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Monte Carlo simulation of the risk of contamination of apples with *Escherichia coli* O157:H7

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Abstract

Quantitative descriptions of the frequency and extent of contamination of apple cider with pathogenic bacteria were obtained using literature data and computer simulation. Probability distributions were chosen to describe the risk of apple contamination by each suspected pathway. Tree-picked apples may be contaminated by birds infected with *Escherichia coli* O157:H7 when orchards were located near a sewage source (ocean or landfill). Dropped apples could become contaminated from either infected animal droppings or from contaminated manure if used as fertilizer. A risk assessment model was created in Analytica. The results of worst-case simulations revealed that 6–9 log CFU *E. coli* O157:H7 might be found on a harvest of 1000 dropped apples, while 3–4 log CFU contamination could be present on 1000 tree-picked apples. This model confirms that practices such as using dropped apples and using animal waste as fertilizer increase risk in the production of apple cider, and that pasteurization may not eliminate all contamination in juice from heavily contaminated fruit. Recently published FDA regulations for juices requiring a 5-log CFU/ml reduction of pathogenic bacteria in fresh juices should be a fail–safe measure for apples harvested in all but the worst-case scenarios.

Keywords: *Escherichia coli* O157:H7; Apple cider; Apple; Modeling; Quantitative risk assessment; Manure; Birds; Contamination

1. Introduction

Contamination of fresh, unpasteurized juices with food-borne pathogens is a problem that has received recent attention from government, industry and academic researchers (Dingman, 1999; FDA, 2001). Several outbreaks of hemorrhagic colitis and hemolytic uremic syndrome have been attributed to *Escherichia coli* O157:H7 in apple cider and juice in North America (Anonymous, 1996, 1998). This acid-tolerant pathogen is frequently found in livestock and wild animals (Rice and Hancock, 1995; Phillips, 1999), but had not previously been associated with fruit and fruit products prior to its discovery in apple cider. The routes by which apples become contaminated have been the subject of speculation by epidemiologists and food scientists, but since *E. coli* O157:H7 rarely causes an outbreak in apple cider, many suspected sources of contamination have never been confirmed, or even implicated, in an outbreak.

It is assumed that contamination of apple cider is a rare, or at least rarely reported, event because the low infectious dose of *E. coli* O157:H7 would insure that any amount of *E. coli* O157:H7 consumed could cause

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human illness (Dingman, 1999). New regulations from the FDA that call for a 5-log reduction of pathogenic bacteria in fresh juices (FDA, 2001) have increased interest in estimating the effect of various factors on the prevalence and concentration of *E. coli* O157:H7 on apples and in apple cider. Our objective was to describe and model the various sources of contamination that could potentially contribute to this problem, both in the orchard and in subsequent cider processing, using data available in the published scientific literature. Though future research will undoubtedly improve upon our work, this model describes the prevalence and concentration of *E. coli* O157:H7 that may occur on apples, and serves as a useful summary of the research to date on contamination of apples with *E. coli* O157:H7. Both aspects of this work are helpful as part of a full quantitative risk assessment (QRA) for *E. coli* O157:H7 in apple cider (Duffy and Schaffner, 1999).

2. Materials and methods

Potential sources of apple cider contamination were identified from epidemiological reports of *E. coli* O157:H7 outbreaks attributed to cider as well as original research on apples and apple cider in the literature. Research pertaining to the prevalence of other serotypes of *E. coli* O157 was also considered in the literature search, as it was assumed that these serotypes would be similar enough to *E. coli* O157:H7 to be included in subsequent modeling. Additional in-depth literature searches were conducted on each potential source. Expert opinion was solicited to fill in data gaps. Published tabular data were entered into Excel (Microsoft, Redmond, WA) spreadsheets. Figures with relevant data were scanned into ScanPro (Jandel Scientific, San Rafael, CA), which allowed the graphical points to be accurately converted back into numerical data. Sources of contamination were separated into two categories: those affecting apples on the tree and those affecting apples that had fallen to the ground (known in the industry as windfalls or drops). Contamination by land animal feces was divided into two categories, infected animal droppings in the orchard and contamination due to manure, which is used as fertilizer in the orchard.

Data were collated into histograms using Excel and analyzed with BestFit (Palisades Decision, Newfield, NY) to create probability distribution functions (PDFs). Some data were analyzed by correlation in Excel. Data were synthesized into simple PDFs, such as the triangular distribution, when fewer data points were present in the literature. These PDFs were combined using reasonable assumptions in the Monte Carlo simulation software, Analytica (Lumina, Los Gatos, CA), and simulated for at least 1000 iterations and up to 5000 iterations.

