically reserved for those cows with moderate to severe clinical illness, and we assumed that most cows with moderate to severe clinical mastitis will be given both IMM and parenteral antimicrobial agents. The estimated percentage of mastitis cases receiving IMM therapy, parenteral therapy, or both is shown in Table 1. Parameter estimates and model outputs of node 1 are shown in Table 2. We assumed that the probability of moderate to severe clinical mastitis is independent of rbST supplementation *(17, 37, 54),* that 25% of treated cows would be culled and shipped to slaughter after the first treatment immediately following recovery and the end of the antimicrobial agent

TABLE 4. *Antimicrobial-resistant pathogens leave the farm (node 3)*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference
Input					
3a	Probability that antimicrobial-resistant pathogens persist to slaughter in dairy cows that do not return to milking herd after recovery from mastitis (%)	100	100	100	Model assumption
3b	Probability that antimicrobial-resistant pathogens persist to slaughter in dairy cows that return to milking herd after recovery from mastitis (slaughter at a later date) (%); PERT(0.25, 0.625, 0.75)	58.3	58.3	58.3	*(28)*
3c	Probability that antimicrobial-resistant pathogens persist to slaughter in dairy cows not treated for clinical mastitis but carrying resistant pathogens due to commingling (%); PERT(0.25, 0.625, 0.75)	58.3	58.3	58.3	Model assumption
Output					
Parenteral ceftiofur					
3aa	Excess no. of dairy cows carrying resistant pathogens at slaughter due to antimicrobial treatment of mastitis		2 (−1, 8)	36 (−9, 113)	Model calculation: (2aa + 2bb) × (3a)
3bb	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that stay in herd following treatment with an antimicrobial agent		4 (−1, 8)	54 (−14, 175)	Model calculation: (2cc) × (3b)
3cc	Excess no. of dairy cows not treated for clinical mastitis but carrying resistant pathogens at slaughter due to commingling		1 (0, 5)	22 (−6, 72)	Model calculation: (2dd) × (3c)
Parenteral and IMM ceftiofur					
3aa	Excess no. of dairy cows carrying resistant pathogens at slaughter due to antimicrobial treatment of mastitis		10 (−3, 35)	155 (−39, 487)	Model calculation: (2aa + 2bb) × (3a)
3bb	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that stay in herd following treatment with an antimicrobial agent		11 (−3, 38)	161 (−41, 527)	Model calculation: (2cc) × (3b)
3cc	Excess no. of dairy cows not treated for clinical mastitis but carrying resistant pathogens at slaughter due to commingling		7 (−2, 24)	104 (−26, 341)	Model calculation: (2dd) × (3c)
Parenteral oxytetracycline					
3aa	Excess no. of dairy cows carrying resistant pathogens at slaughter due to antimicrobial treatment of mastitis	4 (−1, 14)	2 (−1, 8)	2 (−1, 13)	Model calculation: (2aa + 2bb) × (3a)
3bb	Excess no. of dairy cows with clinical mastitis and carrying resistant pathogens that stay in herd following treatment with an antimicrobial agent	6 (−1, 21)	3 (−1, 12)	3 (−1, 20)	Model calculation: (2cc) × (3b)
3cc	Excess no. of dairy cows not treated for clinical mastitis but carrying resistant pathogens at slaughter due to commingling	2 (−1, 9)	1 (0, 5)	1 (0, 8)	Model calculation: (2dd) × (3c)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.

[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.

TABLE 5. *Antimicrobial-resistant pathogens contaminate the carcass during and after slaughter (node 4)*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference(s)
Input					
4a	Probability that a dairy cow carcass, carrying pathogens or not, is contaminated with pathogens (%)	4.1	0.74	15.8	*(5, 66, 67, 70, 72)*
	s	134	101	270	
	n	3,294	13,784	1,719	
	Beta distribution	(13.5, 315.9)	(10.2, 1,368.2)	(27.1, 144.8)	
4b	% of culled dairy cows that are slaughtered and consumed domestically	99	99	99	*(22)*
4c	Condemnation rate of cull dairy cows (%)	3.49	3.49	3.49	*(22)*
Output					
Parenteral ceftiofur					
4aa	Excess cull dairy cow carcasses contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (carcasses/yr)		0.05 (−0.01, 0.19)	16.5 (−4.2, 55.4)	Model calculation: (3aa + 3bb + 3cc) × (4a) × (4b) × (1 − 4c)
Parenteral and IMM ceftiofur					
4aa	Excess cull dairy cow carcasses contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (carcasses/yr)		0.19 (−0.05, 0.72)	62.3 (−15.8, 207.8)	Model calculation: (3aa + 3bb + 3cc) × (4a) × (4b) × (1 − 4c)
Parenteral oxytetracycline					
4aa	Excess cull dairy cow carcasses contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (carcasses/yr)	0.46 (−0.12, 1.77)	0.05 (−0.01, 0.18)	1.01 (−0.26, 6.31)	Model calculation: (3aa + 3bb + 3cc) × (4a) × (4b) × (1 − 4c)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.

