Modeling the Survival of *Escherichia coli* O157:H7 in Apple Cider Using Probability Distribution Functions for Quantitative Risk Assessment

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ABSTRACT

Outbreaks of foodborne illness from apple cider have prompted research on the survival of *Escherichia coli* O157:H7 in this food. Published results vary widely, potentially due to differences in *E. coli* O157:H7 strains, enumeration media, and other experimental considerations. We developed probability distribution functions for the change in concentration of *E. coli* O157:H7 (log CFU/day) in cider using data from scientific publications for use in a quantitative risk assessment. Six storage conditions (refrigeration [4 to 5°C]; temperature abuse [6 to 10°C]; room temperature [20 to 25°C]; refrigerated with 0.1% sodium benzoate, 0.1% potassium sorbate, or both) were modeled. *E. coli* survival rate data for all three unpreserved cider storage conditions were highly peaked, and these data were fit to logistic distributions: ideal refrigeration, logistic (−0.061, 0.13); temperature abuse, logistic (−0.0982, 0.23); room temperature, logistic (−0.1, 0.29) and uniform (−4.3, −1.8), to model the very small chance of extremely high log CFU reductions. There were fewer published studies on refrigerated, preserved cider, and these smaller data sets were modeled with beta (4.27, 2.37) × 2.2 − 1.6, normal (−0.2, 0.13), and gamma (1.45, 0.6) distributions, respectively. Simulations were run to show the effect of storage on *E. coli* O157:H7 during the shelf life of apple cider. Under every storage condition, with and without preservatives, there was an overall decline in *E. coli* O157:H7 populations in cider, although a small fraction of the time a slight increase was seen.

Apple cider, a traditional, unpasteurized, acidic beverage, has become a vehicle for foodborne illness from *Escherichia coli* O157:H7 [1, 2]. The acid tolerance of this pathogen has been demonstrated in laboratory broth systems [7, 15], but these experiments do not adequately reflect the high survival rate of *E. coli* O157:H7 in apple cider [15]. Due to the pathogen's longevity in cider and its probable low infectious dose [5], several researchers have studied the survival of *E. coli* O157:H7 in apple cider. Because the experimental conditions and bacterial strains differed, it is not surprising that published results often vary considerably.

The eight articles we found relevant to *E. coli* O157: H7 survival in apple cider come from seven laboratories at Connecticut Agricultural Experiment Station [8, 9], University of Wisconsin [12–14], University of Georgia [15, 20], and University of Tennessee [16]. A total of nine different strains of *E. coli* O157:H7 were studied, the most commonly used strain being C7927, a clinical isolate from a human infected by drinking contaminated cider. Only two articles examined the effects of preservatives in cider [12, 20]. Storage temperatures examined were 4 [8, 12, 14], 5 [15], 6 [13], 8 [20], 10 [14], 20 [8, 16], and 25°C [8, 15, 20]. Several studies used only a selective medium for enumerating *E. coli* O157:H7 [14, 16, 20]; the others primarily used recovery media. The inoculum sizes ranged throughout five orders of magnitude, although 10^5 CFU/ml was the

most common inoculum size. The volumes of cider used varied from 10 ml to 1 liter, and the ciders undoubtedly differed in cultivar composition.

One useful tool that has emerged for assessing risk of foodborne illness is the quantitative risk assessment (QRA) [6, 19]. Work on a QRA for *E. coli* O157:H7 in apple cider is ongoing in our laboratory [10, 11], and survival of the pathogen in pressed cider is an important component of our model. The QRA offers a way to organize and combine published data from different laboratories into probability distribution functions (PDFs), which can be more reflective of the survival of *E. coli* O157:H7 than data from any single article. Because the accuracy of a QRA is in part dependent on the appropriateness of the PDFs that describe the various steps and conditions of food processing, the creation of PDFs is a critical part of the risk assessment process. As part of a QRA, these distributions can create a more accurate exposure assessment for *E. coli* O157:H7 in apple cider.

