Available online at www.sciencedirect.com

ScienceDirect

Preventive Veterinary Medicine 77 (2006) 15–30

PREVENTIVE
VETERINARY
MEDICINE

www.elsevier.com/locate/prevetmed

Simulating *Escherichia coli* O157:H7 transmission to assess effectiveness of interventions in Dutch dairy-beef slaughterhouses

Bouda Vosough Ahmadi [a,*], Annet G.J. Velthuis [a],
Henk Hogeveen [a,b], Ruud B.M. Huirne [a]

[a] *Business Economics Group, Wageningen University, Hollandseweg 1,*
6706 KN Wageningen, The Netherlands
[b] *Department of Farm Animal Health, Faculty of Veterinary Medicine,*
Utrecht University, Yalelaan 7, 3584 CL Utrecht, The Netherlands

Received 10 May 2005; received in revised form 23 April 2006; accepted 4 May 2006

Abstract

Beef contamination with *Escherichia coli* O157:H7 (VTEC) is an important food-safety issue. To investigate the effectiveness of interventions against VTEC in Dutch beef industrial slaughterhouses that slaughter 500 dairy cattle per day, a Monte Carlo simulation model was built. We examined seven carcass-antimicrobial interventions, namely: hot-water wash, lactic-acid rinse, trim, steam-vacuum, steam-pasteurization, hide-wash with ethanol and gamma irradiation, and their combinations. The estimated daily prevalence of contaminated beef-carcass quarters as the output of the model was 9.2%. Contaminated was defined as containing one or more CFU on the surface of a carcass quarter at the end of the quartering stage. Single interventions (except irradiation) could reduce the prevalence to from 6.2% to 1.7%, whereas the combination of interventions could lower it to from 1.2% to 0.1%. The most powerful intervention was irradiation, which could reduce the prevalence to <0.1%. The results of this study indicate that application of single interventions might be useful, although not sufficient. Hence, a combination of interventions along the slaughter process is the more promising approach to reduce the prevalence of contaminated beef quarters.

© 2006 Elsevier B.V. All rights reserved.

Keywords: *Escherichia coli* O157:H7; Simulation model; Slaughterhouse interventions; Risk assessment; Food safety; Beef

---

* Corresponding author. Tel.: +31 317 483636; fax: +31 317 482745.
E-mail address: Bouda.Vosough-Ahmadi@wur.nl (B. Vosough Ahmadi).

0167-5877/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.prevetmed.2006.05.011

1. Introduction

Since the first report of *Escherichia coli* O157:H7 (VTEC) as a human pathogen in 1982 (Phillips, 1999), it has been of concern to the beef-processing industry. In the Netherlands, an overall prevalence of 1.1% (6 of 571 samples) of VTEC-contaminated minced-beef products has been reported (Heuvelink et al., 1999). Furthermore, the result of a VTEC risk-assessment study suggests that 0.3% of raw Dutch steak-tartar patties are contaminated with the bacteria (Nauta, 2001). The result of a recent study at the herd-level suggests that 7.2% of Dutch dairy herds are infected with VTEC (Schouten et al., 2004). In a study by Heuvelink et al. (2001) no VTEC was isolated in the slaughterhouses, while >10% of carcasses were visibly contaminated with manure in 11 of the 27 slaughterhouses and >50% of the inspected carcasses were visibly contaminated with manure in six slaughterhouses. These facts imply that beef carcasses might become contaminated with VTEC during the slaughter process in Dutch slaughterhouses.

A variety of interventions that can reduce carcass contamination with VTEC during the slaughter process are available (Huffman, 2002; Juneja and Sofos, 2002). But decision makers must decide which interventions to apply. Cost-effectiveness analysis provides decision makers insight into both the costs and the effectiveness of interventions. One of the ways to perform a credible cost-effectiveness analysis is to build an integrated epidemiological-economic model. This paper describes the epidemiological model to determine the effectiveness of the different slaughterhouse interventions.

The effectiveness of different interventions has been investigated in several studies in a laboratory environment (Phebus et al., 1997; Juneja and Sofos, 2002; Retzlaff et al., 2004). In most such studies, the reduction in the number of colony-forming units (CFU) of VTEC on the meat surface was determined (Phebus et al., 1997; Retzlaff et al., 2004). However, the effectiveness of interventions to reduce the proportion or prevalence of the contaminated end product of the beef slaughterhouse is unknown. To determine this, field studies are needed, but these are difficult to design, hard to apply and in most cases disruptive to the slaughter process. A modelling approach is therefore a good alternative.

Our objective was to present a Monte Carlo model that simulates the dynamics of VTEC in a Dutch industrial beef slaughterhouse. Our aim was to rank the different intervention methods according to their effectiveness in reducing the frequency of VTEC-contaminated beef-carcass quarters at the end of the quartering stage.

2. Materials and methods

2.1. The slaughter process

We modelled a typical Dutch dairy-industrial slaughterhouse, with a capacity of 500 dairy cattle per day. Cattle are loaded onto transport trucks at the farms of origin, transported to the slaughterhouse and unloaded into the lairage. Animals are kept in the lairage before entering the slaughter line. The modelled slaughter process has nine stages (Fig. 1): 1, lairage; 2, de-hiding; 3, evisceration; 4, splitting (producing half carcasses); 5, fat and tail removal; 6, trimming (for decontamination); 7, washing (for lower carcass