[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.

withdrawal period *(70),* and that cows with recurring mastitis in the same lactation would be culled 100% of the time.

Node 2 (Table 3) estimates the likelihood of development of AMR following antimicrobial agent administration and uses the prevalence of each bacterial pathogen in dairy cows, the background susceptibility of each pathogen to each antimicrobial agent, and the probability that AMR will develop in each pathogen following use of the antimicrobial agent. Finally, and perhaps most importantly, the model addresses commingling for the direct transmission of resistant pathogens from treated cows to untreated cows on the farm. This number is actually the number of effective contacts, i.e., the number of actual transmission events from treated to nontreated cows, and was based on published estimates of R_0 for various bacteria in cattle populations *(12, 40).* Node 3 (Table 4) presents estimates of the likelihood that resistant pathogens leave the farm when the culled dairy cows are sent to slaughter. Because of the absence of studies tracking the carriage of *Salmonella, E. coli,* and *Campylobacter* in cattle over time, we used a study that revealed (but did not quantify) the long-term persistence of specific *Campylobacter* strains in some dairy cattle *(28).* Even though the cows in node 3 will not go to slaughter for many months to years after treatment, we estimated (maximum risk) that the most likely probability of carriage to slaughter would be 62.5% (range, 25 to 75%) based on a PERT distribution.

Exposure assessment. The exposure assessment evaluated the probability that humans will be exposed to resistant bacteria released from the farm through the slaughter of culled dairy cows and the subsequent consumption of contaminated beef. Node 4 (Table 5) presents estimates of the likelihood that viable, antimicrobial-resistant pathogens (*Campylobacter, Salmonella,* and *E. coli*) contaminate the carcass after slaughter. Node 5 (Table 6) presents estimates of the total amount of beef produced from the culled dairy cows carrying fecal antimicrobial-resistant foodborne pathogens to slaughter due to the use of antimicrobial agents for the treatment of clinical mastitis. The total amount of beef produced is allocated into two product categories: ground beef and whole intact or nonintact cuts. Although the prevalence of each pathogen differs between the product categories, the prevalence of AMR was assumed to be the same between the product categories. Node 6 (Table 7) quantifies the number of servings of beef products consumed at home or away from home that remain

TABLE 6. *Resistant pathogens remain in beef products through processing (node 5)*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference(s)
Input					
5a	Avg hot carcass weight of cull dairy cow (kg/carcass)	294	294	294	*(22, 45)*
5b	Percentage of cull dairy cow carcass that is processed into ground beef	39.4	39.4	39.4	*(22, 46)*
5c	Percentage of cull dairy cow carcass that is processed into whole intact/nonintact cuts	24.1	24.1	24.1	*(22, 46)*
5d	Probability that ground beef from a carcass contaminated with pathogens is also contaminated (%)	100	100	100	Model assumption
5e	Multiplier due to mixing of meat from carcasses contaminated by pathogens with that of noncontaminated carcasses	2	2	2	*(22)*
5f	Probability that whole intact/nonintact cuts from a carcass contaminated with pathogens are also contaminated (%)	50	50	50	Model assumption
Output					
Parenteral ceftiofur					
5aa	Excess quantity of ground beef contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (kg/yr)		11.55 (−3.01, 44.94)	3,826.7 (−974.2, 12,833.4)	Model calculation: (4aa) × (5a) × (5b) × (5d) × (5e)
5bb	Excess quantity of whole intact/nonintact cuts contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (kg/yr)		1.77 (−0.46, 6.87)	585.2 (−148.9, 1,962.5)	Model calculation: (4aa) × (5a) × (5c) × (5f)
Parenteral and IMM ceftiofur					
5aa	Excess quantity of ground beef contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (kg/yr)		43.71 (−10.98, 167.84)	14,431.2 (−3,662.2, 48,137.0)	Model calculation: (4aa) × (5a) × (5b) × (5d) × (5e)
5bb	Excess quantity of whole intact/nonintact cuts contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (kg/yr)		6.68 (−1.68, 25.67)	2,206.8 (−560.0, 7,361.1)	Model calculation: (4aa) × (5a) × (5c) × (5f)
Parenteral oxytetracycline					
5aa	Excess quantity of ground beef contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (kg/yr)	105.8 (−27.3, 408.9)	10.9 (−2.8, 41.9)	233.7 (−61.3, 1,462.5)	Model calculation: (4aa) × (5a) × (5b) × (5d) × (5e)
5bb	Excess quantity of whole intact/nonintact cuts contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (kg/yr)	16.2 (−4.2, 62.5)	1.7 (−0.4, 6.4)	35.7 (−9.4, 223.6)	Model calculation: (4aa) × (5a) × (5c) × (5f)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.