MATERIALS AND METHODS

A comprehensive literature search identified articles with data on *E. coli* O157:H7 survival in apple cider. No data from fermented or filtered apple cider were used, because they are not reflective of the fresh apple cider available commercially. The published tabular data were entered into an Excel (Microsoft, Redmond, Wash.) spreadsheet. Figures with relevant data were scanned into ScanPro (Jandel Scientific, San Rafael, Calif.), which allowed the graphical points to be accurately converted back into numerical data. These were added to the appropriate spreadsheets.

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Data sets that extended past 1 month were censored to fewer than 35 days, because cider typically has a shelf life of 1 month or less *(18)*. The change in *E. coli* O157:H7 concentration was calculated as change in log CFU/day. Where measurements were taken less frequently than once a day, a single rate was calculated for each gap and then converted to change in log CFU/day by assuming first-order inactivation kinetics.

The data were grouped into six categories: unpreserved cider under refrigeration (4 to 5°C), unpreserved cider under refrigeration abuse conditions (6 to 10°C), unpreserved cider at room temperature (20 to 25°C), refrigerated cider with 0.1% sodium benzoate, refrigerated cider with 0.05% or 0.1% potassium sorbate, and refrigerated cider with 0.1% sodium benzoate and 0.1% potassium sorbate. All data pertaining to each condition were grouped into a histogram and then fit to a probability distribution with Bestfit (Palisade Corp., Newfield, N.Y.).

The PDFs were coded into Analytica (Lumina, Los Gatos, Calif.), and simulations (1,000 iterations) were run to show the growth and decline of *E. coli* O157:H7 over time in cider, starting with an inoculum of 5 log CFU/ml. This inoculum size was chosen because it is frequently used in the literature (Table 1). This method of modeling survival of *E. coli* O157:H7 was chosen so predictions of cider safety can be made over time under various storage conditions. For each day the cider is held at a given storage condition, a unique "change in log CFU/day" value from the PDF describing that storage condition is used by the simulation. These values are added to the inoculum, producing the final number of *E. coli* O157:H7 in apple cider in log CFU/ml.

RESULTS

Most of the studies examined cider without any preservatives, usually under refrigeration or near-refrigeration storage conditions *(8, 12, 15, 20)*. Because that is the most typical storage condition for unpasteurized cider without preservatives *(18)*, this focus is understandable. Four laboratories studied the pathogen's survival in unpreserved cider at room temperature (20 to 25°C) *(8, 9, 15, 16, 20)*. Only two articles discussed preservative use *(12, 20)* and demonstrated varying bactericidal effects of sodium benzoate and potassium sorbate. The experimental conditions of all eight articles are listed in Table 1.

Figure 1 shows the resulting histogram and logistic distribution chosen to describe the data from four laboratories *(8, 12, 14, 15)* for cider held at ideal refrigeration conditions, defined as 4 to 5°C for the purposes of this study. The temperature abuse condition covered 6 to 10°C *(13, 14, 20)*. One of the articles *(20)* looked at two inoculum sizes, 2 and 5 log CFU/ml, but there did not appear to be an inoculum size effect, and data from both conditions were included in the temperature abuse data set. The rates of decline and increase are shown in a histogram with the fitted logistic distribution in Figure 2.

The decline of *E. coli* O157:H7 in cider was usually more rapid at room temperature (Fig. 3). Only Ryu and Beuchat *(15)* reported substantial (more than 56 days) survival—or even growth in some room temperature samples. Zhao et al. *(20)* reported extremely large reductions (greater than 4 log CFU/day) in cider held at room temperature, creating a very large range of possible rates, as indicated in the highly left-tailed Figure 3. Two distributions, a logistic to describe the bulk of the results and a uniform distribution to model the extremely high log reductions (i.e., the tail), were chosen. The logistic distribution would be used 93.7% of the time (reflective of the number of data points from the literature supporting it), and the uniform would be used 6.3% of the time. The line in Figure 3 is the result of an Analytica simulation of these two distributions used together.

