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# Control of human pathogenic *Yersinia enterocolitica* in minced meat: Comparative analysis of different interventions using a risk assessment approach

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ABSTRACT

This study aimed to evaluate the effect of different processing scenarios along the farm-to-fork chain on the contamination of minced pork with human pathogenic *Y. enterocolitica*. A modular process risk model (MPRM) was used to perform the assessment of the concentrations of pathogenic *Y. enterocolitica* in minced meat produced in industrial meat processing plants. The model described the production of minced pork starting from the contamination of pig carcasses with pathogenic *Y. enterocolitica* just before chilling. The endpoints of the assessment were (i) the proportion of 0.5 kg minced meat packages that contained pathogenic *Y. enterocolitica* and (ii) the proportion of 0.5 kg minced meat packages that contained more than $10^3$ pathogenic *Y. enterocolitica* at the end of storage, just before consumption of raw pork or preparation. Comparing alternative scenarios to the baseline model showed that the initial contamination and different decontamination procedures of carcasses have an important effect on the proportion of highly contaminated minced meat packages at the end of storage. The addition of pork cheeks and minimal quantities of tonsillar tissue into minced meat also had a large effect on the endpoint estimate. Finally, storage time and temperature at consumer level strongly influenced the number of highly contaminated packages.



## 1. Introduction

As pork is the second most consumed meat worldwide (OECD, 2016), an effective control of zoonotic agents transferred via pork is of major importance to limit the public health risk of zoonotic diseases. Due to the frequent finding of human pathogenic *Yersinia enterocolitica* in pigs and pork compared to other food producing animals and food products, and the high genetic relatedness of human and porcine strains, pork is considered the main source of human pathogenic *Y. enterocolitica*. As such, 77% of *Y. enterocolitica* cases in Europe may be attributed to the consumption of pork (Fosse et al., 2008). The consumption of raw minced meat may be of particular importance in transmitting pathogenic *Y. enterocolitica* to humans as Rosner et al. (2012) found that 34% of yersiniosis cases in Germany had consumed raw minced pork in the seven days preceding illness compared to 12% of the control group.

With 6471 confirmed cases in 2013, yersiniosis remains the third most commonly reported zoonosis in the European Union. Over 98% of cases is caused by human pathogenic *Yersinia enterocolitica* (EFSA and ECDC, 2015), the majority of strains belonging to bioserotype 4/O:3 (EFSA, 2009). The main reservoirs of these strains are domestic pigs, which can asymptomatically carry the pathogens in lymph nodes, tonsils and the intestinal tract (Laukkanen-Ninios et al., 2014a), resulting in the spread to the carcass during different steps in the slaughter process (Borch et al., 1996). The presence of pathogenic *Y. enterocolitica* in the intestines and especially the tonsils is strongly associated with carcass contamination (Van Damme et al., 2015; Vilar et al., 2015) and carcass contamination has been shown to differ according to the location on the carcass, with more positive samples found near the head region and sternum than other areas of the carcass

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(Laukkanen et al., 2010; Van Damme et al., 2015).

Although the species *Y. enterocolitica* is very heterogeneous, the presence of virulence genes in the most common types of pathogenic *Y. enterocolitica* seems to be homogeneous (Murros et al., 2016; Schneeberger et al., 2015). As a result, exposure to these pathogenic types may be more relevant for public health, rather than specific virulence traits of certain strains. Therefore, identification of the process steps along the farm-to-fork pathway that have the largest influence on this exposure may be the most effective way in reducing the public health risk of yersiniosis, prospecting the development of targeted control measures. Quantitative microbial risk assessment (QMRA) has emerged in the area of food safety as a comprehensive and systematic approach for addressing the risk of microbial hazards in the food chain and can be used to assess the impact of control strategies or interventions (Havelaar et al., 2008; Møller et al., 2015). Using the Modular Process Risk Model (MPRM) methodology as proposed by Nauta (2008), the food production pathway is described by subdividing the chain in different modules that each represent a basic process. These basic processes include microbial (growth or inactivation) and food handling processes (cross-contamination, removal, partitioning and mixing), by which the changes in prevalence, concentration and unit can be modelled. The output of one module then serves as the input for the following module. This structured approach allows a structured analysis of the food chain, which gives new insights in the complex process of food production and can identify crucial data gaps.

The objective of this study was to model the spread of pathogenic *Y. enterocolitica* contamination during the production of minced meat and to evaluate the effect of different intervention scenarios during minced meat production on human exposure via raw minced pork. Therefore, a food chain modelling approach was applied to assess the exposure of human pathogenic *Y. enterocolitica* through industrially produced minced meat using the MPRM methodology. First a baseline model was built describing the current processing practices and changes in prevalence and concentrations during the process. Next, alternative scenarios were defined to evaluate the effects of potential interventions. As, to our knowledge, there is no dose response model available for *Y. enterocolitica* and no accurate data on raw minced meat consumption could be found, the endpoint of the assessment was not the exposure or the health risk but (A) the proportion of contaminated 0.5 kg minced meat packages with pathogenic *Y. enterocolitica* and (B) the proportion of 0.5 kg minced meat packages that contained more than $10^3$ pathogenic *Y. enterocolitica* at the end of storage, just before consumption of raw minced pork or preparation. To identify the most important data gaps, uncertainties were studied by comparative scenario analyses.

## 2. Material and methods

### 2.1. Description of the food pathway and model implementation

An overview of the pathway used in the model is shown in Fig. 1. A general overview of the model and a detailed description of the distributions and parameters used are shown in Tables 1 and 2, respectively.