3. Results and discussion

3.1. Model development

A paucity of published data exists describing the contamination of apples destined for apple cider production. No survey that has set out to quantify the prevalence of *E. coli* O157:H7 in orchards has ever found it (Dingman, 1999; Riordan et al., 2000). *E. coli* O157:H7 has been found in animal vectors (but not on the fruit itself) near orchards that produced apples implicated in outbreaks (Trevena et al., 1996). The major routes of contamination of apples with *E. coli* O157:H7 are thought to be through contact with feces from infected animals (Naylor et al., 1998). This can occur when apples drop to the ground if the orchard was fertilized with manure, or if there are animal droppings on the ground in the orchard; apples on the tree can be contaminated by bird droppings. The routes of contamination for tree-picked apples were examined first.

3.1.1. Tree-picked apples

It has been shown that birds, specifically gulls, can carry and shed *E. coli* O157:H7, but only when there is an infected food source nearby on which the gulls can feed (Wallace et al., 1997). Proximity to sewage sources has also been associated with *Listeria* carriage in birds (Fenlon, 1998). Whether or not an orchard is near such contamination sources, impacts the probability that birds flying over the orchard will be shedding *E. coli* O157:H7. While many theoretical sources of contamination are possible, few are documented in the literature. The locations of landfills and oceans were specified in the model because they were implicated in the literature. This is one of several user-defined inputs used in this simulation, shown in Table

1. Whether or not an orchard is located close to an ocean or landfill is used in the model (see the first line of Table 2); if not, then no birds will be infected with *E. coli* O157:H7, and there will be no contamination of tree-picked apples. The prevalence of *E. coli* O157:H7 in birds around sewage sources was described by a triangular distribution (Table 2) where both the maximum (2.9% from gulls on a beach) and most likely (0.9% from gulls near a landfill) values were taken from Wallace et al. (1997). It was assumed that birds (having the same prevalence of *E. coli* O157:H7 as gulls) would relieve themselves on each acre of the orchard, and 1 acre was modeled as representative of the contamination of the whole orchard. No studies on the concentration per gram of *E. coli* O157:H7 per gram of bird feces were identified in our literature search, but there are such data for cow feces. We assumed that this was an adequate substitute, and thus fit data on *E. coli* O157:H7 per gram of cow feces from Zhao et al. (1995) to a logistic distribution (RMS error = 0.0028) to describe the assumed concentration of *E. coli* O157:H7 per gram of bird feces (Table 2).

It was assumed that each bird's droppings would weigh 10 g. Since we were unable to locate the average weight of a gull dropping from our literature search, this weight was derived from daily feces weight of broiler chickens (~ 64 g/day) (Hermanson et al., 2001) and frequency of defecation of horses (6–8 times/day), the only animal for which we obtained defecation frequency data (Malinowski, personal communication). We assumed that the gulls would defecate seven times a day, giving an average of 9.14 g/dropping, which was rounded to 10 g/dropping. The total contamination on apples remaining on the tree was then calculated. It was assumed that all contamination from birds would land on apples and not on leaves, branches or onto the ground. The contamination is assumed to be evenly spread throughout the apples in the orchard for simplicity.

Table 1
The variables that the user of the model can control

User-defined input	Possible responses
How many acres is the orchard?	1–100 by 5
How many apples per acre?	100,000[a]
How many apples harvested at one time?	1000[a]
Is the orchard near a landfill or the ocean?	yes/no
Is the orchard manured with animal waste?	yes/no
How many days after applying manure do you harvest the apples?	100[a]
Number of animals[b] in the orchard last month	0–100 by 5
Number of dogs in the orchard last month	0–10
What percentage of drops do you use?	0–100 by 5

[a] This value was fixed for the simulations presented in this paper, but can be altered in the computer model.

[b] In the computer model, there are separate questions for cows, horses, sheep, pigs and deer (each from 0 to 100). A representative question has been presented here.

3.1.2. Dropped apples

Though nonpathogenic *E. coli* has been found on both tree-picked and dropped apples (Dingman, 1999), dropped apples are more often and more heavily contaminated with fecal coliforms (Goverd et al., 1979; Lang et al., 1999). Dropped apples are also more likely to become colonized by the bacterium; when an apple is bruised by a fall to the ground, the pH of the bruised tissue becomes more neutral and the sugar content is lowered—conditions which are more permissive to *E. coli* O157:H7 growth (Dingman, 2000). It is not surprising, therefore, that dropped apples have been implicated or suspected in at least three outbreaks of hemorrhagic colitis from apple cider (Anonymous, 1998; Besser et al., 1998; Cody et al., 1999). Recent surveys of apple cider producers show that it is well known that dropped apples are associated with outbreaks of food-borne illness (Uljas and Ingham, 2000), but 16% of surveyed cider producers in Wisconsin (Uljas and Ingham, 2000), 27% in Maryland (Senkel et al., 1999) and 32% in Virginia (Wright et al., 2000) still used dropped apples in their cider at the times of the respective studies.