Analytica simulations for ideal refrigeration (Fig. 4) and room temperature (Fig. 5) storage for cider for 9 days with an initial inoculum of 5 log CFU/ml are shown. Despite some positive tailing (i.e., values greater than zero) in all three logistic distributions mentioned, there is an overall net decline of *E. coli* O157:H7 in cider over time. In Figure 4A, the mean of the distribution is about 5 log CFU/ml, but after 5 days (Fig. 4B) the mean of the increasingly wide distribution is shifted to approximately 4.7 log CFU/ml. After 9 days (Fig. 4C), there is an average reduction of 0.5 log CFU/ml in *E. coli* O157:H7 in apple cider under ideal refrigeration conditions. In general, the distribution widens over time and shifts in the direction of log CFU/ml reductions.

A similar trend is seen for room temperature storage (Fig. 5). After 1 day at room temperature (Fig. 5A), the average *E. coli* O157:H7 population was approximately 5 log CFU/ml, with a wide range of less likely populations from 0.5 to 7 log CFU/ml. The peculiar left tail seen after 1 day disappears by the fifth day of the simulation (Fig. 5B). Over time the mean population decreases to less than 4 log CFU/ml after 9 days (Fig. 5C). A very large lower tail of higher log reductions develops over time, with a smaller higher tail of growth of *E. coli* O157:H7 in apple cider at room temperature. These higher tails in Figures 4 and 5 do not outweigh the bulk of the results for 5 and 9 days, which show a decline in bacterial counts. In general, the longer *E. coli* O157:H7 incubates in cider, the more likely it is to die off at any temperature. This is also true of cider at room temperature with any preservative (data not shown).

Zhao et al. *(20)* reported favorably on the efficacy of sodium benzoate and had great success using both 0.1% sodium benzoate and 0.1% potassium sorbate. Garland-Miller and Kaspar *(12)*, conversely, found that neither preservative was fast acting or effective against one strain, *E. coli* O157:H7 ATCC 43895. A beta distribution was fit to the data for cider with sodium benzoate in Figure 6A. The efficacy of potassium sorbate, shown in Figure 6B, was fit to a normal distribution with a small range of possible rates. Figure 6C contains data from only the study by Zhao et al. *(20)*, because the combination of two preservatives is not commonly practiced by cider producers or commonly researched by cider researchers. A gamma distribution fit these data.

DISCUSSION

The three unpreserved cider data sets were highly peaked around zero. Ideal refrigeration was the least variable PDF, with a range of results less than 2 log CFU/ml. As the storage temperature increased, so did the variation in the published data and the range of possible outputs of

TABLE 1. *The experimental conditions for relevant articles on E. coli O157:H7 growth and decline in apple cider[a]*

Source	E. coli O157:H7 strains	Temperature (°C)	Volume of cider	pH of cider on first day	Agar used for enumeration	Inoculum size (log CFU)
Dingman (8)	Gfp-72ec (USDA)	4; 20–25	Unpasteurized (not listed in article)	3.8	LEMB, TPAP, and SMAC for confirmation of O157	10^5/ml
Dingman (9)	Gfp-72ec (USDA)	Room temperature	2 apples' worth, unpasteurized	3.65–4.17	(Not explicitly listed in article for cider experiment, but probably TPAP)	2.2×10^4 (probably for the entire volume)
Garland-Miller and Kaspar (12)	ATCC 43889 (human), ATCC 43895 (human isolate from hamburger outbreak)	4	100 ml (probably unpasteurized)	3.7–4.1	TSA	3×10^4/ml
Leyer et al. (13)	932 (human isolate), 933 (hamburger), 505B (hamburger), 204P (pork), C7927 (human isolate from cider outbreak)	6	Commercially pasteurized (not listed in article)	3.46	TPAP	10^5/ml
Reoring et al. (14)	C7927 (human isolate from cider outbreak)	4; 10	100 ml unpasteurized and laboratory pasteurized	3.3–3.5	SMAC	10^7/ml
Ryu and Beuchat (15)	EO139 (deer jerky)	5; 25	10 ml commercially pasteurized	3.56–3.98	TSA; if necessary, then enriched and plated on SMAC	10^5–10^6/ml
Semanchek and Golden (16)	7927 (human isolate from cider outbreak)	20	1 liter unpasteurized	3.62	TSA plus chloramphenicol SMAC	10^6/ml
Zhao et al. (20)	7927 (human isolate from cider outbreak)	8; 25	50 ml unpasteurized	3.6–4.0	SMAC	10^2/ml, 10^5/ml