[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.

TABLE 7. *Resistant pathogens remain in beef products through distribution (node 6)*

Model node[a]	Description	At home		
		Campylobacter[b]	*Salmonella*	*E. coli*
Input				
6a	% of ground beef distributed for consumption	10	10	10
6b	% of whole cuts distributed for consumption	2.9	2.9	2.9
6c	Avg serving size of ground beef (kg)	0.071	0.071	0.071
6d	Avg serving size of whole cuts (kg)	0.136	0.136	0.136
6e	Probability that a serving of ground beef remains contaminated after preparation (%)	0.5	0.5	0.5
6f	Probability that a serving of whole cuts remains contaminated after preparation (%)	0.01	0.01	0.01
Output				
Parenteral ceftiofur				
6aa	Excess servings of ground beef contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (servings/yr)		0.08 (−0.02, 0.31)	27.04 (−6.85, 90.34)
6bb	Excess servings of whole intact or nonintact cuts contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (servings/yr)		0.00004 (−0.00001, 0.0001)	0.01 (−0.003, 0.04)
Parenteral and IMM ceftiofur				
6aa	Excess servings of ground beef contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (servings/yr)		0.31 (−0.08, 1.19)	101.74 (−25.91, 338.59)
6bb	Excess servings of whole intact or nonintact cuts contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (servings/yr)		0.001 (−0.00004, 0.005)	0.05 (−0.01, 0.16)
Parenteral oxytetracycline				
6aa	Excess servings of ground beef contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (servings/yr)	0.75 (−0.19, 2.88)	0.08 (−0.02, 0.29)	1.65 (−0.43, 10.00)
6bb	Excess servings of whole intact or nonintact cuts contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (servings/yr)	0.0003 (−0.00009, 0.001)	0.00004 (−0.00001, 0.0001)	0.0008 (−0.0002, 0.005)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.

[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.

contaminated with the antimicrobial-resistant foodborne pathogens (*Campylobacter, Salmonella,* and *E. coli*).

Consequence assessment. The consequence assessment considers the probability and magnitude of human health harm that might result from antimicrobial-resistant *Campylobacter, Salmonella,* or *E. coli*. To estimate the probability of human illness following consumption of beef originating from dairy cows treated with antimicrobial agents for mastitis, an approach similar to that used by the FDA Center for Veterinary Medicine and others was utilized *(3, 31, 32)*. This approach uses a parameter to link human illnesses attributed to beef to the estimated number of contaminated servings. Node 7 (Table 8) presents estimates of the number of human illnesses caused by the consumption of beef products contaminated with foodborne pathogens that are resistant to antimicrobial agents because of the treatment of clinical mastitis in dairy cows and relies on a parameter that enables us to estimate the number of resulting human illnesses, connecting contaminated servings from node 6 to human illness. This parameter is best defined as the probability of illness given a contaminated serving of beef, regardless of dose; the approach uses an assumed linear relationship between servings of beef consumed in the United States annually that are contaminated with antimicrobial-resistant pathogens and the number of human illnesses in the United States annually caused by antimicrobial-resistant foodborne pathogens that are attributable to domestic beef consumption. The parameter implicitly assumes that all servings deemed contaminated in node 6 have a sufficient dose to cause illness. This parameter is modeled in

