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Development of a quantitative risk assessment model for *Salmonella enteritidis* in pasteurized liquid eggs[1]

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Abstract

The performance of hazard analyses and the establishment of critical limits by the food industry are both hampered by the inability to directly relate food processing operations from farm-to-table with their public health impact. Using a 'unit operations' and stochastic simulation approach, data on the frequency of pathogens in raw ingredients, predictive microbiology models for growth and inactivation (thermal and non-thermal), and dose-response models for infectivity were integrated to create a quantitative risk assessment model for a *Salmonella enteritidis* infection from thermally processed liquid whole eggs made into mayonnaise in the home. The risk assessment indicated pasteurization provides sufficient consumer protection from a high incidence of infected birds and from temperature abuse between the farm and the egg breakers. However scenarios showed how inadequate pasteurization temperatures and/or temperature abuse during storage leads to a hazardous product. This dynamic approach to modeling risk should aid in identification and setting critical control points and assessing the impact of altering food formulations or processes. © 1997 Elsevier Science B.V.

Keywords: Models; Modeling; Pathogens

1. Introduction

Food microbiologists are called upon daily to make decisions concerning both the quality and safety of foods, optimizing the dual needs to provide wholesome products while maintaining high quality at the least possible cost. Various conceptual tools have been developed to help microbiologists make decisions of this nature. The most widely recognized and used system is 'Hazard Analysis Critical Control Point' (HACCP), which focuses on identifying and controlling the key process steps most significantly affecting the safe production of a food. However, as HACCP has become more widely adopted, it has become evident there are areas within this approach that could be strengthened if microbiologists were better able to quantitatively link product attributes with public health concerns (Buchanan, 1995). In particular, conducting the initial hazard analysis and

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establishing critical limits would benefit from more quantitative means of assessing the risks associated with the hazards identified for a food product.

The desire to better estimate the potential impact of both safety- and quality-related issues on the public health and economic consequences associated with foods has stimulated interest in the development of quantitative risk assessment techniques that would allow the food industry to perform reliable risk analyses (Roberts et al., 1995b). Risk assessment techniques have been used extensively in the areas of engineering, economic forecasting, chemical/pharmaceutical toxicology, and environmental impact assessment; however, application to food microbiological issues has been largely non-existent (Roberts et al., 1995a). This probably reflects our lack of knowledge and techniques in two areas (Buchanan and Whiting, 1996): questions related to the dose-response relationship for many food-borne pathogens, particularly when present at low levels, and difficulty in estimating the actual levels of pathogenic microorganisms ingested by consumers because levels of microorganisms in foods can change drastically within a short time.

During the last several years, a range of predictive microbiology models have been developed to mathematically describe the growth, survival and thermal death for a variety of food-borne pathogens (Whiting and Buchanan, 1994). Buchanan and Whiting (1996) proposed that such models could be coupled with available dose-response models to develop a 'unit operations' approach for conducting quantitative microbial risk assessments. Another prototype risk model calculated the frequency distributions of the probabilities that a cooked poultry patty will result in an infection from a single serving, illustrating the model's stages and the type of data necessary in a microbial model (Whiting, 1997).

The purpose of this manuscript is to construct a viable microbial risk assessment model to illustrate how modeling techniques in both dose-response relations and predictive food microbiology have advanced to the point where we can begin to make reasonably sophisticated quantitative analyses of the hazards associated with a food process. It is further proposed that by using this unit operations approach to assessing changes in the number of food-borne microorganisms during a series of processing operations, it should be possible for food manufacturers to assemble risk assessment protocols and establish the risk characteristics of their products. This, in turn, can be used to provide a stronger scientific rationale for the degree of stringency that must be built into HACCP programs. We developed a quantitative, 'farm-to-table' risk assessment model of the potential for acquiring *Salmonella enteritidis* infections from pasteurized liquid eggs to provide an example of the different types of data and models needed and to emphasize the important role that predictive food microbiology can play.

2. Risk analysis

Risk analysis is usually divided into three separate parts: risk assessment, risk management, and risk communication. These are often described as separate activities carried out by different groups. However, when developing microbial risk assessments for food processes and HACCP design, there are more typically a series of risk assessment/risk management iterations. A process is assessed, the results interpreted, the process modified, and the new risk assessed until the process provides an acceptable level of control.

Microbial risk assessment is divided into several components. One measures the dose-response relationship between pathogen and host. This estimates the relationship between the numbers of a pathogen ingested and the probability of a specified human population (e.g., everyone, children, aged, pregnant women) responding. This response may be an infection (colonization and reproduction in the intestinal tract), morbidity (illness), or mortality. Dose-response models based upon the number of pathogens that cause an infection will indicate a greater hazard than models for the other two responses, because infection may require fewer pathogens and includes persons who are asymptomatic carriers of the pathogen. Another principle component of risk assessment is a population's exposure to the pathogen. Microbial risk assessment differs from more traditional chemical risk assessments in that exposure is not cumulative; exposure to a pathogen in one meal does not change the probability of infection of an exposure at a later time. Further, chemical risk assessment does not generally need to consider changes in the amount of the chemical, other than

simple dilution. However, a major part of microbial risk assessment is taking into account changes in the microbial population during processing and storage.

3. Elements of a microbial risk assessment model

We propose that microbial risk assessment modeling be divided into four stages. The first is quantitative information on the incidence and prevalence of pathogens in raw ingredients. This information cannot be merely the percentage of positive samples. Quantitative information described by a mean and distribution or a histogram with the percentage of samples that contain various levels of pathogens/g, (e.g., 5% of the samples have from 0.1 to 1.0 cfu/g) is necessary.