[a] USDA, U.S. Department of Agriculture; LEMB, Levine eosin-methylene blue agar; TPAP, tryptose-phosphate agar + 0.1% sodium pyruvate; SMAC, sorbital MacConkey agar; and TSA, tryptic soy agar.
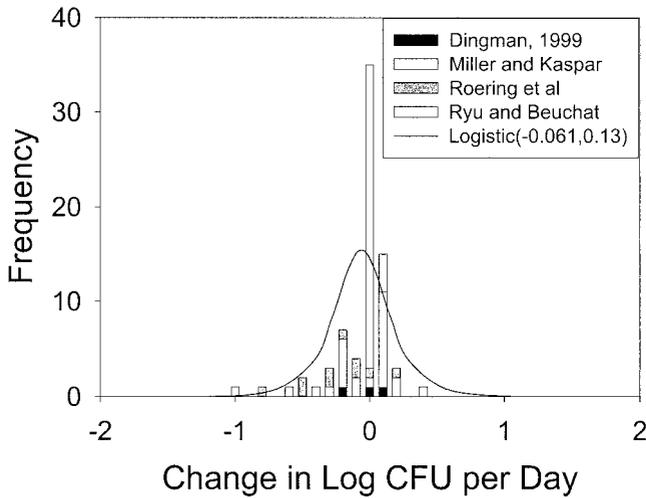
FIGURE 1. *The change in log CFU/day of* E. coli *O157:H7 in apple cider under ideal refrigeration conditions (4 to 5°C). The bar graph represents the histogram of data collected from the literature, with data points from each article in different shades. The solid line is a logistic distribution (−0.061, 0.13).*

FIGURE 2. *The change in log CFU/day of* E. coli *O157:H7 in apple cider under temperature abuse refrigeration conditions (6 to 10°C). The solid circles represent the histogram of data collected from the literature, and the solid line is a logistic distribution (−0.0982, 0.23).*

the resulting PDFs. The logistic distribution was chosen for its ability to emphasize these highly peaked data sets, while still accounting for any tailing in the data set. Short of using as yet undetermined polynomial equations, the logistic equation reflects the peaking trend best. If this extreme peaking is found to be common in food microbiology data sets, perhaps other distributions should be created to account for this phenomenon. The popular beta-pert distribution *(4)* was not considered, because it would not reflect the strong peak of any of the three conditions for unpreserved cider.

One potential explanation for the increased variability at warmer temperature conditions is in the enumeration methods used in these studies. It has been shown that *E. coli* O157:H7 from apple cider is acid stressed and does not grow as well on selective agar *(17)*. Of the four articles that examined ideal refrigeration, only one *(14)* used only a selective agar, sorbitol MacConkey agar (SMAC), for their studies; the other laboratories chose to enumerate on recovery media (most commonly tryptic soy agar or tryptose-phosphate agar with 0.1% sodium pyruvate). However, of the laboratories that studied temperature abuse conditions, two used SMAC *(14, 20)* and only one used a recovery agar *(13)*; therefore, the resulting differences in enumeration may have caused the greater variability in the results. At room temperature, the even wider range of results could again be due to differences in agar, since the study by Ryu and Beuchat *(15)* that shows the greatest persistence and growth of *E. coli* O157:H7 used recovery media and the study by Zhao et al. *(20)* that showed the fastest death rates used a selective agar.