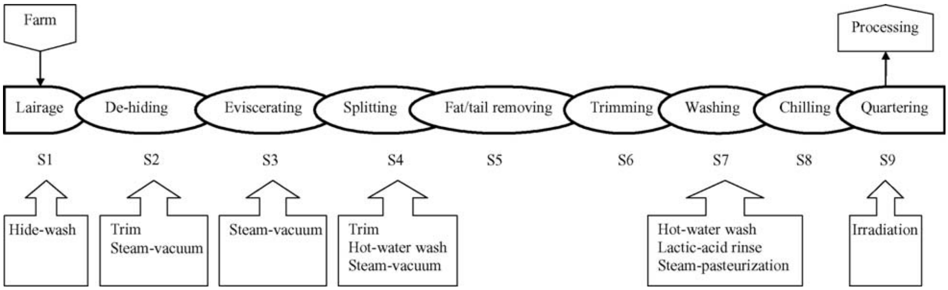

Fig. 1. Schematic overview of the stages of the slaughter process and related interventions that can be used at each stage.

temperature); 8, chilling; 9, quartering (producing quarters). In this model, until S4 (splitting), the whole carcasses is used as basic unit. The output until this stage is the prevalence of infected carcasses (500 carcasses). At S4 the carcass is split into two half carcasses by a transverse cut. The basic unit from this stage on is the half carcass (1000 halves). At stage S9, each half carcass is broken down into two quarters. From that moment the basic unit is a quarter (2000 quarters). These fore- and hindquarters are considered the end product of our model.

2.2. VTEC sources and animal status

Most enteric pathogens (such as *Salmonella*, *Campylobacter* and VTEC) are most likely brought into the slaughterhouse by either the interior (gastrointestinal tract) or exterior (hide) of live animals or both (Small et al., 2002). The gastrointestinal (GI) tract is considered the main source of VTEC beef contamination (Chapman et al., 1994). In this study GI-positive (GI^+) refers to the animals that carry VTEC in their GI tract and shed it in their faeces. Cattle that carry VTEC on their hide (Sofos et al., 1999) are denoted as hide-positive (H^+). With respect to the bacterial sources mentioned, live cattle on the farm and at the slaughterhouse can be put into four categories: GI^+H^+; GI^+H^-; GI^-H^+; GI^-H^-.

When a GI^+ animal enters the slaughter process, it poses the risk of leaking faeces with VTEC from the anus into the environment or onto the carcass. During the evisceration stage faeces can be leaked in the environment because of a rupture. On the other hand the contamination risk posed by an H^+ animal relates to the direct contact of the contaminated hide with the surface of the carcass, personnel, tools and surfaces in the slaughterhouse environment. This is mainly due to the large hide surface and its direct and frequent contact with personnel and tools (Bell, 1997; Hudson et al., 1998). In the following section the assumed transmission dynamics are described in detail.

2.3. Model structure

The model described in this paper was built using Microsoft Excel with @Risk add-in software (Palisade, 2002). Monte Carlo simulation was used to compute the average number of VTEC-contaminated carcass quarters per day. In the Netherlands, culled dairy cattle are the main source of beef. The slaughter cows each have a specific GI and H status, and are from many different herds (each with a different VTEC prevalence). Therefore, the process of entering the slaughter line is assumed to be binomial for each animal. One iteration of the model represents one slaughter day, on which 500 cows enter the slaughterhouse. Quarters contaminated with no bacteria (zero CFU) are defined as negative (i.e. not-contaminated; N) and quarters with at least one CFU on their surface are defined as positive (i.e. contaminated; P). Within each stage of the slaughter process modelled, the status of a carcass can change from P to N, or the other way around. Therefore, the binomial distribution was also used as the basic stochastic process of the model (Vose, 2000). This stochastic process is outlined in Fig. 2.

Two contamination routes and one decontamination route (Fig. 2) per stage determine the contamination status of a quarter in each of the nine stages of the slaughter process (Fig. 1). Corresponding to these routes three probabilities can be recognized. The first one is the probability of transferring VTEC onto the carcass by means of the main risk factor of that specific stage (Pr). Examples of these probabilities are the probability of GI rupture during the eviscerating operation, and the probability of getting infected by the contaminated splitter saw during the splitting stage. The second probability is the probability of transferring the bacteria from the environment onto the carcass (Pe). This probability depends on the risk profile of the slaughterhouse and we assumed that this probability is equal for all stages. Because this probability is unknown for a typical Dutch dairy slaughterhouse, we assumed the same probability (1%) as used in a model about the spread of *Salmonella* in a typical Dutch pig slaughterhouse (Van der Gaag et al., 2004). The third probability is the probability of eliminating the bacteria from the carcass (Pd) by a decontamination intervention.

The three routes are modelled as follows. Let T denotes the total number of quarters entering a certain stage, $S^{+}_{(j)}$ the number of positive quarters after modelling the stochastic

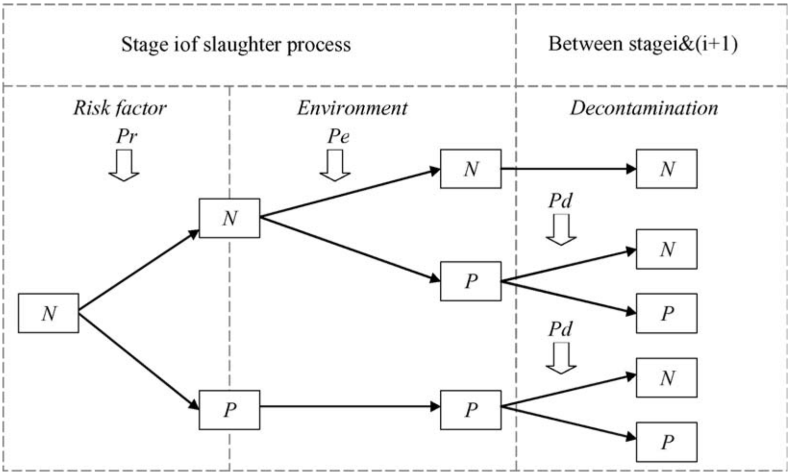

Fig. 2. Contamination and decontamination processes modelled in each stage of the slaughter process. N, negative quarters; P, positive quarters (i.e. CFU > 0); Pr, probability of bacterial transmission by the main risk factor of each individual stage; Pe, probability of bacterial transmission by the environment of each stage; Pd, elimination probability by decontamination methods after each stage.