The next stage evaluates the changes in cfu/g during the defined processing operations. This may be from farm-to-table or be restricted to operations occurring within a specific food plant. These population changes are sequentially modeled for each unit operation using the appropriate growth, thermal death or survival models. While each step in a process influences microbial levels, typically a limited number of operations determine most variation in pathogen levels and, as such, are the key parameters in a microbial risk assessment model.

The third stage is the consumption of the food. The quantity consumed at a meal determines the total number of pathogens ingested. If the cumulative risk per month or year is being modeled, the frequency of consumption is a factor.

The first three stages fit into the exposure assessment portion of a traditional risk assessment. The final stage of the microbial model is the dose-response relationship. The beta-poison and exponential models have been proposed for *Salmonella*, *Shigella*, and other infectious agents to link the consumption of a specific number of microorganisms to the probability they will cause infection (Haas, 1983). The two models are similar; the beta-poison is

$$P = 1 - (1 + N/\beta)^{-\alpha} \quad (1)$$

where P is the probability of infection (0 to 1), N is the number of pathogens consumed (cfu), and α and β are parameters specific to the pathogen. The exponential model is

$$P = 1 - e^{-rN} \quad (2)$$

where r is the specific constant for the pathogen. Many factors affect the parameter values for these dose-response relationships including the susceptibility of various subpopulations to the pathogen, the food matrix around the pathogens and expression of virulence factors. The exponential model r parameter value for *Salmonella* was estimated to be 0.00752 (Rose et al., 1996). Plotting P vs log N yields a sigmoidal relationship that asymptomatically approaches zero when fewer than 10 cells are ingested (Fig. 1a). However, a food process that resulted in a probability of infection after a single consumption of 10^{-1} or 10^{-2} (10% and 1% of the consumptions

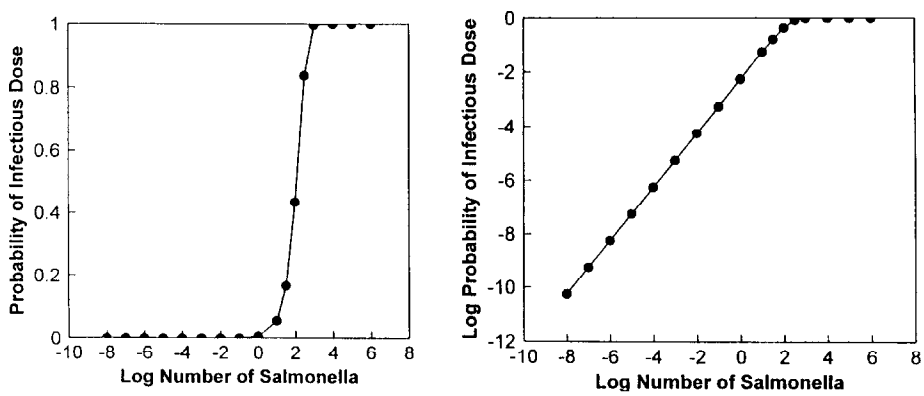

Fig. 1. Exponential model for the infective dose from *Salmonella*. Fig. 1a is a plot of the probability of infective doses and Fig. 1b is a plot of the log probability of infective dose. Rose et al. (1996).

causing infection) would be grossly unsafe. Plotting log P shows the relationship for probability ranges of concern (Fig. 1b). Current estimates of approximately 6.5 to 33 million cases of illness from food-borne pathogens per year in the United States (CAST, 1994) indicate a yearly risk of approximately 10^{-1} or a risk per meal of 10^{-4} to 10^{-5}. Establishment of acceptable or tolerable levels of risk is a complex process, reflecting the need to integrate scientific judgement and societal values. The minimum infectious unit is a single viable cell. However, fractional microbial populations such as 10^{-2}/g can be interpreted as one cell per ten 10-g packages. An alternative approach for the dose-response step is to have a defined accept/reject number, such as the 100 *Listeria monocytogenes*/g proposed for certain foods (ICMSF, 1994). It is worth noting that the dose-response models described above are for infectious and toxico-infectious agents. Toxigenic food-borne microorganisms require a somewhat modified approach.

Conventional models usually estimate the mean values. Linked models give the mean probability of risk for the entire process. However, variability has a critical impact on the risk associated with a process and the calculation and interpretation of that risk (Roberts et al., 1995a). Outbreaks often occur when more resistant strains, higher contamination levels in raw ingredients, greater abuse, inadequate cooking, or more than one of these conditions are encountered. To effectively model the risks of a process, the complete distribution including tails of the various data must be included in the assessment. To do this, simulation (Monte Carlo) modeling is used. The entire model is repeatedly calculated; each time a distribution or variation is encountered a 'possible' value is picked according to the specified distribution. This means that each simulation will give a different estimate of the final probability of infection. After 1000 or more simulations are made, frequency patterns for the values at various steps in the model emerge. These patterns are not necessarily smooth, bell-shaped curves. Depending upon the input variations, skewed, multi-peaked or even discontinuous patterns may emerge. These patterns are then interpreted to make judgements pertaining to the safety of the food. In the current model, the 'result' is a probability distribution that a serving will produce a *S. enteritidis* infection. The average or median probability of an infective dose may have little relevance if a portion of the simulations yield an unacceptably high risk.

4. Example of a microbial risk assessment model: *Salmonella enteritidis* in pasteurized liquid whole eggs

To provide an example of the principles described above, a quantitative risk assessment model was developed for estimating the probability of acquiring a *Salmonella enteritidis* infection from home-made mayonnaise made from commercially pasteurized liquid whole egg. While the model cannot be considered definitive, it considers key aspects of farm production, food processing, and consumer use that would affect the safety of the product ultimately consumed. The model is based on an extensive review of published epidemiological surveys and experimental studies. The rationale for the specific relationships used and the key assumptions made in developing the model are described below to make the model appropriately 'transparent'.