Cows are thought to be the natural reservoir for *E. coli* O157:H7, and other animals have been shown to carry and shed the pathogen (Beutin et al., 1996; Phillips, 1999), so the prevalence of *E. coli* O157:H7 in animals that could be present in an orchard was estimated. Proximity to cattle pastures has been identified as a potential source of *E. coli* O157:H7 contamination in at least two outbreaks (Anonymous, 1998; Besser et al., 1998), and 22% of cider producers in Wisconsin and 54% in Virginia have livestock farms adjacent to their orchards (Wright et al., 2000; Uljas and Ingham, 2000). Five percent of Virginia cider producers even allow animals to graze in their orchards (Wright et al., 2000). Data from numerous papers on *E.*

Table 2
Variables and distributions that affect the levels of contamination with *Escherichia coli* O157:H7 on tree-picked apples

Name of node	Description	Ref.
Gulls per acre	If (**Orchard near landfill or ocean** = 'Yes') Then Triangular(1, 3, 10) Else 0	Fenlon, 1998; Wallace et al., 1997
%O157 in gulls	Triangular(0, 0.009, 0.029)	Wallace et al., 1997
Infected gulls per acre (array 0...10)	If (*X* <= **Gulls per acre**) Then Bernoulli(**%O157 in gulls**) Else 0	
Infected gulls in orchard	Sum(**Infected gulls per acre**) * **Acreage**	
O157/gram from gulls in orchard (array 1...300)	If (*X* <= **Infected gulls in orchard**) Then Logistic(4.09128, 0.31460) Else 0	Zhao et al., 1995[a]
Convert from logs	If (**Orchard near landfill or ocean** = 'Yes') Then 10^**O157/gram from gulls in orchard** Else 0	
Total O157 from gulls on undropped apples	Sum(**Convert from logs**) * 10 g/dropping	

Names of nodes are bolded.

[a] No data were available for birds, so this reference, which pertains to cows, was used.

"<=" signifies "less than or equal to."

coli O157:H7 in cows (Zhao et al., 1995; Dargatz et al., 1997; Chapman et al., 1997, 2001; Hancock et al., 1997a,b; Wells et al., 1998; Heuvelink et al., 1998; Johnsen et al., 2001) were pooled into a histogram and fit with BestFit. Studies which reported percentages of cattle shedding verocytotoxigenic *E. coli*, but not the prevalence of *E. coli* O157 serotypes (Phillips, 1999), were not included. A lognormal distribution (Table 3) fits the data well (RMS error = 0.0000514).

Fewer researchers have examined the prevalence of *E. coli* O157:H7 in wild deer, though deer have been shown to carry and shed *E. coli* O157:H7 (Keene et al., 1998) and are suspected contaminators of apple orchards (Rice and Hancock, 1995; Cody et al., 1999). Two papers on the subject reported prevalence as a single number, so a triangular distribution was used (Table 3), where the most likely value was published in a study of prevalence in deer unrelated to any outbreak of *E. coli* O157:H7 (Rice and Hancock, 1995), and the maximum value is from a study conducted in the area of an outbreak of hemorrhagic colitis associated with deer jerky (Keene et al., 1998).

Fewer data exist regarding prevalence of *E. coli* O157:H7 in other livestock and companion animals. Two studies we identified each had one prevalence of *E. coli* O157:H7 in pigs (Chapman et al., 1997; Johnsen et al., 2001) and so prevalence was modeled as a triangular distribution (0%, 0.04%, 0.1%). Prevalence in sheep was found in one study to be 1.4% (Chapman et al., 2001) and 2.2% in another study by the same researchers (Chapman et al., 1997), and was also modeled with a triangular distribution (Table 3). There were no data available on the prevalence of *E. coli* O157:H7 in horses or dogs, though it has been confirmed that both of these animals can carry and shed the pathogen (Trevena et al., 1996). Consequently, the prevalence distribution developed for cows was used for both horses and dogs in this model, so that the effect of the presence of these animals on contamination of apples could be assessed. Since cows have the highest reported prevalence of *E. coli* O157:H7, using a cow distribution to represent prevalence in other animals is a conservative assumption.