process j, where ($j = r$) denotes the main risk factor, ($j = e$) the environment and ($j = d$) the decontamination process. $S^{-}_{(j)}$ is the number of negative quarters after each stochastic process. $S^{+}_{(0)}$ denotes the contaminated quarters coming from a previous stage. Pr and Pe are the probabilities of changing the status of a quarter from negative to positive due to the risk factors and/or environment, and Pd is the probability of change of status from positive to negative (i.e. elimination of bacteria) by decontamination. The three stochastic processes per stage in the slaughterhouse are then written as the following equations:

1. Contamination due to the *risk factor*

$$S^{+}_{(r)} = \text{Binomial}\,(S^{-}_{(0)}; Pr) + S^{+}_{(0)} \tag{1a}$$

$$S^{-}_{(r)} = T - S^{+}_{(r)} \tag{1b}$$

2. Contamination by the *environment*

$$S^{+}_{(e)} = \text{Binomial}\,(S^{-}_{(r)}; \text{Pe}) + S^{+}_{(r)} \tag{2a}$$

$$S^{-}_{(e)} = T - S^{+}_{(e)} \tag{2b}$$

3. Decontamination

$$S^{-}_{(d)} = \text{Binomial}\,(S^{+}_{(e)}; \text{Pd}) + S^{-}_{(e)} \tag{3a}$$

$$S^{+}_{(d)} = T - S^{-}_{(d)} \tag{3b}$$

In practice, environmental risks might come before the risk factors of each stage or vice versa. In this model we simplified the process by separating the process into three parts following each other in a fixed order: contamination by a risk factor, contamination by the environment and decontamination.

2.4. Input data and distributions

2.4.1. Prevalence of VTEC-contaminated cattle

Table 1 summarizes the input data, distributions and the source of the data that are used in the model. Both the herd-level prevalence and animal-level prevalence are needed to simulate the number of GI^{+} or H^{+} animals entering the slaughterhouse. The number of GI^{+} animals is simulated based on the herd and animal-level prevalence at negative-tested (i.e. assumed by us to be false-negative) and positive-tested herds (Nauta, 2001). If P denotes probability of being infected, HP herd-level prevalence, AP^{+} animal-level prevalence in positive-tested herds and AP^{-} animal-level prevalence in negative-tested herds, the probability of a positive animal entering the slaughterhouse was calculated as $P = HP\,AP^{+} + (1 - HP)AP^{-}$.

In the Netherlands herd-level prevalence is 7.2% (90% CI: 5.6–8.8) (Schouten et al., 2004). We considered a beta distribution for herd-level prevalence to take care of the uncertainty. The animal-level prevalence of GI^{+} cattle coming from positive-tested herds was modelled using a uniform distribution with values between minimum 0.8% and

Table 1

Description of variables used in the model to estimate the VTEC contamination in Dutch industrial beef slaughterhouses

Variable	Distribution	Values/formulas	Source
Animal-level prevalence on positive-tested herds	Uniform	Minimum: 0.8%; maximum: 22.4%	Heuvelink et al. (2001)
Animal-level prevalence on negative-tested herds	Constant	0.45%	Heuvelink et al. (1998)
Concentration of bacteria (log CFU) in 1 g of manure	Cumulative[a]	Minimum: 0; maximum: 6; $\{x_\mathrm{i}$: 2, 3, 4, 5$\}$; $\{p_\mathrm{i}$: 0.46, 0.53, 0.87, 0.96$\}$	Nauta (2001), Vose (2000)
Elimination probability	Poisson[b]	x: 0 (no. of CFU); λ: expected number of CFU on quarters	Expertise of authors
Herd-level prevalence	Beta	α: 50, β: 628	Schouten et al. (2004)
Gram of manure on each carcass	Beta[c]	Maximum: 10.1; α: 0.395; β: 2.47	Nauta (2001)
Hide-level prevalence in lairage	Triangular[d]	Minimum: 6.7%; mode: 32.9%; maximum: 42.3%	Avery et al. (2002)
Probability of GI rupture (Pr)	Constant	1%	Ebel et al. (2004)
Probability of infection via splitter saw (Pr)	Constant	1%	Expertise of authors
Probability of infection via environment (Pe)	Constant	1%	Van der Gaag et al. (2004)
Slaughtered animals/day	Constant	500	Expertise of authors
Total surface of a carcass (cm^2)	Constant	32000	Ebel et al. (2004)
UK animal-level prevalence	Constant	14%	Mechie et al. (1997)

[a] Function: cumulative (min, max, $\{x_i\}$,$\{p_i\}$).

[b] Excel function: Poisson (x, λ, false).

[c] @Risk function: Max × RiskBeta (α, β).

[d] We scaled hide-level prevalence to reflect the GI prevalence of Dutch cattle.

maximum 22% due to the seasonal effect and sensitivity of the tests used (Heuvelink et al., 1998). The animal-level prevalence for the negative-tested herds is included as a constant value that was reported as 0.45% (Heuvelink et al., 1998) indicating that these herds might be false negative.