S. enteritidis in pasteurized liquid eggs was selected as the subject of this example for several reasons. Obviously one is the recent emergence of *S. enteritidis* as an important public health concern. Beginning in the mid-1980s, the incidence of human *S. enteritidis* outbreaks increased dramatically in both North America and Europe (St. Louis et al., 1988; Humphrey, 1990, 1994); the majority of outbreaks were associated with the consumption of raw or undercooked hen's eggs. A second reason is that the quality attributes of liquid egg products are very heat sensitive, and the time–temperature margins for thermal pasteurization to produce a product that is both high quality and microbiologically free of infectious pathogens are very narrow, particularly for several of the more heat resistant egg-borne human pathogens (Foegeding and Leasor, 1990; Foegeding and Stanley, 1990; Shah et al., 1991; Humphrey et al., 1995; Palumbo et al., 1995). A third reason is that the United States regulatory programs require that eggs from flocks identified as being *S. enteritidis* positive be diverted to pasteurized products (Mason, 1994). Finally, surveys found a high prevalence of consuming raw eggs (53% of

the population) including home-made mayonnaise (5.2%) (Klontz et al., 1995)

Clearly stating the assumptions used in a risk assessment model is critical for its development, refinement, and interpretation. Accordingly, we have attempted to outline below the assumptions and factors used in the model. Fig. 2 depicts a flow chart of the steps involved in getting pasteurized liquid eggs to a consumer. The first part determines the number of *S. enteritidis* in the blended lot of eggs, the reduction in numbers with pasteurization, and the probability that a specified quantity of eggs (the retail package) will contain a viable cell. Within the dotted section, the changes in numbers in the package containing a *S. enteritidis* cell are calculated for a series of storage and home preparation steps. From the amount consumed the total number of cells is determined and the probability of infection from that package calculated. Finally, the overall probability of infection from one serving is computed.

4.1. Percentage of eggs contaminated

The percentage of contaminated eggs is a function of the portion of flocks that harbor *S. enteritidis*, the portion of birds within the flock that actually carry the microorganism, and the portion of eggs produced by those birds that contain the pathogen. However, these values are confounded by the fact that eggs can acquire *S. enteritidis* by two routes, transovarian or trans-shell transmission. *Salmonella* acquired from infected ovaries or oviduct tissue are introduced before shell formation and as such are present in the egg's interior. Alternatively, trans-shell transmission involves the deposition of fecally-derived *Salmonella* on the eggshell, with or without subsequent penetration into the egg's interior. To derive the terms used in the model, we made two assumptions concerning the route of transmission. The first is that *S. enteritidis* outbreaks are largely a function of transovarian transmission. The rationale for this assumption is that if trans-shell transmission was a major factor in egg associated outbreaks we would not observe an increased rate of *S. enteritidis* infections in relation to other *Salmonella* serovars. Studies have indicated that *S. enteritidis* is a minor serovar on the egg's surface (Barnhart et al., 1991). Unless it possessed either substantially greater ability to penetrate the egg or infected humans at a much greater rate than other *Salmonella*, the incidence of *S. enteritidis* outbreaks should be proportional to its relative incidence on the egg surface and thus would be expected to be a relatively minor serovar in human infections.

A second assumption, which follows from the first, is the best estimate of the percentage of infected flocks is based on examining the birds for the presence of the pathogen in the ovaries or oviduct tissue, and not the intestinal tract. However, while the relationship does not appear to have been tested rigorously, the presence of *S. enteritidis* in the laying hens' environment does seem to be related to the prevalence of flocks with ovarian infections. When available surveys were reviewed, it was apparent that the frequency of *S. enteritidis* in flocks varied greatly among geographical regions. Typically, it was present in approximately 3% of the flocks, but in epidemic areas this frequency was substantially greater. In one survey the percentage of infected flocks in the northeastern region of the United States was estimated to be 45% (Ebel et al., 1992). Because of the wide region-to-region variation, a value for the percentage of infected flocks is the first input in the risk assessment model.

We decided it was not necessary to include a term for the percentage of *S. enteritidis* positive birds in an infected flock. The results from studies with experimentally infected flocks (Barrow and Lovell, 1991) and commercially reared birds (Humphrey et al., 1989) indicated that 20–30% of the birds within an infected flock will harbor *S. enteritidis*. This value appears to be reasonably constant. Further, most available surveys and studies of the incidence of *S. enteritidis* have not attempted to acquire data on a bird-by-bird basis, but instead have simply determined the incidence on a flock basis.

The rate at which *S. enteritidis*-positive *eggs* are laid by infected flocks is generally low; typically the rate is less than 1%. However, this has varied greatly among both surveys and trials with artificially infected flocks. Isolation rates from 0 to 19% have been reported (Humphrey et al., 1989; Perales and Audicana, 1989; Timoney et al., 1989; Shivaprasad et al., 1990; Gast and Beard, 1990, 1992a,b; Buchner et al., 1991; Barrow and Lovell, 1991; Poppe et al., 1992; de Louvois, 1993; Vugia et al., 1993; Humphrey, 1994; Poppe, 1994). The percentage of contaminated eggs appears to be dependent on a number of