It was assumed that a maximum of 100 each of cows, deer, pigs, sheep and horses would pass through the model orchard (the maximum size of the orchard is 100 acres) in a month, and a smaller amount (up to 10) of dogs would pass through the orchard in a month. A simplifying assumption was made that only animal droppings from the previous month could affect apples on the ground, though the longevity of this pathogen in feces may be over 1 month (Wang et al., 1996; Bolton et al., 1999). The number of each type of infected animal is multiplied by how often the infected animals defecate in the orchard. The values shown in Table 3 are not based on scientific literature, but rather are

Table 3

Variables and distributions that affect the levels of contamination with *Escherichia coli* O157:H7 on dropped apples

Name of node	Description
%O157 in cows[a]	Lognormal(7.2592, 3.7124) − 2.3470
%O157 in deer	Triangular(0, 0.01851, 0.11)
%O157 in pigs	Triangular(0, 0.0004, 0.001)
%O157 in sheep	Triangular(0, 0.014, 0.022)
Infected *animals*[b] (array 1...100)	(If (X<=***animals* in orchard**) Then Bernoulli(**%O157 in *animals***) Else 0)
Infected horses (array 1...100)	(If (X<=**Horses in orchard**) Then Bernoulli(**%O157 in cows**) Else 0)
Infected dogs (array 1...10)	(If (X<=**Dogs in orchard**) Then Bernoulli(**%O157 in cows**) Else 0)
Chance of livestock droppings	Discrete probabilities for 0–6 droppings, maximum probability 0.25 at 1 dropping
Chance of housepet droppings	Discrete probabilities for 0–10 droppings, maximum probability 0.25 at 5 droppings
Chance of wild animal droppings	Discrete probabilities for 0–3 droppings, maximum probability 0.5 at 1 dropping
Infected *animal*[b] **droppings**	Sum(**Infected *animals***) * **Chance of livestock droppings**
Total infected droppings	**Infected cow droppings** + **Infected sheep droppings** + **Infected horse droppings** + **Infected dog droppings** + **Infected pig droppings** + **Infected deer droppings**
Proportion of 9 in.^{2} with infected droppings	**Total infected droppings/Total 9 in.^{2} in orchard**
Infected drops due to droppings (array)	Bernoulli(**Proportion of 9 in.^{2} with infected droppings**) * Triangular($10^{4.9}$, $10^{6.1}$, $10^{6.9}$)
Total amount of manure	**Acreage** * 1,000,000 g/acre
Contamination of cow feces	Logistic(4.09128, 0.31460) log CFU/g
Decline per day in feces	Logistic(− 0.0633981, 0.0087256) log CFU/g/day
Concentration at harvest	**Contamination of cow feces** + **Decline per day in feces**
Total contamination by manure	If (**Is the orchard is manured with animal waste** = ‘yes’) then **Total amount of manure** * **%O157 in cows** * **10^Concentration at harvest** else 0
Manure contamination per 9 in.^{2}	**Total contamination by manure/Total 9 in.^{2} in orchard**
Total contamination on dropped apples	**Number of drops** * **Manure contamination per 9 in.^{2}** + Sum(**Infected drops due to droppings**)
Total contamination on all apples	Log(**Total O157 from gulls on undropped apples** + **Total contamination on dropped apples**)

Names of nodes are bolded.

[a] This distribution can produce responses that are less than zero, so it is error-trapped in the model. When this distribution produces a negative number, it is changed to zero.

[b] In the computer model, each animal has its own equations, however, generic ‘animal’ nodes have been presented where possible.

“<=” signifies “less than or equal to.”

scaled against each other, so that housepets, like dogs, are more likely to leave more droppings in the orchards than wild animals, like deer, which are just passing through. These values were also based on the frequency of horse defecation (Malinowski, personal communication).

The numbers of infected droppings from all animals were summed. We assumed that each dropping occupies its own square foot (no two droppings in the same square foot) and calculated the proportion of square feet in the orchard containing infected animal droppings. Any apple that falls onto a 9-in.^{2} area with an animal dropping on it becomes colonized by *E. coli* O157:H7 to the maximum CFU/wound levels derived from those reported by Janisiewicz et al. (1999). Each dropped apple that contacts infected animal droppings receives its own unique population from this distribution. Other data on the colonization of bruised apple tissue with *E. coli* O157:H7 (Dingman, 2000) were not used because of the isolation method (plated juice squeezed manually from infected tissue) which relied on an arbitrary multiplicative factor to back-calculate the true populations of *E. coli* on the apples, and was less standard than the method used by Janisiewicz et al. (1999) (infected tissue stomached with phosphate buffer).