TABLE 7. *Extended*

Away from home			
Campylobacter	*Salmonella*	*E. coli*	Reference(s)
90	90	90	*(22)*
97.1	97.1	97.1	*(22)*
0.099	0.099	0.099	*(41, 43, 44, 62)*
0.1	0.1	0.1	*(41, 43, 44, 62)*
0.01	0.01	0.01	*(10, 11)*
0.01	0.01	0.01	*(10, 11)*
	0.01 (−0.003, 0.04)	3.49 (−0.88, 11.66)	Model calculation: (5aa) × (6a)/(6c) × (6e)
	0.002 (−0.0004, 0.007)	0.57 (−0.14, 1.90)	Model calculation: (5bb) × (6b)/(6d) × (6f)
	0.04 (−0.01, 0.15)	13.13 (−3.34, 43.71)	Model calculation: (5aa) × (6a)/(6c) × (6e)
	0.006 (−0.002, 0.03)	2.15 (−0.55, 7.14)	Model calculation: (5bb) × (6b)/(6d) × (6f)
0.10 (−0.02, 0.37)	0.01 (−0.002, 0.04)	0.21 (−0.06, 1.29)	Model calculation: (5aa) × (6a)/(6c) × (6e)
0.02 (−0.004, 0.06)	0.002 (−0.0004, 0.006)	0.03 (−0.01, 0.21)	Model calculation: (5bb) × (6b)/(6d) × (6f)

a separate spreadsheet and requires information about the estimated total of U.S. illness cases in humans caused by *Campylobacter, Salmonella,* or *E. coli,* an underdiagnosis factor for cases missed by the observation system, and the proportion of these cases that are attributable to beef consumption.

Node 8 (Table 9) quantifies the number of human illnesses caused by antimicrobial-resistant pathogens (node 7 output) that are subsequently treated with an antimicrobial agent in a class that matches that of the antimicrobial agent used on the dairy farm to treat clinical mastitis. For *Campylobacter, Salmonella,* or *E. coli* infections to cause harm as a result of the development of AMR, an ill person must (i) seek medical care and (ii) be administered an antimicrobial treatment. Ideally, this individual would have a culture performed on a clinical specimen and then receive a positive test result for the pathogen, but these steps are not always performed. Therefore, the model addressed the probability of an individual seeking medical care and subsequently receiving treatment with an antimicrobial agent.

In node 9 (Table 10), the number of human illnesses that result in an adverse health outcome (e.g., prolonged diarrhea) is estimated. Rather than attempting to determine the fraction of cases that might exhibit an adverse outcome due to AMR and antimicrobial treatment of the infection, we decided to use the maximum risk assumption by assuming 100% of humans who became ill due to infection with any of the modeled resistant pathogens and who received antimicrobial therapy would experience an adverse health outcome.

Sensitivity analysis. A sensitivity analysis was conducted to identify input parameters that significantly impact the output values of the model. The relationship between the stochastic input parameters and output values was quantified with a Spearman rank correlation coefficient to determine the amount of change in the model outputs for percentile changes in the model inputs. First, we ran a global, crude sensitivity analysis on all stochastic parameters simultaneously to determine their importance to the outcome

TABLE 8. *Human illness caused by consumption of beef contaminated with antimicrobial-resistant pathogens (node 7)*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference(s)
Input					
7a	Ratio of illnesses attributed to beef products per contaminated serving of beef following food preparation	0.07688	2.6799	0.000208	*(5, 20, 21, 27, 45, 46, 51, 58, 61, 66, 67, 70, 72)*
Output[c]					
Parenteral ceftiofur					
7aa	Excess no. of human illnesses caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)		0.18 (−0.05, 1.20)	0.005 (−0.001, 0.03)	Model calculation: (6aa + 6bb) × (7a)
Parenteral and IMM ceftiofur					
7aa	Excess no. of human illnesses caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)		0.69 (−0.18, 4.45)	0.02 (−0.005, 0.10)	Model calculation: (6aa + 6bb) × (7a)
Parenteral oxytetracycline					
7aa	Excess no. of human illnesses caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)	0.04 (−0.01, 0.33)	0.17 (−0.05, 1.12)	0.0003 (−0.0001, 0.0027)	Model calculation: (6aa + 6bb) × (7a)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.
[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.
[c] Assumed 1 in 200 cows would develop resistance following antimicrobial agent administration.

parameter 9aa: excess cases of human illness where symptoms persist. Based on the Spearman rank correlation coefficients (correlation to outcome variable 9aa), the most important individual parameters were selected for a targeted sensitivity analysis. In this analysis, each individual parameter was evaluated over its range for its influence on the outcome variable (9aa).