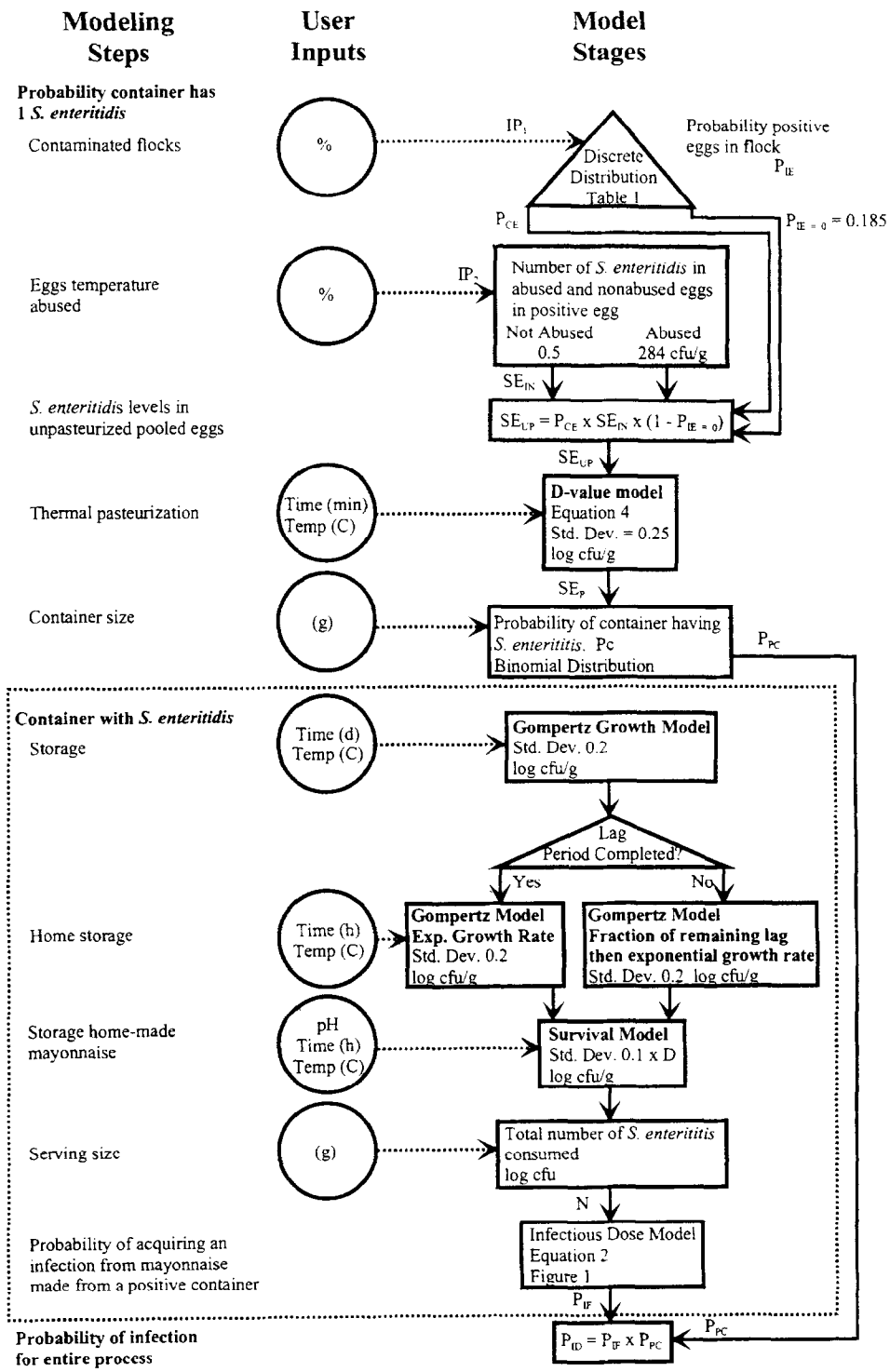

Fig. 2. Protocol of the risk assessment model. Individual steps are on the left and proceed from top to bottom. The center column has parameters entered by the user and the right column lists parameters, models and distributions. Bold type indicates a step with a distribution. Area within dotted box indicates portion of the model that calculates changes within a *S. enteritidis* positive container.

different factors including the age of birds, the duration of time after initial infection of the animals, the strain of *S. enteritidis*, whether the birds are molting, and the temperature at which the eggs are stored. Because of the large number of variables associated with this step, it was decided that the most effective approach with the current data was to use an isolation rate based on the distribution of values reported in 27 surveys and experimental trials (Table 1). The logarithm of the fraction of contaminated eggs was calculated and a histogram distribution was entered into the model. In 5 of the 27 surveys, no contaminated eggs were detected in a positive flock. Because the risk assessment uses logarithms, the undefined logarithm of zero positive eggs per flock must be circumvented. Therefore, the 5/27 samples ($P_{IE=0}=0.185$) were removed from the simulations and the probability of eggs being non-zero was multiplied with the probability of a container having one or more *S. enteritidis* later in the model.

An alternative approach is to fit a mathematical distribution (eg., log normal) to the data and enter the mean and standard deviation into the model. The occurrence of eggs with no *S. enteritidis* raises the question of sample sensitivities and false-negatives. The usual distribution of microorganisms in foods appears to be log normal (Kilsby and Pugh, 1981), but low levels near the detection limit give censored or truncated distributions and imprecise estimates of the true microbial counts.

