COLONY FORMING UNITS OR PREVALENCE: HOW TO USE EXPERIMENTAL DATA IN PREVALENCE SIMULATION MODELLING

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SUMMARY

The effectiveness of antimicrobial decontamination methods in slaughterhouses can be expressed as reduction in number of colony forming units (CFU) counts and as a reduction in prevalence of contaminated end products. In many risk assessments the contamination status of the food products are modelled, with prevalence as output parameter. To use experimental microbiological data in these models, indicating the CFU reduction after an applied intervention, these data should be translated into a probabilistic input parameter. A methodology is presented to calculate such a probability from experimental data. Using this methodology it is demonstrated that the effectiveness of decontamination methods varies with the initial number of bacteria present on the carcase. And in case of a high initial concentration of bacteria (>log 5), the elimination probability will be zero even if a very powerful decontamination method is applied.

INTRODUCTION

Carcase antimicrobial-decontamination methods are considered as slaughterhouse interventions against enteric pathogens such as *E.coli* O157:H7 (VTEC) (Koohmaraie et al., 2005). The effectiveness of decontamination methods is an element that should be considered in a cost-effectiveness analysis. Two measures of effectiveness of decontamination methods at the slaughterhouse can be distinguished: (i) reducing the fraction (i.e., prevalence) of contaminated carcasses and (ii) reducing the number of bacterial colony forming units (CFU) on a carcase. When focusing on food safety problems related to the enteric pathogens that may contaminate meat, models that predict the number of CFU counts (see for example Ebel et al., 2004; Nauta, 2001) are suggested. However, such models require a large number of input variables and thus many assumptions. Prevalence simulation models are often used to estimate the effectiveness of intervention strategies to reduce the fraction of carcasses contaminated by enteric pathogens (see for example Alban & Stärk, 2005; van der Gaag et al., 2004b). The advantage of prevalence simulation models is that there are less input variables and thus fewer assumptions are needed.

Results of experimental studies are often expressed in terms of log reduction of CFU counts on the surface of the meat (Juneja & Sofos, 2002; Phebus et al., 1997; Retzlaff et al., 2004). If it is desirable to use these data in a prevalence simulation model, an approach needs to be developed to convert the reported log reduction to an elimination probability, which is the

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probability of eliminating all bacteria from surface of the meat using decontamination methods. Although, there have been some efforts to translate a pathogen reduction into an elimination probability (SCVPH, 1998, 2003) they were not satisfying. Their focus was mainly on converting percentage reduction of CFU counts by decontamination methods into the proportion of positive carcasses and not on translating the experimental data to an elimination probability. More studies are therefore needed to introduce and examine other approaches. In this paper, the authors demonstrate a modelling approach that can be used to translate an experimentally measured log reduction (of decontamination methods) to an elimination probability. Such elimination probability can be used in a prevalence simulation model to evaluate the effectiveness of decontamination methods. In the following sections first the modelling approach is presented and then, using some published data for the initial number of bacteria and the antimicrobial effects of the decontamination methods, this modelling approach is illustrated in a prevalence model.

MATERIALS AND METHODS

Modelling approach

The aim of this modelling approach is to estimate the elimination probability for antimicrobial decontamination methods, given different values of the initial number of pathogens on the surface of the carcasses. The reduced number of CFU resulting from implementing a decontamination method is translated into the probability of having zero bacteria (i.e. the elimination probability) using the first element of a poisson distribution. The expected number of CFU per carcase after intervention equals the initial number of CFU on each carcase minus the reported CFU reduction due to that intervention. Let EP denote the estimated elimination probability, μ the initial number of CFU on the whole carcase and λ the reduction in number of CFU on the whole carcase. The elimination probability can be calculated using following equation:

$$EP = e^{-(\mu-\lambda)} \tag{1}$$

Using Eq. (1) the relation between EP, μ and λ has been illustrated (Fig 2). For this illustration, seven different decontamination methods with antimicrobial effectiveness varying from one to seven log reduction of CFU (log 1 to log 7) were assumed. The results of this methodology are given in the result and discussion section.