The entire model was simulated with Monte Carlo techniques (100,000 iterations) using @Risk software (version 7.5.0., Palisade Corporation, Newfield, NY, US). By the lack of a health risk estimate, the alternative main outputs of the model were point estimates of the prevalence (proportion of 0.5-kg packages containing one or more pathogenic *Y. enterocolitica*) and/or the proportion of highly contaminated minced meat packages (containing > $10^3$ pathogenic *Y. enterocolitica* per 0.5-kg package). To evaluate the effect of alternative scenarios, the value of one or more model parameters was changed and the corresponding endpoint estimate was compared to that of the baseline scenario. Different scenarios were compared by calculating the $\log_{10}$ of the relative proportions (the quotient of the endpoint estimate of an alternative scenario and the endpoint estimate of the baseline scenario), as e.g. in Møller et al. (2015).

### 2.2. The baseline model

#### 2.2.1. Input data - initial contamination of carcasses

The prevalence and concentration of human pathogenic *Y. enterocolitica* on pig carcasses were used as input for the model and were based on the results of a Belgian study describing the contamination of pork carcasses with pathogenic *Y. enterocolitica* after evisceration before cooling (Van Damme et al., 2015). The study detected *Y. enterocolitica* bioserotype 4/O:3 on the sternal region (breast cut and surrounding skin) of 16.4% of the carcasses, which was the value used as the initial prevalence of carcasses ($P_{initial}$). Quantitative and semi-quantitative concentration data of pathogenic *Y. enterocolitica* at the sternal region were obtained by analysing different subsamples with different isolation methods. The R package "fitdistrplus" was used to fit a normal distribution to the censored data using the "fitdistcens" function (Pouillot and Delignette-Muller, 2010). The resulting normal distribution of the *Y. enterocolitica* concentration on pork carcasses was used as input for the model ($C_{initial}$ ~ Normal(-2.565; 0.736) in $\log_{10}$ CFU/cm$^2$, with ~ meaning that it is a random sample from the distribution). As $P_{inital}$ was based on the combined results of different detection methods from which the $C_{initial}$ distribution was derived, the distribution was truncated at a minimum value of −1.85 $\log_{10}$ CFU/cm$^2$, which was the limit of detection of the most sensitive detection method. The final (truncated) distribution had a mean of −1.46 $\log_{10}$ CFU/cm$^2$ and standard deviation of 0.33.

#### 2.2.2. Inactivation and growth during carcass chilling and cold storage

Blast chilling, during which the carcass surface is frozen, was considered to cause a 0.6 $\log_{10}$ reduction in pathogenic *Y. enterocolitica* concentrations ($I_{cc}$), according to data of King et al. (2012) who evaluated the effect of freezing on *Y. enterocolitica* numbers on pig organs. When the concentration after inactivation ($N_{cci}$) was below 1 CFU/2000 cm$^2$, the carcass was considered to be pathogenic *Y. enterocolitica* negative and growth after the blast chilling step was not allowed in the model.

After inactivation during blast chilling, *Y. enterocolitica* was assumed to grow during conventional air chilling and cold storage of carcasses at 4 °C. The doubling time for the growth model during carcass cold storage ($D_{ccg}$) was set at 10.0 h, based on ComBase Predictor results (http://combase.cc) using a pH of 5.8, Aw value of 0.997, and temperature of 4 °C as input values. The lag phase ($\lambda_{ccg}$) for the growth model was set at 24 h and the maximum growth was never allowed to result in concentrations higher than 7 $\log_{10}$ CFU/cm$^2$ (van Netten et al., 1997). Carcasses from pigs that were slaughtered on Mondays to Thursdays were assumed to be processed the next day and pigs slaughtered on Fridays were processed on Monday, resulting in a cold storage time ($Time_{ccg}$) of respectively 20 h and 68 h in 80% and 20% of the iterations. The concentration of pathogenic *Y. enterocolitica* on carcasses after growth during cold storage, $N_{ccg}$, was determined:

$$N_{ccg} = N_{cci} \times 2^{\frac{Time_{ccg} - \lambda_{ccg}}{D_{ccg}}}$$

When $\lambda_{ccg}$ was higher than $Time_{ccg}$, no growth was allowed, so $N_{ccg}$