The number of contaminated eggs can be calculated on the basis of the simple relationship:

$$P_{CE} = IP_I \times P_{IE} \quad (3)$$

Where P_{CE} is the proportion of *S. enteritidis* contaminated eggs, IP_I is the portion of flocks in a region that are infected and P_{IE} is the portion of eggs in a contaminated flock that actually contains *S. enteritidis*. Thus, in a production lot of 10,000 eggs from a region where 25% of the flocks are contami-

Table 1
Summary of published studies of the frequency of positive eggs in *Salmonella enteritidis* positive flocks

Reported fraction of positive eggs	Log fraction of positive eggs	Grouped values for distribution
0.0000		[a]
0.0000		[a]
0.0000		[a]
0.0000		[a]
0.0000		[a]
0.0006	−3.22	−3.0
0.0008	−3.10	−3.0
0.001	−3.00	−3.0
0.002	−2.70	−2.5
0.003	−2.52	−2.5
0.004	−2.40	−2.0
0.004	−2.40	−2.0
0.006	−2.22	−2.0
0.009	−2.05	−2.0
0.01	−2.00	−2.0
0.01	−2.00	−2.0
0.01	−2.00	−2.0
0.01	−2.00	−2.0
0.014	−1.85	−1.5
0.019	−1.72	−1.5
0.029	−1.54	−1.5
0.029	−1.54	−1.5
0.043	−1.37	−1.0
0.075	−1.12	−1.0
0.081	−1.09	−1.0
0.086	−1.07	−1.0
0.19	−0.72	−0.5

[a] These are the probability of zero infectious eggs in a positive flock $P_{IE=0}$ of 0.185.

nated and 1% of the eggs from infected flocks are contaminated, we would expect 25 of the eggs to contain *S. enteritidis*.

4.2. Levels of *S. enteritidis* in contaminated eggs

Under the right circumstances (e.g., *S. enteritidis* contamination or penetration into the yolk in combination with holding the egg at an elevated temperature) the level of *S. enteritidis* in an intact shell egg can reach levels approaching $>10^8$ (Bradshaw et al., 1990). However, in most instances the multiple antimicrobial systems present in eggs prevent pathogens from reaching such levels. There appear to be three key factors that interact to determine the extent and duration of this protection: location of *S. enteritidis* within a contaminated egg, storage temperature, and length of storage.

The egg yolk is a nutrient rich environment that has few antimicrobial attributes to prevent bacterial growth. Conversely, the albumen is a much more restrictive environment that contains several antimicrobial systems (e.g., conalbumin, alkaline pH). With transovarian transmission, the location of bacterial deposition in the newly forming egg is dependent on the site of infection within the bird. An infection restricted to the ovaries results in an infected yolk; whereas an infection of the oviduct leads to the deposition of the bacterium in the albumen. Studies examining eggs from both naturally and experimentally infected flocks indicate that at the time of laying the levels of *S. enteritidis* in contaminated eggs are low (generally less than 1 cfu/ml) and largely restricted to the albumen (Humphrey et al., 1989, 1991).

The temperature and duration of the subsequent storage of contaminated eggs affects *S. enteritidis* numbers in contaminated eggs. Storage at temperatures $\leq 12°C$ prevents both the replication of the pathogen in the albumen (Bradshaw et al., 1990; Clay and Board, 1991; Ruzickova, 1994) and the migration from the albumen to the yolk (Braun and Fehlhaber, 1995). At room temperature (20°C), *S. enteritidis* levels generally remain static for approximately 3 weeks due to the albumen's antimicrobial systems. However, after that time the membrane separating the yolk and albumen deteriorates, inactivating the antimicrobial systems as the exchange of material between the two compartments increases (Humphrey, 1994). This breakdown is significantly faster when the storage temperatures are greater than 20°C (Gast and Beard, 1992b; Humphrey and Whitehead, 1993).

Several assumptions were made in selecting a value for *S. enteritidis* numbers in contaminated eggs. First, at least in the United States, the majority of eggs destined for liquid egg products are either held at refrigerated temperatures or are processed within a week. Thus, the majority of positive eggs processed have low levels of *S. enteritidis*. A value of 0.5 cfu/ml was assumed based on the study of Humphrey et al. (1991). However, it was also considered that a portion of the eggs are held for 21 days at room temperature or for shorter times at higher temperatures. For those eggs, a distribution of *S. enteritidis* levels based on the study of Humphrey et al. (1991) was assumed: 58.4% = 0.5 cfu/ml, 25.0% = 13 cfu/ml, 8.3% = 375 cfu/ml, and 8.3% = 3000 cfu/ml. Because large numbers of eggs are blended, the different counts in abused eggs are effectively averaged at 284 cfu/ml and a distribution was not used at this step. However, because the portion of temperature abused eggs can vary substantially based on region and season, it was decided to include that as an input value. Thus, the levels of *S. enteritidis* were calculated using the relationship:

$$SE_{IN} = ((1 - IP_2) \times 0.5 \text{ cfu/ml}) + (IP_2 \times 284 \text{ cfu/ml}) \quad (4)$$

where SE_{IN} is the *S. enteritidis* (cfu/ml) in intact eggs and IP_2 is the portion of temperature abused eggs.

Assuming further that washing, shell sanitization, and breaking operations are adequate such that the extent of *S. enteritidis* coming from the surface of the eggshell contributes little to the overall levels of the pathogen in the liquid product (Catalano and Knabel, 1994), then the levels of *S. enteritidis* in a production lot just prior to pasteurization can be estimated by the equation:

$$SE_{UP} = P_{CF} \times SE_{IN} \times (1 - P_{HC-O}) \quad (5)$$

Where SE_{UP} is the *S. enteritidis* (cfu/ml) in a production lot of unpasteurized liquid whole egg.

Apparently, only one study has examined the prevalence of *S. enteritidis* in unpasteurized liquid eggs in commercial bulk tanks (Ebel et al., 1992,

1993). This nationwide survey of the U.S. egg industry found that 15% of the samples were positive (lower limit of detection approximately 1 *S. enteritidis* per 10 g). The rate of isolation varied regionally, ranging from 6 to 20%. Therefore, our model's calculated average values of 0.024 to 0.41 cfu/g for the low and high combinations of percentage of positive flocks and percentage of temperature abused eggs appear to be reasonable levels to give 15% positive samples.