E. coli O157:H7 can also contaminate dropped apples if infected animal waste is used as fertilizer in the orchard. If an apple falls onto ground that has been manured, the simulation assumes that the apple becomes contaminated with all the bacteria in an assumed 9 in.^{2} of ground that the apple contacts. It was assumed that any manure used came from cattle because more data exist on the survival of *E. coli* O157:H7 in bovine feces than for any other animal (Wang et al., 1996; Kudva et al., 1998; Bolton et al., 1999; McGee et al., 2001). A logistic distribution for initial population of *E. coli* O157:H7 in bovine feces was fit to data from Zhao et al. (1995) (RMS error = 0.0028). Data on survival of *E. coli* O157:H7 in bovine feces at moderate temperatures (10–23 °C) were taken from four papers (Wang et al., 1996; Kudva et al., 1998; Bolton et al., 1999; McGee et al., 2001) by calculating a single change in log CFU *E. coli* O157:H7/day for each published replicate, as shown in Eq. (1).

$$\frac{\log(\text{Ending Population}) - \log(\text{Starting Population})}{\text{Ending Day} - \text{Starting Day}} \tag{1}$$

These data were then fit to a PDF with BestFit and a logistic distribution was chosen to describe the decline of *E. coli* O157:H7 over time in bovine feces. The number of days prior to harvest that the field was manured can be input (Table 1), and a single value from this logistic distribution is used to estimate the population of *E. coli* O157:H7 on the manured ground at the time of harvest. Quantities of manure typically used for agricultural fertilization vary widely depending on soil type, crop and expert opinion. It was very difficult to determine an appropriate value for use in the simulation. The quantity of manure was assumed to be 1000 kg/acre (~ 3000 kg/ha), but this could easily be modified to any appropriate value. The contamination was assumed to be spread evenly throughout the orchard (Table 3).

E. coli O157:H7 contamination of dropped apples may be both internal and external to the fruit, though this model does not distinguish between the two. Internal contamination of apples has implications for cider processing (Buchanan et al., 1999), but not for total contamination of harvested apples—the endpoint of this model (Table 3).

3.2. Simulation

3.2.1. Tree-picked apples

The contaminating effect of birds was simulated for 1000 tree-picked apples, assuming that the orchard was near an ocean or landfill (Fig. 1). Most of the time (96.7%), the apples are not contaminated with any *E. coli* O157:H7, even though the orchard is near a source of raw sewage. There is a distribution of potential levels of contamination, as shown in the inset in Fig. 1. Contamination of tree-picked apples with pathogenic *E. coli* is uncommon, and when tree-picked apples do become contaminated, it is at a very low level. Fig. 1 inset shows a contamination level of 3–4 log CFU per 1000 apples which (assuming even levels of contamination) is 1–10 CFU/apple.

The two factors that affect the frequency of contamination of tree-picked apples are the prevalence of *E. coli* O157:H7 in birds and the number of birds that defecate per acre of orchard. While the *E. coli* O157:H7 prevalence in birds was based on values found in the literature, the number of birds per acre (set at 10) was an assumption—as there are no published data on this topic. If more birds defecate on the orchard, the frequency of contamination of tree-picked apples near a sewage source would increase. Future research may show how many birds defecate on an acre of orchard in

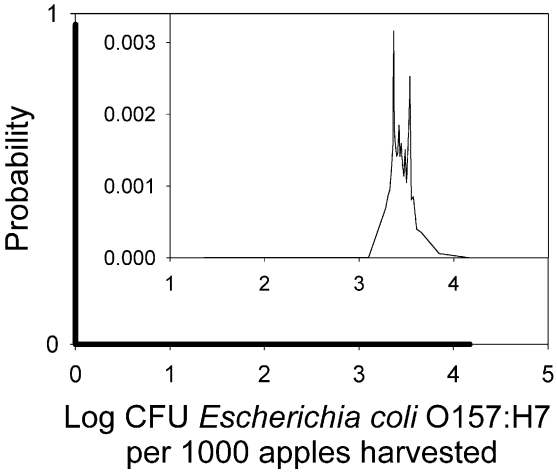

Fig. 1. The probability of *E. coli* O157:H7 contamination on 1000 tree-picked apples in a 100-acre orchard near an ocean or landfill. There are no *E. coli* O157:H7 on the apples (thick lines on the axes in the large graph) 96.7% of the time. The range of levels of contamination is enlarged in the inset graph.

a given time span, and then the accuracy of the assumption of 10 birds/acre and the resulting simulation can be assessed. It is also important to note that the phrase “near to an ocean or landfill” is not yet defined with a specific distance, but should be in the future to determine which orchards are susceptible to contamination of tree-picked apples and which are not. The model also does not account for decline in *E. coli* O157:H7 populations in the bird feces on the apples over time, which may mean that the model overestimates the amount of bacteria on the apples at time of harvest. Again, future research can refine the model and reduce the number of assumptions in the simulation.

3.2.2. Dropped apples

To investigate the effect of manure and animal droppings on dropped apples, several worst-case scenarios were run. These scenarios were chosen to both set an upper boundary on the extent and frequency of contamination of apples with *E. coli* O157:H7 and because most non-worst-case simulations of our model do not predict any contamination in 1000 iterations.