Application

The modelling approach described above, was developed to investigate the effectiveness of interventions (in terms of reduction in prevalence) against *E.coli* VTEC in Dutch dairy-beef industrial slaughterhouses (Vosough Ahmadi et al., YEAR?). Five carcase-antimicrobial decontamination methods, hot-water wash, lactic-acid rinse, steam vacuum, steam pasteurization and gamma irradiation including their combinations were examined. With a Monte Carlo simulation the elimination probabilities for the decontamination methods were calculated using published data for antimicrobial effectiveness of the decontamination methods and the initial number of bacteria on the surface of the beef carcase (Fig 1). The area separated by the dashed line in Fig. 1 is the model to estimate the elimination probability presented in this paper. The output of this model serves as input in the prevalence simulation model, which uses binomial processes (Vosough Ahmadi et al., YEAR?). The initial number of bacteria (CFU) on each

carcase was simulated by multiplying two distributions: the amount of transferred manure in grams to the carcase (beta distribution) and the concentration of VTEC in one gram of manure (cumulative distribution). The used data and distributions were based on a VTEC risk assessment (Table 1, Nauta, 2001). A beta distribution was chosen to describe the carcase contamination with manure after fitting the results of expert estimates to a series of probability distributions (Nauta, 2001). The parameters α and β were used to express the level of carcase contamination with manure and its variability per carcase. A cumulative distribution was used to include the uncertainty related to the concentration of VTEC in a gram of manure, based on data reported by Zhao et al. (1995). In the mentioned study, VTEC concentrations in the faeces of 31 positive calves were measured from a survey of dairy herds in the U.S.

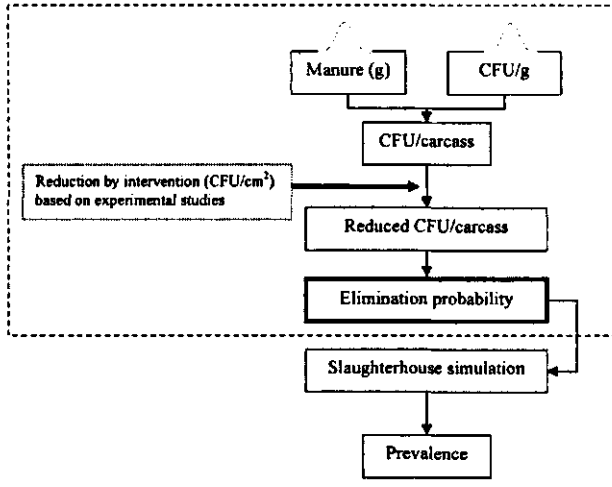

Fig. 1 Schematic view of VTEC simulation model for beef-carcasses at slaughter

For the simulations it was assumed that each carcase has a total surface of 32,000 cm^2 and that each quarter receives equally one fourth of the total faeces. The expected number of CFU per quarter when interventions are applied equals the initial number of CFU (μ) on each quarter minus the reported reduction (λ) due to a specific intervention (Phebus et al., 1997). The reduced number of bacterial counts resulting from a reduction due to intervention is calculated by Eq. (1). The mean elimination probabilities were determined using 10,000 iterations and were used as inputs in the VTEC prevalence simulation. The model was built in Microsoft Excel spreadsheet using @Risk add-in software.