4.3. Inactivation due to thermal processing

The thermal death model was calculated from the data for the inactivation of 17 *S. enteritidis* strains in whole eggs presented by Shah et al. (1991). The equation for the D-value was

$$\log_{10} D_{(\mathrm{sec})} = 19.104 - 0.2954T \quad (6)$$

where T is the temperature (°C). The reported standard deviations between strains for the D values were 27% of the D value, 103±27 s and 19.0±5.26 s at 57.2 and 60°C, respectively. *S. enteritidis* data presented by Baker (1990) had very similar D values and standard deviations. This variation between strains was included in the risk assessment model with σ approximated as 0.25. The reduction in *S. enteritidis* is calculated by

$$SE_{\mathrm{P}} = SE_{\mathrm{UP}} - D \times t \quad (7)$$

where D is the calculated time for 1 log decline, t is time and SE_{P} is the population after pasteurization. Treating the eggs with the standard pasteurization of 60°C for 3.5 min (U.S. Code of Federal Regulations §59.570) results in an 8.8 log reduction in viable *S. enteritidis* and in counts as low as 10^{-11} cfu/g. However, the eggs are in bulk storage and may contain a single blending of over 40,000 eggs (10^6 g).

4.4. Number of consumer packages containing S. enteritidis

After pasteurization, the liquid eggs are pumped into containers of 100 to 1000 g for consumer purchase. This means that most containers will not have any *S. enteritidis* and subsequent abuse cannot lead to an infection. It also implies that the infrequent positive container will start with 10^{-2} or 10^{-3} cfu/g, respectively, and relatively minor abuse leads to a high probability of infection associated with that specific container. The risk model takes into account the population of *S. enteritidis* after pasteurization and the container size to calculate the fraction of *S. enteritidis* positive containers. A binomial distribution is assumed (container size (10^3 g) is n, the count after pasteurization is p and x is 1 cell). The occurrence of containers with two or more *S. enteritidis* is negligible unless an obviously high risk scenario is proposed.

4.5. Growth modeling during storage

After blending and pasteurizing the eggs, the pH is lower, the inhibitors are inactivated and the liquid egg has increased potential for supporting pathogen growth. The *Salmonella* growth model of Gibson et al. (1988) can calculate the lag times and exponential growth rates under these conditions. Temperature and storage times are entered by the user, while pH and salt parameter values of the blended whole eggs were set at 7.0 (Powrie and Nakai, 1990) and 0.5%, respectively. If the temperature is less than 10°C, the minimum for the model, no growth is estimated. If the lag period is not completed during the initial storage period, the fraction of the lag remaining is calculated and subtracted from the abuse time of the second storage period (Zwietering et al., 1994). If no lag time remains or the fraction remaining is completed, *S. enteritidis* growth is calculated using the exponential growth rate. The first storage period is for relatively long times (days) at the mild abuse temperatures associated with commercial distribution, while the home storage period focuses on shorter times (hours) of higher abuse temperatures. The variation for the lag and growth rate estimates were not determined by the published model, therefore, a standard deviation of 0.2 log cfu/g is assumed at the end of each growth period.

4.6. Survival in mayonnaise

After the home storage period, the consumer uses the eggs to make mayonnaise for a salad dressing. A typical pH would be 3.9 which would ultimately inactivate salmonellae. A *Salmonella* survival model (Whiting, 1993, 1996) uses pH, temperature and

time to calculate the expected decline in population. A standard deviation of 0.2×log decline is estimated based upon experience.

4.7. Consumption

The quantity of mayonnaise consumed during the meal is the final factor determining exposure. The serving size in the current example is estimated to be 10 g and entered to determine the total number of *S. enteritidis* consumed.

4.8. Probability of infection

The exponential model for probability of infection is used, assuming the infectivity of *S. enteritidis* is typical of other *Salmonella* (Fig. 1). The probability that a serving of mayonnaise made from a container of pasteurized liquid eggs initially containing 1 *S. enteritidis* will lead to an infection (P_{IF}) is determined by entering the total number of *S. enteritidis* consumed (N) into the exponential model.

4.9. Calculation of risk

The probability of acquiring an infection for the entire process (P_{ID}) is the probability that the specific container has 1 *S. enteritidis* (P_{pc}) times the probability of infection after the storage, survival and consumption steps (P_{IF}). The frequency distribution of P_{ID} contains the primary information for interpreting the hazard associated with entire process, however, the frequency graphs for probability of a positive container (P_{pc}) and the probability of a serving of mayonnaise from a positive container causing an infection (P_{IF}) can also be evaluated.

4.10. Simulations

After entering the thirteen required process and environmental input values (Fig. 2 and Table 2), this entire model is simulated 2000 times using @RISK (Palisade Corp. Newfield, NY) running with LOTUS for Windows (Lotus Development Corp., Cambridge, MA). Preliminary simulations showed that this number of simulations exceeded the number necessary for achieving the convergence requirements for all three output distributions (<1.5% change). Individual simulations, summary statistics, sensitivity analyses, and graphs provide an understanding of the sources and degree of risk inherent in the specific process being evaluated. Four scenarios are provided as examples (Table 2). The first scenario represents a relatively low level of *S. enteritidis* contamination in the flocks and low frequency of abuse of the eggs (resulting in estimated mean of 0.024 cfu/g in the pooled, blended eggs), standard thermal processing (8.8 logs decline), and good storage practices (0.6 log decline overall). As expected, this scenario has a low probability of 1 *S. enteritidis* in a container of $10^{-6.7}$, a $10^{-4.8}$ risk of a serving of mayonnaise from a *S. enteritidis*-positive container producing an infection, and a median probability of infection for the entire process of only $10^{-11.5}$. This would be considered a safe process. The second scenario determines whether high levels of infected flocks (45%) and abuse of eggs (10%) would make the pasteurization inadequate for protecting the consumer. Even though the contamination levels in the blended eggs increases to 0.41 cfu/g, the median probability of infection of $10^{-10.2}$ indicates that the overall process remains safe. The third scenario tests whether a failure to achieve the intended pasteurization constitutes a hazard. When the pasteurization temperature was reduced from 60 to 59°C, the pasteurization now reduces the *Salmonella* by only 4.5 logs and the median probability of an infection increases from $10^{-10.2}$ to $10^{-6.5}$. This would probably be judged a marginal process. In the last scenario, the first storage period has the temperature increased to 11°C which permits the *Salmonella* to grow slowly. The final *Salmonella* population in the mayonnaise increases from 0.00025 cfu/g ($10^{-3.6}$) to 0.2 cfu/g ($10^{-0.68}$). The probabilities of infection for a container with *Salmonella* is 30% ($10^{-0.53}$) and the median overall probability of an infectious dose now jumps to $10^{-2.3}$, unquestionably an unsatisfactory situation.