RESULTS

The estimated risk of human infection with antimicrobial-resistant bacteria and the subsequent adverse health outcome in humans as a result of use of antimicrobial agents in dairy cattle for the treatment of clinical mastitis are presented in Tables 2 through 10; Table 10 shows the final risk estimates of an adverse human health outcome in the United States. The model predicts that the probability of an adverse human health outcome is less than 1 in 1 billion persons per year in the U.S. population. The highest risk was associated with the third- or fourth-generation cephalosporins for a foodborne *Salmonella* infection in the model that included both parenteral and IMM administration of ceftiofur to dairy cows; the estimated median was one excess adverse health outcome every 21.7 years under the assumptions of the model. The model predicts an excess of 0.046 adverse outcomes for *Salmonella* infections per year in the United States due to rbST usage in 30% of the lactating U.S. dairy cows. The risks associated with the remaining antimicrobial agent–pathogen combinations are all significantly lower (Table 10).

The sensitivity analysis revealed that the main model output of human illnesses that develop persistent symptoms (adverse health outcomes) following antimicrobial treatment was highly sensitive to (i) the excess percentage of lactating dairy cows with clinical mastitis when rbST is used (parameter 1c), (ii) the probability of illness given a contaminated serving of beef (parameter 7c), (iii) the probability that an ill human is prescribed an antimicrobial agent (parameter 8b), and (iv) the probability that a dairy cow carcass becomes contaminated with the pathogen (parameter 4a). The targeted sensitivity analysis was conducted on these four input parameters, and each individual parameter was evaluated over its entire range. A spider plot depicting the influence that each of these four parameters has on the outcome variable was generated for the model of *Salmonella* when both parenteral and IMM administration of ceftiofur are used to treat clinical mastitis in dairy cows (Fig. 2); the other simulations had very similar results in the targeted sensitivity analysis. After adjusting for all other input variables and when parameter 1c is fixed at its upper 99th percentile, the predicted number of U.S. human illnesses with persistent symptoms associated with *Salmonella* and ceftriaxone resistance is only 0.2 excess cases per year.

TABLE 9. *Human illness caused by consumption of beef contaminated with antimicrobial-resistant pathogens and treated with an antimicrobial agent (node 8)*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference(s)
Input					
8a	Probability that the patient seeks medical help (%)	26; PERT(0.16, 0.26, 0.36)	27; PERT(0.16, 0.26, 0.36)	27; PERT(0.16, 0.26, 0.36)	*(30, 31, 58)*
8b	Probability that an antimicrobial agent is prescribed and is a macrolide (for *Campylobacter*) or a third- or fourth-generation cephalosporin (for *Salmonella* and *E. coli*) (%)	29; PERT(0.1, 0.3, 0.45)	27; PERT(0.1, 0.25, 0.5)	27; PERT(0.1, 0.25, 0.5)	
Output[c]					
Parenteral ceftiofur					
8aa	Excess cases of human illness treated with an antimicrobial agent, where illness was caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)		0.012 (−0.003, 0.89)	0.0003 (−0.00009, 0.002)	Model calculation: (7aa) × (8a) × (8b)
Parenteral and IMM ceftiofur					
8aa	Excess cases of human illness treated with an antimicrobial agent, where illness was caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)		0.046 (−0.012, 0.334)	0.001 (−0.0003, 0.007)	Model calculation: (7aa) × (8a) × (8b)
Parenteral oxytetracycline					
8aa	Excess cases of human illness treated with an antimicrobial agent, where illness was caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)	0.001 (−0.0004, 0.01)	0.011 (−0.003, 0.084)	0.00002 (−0.000006, 0.0002)	Model calculation: (7aa) × (8a) × (8b)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.

[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.