Because of the short period between the transport of animals and moment of slaughter, any change in prevalence of GI^+ animals is ignored. However, grouping animals in the transportation loads and the lairage before slaughter might increase the hide-level prevalence in animals (Small et al., 2002). No Dutch data on hide-level prevalence are available. Therefore, we used a triangular distribution of hide-level prevalence with a minimum of 6.7%, a most likely value of 32.9% and a maximum of 42.3%, based on sampling at the lairage stage in the UK (Avery et al., 2002). Using a UK animal-level prevalence (AP_{UK}) of 14% (Mechie et al., 1997), the hide-level prevalence (HP) was scaled to the Dutch situation: $HP_{Dutch} = (AP_{Dutch}HP_{UK})/AP_{UK}$. Table 1 summarizes the input data used in this model.

2.4.2. *Interventions*

Various hygienic and decontamination measures can be applied along the whole slaughter process. Antimicrobial interventions can reduce the number of bacteria on the carcass surface, and in the case of elimination of all bacteria they can change the contamination status of the quarter (Smulders and Greer, 1998). In this study we considered the seven well-known decontamination methods in the beef industry: hot-water wash (W); trim (T); steam-vacuum (V); steam-pasteurization (S); lactic-acid rinse (L); irradiation (Ir) and hide-wash with ethanol (H). We compared their effectiveness used individually or in combination. In general, applying carcass-decontamination technologies after the most contaminating stages (e.g. de-hiding, evisceration and splitting) seems the most logical. To choose the place of the interventions and the combination of interventions in the slaughter line in this study we followed three guidelines: (1) the place of the interventions suggested by the reference study (Phebus et al., 1997); (2) the place of the interventions based on practices of US beef-slaughter plants (Ebel et al., 2004); and (3) our own experience in slaughter practice and the technical feasibility of applying the interventions. As an example, hot-water wash is technically feasible almost at all stages (except in the chilling room). However, it is usually done after splitting. On the other hand in Dutch beef-slaughter plants there is a washing stage (S7) for the pre-chilling purpose. Therefore hot-water wash was examined at both stages. Irradiation comes at the quartering stage, after carcasses come out of the chilling room and before entering the de-boning and processing stage. Irradiating the meat before the quartering stage is not logical because before this stage there are some highly contaminating stages that can re-contaminate the irradiated meat.

The combinations of interventions were chosen in such a way as to be consistent with the combinations that were mentioned in the reference study (Phebus et al., 1997), and those are combinations that are technically more justifiable. In that study there are some technical reasons (like more bactericidal effects) for choosing these particular combinations.

2.4.3. *Simulation of elimination probabilities*

The elimination probability (Pd) for each intervention was calculated based on results of experimental studies that are expressed as reduction in log CFU/cm^2 of the initial bacterial population. Because the number of CFU on each quarter follows a Poisson distribution, the probability of having zero CFU (i.e. Pd) was calculated from the expected number of CFU on a quarter after applying the intervention (λ). λ equals the initial number of CFU on each quarter minus the reported reduction due to a specific intervention (Phebus et al., 1997). The initial number of bacteria (CFU) on each quarter was simulated by multiplying two distributions: the amount of manure (in grams) transferred to the carcass (beta distribution) and the concentration of VTEC in 1 g of manure (cumulative distribution). The data and distributions used were based on a VTEC risk assessment (Table 1; Nauta, 2001). A beta distribution to describe the carcass contamination with manure was chosen after fitting the results of expert estimates to a series of probability distributions (Nauta, 2001). The parameters α and β express the level of carcass contamination with manure and its variability per carcass. 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). For these simulations we assumed that each carcass has a total surface of 32,000 cm^2 and that each quarter receives equally one fourth of the total faeces. The mean elimination probabilities were based on 10,000 iterations. The last column of Table 2 represents these probabilities. For example, steam-pasteurization can reduce the initial number of bacteria by 3.53 log CFU/cm^2. Given our assumptions, this corresponds to an 83% probability of eliminating all the bacteria from a quarter.

2.5. Sensitivity analysis

Running the model using default input values and without incorporating the interventions was considered the baseline scenario. In the sensitivity analysis, the baseline output, was compared with alternatives (Vose, 2000). We changed only one of the input variables at a time. For the input variables that were described by distributions, such as herd-, animal- and hide-level prevalences, we examined the situations where these distributions shifted upwards or downwards by 50% of their mean in the default situation. In our view ±50% for the mentioned parameters generate such variations in the outputs that can demonstrate the most sensitive inputs of the model. Theoretically we can assume a reduction to zero or a large increase (e.g. 100%) in the value of a probability which in our

Table 2
Reduction of VTEC population from the surface of beef quarters and corresponding elimination probabilities of all CFU counts from carcass quarters

Intervention	Reduction (log CFU/cm^2)		Reference	Estimated elimination probability, Pd (%)
	Mean	S.E.		
Hot-water wash (W)	0.75	0.49	Phebus et al. (1997)	34.69
Lactic-acid (L)	2.70	0.49	Phebus et al. (1997)	68.75
Steam-vacuum (V)	3.11	0.49	Phebus et al. (1997)	76.01
Trimming (T)	3.10	0.49	Phebus et al. (1997)	75.83
Hide-wash with ethanol (H)	5.00	0.20	Mies et al. (2004)	83.33
Steam-pasteurization (S)	3.53	0.49	Phebus et al. (1997)	83.17
Irradiation (Ir)	6.00	0.49[a]	Molins et al. (2001)	99.48

[a] We assumed the same standard error as the other interventions.

opinion in this particular case (i.e. VTEC) is not fully compatible with the real observations. The number of iterations for the sensitivity analysis was 10,000. For the three input parameters for which the input was a single value namely the probability of contaminating the carcass with the splitter saw, due to a rupture or by the environment (in the basic situation all with a value of 1%), a probability of 0.1% and 10% were examined. We think that this range is compatible with natural variations for these values. We also believe that it is unlikely to have > and < of 10-fold change for the mentioned parameters. Finally, a sensitivity analysis was performed to study the effect of different effectiveness of the six single interventions using the standard errors of their CFU reduction.