However, determining the median probability for these distributions does not provide an adequate description of the process. The frequency distributions for scenario #3 (Fig. 3) indicate the dispersion of the individual simulations. The probability of a positive container ranged from $10^{-3.6}$ to 1 with a majority of the probabilities greater than $10^{-1.7}$. The probability of infection for a positive container was more tightly distributed around $10^{-4.8}$, although the highest probability of the 2000 simulations reached

Table 2
Summary of the model parameters and calculated values for the four scenarios of the probability of an infective dose of *Salmonella enteritidis* in liquid eggs

	Scenario			
	1	2	3	4
Input parameter values				
% Contaminated flocks	**10**	**45**	45	45
% Eggs temperature abused	**2.5**	**10**	10	10
Container size (g)	1000	1000	1000	1000
Pasteurization time (min)	3.5	3.5	3.5	3.5
Pasteurization temperature (C)	60	60	**59**	59
Storage temperature (C)	6	6	6	**11**
Storage time (days)	6	6	6	6
Second storage temperature (C)	20	20	20	20
Second storage time (h)	4	4	4	4
Temperature of mayonnaise (C)	20	20	20	20
Storage time of mayonnaise (h)	24	24	24	24
pH of mayonnaise	3.9	3.9	3.9	3.9
Amount mayonnaise consumed (g)	10	10	10	10
Output values				
Initial *S. enteritidis* in pooled eggs (cfu/g)	0.024	0.41	0.41	0.41
Post pasteurization (log cfu/g)	−9.7	−8.4	−4.7	−4.7
Median log probability of *S. enteritidis* Positive container (P_{PC})	**−6.7**	**−5.5**	**−1.7**	**−1.8**
Post storage (log cfu/g)	−3.0	−3.0	−3.0	2.3
Post home storage (log cfu/g)	−0.8	−0.8	−0.8	3.6
Mayonnaise at consumption (log cfu/g)	−3.6	−3.7	−3.6	0.68
Median log probability of positive container being infectious (P_{IF})	**−4.8**	**−4.8**	**−4.8**	**−0.53**
Median log probability of infection from entire process (P_{ID})	**−11.5**	**−10.2**	**−6.5**	**−2.3**

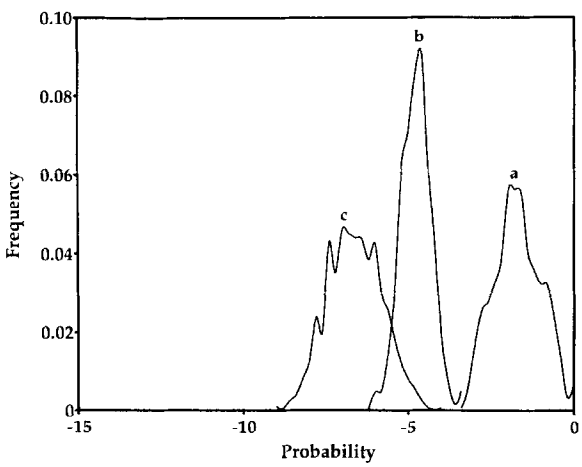

Fig. 3. Plotted distributions for scenario #3. Curve a is the probability of a container having one *S. enteritidis*, curve b is the probability of acquiring a *S. enteritidis* infection from mayonnaise made from a positive container, and curve c is the probability of infection for the entire process.

$10^{-3.5}$. The probabilities for the entire process ranged from $10^{-9.3}$ to $10^{-4.0}$.

The cumulative frequencies for the overall probability of infection for the four scenarios are shown on Fig. 4. The occurrence of some simulations having much greater probability than the median is apparent. Most importantly, in the first two scenarios none of the simulations exceeded an overall probability of infection of 10^{-7}. However, approximately 23% of the simulations in scenario 3 exceed a probability of 10^{-6} while in scenario 4 approximately 32% exceed a probability of 10^{-2}! It is the assessment of the frequency of these high probability events that determines the acceptance of a process, more than the mean or median probabilities. The frequency of high probability simulations depends upon the distributions and variances for the individual steps in the risk model. The frequencies of high probability simulations reflect the likelihood of

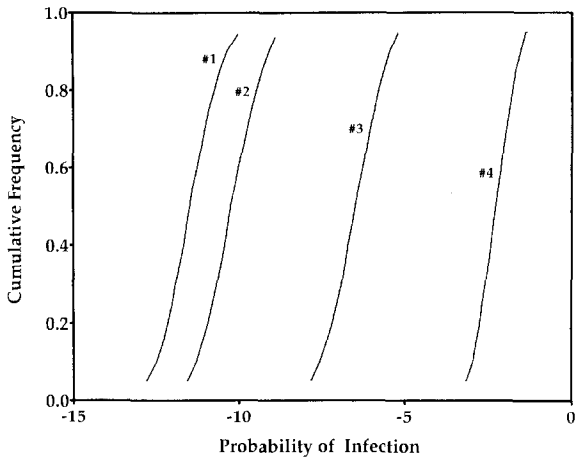

Fig. 4. Probability of infection for the entire process for scenarios #1 through 4, curves #1 to 4, respectively.

the 'worst cases' occurring. This is a more realistic assessment of the risk of a process than using the value for the worst case in every step of the process's model (Cassin et al., 1996).