Other experimental considerations may also have influenced the variability, since both Semanchek and Golden *(16)* and Zhao et al. *(20)* examined the same strain of *E. coli* O157:H7 (7927) in unpasteurized cider at similar temperatures (20 and 25°C, respectively) and both plated on SMAC and yet published very different results. Zhao et al.
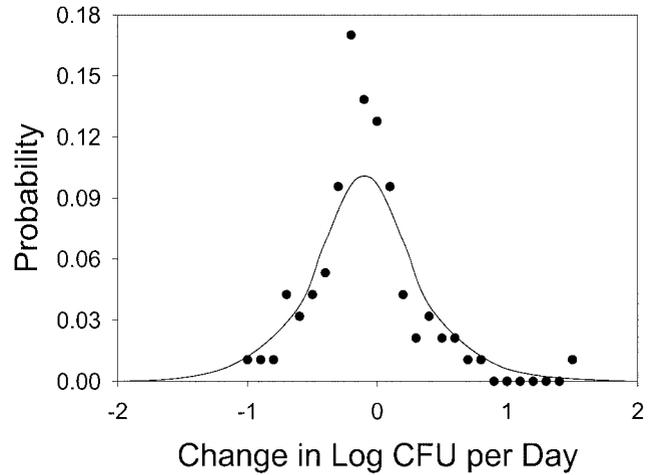
(20) showed a 5-log CFU/ml reduction in room temperature cider after 1 week, and Semanchek and Golden *(16)* reported a less than 4-log CFU/ml inactivation in 10 days at room temperature. The volumes of cider used by the two laboratories were quite different (50 ml and 1 liter, respectively), and sampling variation may account for the differing death rates of *E. coli* in the cider. Since the volume of cider used by Semanchek and Golden *(16)* was 20 times that studied by Zhao et al. *(20)*, one can imagine that Semanchek and Golden's results were an average of 20 samples studied by Zhao et al. It would be understandable, then, that the study by Zhao et al. *(20)* shows larger jumps in growth and decline than the study by Semanchek and Golden *(16)*, which shows a slow, steady decline of *E. coli* O157:H7 in cider. Zhao et al. *(20)* explain their reported
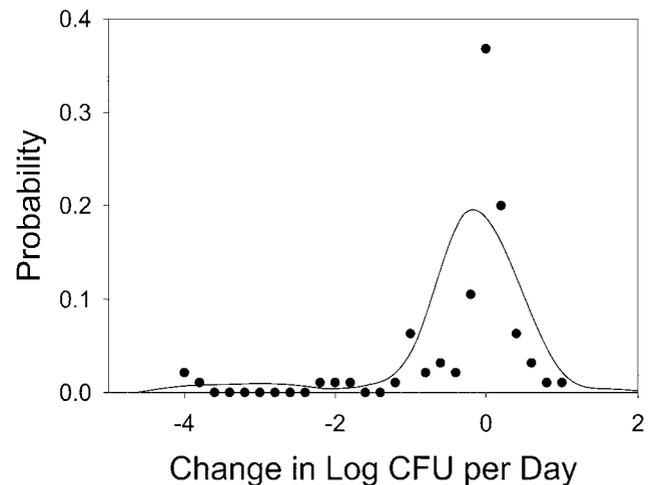

FIGURE 3. *The change in log CFU/day of* E. coli *O157:H7 in apple cider at room temperature (20 to 25°C). The solid circles represent the histogram of data collected from the literature, and the solid line is the result of a simulation in Analytica (1,000 iterations) using two equations: logistic (−0.1, 0.29) and uniform (−4.3, −1.8).*
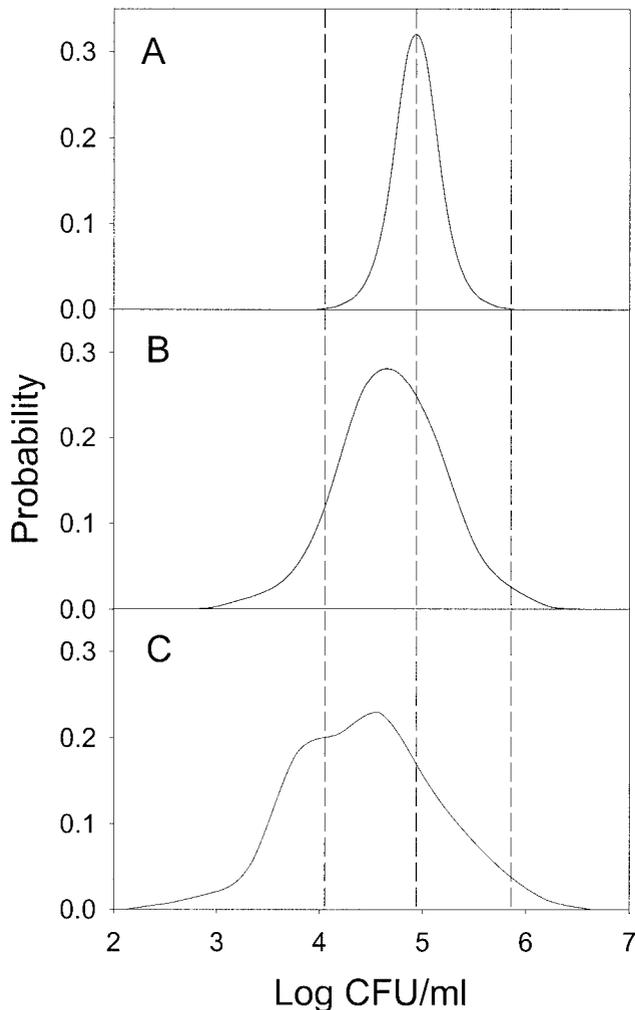
FIGURE 4. *The* E. coli *O157:H7 populations in cider held at ideal refrigeration conditions over time, starting from an inoculum of 5 log CFU/ml, simulated in Analytica (1,000 iterations). (A) Possible range of the population of* E. coli *for cider held for 1 day. (B) Possible populations after 5 days. (C) Possible populations after 9 days.*