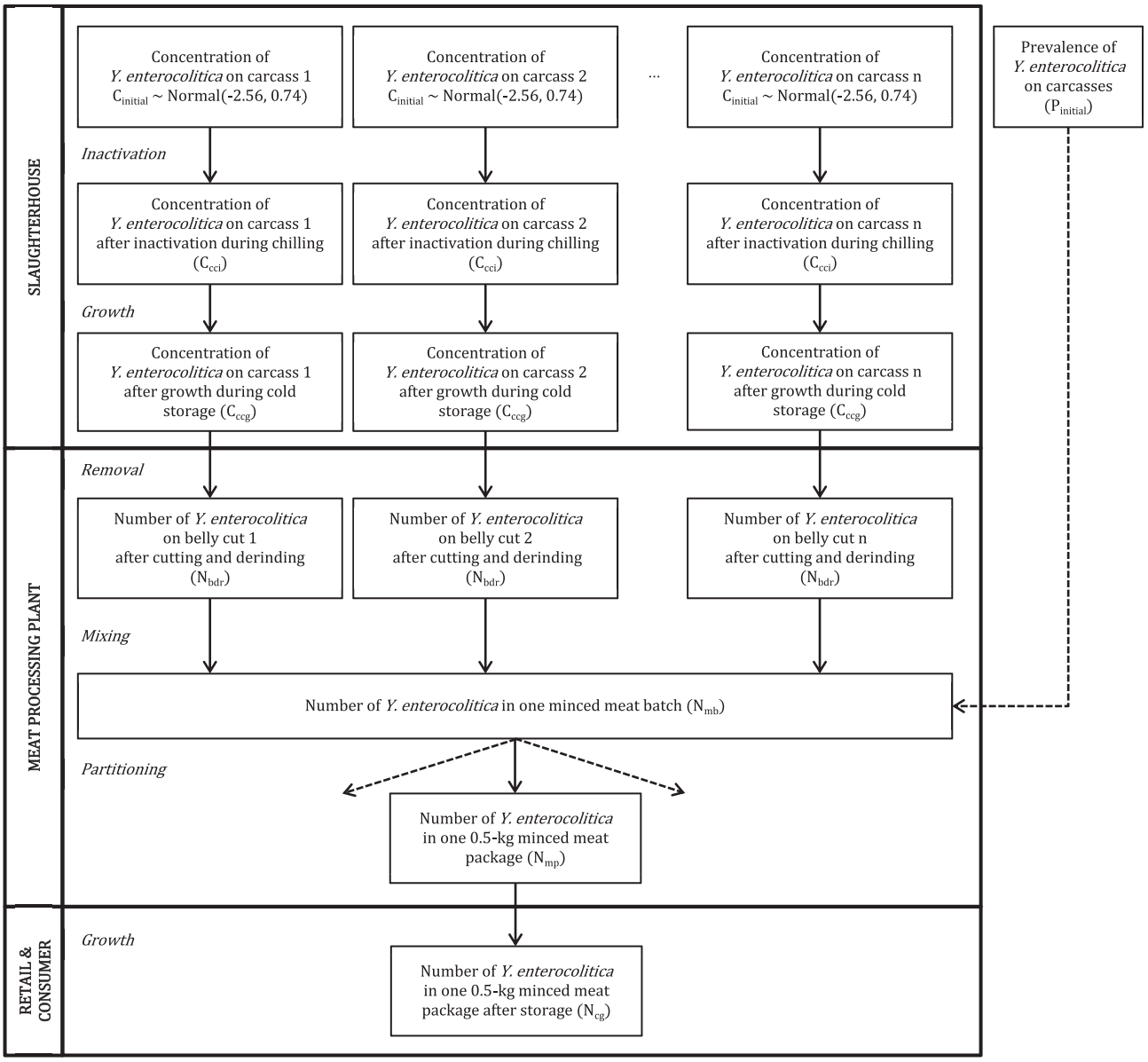


**Fig. 1. Food pathway of the baseline model to describe *Y. enterocolitica* in minced meat produced by an industrial meat processing plant**. The model starts with the contamination of carcasses in the slaughterhouse after evisceration and ends with a 0.5-package of minced pork just before consumption and/or preparation.

**Table 1**
Overview of the different steps, processes and units that were used in the risk assessment model for *Y. enterocolitica* in minced pork.

| Processing step | | Basic process | Unit |
|---|---|---|---|
| 1 | Contamination of carcasses (after evisceration, before chilling) | Initial contamination | Carcass half – belly area (2000 $cm^2$) |
| 2 | Chilling room | Inactivation + growth | Carcass half – belly area (2000 $cm^2$) |
| 3 | Cutting and derinding | Removal | Belly cut (2000 $cm^2$; 7.5 kg) |
| 4 | Grinding and seasoning | Mixing | Batch of minced meat (900 kg) |
| 5 | Packaging | Partitioning | Minced meat package (0.5 kg) |
| 6 | Storage (meat processing plant and retail) | Growth | Minced meat package (0.5 kg) |
| 7 | Storage (consumer) | Growth | Minced meat package (0.5 kg) |

was equal to the number of CFU after blast chilling ($N_{cci}$).

### 2.2.3. Cutting, derinding, grinding and packaging at the meat processing plant

The model for grinding was based on practices of a representative large minced meat producing company in Belgium. In the baseline model, a batch consisted of 900 kg minced meat and contained 34% pork bellies (weight/weight percent, w:w). The remaining ingredients (which may be beef, eggs, herbs, and/or other pork cuts) were assumed to have no contribution to contamination with pathogenic *Y. enterocolitica*. Although other pork cuts, such as shoulder cuts, are also frequently used for the

**Table 2**
Overview of variables and parameters in the baseline Modular Process Risk Model (MPRM) for human pathogenic *Y. enterocolitica* in minced meat.