5. Role in HACCP

The primary focus of risk assessment to date has been to consider risks associated with entire industries and broad groups of products. In fact, some proponents feel that microbial risk assessment should be limited to these broad evaluations. However, when dealing with an industry whose processes and products are as diverse as those encountered with foods, any meaningful translation of risk assessment models into risk management decisions will require individual assessments of specific products and processes. Just as HACCP is only truly effective when applied on a plant-specific basis, ultimately risk assessment models need to be flexible enough to allow the user to modify parameters to reflect the specific attributes of their products, processes and facilities.

After evaluating the risk assessment model presented above, it is apparent to us that dynamic models of this type would greatly enhance the scientific basis for HACCP programs. Of the seven principles of HACCP, quantitative risk modeling has potential roles in the first three: (1) conduct a hazard analysis, (2) identify critical control points (CCP), and (3) establish critical limits for each CCP (ICMSF, 1988; NACMCF, 1990). Performing risk simulations for a standard or proposed food process gives an initial assessment of the magnitude of the hazard and more directly relates the process to public health goals. The use of simulation or related techniques represents an important paradigm shift in that hazard assessments begin to address in a meaningful manner the variability that is inherent in varying degrees in any process, product, and pathogen. The frequency patterns of the simulations can be used to determine if the focus of a HACCP plan needs to be an improvement of an entire process or one operation, or whether a reduction of the incidences of a few extreme combinations of factors (i.e., decreasing the variability of a product or process) will make a process safer.

For pasteurized, refrigerated and semi-stable foods, processing typically keeps the pathogen in the lag phase, limits its growth to less than 1 log, or produces at least a 3 to 5 log decline in pathogen populations. In addition to providing estimates of the distributions that are likely to be encountered with a food product, software of the type used to generate the model can be run with various values to quickly get an indication of the relative importance of a factor. For example, the growth models indicated that *Salmonella* would not grow during commercial storage and transportation if the temperature was properly controlled. Thus, time and temperature for this phase would not have to be modeled if the temperature is below a threshold value. If gross abuse occurred, which a hazard analysis could define, this element then could be inserted into the risk assessment program to determine the consequences of this out-of-process event. This approach could be further enhanced by industry employing historical survey data on conditions during storage, transportation, and marketing to provide distributions of the frequency and extent of abuse. This could then be used to determine first if this segment has a major effect on the overall microbiological safety of the product, and as such may be a critical control point. Second, such analyses can help establish the degree of control that would need to be exercised to control a pathogen, thereby providing a basis for setting critical limits.

In the current risk assessment model, the temperature and time of thermal processing was quickly

identified as a critical point. Testing various combinations with the model emphasized that the thermal processing temperature is particularly important. Being even one degree below the intended temperature greatly altered the risk. Pasteurization time is also important but not nearly as critical as temperature. With this information, it should be much easier to establish quantitative values for critical limits to produce the degree of stringency required, and then design appropriate monitoring systems. The model also indicates that pasteurization will minimize the risk from high contamination in the flocks if the pathogen is not allowed to grow. It also suggests how much abuse the process can withstand, both before and after the product leaves the manufacturer's control. This can assist manufacturers in practising "due diligence" in the design of their process and HACCP program.

6. Conclusions

This paper demonstrates how predictive food microbiology can be incorporated into a more comprehensive assessment of the risk of becoming ill from the consumption of a specific food. Although the individual models and data necessary to construct a quantitative risk assessment model are frequently incomplete at the present time, the approach is feasible and meaningful answers can be found. This is particularly true when the focus of a risk assessment is the relative risks that would be encountered as a result of changes in production, processing, or consumer use of a food. Such analyses are particularly useful for perishable foods that do not have a critical control point that ensures a 'risk-free' product. Safety is the result of a combination of factors that inhibit or reduce the numbers of pathogens, but can not eliminate them.

Assembling a risk assessment model quickly shows where additional research is needed. It is critical that the variation for each of these steps be estimated because it appears that outbreaks occur when probabilities are in the range of 10^{-4} to 10^{-8} This means that an outbreak is often the consequence of several distributions being on the margins. The average number or change in number of pathogens with each step cannot give a complete assessment of the processes' risk. Sources of variation include initial distributions of the pathogen in raw materials or actual times and temperatures of a process. For a specific step in the food process, distributions arise from strain and cultural differences in the microorganism as well as analytical and statistical variations. A modular approach in linking individual models is proposed to estimate the final pathogen numbers and risk of a process. This provides flexibility in defining a process, limiting it to within a food processing plant or expanding it to encompass the complete farm-to-table continuum. Additional understanding of the lag periods that may or may not exist between the various growth, pasteurization and survival steps is needed. The physiological state of a cell including exponential vs stationary cells and acid or temperature adaptations are known to affect lag times as well as D values. The magnitude of the transition and favorableness of the new environment affect the rate cells adjust to an environment. Cellular injury and repair also may be important factors in determining the final pathogen populations. Better understanding of virulence mechanisms and infectious doses is needed to model accurately the final step from the numbers of pathogens consumed to the likelihood of illness in various human populations.

Although these nascent risk assessment models can be very useful in understanding a process, there is an obvious need for techniques to validate them before regulatory decisions and HACCP plans can be based upon them. But partial or incomplete models can still be of great value for evaluating a process, as well as show where additional data needs to be collected.

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