For each scenario, the model was for the basic initial contamination and for a worst-case scenario. In the worst-case scenario the initial number of CFU was considered to be 13,487, while this value was 20 in the basic situation. For each simulation, 10,000 iterations were used, to have <1% change in the distribution statistics of the output variable.

3. Results and discussion

The baseline scenario represents the current slaughterhouse situation where no extra interventions are applied. The results of the baseline and different scenarios are shown in Table 3. In the baseline scenario, 9.2% of the daily produced beef quarters are predicted to be contaminated by VTEC bacteria. The number of bacteria lies mostly between 1 and 20 per carcass quarter. When considering the maximum initial number of bacteria on the surface of the carcasses (i.e. the worst-case scenario), in the basic situation, the bacteria are never eliminated completely from the surface and in this case 34% of the quarters are contaminated (by 1–13,487 CFU per quarter) at the end of the slaughter line.

The single decontamination interventions W, L, T, V and H reduced the baseline prevalence from 9.2% to respectively 6.2%, 3.0%, 2.4%, 2.4% and 2.00%. In the reference study (Phebus et al., 1997) it is mentioned that trimming is more effective in experimental studies than in practice. This is also applies to steam-vacuuming. The reason for this is that in an experimental study, the site of contamination is known to the worker. Moreover trimming and steam-vacuum are focused on the visible contaminations and therefore their effect is not uniform on the whole surface. This has not been considered in our model, so their effectiveness is probably overestimated. The high effectiveness of hide-wash with ethanol confirms the importance of hide-level prevalence and interventions at this level. However, from the animal-welfare point of view, washing the hides of live animals with ethanol is debatable practice (Mies et al., 2004). Two combined interventions; WT (hot-water wash + trim) and WV (hot-water wash + steam-vacuum), have the same effect (1.8%). Carcass steam-pasteurization is more effective (1.7%) than the combined sets mentioned.

The sets of interventions consisting of two to four decontamination measures (also known as "hurdle strategy" (Juneja and Sofos, 2002), could reduce the baseline prevalence to 1.2% and 0.1%. These are applied over the whole slaughter process, so that some major changes in the slaughter process are necessary to apply them, which might not be desirable from an economic point of view. The effectiveness of combined interventions is not additive, as the experimental microbiological studies confirm.

Table 3
Predicted prevalence of VTEC-contaminated dairy-beef quarters in Dutch slaughterhouses

Interventions	Slaughter stage(s)[a]	Baseline assumption for initial CFU counts/quarter[b]			Worst-case assumption for initial CFU counts/quarter[c]		
		Predicted quarter-level prevalence (%)		Predicted most likely CFU counts	Predicted quarter-level prevalence (%)		Predicted most likely CFU counts
		Mean	5th, 95th percentiles		Mean	5th, 95th percentiles	
None (baseline scenario)		9.2	4.4, 13.1	20	34.0	29.8, 39.6	13487
Carcass hot-water wash (W)	S4	6.2	2.9, 9.0	4	34.0	29.8, 39.6	2398
Carcass hot-water wash (W)	S7	6.1	2.8, 8.8	4	34.0	29.8, 39.6	2398
Carcass lactic-acid rinse (L)	S7	3.0	1.4, 4.4	1	34.0	29.8, 39.6	27
Carcass steam-vacuum (V)	S3	2.7	1.2, 4.1	1	34.0	29.8, 39.6	10
Carcass trim (T)	S2	2.5	1.1, 3.8	1	34.0	29.8, 39.6	11
Carcass trim (T)	S4	2.4	1.1, 3.6	1	34.0	29.8, 39.6	11
Carcass steam-vacuum (V)	S4	2.4	1.1, 3.6	1	34.0	29.8, 39.6	10
Hide-wash with ethanol (H)	S1	2.0	0.9, 3.1	1	34.0	29.8, 39.6	4
WT	S4, S3	1.8	0.8, 2.8	1	34.0	29.8, 39.6	1
WV	S4, S3	1.8	0.8, 2.9	1	34.0	29.8, 39.6	1
Carcass steam-pasteurization (S)	S7	1.7	0.7, 2.5	1	33.4	29.3, 37.6	4
WS	S3, S7	1.2	0.5, 1.9	1	33.4	29.3, 37.6	1
WTS	S4, S3, S7	0.3	0.1, 0.6	1	33.4	29.3, 37.6	2
WVS	S4, S3, S7	0.3	0.1, 0.6	1	33.4	29.3, 37.6	1
WLTS	S3, S4, S2, S7	0.1	0.0, 0.3	1	33.4	29.3, 37.6	1
WLVS	S3, S4, S2, S7	0.1	0.0, 0.3	1	33.4	29.3, 37.6	1
WLVHS	S3, S4, S2, S1, S7	0.02	0.0, 0.1	1	00.8	00.5, 01.2	1
Irradiation of quarters (Ir)	S9	0.02	0.0, 0.1	1	00.8	00.5, 01.2	1

The results are ordered according to the mean prevalence of contaminated quarters.

[a] Stages S1–S9 correspond to slaughter stages illustrated in Fig. 1.

[b] Assuming an initial CFU count of 20 per quarter (most likely value).

[c] Assuming an initial CFU count of 13,487 per quarter (based on maximum values).