| Module | Variable | Description | Unit | Value/distribution/equation | Source |
|---|---|---|---|---|---|
| Input (carcasses, sternal region, after evisceration) | $P_{initial}$ | Prevalence of *Y. enterocolitica* on pig carcasses (sternal region) after evisceration | % | 16.39 | Van Damme et al. (2015) |
| | $C_{initial}$ | Concentration of *Y. enterocolitica* on pig carcasses (sternal region) after evisceration (positive carcasses only) | $Log_{10}$ CFU/$cm^2$ | ~ Normal(-2.565; 0.736) truncated at a minimum value of −1.85 | Calculated based on data from Van Damme et al. (2015) |
| Inactivation during carcass chilling | $I_{cc}$ | Inactivation | $Log_{10}$ reduction | −0.6 | King et al. (2012) |
| | $C_{cci}$ | Concentration on pig carcasses after inactivation during chilling | $Log_{10}$ CFU/$cm^2$ | $=C_{initial} + I_{cc}$ | Calculation |
| Growth during carcass cold storage | $Time_{ccg}$ | Cold storage time of carcasses and all head meat and tonsils applied in the same batch | h | ~ Discrete(20, 68), (4, 1) | Company info |
| | $\lambda_{ccg}$ | Lag phase during carcass cold storage | h | 24 | van Netten et al. (1997) |
| | $D_{ccg}$ | Doubling time during cold storage | h | 9.978 | ComBase |
| | $N_{ccg}$ | Number of *Y. enterocolitica* after growth during cold storage | CFU/$cm^2$ | $= 10^{Ccci} \times 2^{(Time_{ccg}-\lambda_{ccg})/D_{ccg}}$ | Calculation |
| Cutting and derinding | $S_{bc}$ | Surface of belly cut | $cm^2$ | 2000 | Assumption |
| | $N_{bc}$ | Number of *Y. enterocolitica* per belly after cutting | CFU/belly | $= N_{ccg} \times S_{bc}$ (rounded to an integer value) | Calculation |
| | $R_{bd}$ | Proportion of *Y. enterocolitica* that remain on the belly cut after derinding | % | 50% | Assumption |
| | $N_{bdr}$ | Number of *Y. enterocolitica* on belly cut after derinding | CFU/belly | ~ Binomial($N_{bc}$, $R_{bd}$) | Calculation |
| Mixing and grounding | $W_b$ | Weight of a batch of minced meat | kg | 900 | Company information |
| | %bellies | Proportion of bellies per batch (w:w) | % | 34 | Company information |
| | $W_{bc}$ | Weight of a belly cut | kg | 7.5 | Company information |
| | $n_{bb}$ | Number of bellies per batch | | $= \frac{W_b \times \%bellies}{W_{bc}}$ | Calculation |
| | $n_{pbb}$ | Number of positive bellies per batch | | ~ Binomial ($n_{bb}$, $P_{initial}$) | Calculation |
| | $N_{mb}$ | Number of *Y. enterocolitica* in one minced meat batch | CFU | $= \sum_{i=1}^{n_{pbb}} N_{bdr,i}$ | Calculation |
| Partitioning/ packaging | $W_{mp}$ | Weight per minced meat package | kg | 0.5 | Company information |
| | $N_{mp}$ | Number of *Y. enterocolitica* in one minced meat package after packaging/partitioning | CFU | $\sim$ Binomial($N_{mb}$, $W_{mp}/W_b$) | Assumption |
| Storage at retail | $Temp_{rg}$ | Temperature during storage in meat processing plant and at retail | °C | 4 | Assumption |
| | $Time_{rg}$ | Time between packaging and selling at retail | h | 48 | Assumption |
| | $\mu_{max,rg}$ | Maximum growth rate (MAP) | $Log_{10}$ CFU/h | $= 0.0003 \times Temp_{rg}^2 + 0.0005 \times Temp_{rg} + 0.0103$ | ComBase |
| | $N_{rg}$ | Number of *Y. enterocolitica* in one package of minced meat after storage at retail | CFU | $= N_{mp} \times 10^{\mu_{max,rg} \times Time_{rg}}$ | Calculation |
| Storage at consumer level | $Temp_{cg}$ | Temperature of home refrigerators | °C | ~ Pert(25% 5; 50% 7; 75% 9) | Devriese et al. (2006) |
| | $Time_{cg}$ | Time between purchase and consumption/ preparation | days | ~ Pert(0; 1;4) | Marklinder et al. (2004) |
| | $\mu_{max,cg}$ | Maximum growth rate (MAP) | $Log_{10}$ CFU/h | $0.0003 \times Temp_{cg}^2 + 0.0005 \times Temp_{cg} + 0.0103$ | ComBase |
| | $N_{cg}$ | Number of *Y. enterocolitica* in one package of minced meat at the end of storage (just before consumption or preparation) | CFU/0.5-kg package | $= N_{rg} \times 10^{\mu_{max,cg} \times Time_{cg} \times 24}$ | Calculation |

production of minced meat, the contribution of these cuts was not included in the model due to the lack of sufficient reliable data. The baseline model thus assumed that bellies were the sole source of pathogenic *Y. enterocolitica* contamination.

The number of pathogenic *Y. enterocolitica* on a contaminated belly cut ($N_{bc}$) was determined using the number of CFU on the carcass after growth during cold storage and assuming a total surface of 2000 $cm^2$ (approximately 20 cm × 50 cm on both sides). After derinding, the baseline model assumed that half of the bacteria were removed. The prevalence of pathogenic *Y. enterocolitica* on belly cuts was assumed to be the same as the initial contamination of carcasses ($P_{bdr} = P_{initial}$).

Assuming a weight of pork bellies of $W_{bc} = 7.5$ kg each, the number of pork bellies within one batch was calculated ($n_{bb}$). The number of pathogenic *Y. enterocolitica* contaminated pork bellies per batch was determined using $n_{pbb}$ ~ Binomial ($n_{bb}$; $P_{bdr}$). The total number of bacteria per contaminated pork belly ($N_{bdr,i}$) was simulated for each positive belly *i* ($i = 1..n_{pbb}$) included in the batch (taking a random sampling from $C_{initial}$ for each positive belly). All bellies that were used within one batch of minced meat were assumed to originate from pigs slaughtered on the same day, so the time between slaughter and cooling ($Time_{ccg}$) remained constant for all bellies within the same batch. The numbers of pathogenic *Y. enterocolitica* on each of the positive bellies were added to determine the total number of pathogenic *Y. enterocolitica* in a batch of minced meat ($N_{mb}$):

$$N_{mb} = \sum_{i=1}^{n_{pbb}} N_{bdr,i}$$

The weight of individual minced meat packages ($W_{mp}$) was assumed to be 0.5 kg. Pathogenic *Y. enterocolitica* were assumed to be homogeneously distributed in a batch to calculate the number of pathogenic *Y. enterocolitica* in one 0.5-kg minced meat package ($N_{mp}$) (Nauta, 2005).