Table 1. Description of variables and distributions used in a VTEC simulation model for beef-carcasses at slaughter

VARIABLE	DISTRIBUTION	VALUES
Concentration of bacteria (log CFU) in gram of manure	Cumulative [a]	$\{X_i: 0, 2, 3, 4, 5, 6\}$ $\{P_i: 0.00, 0.46, 0.53, 0.87, 0.96, 1.00\}$
Gram of manure on each carcase	Beta [b]	Max: 10.1 α: 0.395, β: 2.47

[a] @Risk function: RiskCumul(0,6, {2,3,4,5},{0.469, 0.531, 0.875, 0.969})
[b] @Risk function: Max * RiskBeta(α, β)

RESULTS AND DISCUSSION

Figure 2 shows the elimination probabilities for the seven assumed categories of decontamination methods (log 1 to log 7) with different values for the initial number of bacteria present on the carcase. The elimination probability will be zero when applying a weak decontamination method (log 1 reduction in CFU) on a carcase that is initially contaminated with more that 68 CFU (log 1.8). At that level of initial contamination, more powerful decontamination methods give a high elimination probability of infection. However, with a higher level of initial contamination, also more powerful decontamination methods may give zero elimination probability. The elimination probability for decontamination methods with antimicrobial effects of log 6 and log 7 will be zero only if the initial CFU count is higher than one million. These results imply that in the case of having very high initial concentration of bacteria on the carcasses (>log 5), the elimination probability can be zero even if a powerful decontamination method is applied. This means that interventions will have no effect on the reduction of the prevalence of contaminated carcasses. However, these decontamination methods still give an important improvement of the beef safety by reducing the CFU counts.

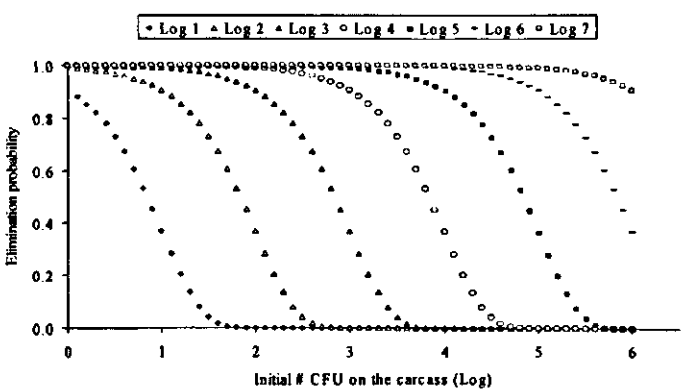

Fig. 2 Elimination probabilities for seven decontamination categories graphed against different levels of initial number of CFU on a beef-carcase at slaughter in log scale

Figure 2 also shows that the elimination probability will be higher than zero when the initial number of bacteria is low (<log 1.8), and therefore a prevalence reduction can be expected using these methods. The majority of the decontamination methods have an elimination probability greater than 90% in the case of having up to 10 CFU (log 1) as initial number of bacteria. These values decline by increasing the initial bacterial load. This result implies that control of the initial contamination of the carcase is effective in two ways. It lowers the prevalence of contaminated carcasses directly and it increases the elimination probability of existing infections.

In the application part of the modelling approach explained in this paper, the data on initial number of bacteria on the carcase and experimental data on antimicrobial effects of decontamination methods were used to estimate the mean elimination probability for each decontamination method. The eventual goal was to use the estimated elimination probabilities in a prevalence simulation model to estimate the effectiveness of decontamination methods in reducing the prevalence of contaminated beef-carcase quarters. To get stable output, the elimination probabilities for the five decontamination methods were calculated with 10,000 iterations. The antimicrobial effects (input) and mean values of elimination probabilities (output) of the five decontamination methods are presented in Table 2. The practical meaning of these values is that, for example, when hot-water wash is used as intervention, a contaminated beef-carcase quarter will have a 34% probability of changing from positive to negative. In this way, these values can be used in prevalence simulation models that are developed based on binomial processes. Because of the low initial number of bacteria coming from the two mentioned distributions, in most cases the most-likely values for the elimination probabilities were close to one. Thus, choosing the mean of the distribution assures us to consider the tail of the distribution.

Table 2. Mean elimination probability for five decontamination methods applied to reduce the amount of VTEC on beef-carcasses obtained through a simulation.....