FIGURE 5. *The* E. coli *O157:H7 populations in cider held at room temperature over time, starting from an inoculum of 5 log CFU/ml, simulated in Analytica (1,000 iterations). (A) Possible range of the population of* E. coli *for cider held for 1 day. (B) Possible populations after 5 days. (C) Possible populations after 9 days.*

increases in bacterial counts to resuscitation of injured cells, but since they plated on selective media and occasional growth of *E. coli* O157:H7 in cider was reported by several laboratories, this may be due to the aforementioned sampling variation rather than recovery of acid-injured *E. coli* O157:H7. Also, it has been suggested that the cultivars of apples used in each batch of cider could have an impact on the rate of decline of *E. coli* O157:H7 *(9)*. This alone may account for large differences between similar studies. Since most studies to date have not reported the cultivars used, this hypothesis can not be tested with the existing data.

Although it is known and accepted that recovery media are preferable to selective media when counting *E. coli* O157:H7 in apple cider, there is no accepted way to adjust the results of studies performed with selective media to account for their consistent undercounting of *E. coli*. Because of the wide variability in experimental conditions, any correction factor that could be created would not be universally applicable and would give inaccurate results to studies that were very different than those the correction
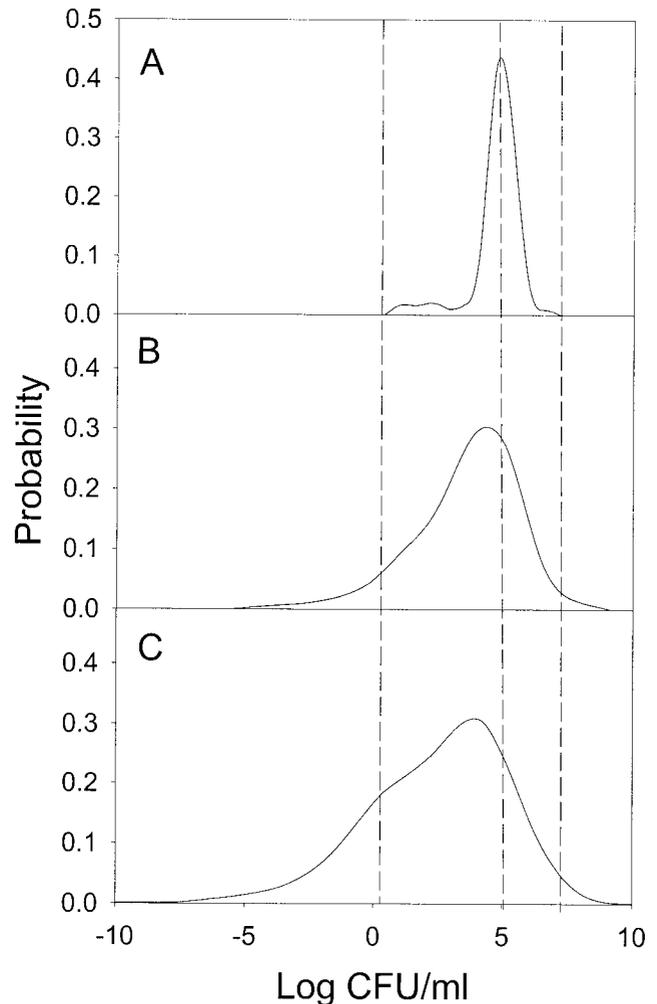
factor was derived from. Similarly, there is no proscribed technique to "weight" studies that have more realistic, cider mill–like conditions (larger volumes of cider, unpasteurized cider). A variety of different experimental conditions may be desirable, because it allows the variability of different strains of *E. coli* O157:H7, varying degrees of acid adaptation of the pathogen, and different formulations of cider to be automatically incorporated into the QRA. This variety must be tempered by the need to reflect the real-life farm-to-fork continuum of a food as accurately as possible.