We investigated the effect of animal waste manure on the contamination of dropped apples through several worst-case simulations. Fig. 2 shows the probability of various levels of *E. coli* O157:H7 contamination on 1000 dropped apples in a 100-acre orchard that has the maximum number of each animal passing through the orchard, and that used animal manure as a fertilizer 1 day prior to harvest (Fig. 2A), 10 days prior to harvest (Fig. 2B) or 100 days prior to harvest (Fig. 2C). This simulated orchard was not near an ocean or a landfill to eliminate contamination of apples while on the tree. When dropped apples are harvested the day after manuring, the levels of contamination are very high and the chance of having apples without any contamination is 7.4% (spike at $x = 0$). There is an 11.2% chance of having no *E. coli* O157:H7 on 1000 dropped apples harvested 10 days after manuring. One hundred days after manuring, there is a 21.7% probability that 1000 dropped apples would not be contaminated (i.e. less than 1 CFU *E. coli* O157:H7), a much larger probability than that immediately following manure application (7.4%). After 100 days, there is also a less than a 0.3% chance of having *E. coli* O157:H7 populations over 5 log CFU on 1000 dropped apples.

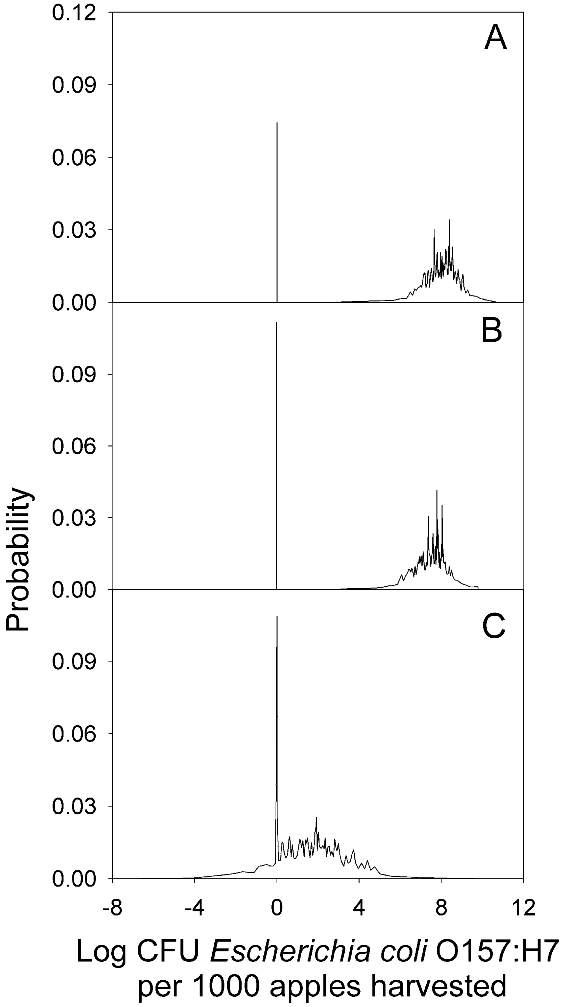

Fig. 2. The probability of *E. coli* O157:H7 contamination on 1000 dropped apples in a 100-acre orchard that is not near an ocean or landfill, has the maximal number of all animals passing through the orchard and was manured with animal waste (A) 1 day, (B) 10 days or (C) 100 days prior to harvest.

Since these three simulations included the maximum chance of contamination by animal droppings, the low level of overall contamination in Fig. 2C reflects how infrequently 1000 apples in a 100-acre orchard will encounter animal droppings in this model. This was verified by simulations of a 100-acre orchard that was not near an ocean or landfill and that was not manured with animal waste, but did have the maximum number of animals passing through and harvested only dropped apples. The frequency of an apple in the 1000 harvested becoming contaminated was less than 0.1%, as repeated 1000 iteration simulations failed to produce any contaminated fruit (data not shown). A 5000-iteration simulation at these conditions, however, showed a 0.082% contamination rate (data not shown). To increase the frequency of contamination of apples from infected animal droppings, a 1-acre orchard was simulated, with the maximum number of animals passing through it. This causes a 100-fold concentration of number of droppings in an orchard. The orchard was not near an ocean or landfill and it was not fertilized with animal waste, but it did harvest only dropped apples. Fig. 3A shows the results of that simulation, and Fig. 3B shows the same scenario with animal manure (applied 10 days prior) on the orchard. The chance of all 1000 dropped apples not landing in any infected animal droppings is 5.8% (Fig. 3A) and 4.6% when the ground is manured (Fig. 3B). The range of potential contamination levels is lower and narrower for the orchard without manure than the orchard with animal manure, and the manured orchard has a much longer right tail. These results are not surprising since it has already been established that manure is a more important contaminant of apples in a 100-acre orchard than infected animal droppings. A comparison between Figs. 2B and 3B (simulations differing only in size of the orchard) reveals that the levels of contamination on 1000 dropped apples on manured ground are similar, again reinforcing the notion that animal manure is a more significant contaminator of dropped apples than infected animal droppings according to the model and assumptions used here.