Two intervention strategies, irradiation and WLVHS (hot-water wash, lactic-acid rinse, steam-vacuum, hide-wash with ethanol and steam-pasteurization) were predicted to reduce the baseline initial prevalence to 0.02%. Even at the highest level of the initial number of CFU (worst-case scenario), irradiation remains the only single decontamination measure that can eliminate almost all the bacterial population and reduce the prevalence. Irradiation cannot be applied in the middle of the process, because then the meat could be newly contaminated through the environment and the risky events of the other stages. Irradiation is recognized as a safe technology for destroying pathogens on the surface of beef (Molins et al., 2001) and is used in the US and elsewhere, though at the time of this study its application to beef (in the EU) is prohibited (EU, 2003). Application of the WLVHS strategy is as powerful as irradiation at the end of the process (ranking 2 in Table 3). The decision whether to invest in more interventions along the slaughter process or in a single intervention at the end of the line is for decision makers.

Under the worst-case scenario, almost all the interventions except a combined set of interventions (i.e. WLVHS) and irradiation were predicted to fail to eliminate all the VTEC bacteria from the surface of the quarters. Carcass steam-pasteurization could slightly reduce the prevalence from 34% to 33.4% and its combinations with other methods fail to reduce the prevalence further. These results imply that in the case of having very high initial concentration of bacteria on the carcasses worst-case scenario (e.g. >log 5), the elimination probability could be zero or close to 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, in the worst-case scenario the interventions mentioned can effectively reduce the number of CFU from the surface of the quarters (Table 3).

3.1. Results of the sensitivity analysis

Hide-level prevalence has a great influence on the number of the contaminated beef-carcass quarters (Fig. 3). Although the importance of hide contamination has been emphasized in previous studies (Avery et al., 2002; Bosilevac et al., 2004), we were surprised at such a strong influence. A reason for this effect might be the high value of the hide-level prevalence in the UK data used as input (Avery et al., 2002), despite the scaling we performed to adjust the data to the Dutch situation. However, an even-higher hide-level prevalence of 76% for animals entering the slaughterhouse has been recently reported from the US (Arthur et al., 2004). An implication of this could be that more attention should be paid to reduce the hide-level prevalence in the pre-slaughter stage to protect beef products against VTEC contamination.

The output is also sensitive to the internal environment of the slaughterhouse. Increasing the probability of transmission from the environment leads to a large increase in output prevalence but decreasing it leads to only a small decrease in the number of contaminated quarters. Thus the current hygienic measures within slaughterhouses should be at least maintained. In general, hardly any field data for estimating the probabilities for the environment, gut rupture and splitter saw contaminations are available. Because the model output is sensitive to these parameters, more field data will be very helpful in improving the results of our model.

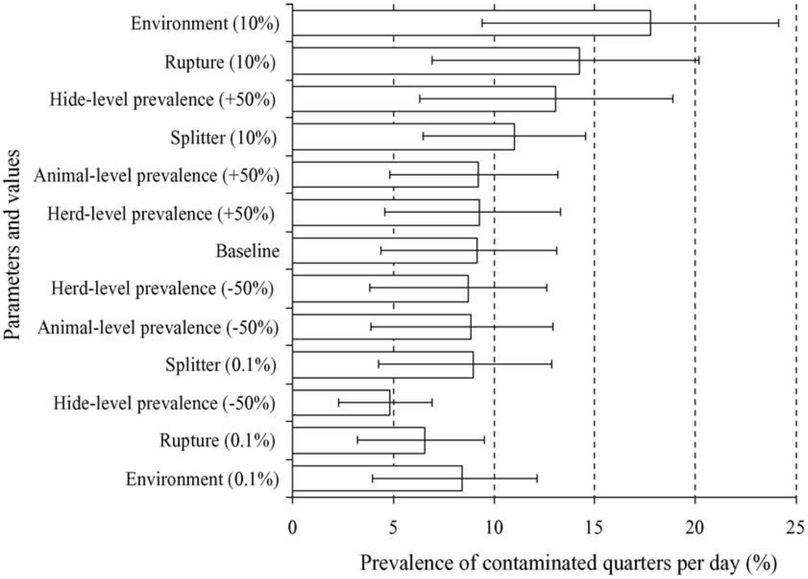

Fig. 3. Results of the sensitivity analysis of the impact of six single input parameters of the model. Given are the mean prevalence and the 5th and 95th percentiles (error bars). For herd-, animal- and hide-level prevalences the ±50% of the default input values were examined, while for environment, GI rupture and splitter saw a minimum value of 0.1% and maximum value of 10% were examined.

The result of a comparison of the effectiveness of six decontamination methods is illustrated in Fig. 4. For these sensitivity analyses, the limits we used for the new runs were based on the mean ± 1 S.E. of the predicted mean of the baseline scenario. These results show that considering the uncertainty of the effectiveness of interventions, particularly of steam-pasteurization, steam-vacuum, trim and lactic-acid rinse, should influence our

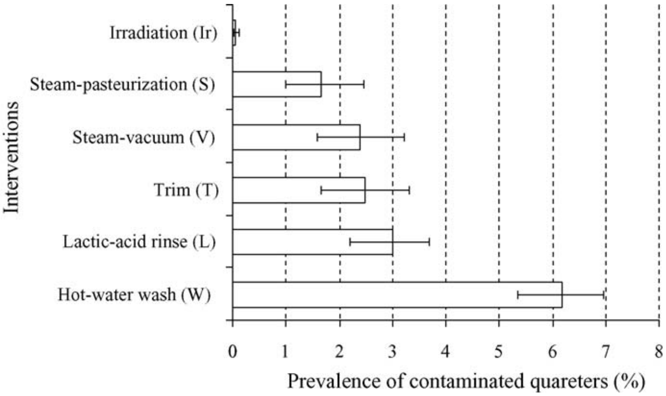

Fig. 4. Predicted quarter-level prevalence and 5th and 95th percentiles (error bars), using default input values ±1 S.E. for six decontamination methods (default values were based on the mean and S.E. of log CFU/cm^2 reduction reported in the reference studies).

judgement. However, considering uncertainty does not affect the outcome of our analysis of hot-water wash and irradiation.