It has not been established whether studies that use only 10 ml of cider are just as reflective of what occurs in a gallon of cider at a farm stand as those that use 1,000 ml, which is much closer to the quart- and gallon-sized containers commonly used for apple cider. The studies that used smaller volumes of cider often produced more data points than studies using larger amounts, perhaps because it was more affordable and less time intensive to do further experimentation with small amounts of cider, which inad-
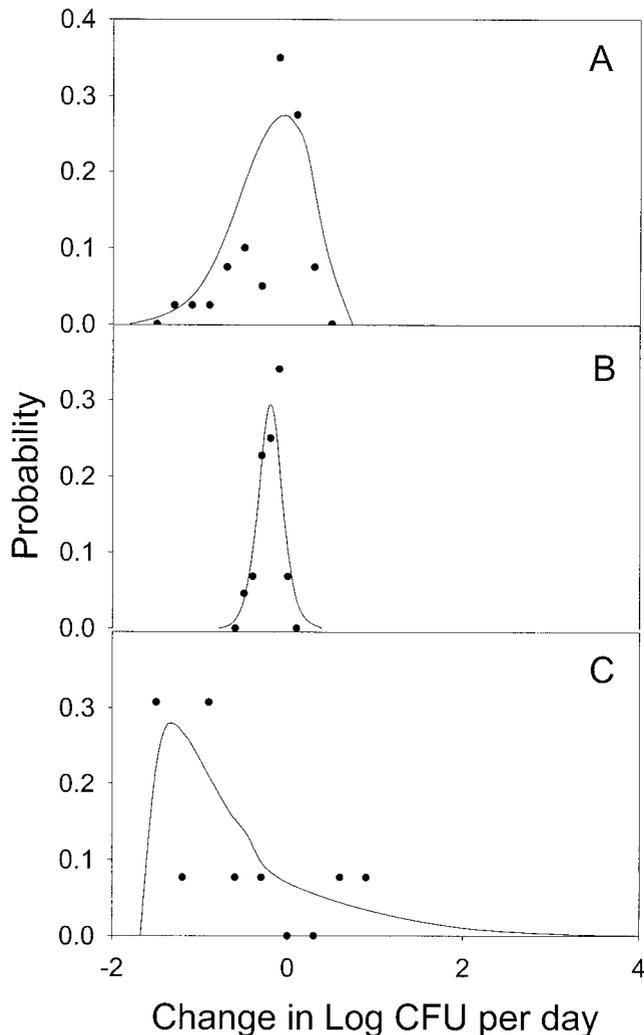
FIGURE 6. *The change in log CFU/day of* E. coli *O157:H7 in refrigerated apple cider (4°C) with different preservatives. The circles represent the histogram of data collected from the literature, and the line is the distribution fit to the data set. (A) Changes in* E. coli *O157:H7 populations per day in cider with 0.1% sodium benzoate, and the PDF is beta (4.27, 2.37) × 2.2 − 1.6. (B) Cider with 0.05% or 0.1% potassium sorbate, and the PDF is normal (−0.2, 0.13). (C) Cider with 0.1% sodium benzoate and 0.1% potassium sorbate, and the PDF is gamma (1.45, 0.6).*

very costly for a laboratory to conduct all of the experiments needed in a farm-to-fork risk assessment themselves, and this approach would limit the variability discussed above.