### 2.2.4. Storage at the meat processing plant, retail and consumer level

As there is no specific secondary growth model available for pathogenic *Y. enterocolitica* in minced meat at different temperatures, the growth at retail and consumer level was modelled using ComBase data (www.combase.cc). Hereby, the maximum growth

rate (in $\log_{10}$ CFU/h) was determined for temperatures varying between 0 and 15 °C (using 1 °C steps) for a pH of 5.8 and NaCl concentration of 1%.The percentage of $CO_2$ was set at 30% to represent MAP packaging. Fitting a regression line through the temperature – growth rate values obtained ($R^2 = 0.9992$), resulted in an equation that was used to calculate $\mu_{max}$ according to the temperature (Table 2).

To represent storage in the meat processing plant, transport and retail, the temperature ($Temp_{rg}$) and time ($Time_{rg}$) was set at 4 °C and 24 h, respectively. To represent storage at consumer level, the temperature ($Temp_{cg}$) was based on data from the Belgian Food Consumption Survey of 2004, in which the temperature of home refrigerators was determined (Devriese et al., 2006), resulting in a Pert distribution defined by the quartiles, 5, 7 and 9 °C. Pathogenic *Y. enterocolitica* were considered not to grow below 0 °C.The time during which minced meat was stored ($Time_{cg}$) was based on results of Swedish consumers (Marklinder et al., 2004), resulting in a Pert distribution with most likely one day, a minimum of zero and maximum of four days. The final number of pathogenic *Y. enterocolitica* in 0.5-kg minced meat packages just before consumption/preparation was calculated as

$$N_{cg} = N_{mp} \times 10^{(\mu_{max,cg} \times Time_{cg} + \mu_{max,rg} \times Time_{rg})}$$

The endpoint estimates were the proportion of 0.5-kg minced meat packages that contained ≥1 pathogenic *Y. enterocolitica* and the proportion of packages that contained ≥1000 pathogenic *Y. enterocolitica* per 0.5-kg minced meat package.

### 2.3. Alternative scenarios

Alternative scenarios of the model were run and compared to the baseline model. Some of these alternative scenarios represent realistic modifications of processing, which can for example be implemented as interventions (2.3.1–2.3.3). Other alternative scenarios are evaluated in an uncertainty analysis, to study the uncertainty attending parameter values and model assumptions (2.3.4; as e.g. in Nauta et al. (2007)). An overview of the different parameters that were modified to evaluate alternative scenarios is shown in Tables 3 to 6.

#### 2.3.1. Initial contamination, chilling and decontamination procedures of carcasses

Alternative scenarios for initial carcass contamination were analysed using a prevalence ($P_{initial}$) of 7.5% and 37.5% and concentrations ($C_{initial}$) that had a mean concentration of 0.5 $\log_{10}$ lower or higher than in the baseline model, to represent the 'best' and 'worst' slaughterhouses regarding pathogenic *Y. enterocolitica* contamination, respectively (Van Damme et al., 2015). Six different scenarios were evaluated: a lower prevalence (7.5%) but baseline concentrations (scenario A1); a lower concentration but baseline prevalence (scenario A2); a lower prevalence and a lower concentration (scenario A3); a higher prevalence but baseline concentrations (scenario A4); a higher concentration but baseline prevalence (scenario A5); and a higher prevalence and higher concentration (scenario A6) (see Table 3).

To simulate a slaughterhouse that only applied conventional air chilling (no prior blast chilling; scenario A7), a 0.1 $\log_{10}$ reduction during chilling was assumed ($I_{cc}$), which is based on the mean reduction of *Y. enterocolitica* after chilling of pig organs to a an internal temperature of 4 °C (King et al., 2012). The use of steam condensation was evaluated based on the reductions observed by Smulders et al. (2012) when applying steam of 65 °C for 18 s on pork skin, and was followed by a reduction to simulate either conventional chilling (scenario A8) or blast chilling (scenario A9).

The effect of applying lactic acid treatment (2% for 10 s at 40–50 °C), combined with blast chilling or conventional air chilling, was simulated using a reduction of 0.7 and 1.6, respectively (King et al., 2012) (scenario A10 and A11). The reduced growth during carcass cold storage after lactic acid treatment was simulated using a lag phase ($\lambda_{ccg}$) of 48 h and doubling time ($D_{ccg}$) of 12.4 h based on results of van Netten et al. (1997), after applying 2% lactic acid (at 37 °C for 120s) on pork skin.

The cold storage time of carcasses ($Time_{ccg}$) was set at either 68 h or 20 h to represent the production of minced meat on Monday (from carcasses slaughtered on Friday; scenario A12) or minced meat produced on Tuesday-Friday (from carcasses