[c] Assumed 1 in 200 cows would develop resistance following antimicrobial agent administration.

We conducted a scenario analysis for the parameter "probability of resistance development" in which the parameter estimate was increased 10-fold (from the baseline value of 0.5% to the new value of 5%) to account for the uncertainty in this parameter. Because the model is based on a linear relationship, all modeled outcomes increased by the same amount. For example, for the model of foodborne *Salmonella* infections that included both parenteral and IMM administration of ceftiofur in dairy cows, there was an estimated median of one excess adverse health outcome every 2.15 years or an excess risk of 1 in 664 million, which would still be considered a negligible risk.

DISCUSSION

In this QRA, the increased (excess) number of cows in the U.S. dairy herd that were predicted to carry resistant bacteria at slaughter due to rbST administration was negligible for all modeled scenarios (node 3 output). The total number of excess human illnesses with resistant bacteria due to rbST administration and subsequent mastitis treatment with antimicrobial agents was also predicted to be negligible. For the third- and fourth-generation cephalosporins, the overall risks of an excess adverse health outcome (e.g., prolonged diarrhea) in human illnesses caused by *Salmonella* and *E. coli* infection were negligible, with estimates of less than 1 event per 1 billion people at risk per year for *Salmonella* and *E. coli* infections. Even when the assumption was made that all uses of ceftiofur, both parenteral and IMM, could enhance the development of resistance in gut bacteria of the dairy cow, the human health risks were still negligible. For the treatment of human *Campylobacter* infections with macrolides, an antibiotic class loosely related to pirlimycin (which is frequently used

TABLE 10. *Adverse human health outcomes due to antimicrobial treatment following illness caused by consumption of beef contaminated with antimicrobial-resistant pathogens (node 9)*

Model node[a]	Description	*Campylobacter*[b]	*Salmonella*	*E. coli*	Reference
Input					
9a	Probability that symptoms persist when human illness is treated with an antimicrobial agent (%)	100	100	100	Model assumption
9b	U.S. 2010 census population	308,745,538	308,745,538	308,745,538	*(61)*
Output[c]					
Parenteral ceftiofur					
9aa	Excess cases of human illness where symptoms persist after treatment with an antimicrobial agent, where illness was caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)		0.012 (−0.003, 0.088); (1 case every 82.2 yr)	0.0003 (−0.0001, 0.002); (1 case every 2,974.1 yr)	Model calculation: (8aa) × (9a)
9bb	Rate of persistent symptoms occurring on an annual basis to the average individual in the U.S. population (illnesses/1,000,000)		0.00004 (−0.00001, 0.0003)	0.000001 (−0.0000003, 0.000006)	Model calculation: (9aa)/[(9b)/ 1,000,000]
9cc	Probability of cases with persistent symptoms occurring on an annual basis to the average individual in the U.S. population		1 in 25,369 million	1 in 918,236 million	Model calculation: 1/(9bb)
Parenteral and IMM ceftiofur					
9aa	Excess cases of human illness where symptoms persist after treatment with an antimicrobial agent, where illness was caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)		0.046 (−0.012, 0.334) (1 case every 21.7 yr)	0.001 (−0.0003, 0.007) (1 case every 787.8 yr)	Model calculation: (8aa) × (9a)
9bb	Rate of persistent symptoms occurring on an annual basis to the average individual in the U.S. population (illnesses/1,000,000)		0.00015 (−0.00004, 0.001)	0.000004 (−0.000001, 0.00002)	Model calculation: (9aa)/[(9b)/ 1,000,000]
9cc	Probability of cases with persistent symptoms occurring on an annual basis to the average individual in the U.S. population		1 in 6,698 million	1 in 243,227 million	Model calculation: 1/(9bb)
Parenteral oxytetracycline					
9aa	Excess cases of human illness where symptoms persist after treatment with an antimicrobial agent, where illness was caused by consumption of beef products contaminated with antimicrobial-resistant pathogens due to antimicrobial treatment for clinical mastitis (U.S. cases/yr)	0.001 (−0.0004, 0.013); (1 case every 731.7 yr)	0.011 (−0.003, 0.083); (1 case every 87.2 yr)	0.00002 (−0.00001, 0.00020) (1 case every 47,181 yr)	Model calculation: (8aa) × (9a)
9bb	Rate of persistent symptoms occurring on an annual basis to the average individual in the U.S. population (illnesses/ 1,000,000)	0.000004 (−0.000001, 0.00004)	0.00004 (−0.00001, 0.0003)	0.00000007 (−0.00000002, 0.0000006)	Model calculation: (9aa)/[(9b)/ 1,000,000]
9cc	Probability of cases with persistent symptoms occurring on an annual basis to the average individual in the U.S. population	1 in 225,903 million	1 in 26,908 million	1 in 14,567,063 million	Model calculation: 1/(9bb)