3.2. Precision of detection

Baseline scenario (Table 3) shows 9.2% of the 2000 daily produced beef quarters are contaminated mostly with one up to around 20 VTEC bacteria. This is in contrast to measurements done in slaughterhouses in the Netherlands. In a study by Heuvelink et al. (2001) no VTEC was isolated, even thought >10% of carcasses were visibly contaminated with faeces (in 11 of the 27 slaughterhouses). In 6 out of the 11 slaughterhouses, >50% of inspected carcasses were visibly contaminated. Other Dutch measurements confirm VTEC contamination at the retail stage from 0.5% for minced mixed beef and pork, to 1.1% for raw minced beef (Heuvelink et al., 1999). This inconsistency can be explained by the fact that the model estimates the true prevalence, while in epidemiological studies the apparent prevalence is estimated. In the simulation model a carcass is positive if it is contaminated with at least one bacterium, whereas in practice, the detection limit of the test limits the number of positive carcasses found. There are many factors, such as characteristics of the model, which determine the current output of the model. In a comparison of this output with real data, we feel that it is reasonable that the model predicts a higher prevalence than currently is recognized in the field.

3.3. Limitations of modelling

Because the results of this study are based on a simulation model, one should remember the fact that models are always a simplification of reality. The main focus of our model was on simulating the prevalence of contaminated beef-carcass quarters; the actual number of VTEC CFU on the surface of the contaminated beef quarters was calculated based on a relatively simple approach. We chose not to model the exact number of bacteria transferred from sources to the beef surfaces along the slaughter line because this requires a lot more assumptions and data. Decision makers currently seem to focus on the prevalence of contaminated carcasses, and this focus was used in other research (Van der Gaag et al., 2004; Alban and Stark, 2005). However, we acknowledge that the ignorance of the number of bacteria transmitted to the end product could be undesirable from the public-health point of view. In the model we assumed that each quarter receives a fourth of faecal contamination. This might have an impact on the output, but to avoid adding more assumptions, the process of the distribution of manure between the quarters was not modelled. Furthermore, assumptions had to be made to calculate the reduction of the probability that the bacteria were eliminated from the carcasses through different decontamination measures. This depends highly on the initial number of bacteria. The initial number of CFU was determined on the basis of distributions of the amount of manure and the concentration of CFU in the manure. These distributions were based on a Dutch expert's opinion and literature (Zhao et al., 1995; Nauta, 2001). The distribution of the bacterial concentration might vary for the Netherlands and the distribution of the transferred manure might be different than in the conditions considered in the reference study. Therefore, the elimination probability used in our model could under or overestimate the effectiveness of interventions.

Culled dairy cattle are the main source of beef in the Netherlands and consequently the population of beef cattle is rather low (119,000 beef cows versus 1,500,000 dairy cows). Therefore, the model focuses only on dairy cattle. However, there is a relatively large veal-production sector in the Netherlands. The prevalence of VTEC is different in the dairy sectors and the veal. Also, the veal sector has separate slaughterhouses. Thus, the model needs some adjustment to be used for veal slaughterhouses.

A last point to be noted is that, under the current EU policy and regulation (February 2006), washing carcasses with organic acids (e.g. lactic-acid rinse) and irradiating red meat is prohibited (Heuvelink, 2000; Duffy et al., 2002; EU, 2003). On the other hand, steam-pasteurization and steam-vacuuming of cattle carcasses are not methods commonly being used in European countries. In case of possible changes in EU policies and more demand for implementing extra decontamination measures in the current slaughter process, the results of this study could be useful in a future discussion of which interventions should be allowed and applied.

4. Conclusions

We predict that the prevalence of VTEC-contaminated quarters of dairy beef can be decreased by roughly one-third to one-sixth by implementing any one of six decontamination methods. However, we predict that using multiple methods generally would decrease quarter-level prevalence by substantially more than most single-method strategies. Under our assumptions, irradiation at the end of the process would decrease prevalence by >99%, only reachable otherwise by combing five of the six other methods.

Acknowledgements

The authors thank the following individuals for their advice and comments on this work: Annet Heuvelink, Klaas Frankena and Marije Schouten.

References

Alban, L., Stark, K.D.C., 2005. Where should the effort be put to reduce the Salmonella prevalence in the slaughtered swine carcass effectively? Prev. Vet. Med. 68, 63–79.

Arthur, T.M., Bosilevac, J.M., Nou, X.W., Shackelford, S.D., Wheeler, T.L., Kent, M.P., Jaroni, D., Pauling, B., Allen, D.M., Koohmaraie, M., 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, Enterobacteriaceae, and *Escherichia coli* O157 at various steps in commercial beef processing plants. J. Food Prot. 67, 658–665.

Avery, S.M., Small, A., Reid, C.A., Buncic, S., 2002. Pulsed-field gel electrophoresis characterization of Shiga toxin-producing *Escherichia coli* O157 from hides of cattle at slaughter. J. Food Prot. 65, 1172–1176.

Bell, R.G., 1997. Distribution and sources of microbial contamination on beef carcasses. J. Appl. Microbiol. 82, 292–300.

Bosilevac, J.M., Arthur, T.M., Wheeler, T.L., Shackelford, S.D., Rossman, M., Reagan, J.O., Koohmaraie, M., 2004. Prevalence of *Escherichia coli* O157 and levels of aerobic bacteria and Enterobacteriaceae are reduced when hides are washed and treated with cetylpyridinium chloride at a commercial beef processing plant. J. Food Prot. 67, 646–650.

Chapman, P.A., Wright, D.J., Siddons, C.A., 1994. A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine feces. J. Med. Microbiol. 40, 424–427.