**Table 3**
Overview of variables and parameters to evaluate alternative scenarios at slaughterhouse level.

| Code | Description of the scenario | Variable | Alternative value/distribution/model | Source |
|---|---|---|---|---|
| A1 | Lower initial prevalence on carcasses | $P_{initial}$ | 7.5 | Van Damme et al. (2015) |
| A2 | Lower initial concentration on carcasses | $C_{initial}$ | ~ Normal(−3.065; 0.736) truncated at a minimum value of −1.85 | Based on data from Van Damme et al. (2015) |
| A3 | Lower initial prevalence and concentration on carcasses | $P_{initial}$ | 7.5 | Van Damme et al. (2015) |
| | | $C_{initial}$ | ~ Normal(−3.065; 0.736) truncated at a minimum value of −1.85 | Based on data from Van Damme et al. (2015) |
| A4 | Higher initial prevalence on carcasses | $P_{initial}$ | 37.5 | Van Damme et al. (2015) |
| A5 | Higher initial concentration on carcasses | $C_{initial}$ | ~ Normal(−2.065; 0.736) truncated at a minimum value of −1.85 | Based on data from Van Damme et al. (2015) |
| A6 | Higher initial prevalence and concentration on carcasses | $P_{initial}$ | 37.5 | Van Damme et al. (2015) |
| | | $C_{initial}$ | ~ Normal(−2.065; 0.736) truncated at a minimum value of −1.85 | Based on data from Van Damme et al. (2015) |
| A7 | Only conventional air chilling (no blast chilling) | $I_{cc}$ | −0.1 | King et al. (2012) |
| A8 | Steam condensation followed by conventional chilling | $I_{cc}$ | ~ -Pert(0.7, 2.2, 4) −0.1 | Smulders et al. (2012) and King et al. (2012) |
| A9 | Steam condensation followed by blast chilling and conventional chilling | $I_{cc}$ | ~ -Pert(0.7, 2.2, 4) −0.6 | Smulders et al. (2012) and King et al. (2012) |
| A10 | Lactic acid treatment followed by conventional chilling and cold storage | $I_{cc}$ | −0.7 | King et al. (2012) |
| | | $\lambda_{ccg}$ | 48 | van Netten et al. (1997) |
| | | $D_{ccg}$ | 12.4 | van Netten et al. (1997) |
| A11 | Lactic acid treatment followed by blast chilling and conventional chilling and cold storage | $I_{cc}$ | −1.6 | King et al. (2012) |
| | | $\lambda_{ccg}$ | 48 | van Netten et al. (1997) |
| | | $D_{ccg}$ | 12.4 | van Netten et al. (1997) |
| A12 | Minced meat produced using carcasses stored over weekend | $Time_{ccg}$ | 68 h | Company information |
| A13 | Minced meat produced using carcasses the day after slaughter | $Time_{ccg}$ | 20 h | Company information |

**Table 4**
Overview of variables and parameters to evaluate alternative scenarios during grinding.

| Scenario | Variable | Description | Alternative value/distribution/model | Source |
|---|---|---|---|---|
| B1-B3: Addition of head meat | $P_{initial,m}$[a] | Prevalence of *Y. enterocolitica* on pig carcasses (mandibular region) after evisceration | 28.89% | Van Damme et al. (2015) |
| | $C_{initial,m}$[a] | Concentration of *Y. enterocolitica* on pig carcasses (mandibular region) after evisceration (positive carcasses only) | ~ Normal (−0.578; 1.256) truncated at a minimum of 0.15 (in $\log_{10}$ CFU/100 $cm^2$) | Based on data from Van Damme et al. (2015) |
| | $C_{mci}$[a] | Concentration on pig carcasses (mandibula) after inactivation during chilling | $=C_{initial,m} + I_{cc}$ (in log10 CFU/100 $cm^2$) | Calculation |
| | $N_{mcg}$[a] | Number of *Y. enterocolitica* after growth during cold storage | $= 10^{C_{mci}} \times 2^{(Time_{ccg}-\lambda_{ccg})/D_{ccg}}$ (in CFU/100 $cm^2$) | Calculation |
| | % headmeat[a] | % of head meat in a batch of minced meat (w:w) | 1% (B1), 10% (B2) or 50% (B3) | Assumption |
| | $W_{hm}$[a] | Weight of a piece of head meat | 0.075 kg | Company information |
| | $n_{hb}$[a] | Number of head meat cuts per batch | $n_{hb} = \frac{W_b \times \%headmeat}{W_{hm}}$ | Calculation |
| | $n_{phb}$[a] | Number of positive head meat cuts per batch | ~ Binomial ($n_{hb}$, $P_{initial,m}$) | Assumption |
| | $N_{mb}$ | Number of *Y. enterocolitica* in one minced meat batch | $N_{mb} = \sum_{i=1}^{n_{pbb}} N_{bdr,i} + \sum_{i=1}^{n_{phb}} N_{mb,i}$ (in CFU) | Calculation |
| B4-B6: Addition of tonsillar tissue | $P_{initial,t}$[a] | Prevalence of *Y. enterocolitica* in pig tonsils at time of evisceration | 44.33% | Van Damme et al. (2015) |
| | $C_{initial,t}$[a] | Concentration of *Y. enterocolitica* in pig tonsils at time of evisceration | Pert(1.00; 4.00; 5.91) in $\log_{10}$ CFU/g | Based on data from Van Damme et al. (2015) |
| | $C_{tci}$[a] | Concentration during chilling (after inactivation) | $=C_{initial,m} + I_{cc}$ (in $\log_{10}$ CFU/g) | Calculation |
| | $N_{tcg}$[a] | Number of *Y. enterocolitica* after growth during cold storage | $N_{tci} \times 2^{(Time_{ccg}-\lambda_{ccg})/D_{ccg}}$ (in CFU/g) | Calculation |
| | $n_{tb}$[a] | Number of tonsil pieces per batch | 1 (B4 and B5) or 10 (B6) | Scenarios |
| | $W_t$[a] | Weight of a tonsil piece | 1 g (B4 and B6) or 10 g (B5) | Scenarios |
| | $n_{ptb}$[a] | Number of positive tonsil pieces per batch | ~ Binomial ($n_{tb}$, $P_{initial,t}$) | Calculation |
| | $N_{mb}$ | Number of *Y. enterocolitica* in one minced meat batch | $N_{mb} = \sum_{i=1}^{n_{pbb}} N_{bdr,i} + W_t \sum_{i=1}^{n_{ptb}} N_{tcg,i}$ (in CFU) | Calculation |
| B7: Smaller batch of minced meat | $W_b$ | Weight of a batch of minced meat | 140 kg | Company information |
| B8: Larger batch of minced meat | $W_b$ | Weight of a batch of minced meat | 1500 kg | Assumption |

[a] New variable.