[a] All input values are the same for no-rbST and rbST simulations for a given pathogen. Model output values are the median (95% prediction interval) for the parenteral administration of ceftiofur, the parenteral-IMM administration of ceftiofur, and the parenteral administration of oxytetracycline.

[b] *Campylobacter* is intrinsically resistant to ceftiofur and thus was excluded from ceftiofur simulations.

[c] Assumed 1 in 200 cows would develop resistance following antimicrobial agent administration.

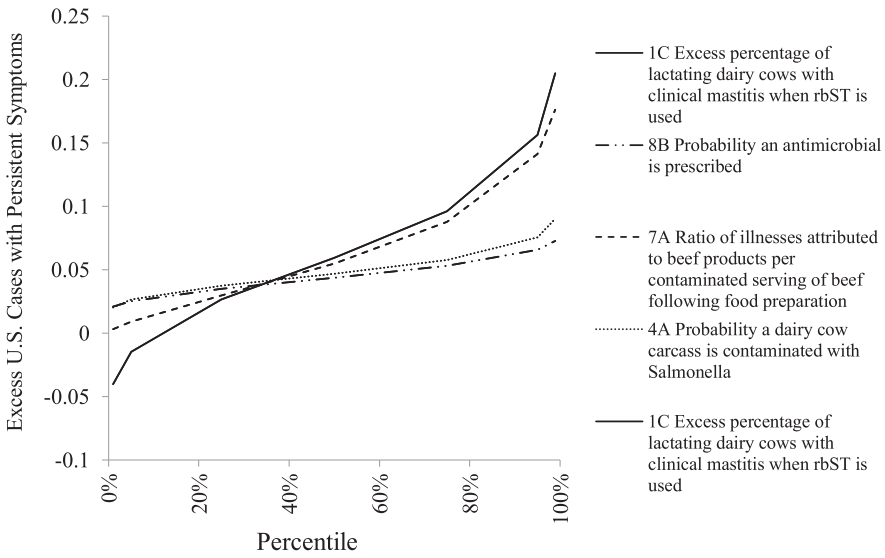

FIGURE 2. *Spider plot of the parameters that contribute most to the model output "excess cases with persistent symptoms" (9aa). The example shown is for the simulation of* Salmonella *and in which ceftiofur is used parenterally and IMM for the treatment of clinical mastitis in dairy cows. The* x *axis represents the percentile of the range of each modeled parameter. In this targeted sensitivity analysis, the plotted simulations were performed by varying each of the parameters one at a time.*

for treatment of clinical mastitis in dairy cows), the risks again were negligible (less than 1 illness in 225 billion people). Overall, the predicted excess risk to the human population of a resistant infection and subsequent adverse health event associated with the use of rbST is negligible. In 1990, the FDA evaluated the human safety issues related to use of rbST and concluded that its use in dairy cows presents no increased health risk to consumers *(38)*; commercial use of rbST then began in 1994 *(4)*. Human safety issues concerning the use of rbST have been reevaluated at periodic intervals, and conclusions have remain unchanged; the use of rbST represents no significant human health risk *(16, 34–36)*.

This risk assessment was designed to estimate excess human health adverse outcomes associated with the use of rbST. The model assumptions were set to maximize potential risk, and even though the risk estimates are rough estimates, the magnitude of the risk estimates reveals that use of rbST only negligibly affects the modeled outcomes. A major limitation of this risk assessment is the reliance on the prevalence of pathogen-positive samples as an indicator of exposure. Ideally, this model would include microbial levels, both for the total amount of bacteria in each compartment and the fraction of the total that is resistant to the antimicrobial agent. These levels could then be tracked from farm to fork. Given current data limitations, this modeling approach was not possible, and future studies should aim to enumerate microbial levels and the resistant subpopulations so that more precise QRAs can be developed. The model also assumes that resistance does not transfer to other bacteria once it leaves the farm and that the resistant strains are not preferentially selected over the susceptible strains through processing and distribution. These limitations could lead to an underestimate of potential risk. Nevertheless, the number of excess cows developing resistance due to antimicrobial agents used to treat clinical mastitis is negligible, and therefore these model limitations are unlikely to significantly impact the risk associated with rbST supplementation.