DECONTAMINATION	MEAN REDUCTION[a] (log CFU/cm^2)	MEAN ELIMINATION PROBABILITY (%)
Hot-water wash (W)	0.75 ± 0.49	34.69
Lactic-acid rinse (L)	2.70 ± 0.49	68.75
Steam vacuum (V)	3.11 ± 0.49	77.00
Steam pasteurization (S)	3.53 ± 0.49	83.17
Irradiation (Ir)	6.00 ± 0.49 [b]	99.48

[a] Mean reduction in VTEC population (log CFU/cm^2) ± standard error of mean (Phebus et al., 1997).
[b] Molins et. al.(Molins et al., 2001), the same standard error as the other methods is assumed.

In general, the reduction in prevalence depends highly on the initial number of bacteria on the surface as well as the antimicrobial power of decontamination method used. The antimicrobial power of decontamination methods depends on different factors such as the technical strength of decontamination methods to destroy the bacterial germ, time and place of intervention (in the slaughter line) as well the type of the bacteria and its adherence characteristics to the meat surface. Therefore, both prevalence reduction and CFU reduction effects should be considered when the "effectiveness" of decontamination methods is concerned. In the majority of the cost-effectiveness analyses on the interventions against enteric bacteria, the main focus is only on one of the mentioned effects. For example Jensen et al., (1998) consider only CFU reduction and van der Gaag et al., (2004a) consider only prevalence reduction as the basis of their economic analysis. This may lead to an underestimation of

effectiveness (in case of focusing only on experimentally measured CFU reduction) or overestimation (in case of focusing only on prevalence reduction). Thus efforts should be done to consider these factors together in such studies.

In the relatively simple simulation model described in this paper, the initial number of CFU was determined based on distributions for the amount of manure and the concentration of CFU in the manure. These distributions were based on the Dutch expert's opinion and literature (Nauta, 2001; Zhao et al., 1995). Because the type of the distributions was based data fitting, as explained by Nauta (2001), these distributions may vary in other countries and conditions. Therefore the elimination probability calculated for each decontamination method might be different for different countries and conditions. This is mainly due to the hygienic measures in the slaughterhouses that allows or prevents the transmission of manure to the carcasses. Also this depends on the concentration of CFU bacteria shed into the manure. Farming practice and the situation of different countries are important factors for concentration of bacteria shed in the manure.

CONCLUSIONS: PREVALENCE VERSUS CFU MODELLING

Considering the prevalence versus CFU modelling issue, on one hand it can be observed that industry, regulatory agencies and consumers focus on the fraction (prevalence) of contaminated end products. Also many scientific studies focus only on prevalence. As it was mentioned before, in the case of a low initial contamination (i.e. lower than 1.8 log CFU count), focusing on prevalence can be a good approach without modelling or considering the CFU counts. This seems a valid assumption for the common slaughter practice in most of the developed countries. However risky events such as gut rupture during the evisceration, which can lead to the release of a large number of bacteria on the carcase, can make this assumption invalid even in the best manufacturing practices at slaughterhouses.

On the other hand public health authorities and farm-to-fork risk assessors are very much concerned about the exact number of CFU present on the surface of the meat. As the infectious dose for some of the enteric pathogens such as *E.coli* VTEC is very low, even one bacterium has a great importance. Therefore, from this point of view studies that consider prevalence as their main criterion do not sufficiently address the problem. In this case the result of the effectiveness analysis may become biased because of the overestimation of the effectiveness.

Thus, it can be concluded that in the effectiveness analysis of decontamination methods the expected number of CFU on the carcasses along with the consideration of the expected prevalence of contaminated carcasses should come together. The best way to this is to develop a CFU model that estimates the number of transmitted bacteria to the end product and thus implicitly estimates the prevalence of contaminated product as well. An alternative way that presented in this paper is modelling the elimination probabilities based on initial CFU contamination and feed them as input to a prevalence simulation model to calculate the prevalence reductions due to specific decontamination methods.

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