**Table 5**
Overview of variables and parameters to evaluate alternative scenarios at consumer level.

| Code | Description | Parameter | Value | Source |
|---|---|---|---|---|
| C1-4 | Consumer storage temperature of 4 °C, 7 °C, 10 °C or 15 °C | $Temp_{cg}$ | 4 °C (C1), 7 °C (C2), 10 °C (C3) or 15 °C (C4) | Scenarios |
| C5-9 | Consumer storage for 0, 1, 2, 3 or 4 days | $Time_{cg}$ | 0 days (C5), 1 day (C6), 2 days (C7), 3 days (C8) or 4 days (C9) | Scenarios |
| C1-9 at ambient atmosphere | Storage at ambient atmosphere | $\mu_{max,rg}$ | $0.0004 \times Temp_{rg}^2 + 0.0012 \times Temp_{rg} + 0.0174$ (in $\log_{10}$ CFU/h) | ComBase |
| | | $\mu_{max,cg}$ | $0.0004 \times Temp_{cg}^2 + 0.0012 \times Temp_{cg} + 0.0174$ (in $\log_{10}$ CFU/h) | ComBase |
| C10 | Consumer storage until the use-by date | $Time_{cg}$ | 7 days | Company info |
| C11 | Purchase and consumption at use-by-date | $Time_{rg}$ | 9 days | Company info |
| | | $Time_{cg}$ | 0 days | Assumption |

slaughtered on Monday-Thursday; scenario A13).

### 2.3.2. Addition of head meat and tonsillar tissue during grinding and batch size effect

The effect of the inclusion of head meat for the production of minced meat was simulated at different levels (1%, 10%, and 50% w:w; scenarios B1, B2, and B3, respectively; Table 4). As input data, prevalence and count data of human pathogenic *Y. enterocolitica* on the mandibular region of carcasses before chilling were obtained from Van Damme et al. (2015). A distribution was fitted through the censored count data (see 2.2.1), resulting in a lognormal distribution for $C_{initial,m}$ with a mean of −0.578 and standard deviation of 1.26 $\log_{10}$ CFU/100 $cm^2$. The distribution was truncated at 0.15 $\log_{10}$ CFU/100 $cm^2$ (the lower limit of the most sensitive isolation method), yielding a new distribution with a mean of 0.93 $\log_{10}$ CFU/100 $cm^2$ and standard deviation of 0.64. All pathogenic *Y. enterocolitica* on one head meat cut were assumed to originate from the carcass at the surface (100 $cm^2$) of the mandibular region. The same steps during the chilling and cold storage of carcasses were applied as for the sternal region. Carcasses containing less than 0 $\log_{10}$ CFU/100 $cm^2$ after blast chilling ($C_{mci}$) were considered negative. The number of pathogenic *Y. enterocolitica* positive head meat cuts per batch ($n_{phb}$) was calculated similar to the pork bellies, assuming a weight of an individual cheek of 75 g ($W_{hm}$), and a prevalence of 28.9% ($P_{initial,m}$). The number of cfu per head meat cut was simulated for each positive cut separately, starting each time from $C_{initial,m}$. The numbers of pathogenic *Y. enterocolitica* on positive head meat cuts were added to the numbers on pork bellies to determine the total number of pathogenic *Y. enterocolitica* per batch of minced meat ($N_{mb}$).

The addition of tonsillar tissue (scenarios B4-B6) was simulated using a prevalence ($P_{initial,t}$) of pathogenic *Y. enterocolitica* in pig tonsils during slaughter of 44.3% and an initial concentration ($C_{initial,t}$) with a minimum of 1.00 $\log_{10}$ CFU/g, most likely of 4.00 $\log_{10}$ CFU/g and a maximum of 5.91 $\log_{10}$ CFU/g (Van Damme et al., 2015). Inactivation and growth during carcass chilling and cold storage was included as described before. Numbers were modelled for each individual positive tonsil and were added to the total

**Table 6**
Overview of the variables and parameters to evaluate uncertainty.

| Code | Variable | Alternative value/distribution | Source |
|---|---|---|---|
| U1 | $C_{initial}$ | ~ Normal(−2.065; 0.736) (in $\log_{10}$ CFU/cm$^2$) | Assumption |
| U2 | $C_{initial}$ | ~ Normal(−3.065; 0.736) (in $\log_{10}$ CFU/cm$^2$) | Assumption |
| U3 | $C_{initial}$ | ~ Normal(−2.565; 1.236) (in $\log_{10}$ CFU/cm$^2$) | Assumption |
| U4 | $C_{initial}$ | ~ Normal(−2.565; 0.236) (in $\log_{10}$ CFU/cm$^2$) | Assumption |
| U5 | $P_{initial}$ | 23.1% | Van Damme et al. (2015) |
| U6 | $P_{initial}$ | 13.3% | Van Damme et al. (2015) |
| U7 | $I_{cc}$ | −0.03 $\log_{10}$ reduction | El-Zawahry and Grecz (1981) |
| U8 | $I_{cc}$ | −0.8 $\log_{10}$ reduction | King et al. (2012) |
| U9 | $L_{ccg}$ | 77 h | Greer and Dilts (1995) |
| U10 | $L_{ccg}$ | 0 h | Greer and Dilts (1995) |
| | $D_{ccg}$ | 10.36 h | ComBase |
| U11 | $R_{bd}$ | 25% | Assumption |
| U12 | $R_{bd}$ | 75% | Assumption |
| U13 | $W_b$ | 850 kg | Assumption |
| U14 | $W_b$ | 950 kg | Assumption |
| U15 | %bellies | 29% | Assumption |
| U16 | %bellies | 39% | Assumption |
| U17 | $W_{bdr}$ | 7 kg | Assumption |
| U18 | $W_{bdr}$ | 8 kg | Assumption |
| U19 | $Temp_{rg}$ | 2 °C | Assumption |
| U20 | $Temp_{rg}$ | 6 °C | Assumption |
| U21 | $Timer_{rg}$ | 1d | Assumption |
| U22 | $Time_{rg}$ | 3d | Assumption |
| U23 | $\mu_{max,rg}$ | $\frac{0.0003 \times Temp_{rg}^2 + 0.0005 \times Temp_{rg} + 0.0103}{2}$ | Assumption |
| | $\mu_{max,cg}$ | $\frac{0.0003 \times Temp_{cg}^2 + 0.0005 \times Temp_{cg} + 0.0103}{2}$ | Assumption |