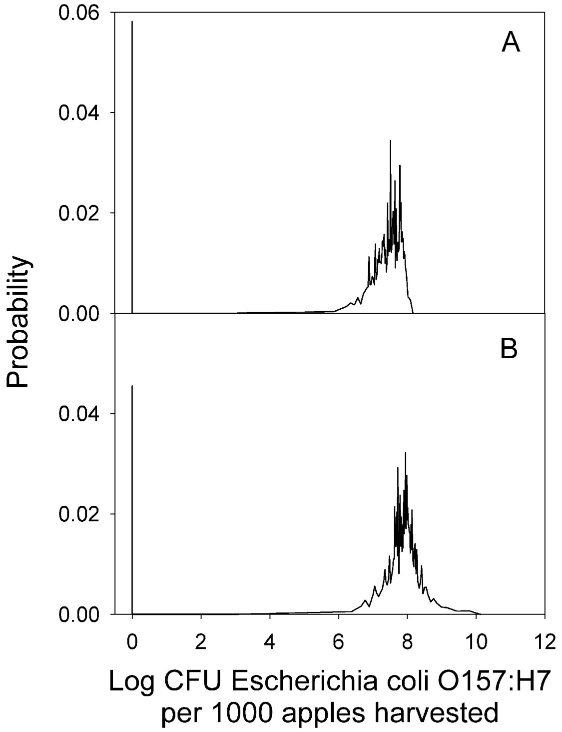

Fig. 3. The probability of *E. coli* O157:H7 contamination on 1000 dropped apples in a 1-acre orchard that is not near an ocean or landfill and has the maximal number of all animals passing through the orchard; (A) not manured with animal waste and (B) manured 10 days prior to harvest.

Although the effect of contaminated animal droppings is less important than animal manure when harvesting dropped apples, every effort should be maintained to keep animals out of the orchard. Since our model assigns such a high population of *E. coli* O157:H7 in an apple wound if an apple falls into an infected animal dropping, a single apple or two can skew the microbial population of the whole 1000-apple harvest. Fig. 4 shows a simulation of a 1-acre orchard that does not use animal manure, is not near an ocean or landfill and keeps all animals except 100 deer out of the orchard. Again, only dropped apples were harvested. Even in this concentrated, 1-acre orchard, there are only 20 infected batches of apples in 1000 iterations (2% of harvests). These contaminated batches of 1000 apples occurred when the number of infected animal droppings was high (at least 24 infected droppings)—though high numbers of infected droppings did not correlate well with log CFU counts on 1000 apples ($r^2 = -0.04455$). The log CFU counts in Fig. 4 indicate that often more than one apple has fallen into an infected deer dropping in this scenario, as the counts on 1000 apples often fall outside the distribution of maximum *E. coli* O157:H7 CFU/wound. Even though this is still a worst-case scenario—only dropped apples were harvested, the orchard has an inordinately high concentration of *E. coli* O157:H7 infected deer droppings—it is more realistic than the previous simulations because it only includes an animal that many apple growers have a hard time excluding from their orchards (Naylor et al., 1998), and the microbial counts are lower than those obtained in the previously presented simulations. Still, since a single apple or two can be contaminated to high

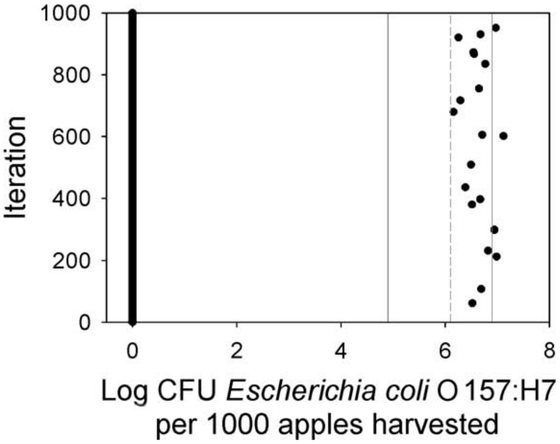

Fig. 4. The amount of *E. coli* O157:H7 contamination on 1000 dropped apples harvested in a 1-acre orchard that is not near an ocean or landfill, is not manured and that has the maximum number (100) of deer passing through it. The solid circles represent the levels of contamination achieved in 1000 iterations of a simulation. The solid gray lines represent the minimum and maximum of the triangular distribution for *E. coli* O157:H7 populations in apples that have dropped into infected animal droppings (Table 3); the dashed gray line is the most likely value of that triangular distribution.

levels, this highlights the importance of careful culling of dropped apples, though this alone cannot assure the safety of apples and apple products.