Duffy, G., Garvey P., Sheridan J.J., 2002. A European study on animal food & biomedical aspects of *E. Coli* 0157:H7. Internet: http://www.teagasc.ie/research/reports/foodprocessing/4545/eopr-4545.htm.

Ebel, E., Schlosser, W., Kause, J., Orloski, K., Roberts, T., Narrod, C., Malcolm, S., Coleman, M., Powell, M., 2004. Draft risk assessment of the public health of *Escherichia coli* O157:H7 in ground beef. J. Food Prot. 67, 1991–1999.

EU, 2003. List of Member States' authorisations of food and food ingredients which may be treated with ionising radiation. Official Journal of the EU. 2003/C 56/03 2003. Internet: http://europa.eu.int/eur-ex/pri/en/oj/dat/2003/c_056/c_05620030311en00050005.pdf.

Heuvelink, A.E., Roessink, G.L., Bosboom, K., de Boer, E., 2001. Zero-tolerance for faecal contamination of carcasses as a tool in the control of O157VTEC infections. Int. J. Food Microbiol. 66, 13–20.

Heuvelink, A.E., 2000. Verocytotoxin-producing *Escherichia coli* in Humans and the Food Chain. Nijmegen University, Nijmegen.

Heuvelink, A.E., Zwartkruis-Nahuis, J.T.M., Beumer, R.R., de Boer, E., 1999. Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meats obtained from retail outlets in the Netherlands. J. Food Prot. 62, 1115–1122.

Heuvelink, A.E., van den Biggelaar, F., Zwartkruis-Nahuis, J.T.M., Herbes, R.G., Huyben, R., Nagelkerke, N., Melchers, W.J.G., Monnens, L.A.H., de Boer, E., 1998. Occurrence of verocytotoxin-producing *Escherichia coli* O157 on Dutch daily farms. J. Clin. Microbiol. 36, 3480–3487.

Hudson, W.R., Mead, G.C., Hinton, M.H., 1998. Assessing abattoir hygiene with a marker organism. Vet. Rec. 142, 545–547.

Huffman, R.D., 2002. Current and future technologies for the decontamination of carcasses and fresh meat. Meat Sci. 62, 285–294.

Juneja, V.K., Sofos, J.N., 2002. Control of Foodborne Microorganisms. Dekker, New York.

Mechie, S.C., Chapman, P.A., Siddons, C.A., 1997. A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd. Epidemiol. Infect. 118, 17–25.

Mies, P.D., Covington, B.R., Harris, K.B., Lucia, L.M., Acuff, G.R., Savell, J.W., 2004. Decontamination of cattle hides prior to slaughter using washes with and without antimicrobial agents. J. Food Prot. 67, 579–582.

Molins, R.A., Motarjemi, Y., Kaferstein, F.K., 2001. Irradiation: a critical control point in ensuring the microbiological safety of raw foods. Food Control 12, 347–356.

Nauta, M.J., 2001. Risk assessment of Shiga-toxin producing *Escherichia coli* 0157 in steak tartare in the Netherlands, RIVM report 257851003. RIVM, Bilthoven. Internet: http://www.rivm.nl/bibliotheek/rapporten/257851003.pdf.

Palisade, C., 2002. Guide to Using @Risk. Palisade Corporation, Newfield, NY.

Phebus, R.K., Nutsch, A.L., Schafer, D.E., Wilson, R.C., Riemann, M.J., Leising, J.D., Kastner, C.L., Wolf, J.R., Prasai, R.K., 1997. Comparison of steam pasteurization and other methods for reduction of pathogens on surfaces of freshly slaughtered beef. J. Food Prot. 60, 476–484.

Phillips, C.A., 1999. The epidemiology, detection and control of *Escherichia coli* O157. J. Sci. Food Agric. 79, 1367–1381.

Retzlaff, D., Phebus, R., Nutsch, A., Riemann, J., Kastner, C., Marsden, J., 2004. Effectiveness of a laboratory-scale vertical tower static chamber steam pasteurization unit against *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria innocua* on prerigor beef tissue. J. Food Prot. 67, 1630–1633.

Schouten, J.M., Bouwknegt, M., van de Giessen, A.W., Frankena, K., De Jong, M.C.M., Graat, E.A.M., 2004. Prevalence estimation and risk factors for *Escherichia coli* O157 on Dutch dairy farms. Prev. Vet. Med. 64, 49–61.

Small, A., Reid, C.A., Avery, S.M., Karabasil, N., Crowley, C., Buncic, S., 2002. Potential for the spread of *Escherichia coli* O157, Salmonella, and Campylobacter in the lairage environment at abattoirs. J. Food Prot. 65, 931–936.

Smulders, F.J.M., Greer, G.G., 1998. Integrating microbial decontamination with organic acids in HACCP programmes for muscle foods: prospects and controversies. Int. J. Food Microbiol. 44, 149–169.

Sofos, J.N., Kochevar, S.L., Bellinger, G.R., Buege, D.R., Hancock, D.D., Ingham, S.C., Morgan, J.B., Reagan, J.O., Smith, G.C., 1999. Sources and extent of microbiological contamination of beef carcasses in seven United States slaughtering plants. J. Food Prot. 62, 140–145.

Van der Gaag, M.A., Vos, F., Saatkamp, H.W., van Boven, M., van Beek, P., Huirne, R.B.M., 2004. A state-transition simulation model for the spread of Salmonella in the pork supply chain. Eur. J. Operat. Res. 156, 782–798.

Vose, D., 2000. Risk Analysis: A Quantitative Guide. Wiley, New York.

Zhao, T., Doyle, M.P., Shere, J., Garber, L., 1995. Prevalence Of Enterohemorrhagic *Escherichia coli* O157-H7 in a survey of dairy herds. Appl. Environ. Microbiol. 61, 1290–1293.