In this model, we kept the total number of dairy cows in lactation in the United States at a fixed value, even though milk yield increases with rbST supplementation allowing milk demand to be met with fewer cows. Use of rbST increases a cow's daily milk yield by 4 to 5 kg; thus, across a lactation cycle the same amount of milk can be produced with 8 to 10% fewer cows *(8, 9)*. By keeping the total number of dairy cows fixed and by not adjusting the risk estimates to account for total milk demand by U.S. consumers, the model likely overestimates the number of mastitis cases in the rbST-supplemented cows.

The model estimates should be considered maximum risk and upper bounded owing to the multiple risk-maximizing parameters implemented. These maximum risk assumptions include (i) a significant transmission of resistant bacteria from the treated to untreated contact cattle, (ii) both treated and untreated cows acquiring resistant bacteria due to contact with treated animals will carry this resistance to slaughter with the same probability, (iii) parenteral and IMM administration of ceftiofur have the same impact on gut bacteria, (iv) resistance develops in susceptible bacteria after antimicrobial treatment, even though published work has rarely indicated any effect of treatment of individual animals on resistance development, (v) all of the *Campylobacter* strains found in cattle are capable of causing human disease (even though *Campylobacter coli* is most frequently isolated), (vi) all cows are carrying *E. coli* strains that can cause human illness, including enterotoxigenic *E. coli,* (vii) all ground beef from a pathogen-positive carcass will be contaminated, (viii) co-resistance to an antimicrobial agent used in human medicine occurs in 100% of antimicrobial-resistant isolates, and (ix) 100% of humans ill with antimicrobial-resistant bacteria and treated with an antimicrobial agent will experience an adverse outcome. Most cases of clinical mastitis are treated using IMM antimicrobial agents, and no research has indicated that this route results in increased AMR in gut bacteria; the model assumes that IMM and parenteral administration of antimicrobial agents have the same effect on gut bacteria.

The sensitivity of the model to the parameter of the probability of resistance developing following antimicrobial treatment was not high, but there is still considerable uncertainty regarding this parameter. We found little to no

evidence that resistance to the modeled antimicrobial agents develops in *Campylobacter, Salmonella,* or *E. coli* following the administration of the modeled antimicrobial agents to individual cows. A recently published systematic review of the literature evaluating the causal relationship between antimicrobial agent use in agriculture and resistance in *Campylobacter* highlighted the absence of studies to inform this relationship *(42)*. For example, Daniels et al. *(19)* found that resistance to the third-generation cephalosporins was widespread in both ceftiofur-treated and untreated calves, but no transfer of resistance was directly associated with ceftiofur treatment. In another study, Davis et al. *(21)* also found no relationship between ceftiofur use and emergence of resistance, primarily mediated by $bla_{\text{CTX-M}}$ in *E. coli*. When resistance has been observed following treatment, such as ceftiofur resistance in *E. coli* following treatment with ceftiofur, this effect was due to the elimination of the susceptible bacterial population; after a few days posttreatment, the susceptible bacterial population returned *(59)*. However, a 0% probability of resistance development cannot be modeled because the outcome of the model would indicate zero risk. Therefore a nonzero probability distribution for the probability of resistance development was used in the model, and it was assumed that 1 of every 200 treated cows will develop resistant bacteria following treatment. The scenario analysis for this parameter, in which the parameter estimate was increased up to 10-fold to account for uncertainty, increased the risk proportionately but still predicted a negligible risk associated with rbST supplementation.

All quantitative models have limitations that can systematically over- or underestimate (bias) the risk estimate. The estimates included in this model are based on maximum-risk assumptions and should therefore overestimate the actual risk. Given this approach, the results of this QRA indicate a high probability that the use of rbST according to FDA-approved label instructions presents a negligible risk for increasing the number of human illnesses associated with *Campylobacter, Salmonella,* or *E. coli* infection for which there is an adverse health outcome due to AMR.

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