The simulations presented have all been worst-case scenarios and the populations of *E. coli* O157:H7 on apples harvested on an actual orchard are likely to be lower than those predicted here. For example, when an orchard is not near an ocean or landfill and no dropped apples are used, the model predicts that the apples will never be contaminated—even if the orchard is manured with animal waste and animals graze in the orchard. While this scenario is the opposite extreme, the majority of cider producers do not use dropped apples (Senkel et al., 1999; Uljas and Ingham, 2000) and animal manure is rarely used as a fertilizer (Wright et al., 2000). Therefore, these worst-case simulations are not reflective of typical orchard conditions. They do, however, set an upper boundary for the levels of contamination possible on 1000 apples in an orchard. The simulation nonetheless reinforces what food science and pomology extension specialists have been advocating for the past decade—do not harvest dropped apples, keep animals out of the orchard and do not use animal waste to fertilize your orchard (Naylor et al., 1998). It is also advisable to not have an orchard located near a sewage source, such as a landfill, or near the ocean.

It is also important to note that this model is built upon the existing literature base and a number of assumptions were used when data were not available. It is virtually impossible to validate this model in its entirety, and so its predictions should not be trusted as fact, but rather as an estimate—especially as an estimate of worst-case scenarios. This risk assessment is useful as a rough quantitative description of the contamination of apples with *E. coli* O157:H7 based on current knowledge, which organizes and synthesizes that knowledge together and that can direct future research into apple contamination in the future.

3.3. Implications for apple cider

The population of *E. coli* O157:H7 on the apples once they reach the cider mill may not foretell the safety of the resulting apple cider. Many bactericidal processes used in cider production cause at least a 5-log reduction of *E. coli* O157:H7/ml of cider (McLellan and Splittstoesser, 1996; Uljas and Ingham, 1999; Duffy et al., 2000), which should be sufficient to inactivate the levels of bacteria predicted on 1000 apples here. Previously untainted apples could also become contaminated in cider processing; the use of contaminated well water has been implicated in one cider-borne outbreak of *E. coli* O157:H7 (Anonymous, 1998). Even in studies of cider safety that did not survey pasteurized cider, the relationship between microbial counts on apples and the microbial counts of apple cider has been questioned (Dingman, 1999).

Since *E. coli* O157:H7 has a low infectious dose (Buchanan and Doyle, 1997), it is safe to assume that when apples are contaminated, the resulting cider could cause illness if bactericidal interventions are not employed. There have only been a handful of outbreaks of *E. coli* O157:H7 in apple cider, and so it is probable that the frequency of contaminated apples is much lower than the worst-case scenarios presented here. Our analysis also indicates that the recommendations put forth by the FDA (2001) are probably adequate for most of the worst-case contamination levels of *E. coli* O157:H7 that could occur on dropped apples, but could occasionally fail when the apples are very highly contaminated. These regulations seem more than sufficient to inactivate the levels of bacteria present on tree-picked apples.

3.4. Additional factors

There are other factors that could influence the contamination of apples with *E. coli* O157:H7 that have not been included in this model. One particularly difficult correlation to add to the QRA is that of time of harvest. Dingman (1999) found that cider was more often contaminated with nonpathogenic *E. coli* when pressed from apples harvested between mid-October and mid-November. However, the highest levels of *E. coli* O157:H7 shedding in livestock have been reported to be in the spring (Chapman et al., 1997). Dingman (2000) has also studied another variable not yet included in this QRA: the differences inherent to different apple cultivars. Due to the high number of apple cultivars used in cider production, this would be a complicated addition to a risk assessment; however, if further research is conducted in this field, it would be possible to include the apple cultivar composition of cider in a QRA. Future research in these areas, and all areas of preharvest contamination of apples with *E. coli* O157:H7, will improve and refine the model presented here.

Interested readers are invited to run their own simulations of our model by downloading the Analytica file from http://www.foodsci.rutgers.edu/schaffner/cider and the Analytica browser (available free at http://www.lumina.com).

4. Conclusions

A quantitative assessment of the risk of *E. coli* O157:H7 on apples as a function of orchard and harvest conditions was created. This model can be customized to predict the risk for any given orchard. In the worst-case scenarios presented here, dropped apples were more often contaminated than tree-picked apples. The number of animals passing through an orchard and the number of days between fertilizing an orchard with animal manure and harvest were critical in determining the extent of contamination on dropped apples. A 5-log CFU/ml reduction in apple cider would probably inactivate all of the contaminating *E. coli* O157:H7 predicted on the dropped apples except in the most worst-case scenarios, but these levels of contamination seem improbable in a real orchard.

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