The only control a risk assessor has over the data is the power to censor data sets or to choose which articles to include. Because all of the articles discussed herein were published in peer-reviewed journals and did not contain any glaring systematic experimental errors, all articles were included and no data were excluded from the distributions. The choice not to censor data points that seemed to be outliers led to distributions with longer and more significant tails than the actual data set implied. This is not an undesirable consequence, since the goal is first to model the data set and then to model the reality of the process. The published data points are means: averages of duplicate plate counts and the mean of triplicate experiments, etc. Some of the potential range of results has been lost in the reporting process (the authors of these studies did not know their work would be incorporated into a QRA and that their variability would be important), and modeling with a PDF can recover this. The largest change in log CFU of *E. coli* O157:H7 in cider in a day reported in eight articles is smaller than the largest possible change in log CFU that might occur in the real world, and the tails of these distributions account for this.

The simulations in Figures 4 and 5 show that storage of apple cider, under any condition, usually causes a decline in the population of *E. coli* O157:H7. This is the same conclusion as the published results used to create this model. The decline due to storage does not approach the 5-log CFU/ml reduction required by the Food and Drug Administration to ensure cider safety *(3)* and could not be used as the sole method of preventing outbreaks of *E. coli* O157:H7 in apple cider under such regulations. However, since contamination of cider with this pathogen is not a common event and levels of contamination are not expected to be as high as 5 log CFU/ml or even 2 log CFU/ml, the effects of cider storage are an important component in a QRA for *E. coli* O157:H7 in apple cider. Storage may significantly affect the amount of viable *E. coli* O157:H7 in the cider on consumption when the amount of contamination is low.

CONCLUSIONS

The survival of *E. coli* O157:H7 in apple cider has been examined throughout the last decade, and probability distribution functions were created that accurately summarize trends in the literature. *E. coli* O157:H7 in cider without preservatives stored at 4 to 25°C showed a strong tendency to survive but a wide range of rates of decline, and even slight growth was reported under various conditions and modeled appropriately. Simulations revealed a general decline in *E. coli* O157:H7 in apple cider over time under any storage condition. Conflicting published results about the efficacy of preservatives in cider were reflected in the resulting PDFs. The problems of comparing different experimental designs and the incorporation of contradictory results into PDFs are not insignificant. As microbial QRA for food producers becomes better known and understood,

vertently weights the risk assessment toward studies done with small tubes of cider. Sampling variation may become an issue with smaller volumes of cider, as discussed above, but it would be inappropriate to simply discard studies for this reason alone.

Data from the literature do not support ignoring studies performed with pasteurized cider *(14)*, because these results are similar to unpasteurized cider. Despite the slow growth seen in Ryu and Beuchat's high pH (3.98), commercially pasteurized apple cider at room temperature (*E. coli* O157:H7 populations increased almost 2 log CFU/ml in a month), the change in log CFU/day was comparable to studies that showed an overall decline in *E. coli* O157:H7 (Fig. 1). Since these articles are the extant literature on the increase or decline of *E. coli* O157:H7 in apple cider, they are what a QRA for apple cider must be based on; it would prove

it is our wish that all food microbiologists will publish data in such a way as to facilitate their use in risk assessments.

ACKNOWLEDGMENTS

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ERRATUM

In the article "Bacterial Thermal Death Kinetics Based on Probability Distributions: The Heat Destruction of *Clostridium botulinum* and *Salmonella* Bedford," *Journal of Food Protection* 63(9):1197–1203, in the experiment with *C. botulinum,* seconds were quoted as the unit of time, but the actual units were minutes. Consequently all values and graphical scales quoted in seconds should now be read as minutes, and all values quoted in minutes:seconds, should now be understood as hours:minutes. This error does not occur in the *Salmonella* data, and does not affect *z*-values, because they are time-independent.

The derivation of an equation for *D*-values was intended as a link between the distribution-based model and commonly understood concepts of thermal inactivation. It is clear that extrapolation from the *D*-value model is heavily biased. To compare results of the model with common processing conditions, it is best to extrapolate from the basic model equation (4) in the paper, which gives:

Time for a 12-log reduction at 121°C = 50 min, 9 s
Log reduction at 121°C if heated for 147 s = 5.93