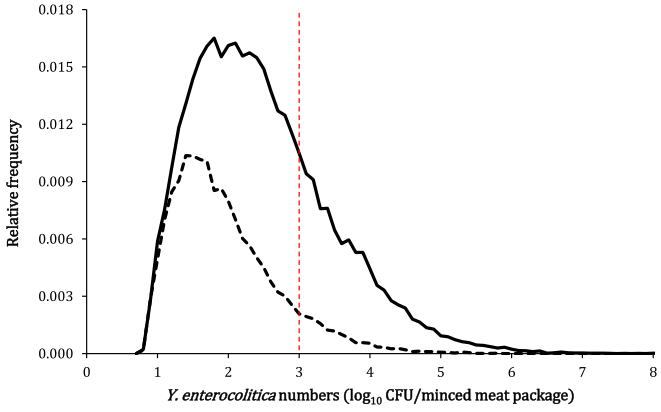


**Fig. 2. Distributions of concentrations of *Y. enterocolitica* in 0.5-kg minced meat packages after storage at consumer level (based on 100 000 iterations) using (1) the baseline scenario that only assumed pork bellies as a source of contamination (dashed line) and (2) the alternative scenario in which 1 g of tonsillar tissue is added to a 900-kg minced batch (solid line)**. Concentrations of *Y. enterocolitica* are given for contaminated packages only; the areas under the curves reflect the prevalence of 15.4% in the baseline scenario and 37.9% in the alternative scenario.

number of pathogenic *Y. enterocolitica* from pork bellies to calculate the total number of pathogenic *Y. enterocolitica* per batch of minced meat ($N_{mb}$). As alternative scenarios, we evaluated the addition of one piece of tonsillar tissue of 1 g (scenario B4), one piece of tonsillar tissue of 10 g (scenario B5), and 10 pieces of tonsillar tissue (of 10 different pigs) of 1 g each (scenario B6).

Besides a batch weight of 900 kg in the baseline scenario, the effect of smaller and larger minced meat batches were simulated by changing $W_b$ to 140 kg and 1500 kg in the alternative scenarios B7 and B8, respectively.

### 2.3.3. Consumer storage practices

Alternative scenarios for consumer storage (C1-C9) were evaluated by replacing $Temp_{cg}$ or $Time_{cg}$ by different fixed values (4 °C, 7 °C, 10 °C and 15 °C for $Temp_{cg}$ and 0, 1, 2, 3, and 4 days for $Time_{cg}$). The effect of consumer storage scenarios was evaluated for both MAP packaging and storage under ambient atmosphere. Storage under ambient atmosphere was simulated by changing the formulas for $\mu_{max}$ both at retail and consumer level (Table 5). The formula was created using ComBase data as described before, but omitting the parameter "$CO_2$".

For simulation of MAP packages that are consumed at the use-by date (scenarios C10 and C11), a shelf-life of 9 days was assumed based on company information. Storage of minced meat at consumer level until the use-by date (scenario C10) was simulated setting the storage time at consumer level at 7 days. For simulation of MAP packages that are sold and consumed/prepared at the use-by date (scenario C11), the storage time at retail ($Time_{rg}$) was set at 9 days and storage time at consumer level ($Time_{cg}$) was set at 0 days.

### 2.3.4. Uncertainty analysis

Uncertainty analyses were performed by estimating the prevalence and proportion of packages containing more than 3 $\log_{10}$ CFU by changing one parameter value in the model to a value that represents the low or high end of the uncertainty interval around the value chosen in the baseline model. The parameter values that were changed are shown in Table 6.

The uncertainty regarding the initial concentration on carcasses ($C_{initial}$) was evaluated by changing the mean or standard deviation with+/-0.5 $\log_{10}$ (U1-U4). For the prevalence ($P_{initial}$), the upper (U5) and lower limit (U6) of the 95% confidence interval for the prevalence at the sternal region were used (Van Damme et al., 2015). A different value for the reduction during blast chilling ($I_{cc}$) was based on the 7% cell inactivation that was observed by El-Zawahry and Grecz (1981) when freezing pathogenic *Y. enterocolitica* in broth at −18 °C for 1 h (U7). A larger reduction during blast chilling (U8) was simulated using the −0.8 log reduction of *Y. enterocolitica* that was observed by King et al. (2012) when applying a water wash before freezing pig organs. Scenario U9 assumed no growth of pathogenic *Y. enterocolitica* during carcass cold storage, which was based on the results of Greer and Dilts (1995), who found no growth of pathogenic *Y. enterocolitica*

O:4,32 during storage at 4 °C for over ten days after artificial inoculation of lean pork tissue. As Greer and Dilts (1995) observed immediate growth of *Y. enterocolitica* O:4,32 on pork fat at 4 °C, a lag phase of 0 h was assumed in scenario U10. The doubling time in scenario U10 was based on ComBase results assuming a temperature of 4 °C, pH of 6.5 (Greer and Dilts, 1995), and $A_w$ of 0.990 (van Netten et al., 1997). The percentage of pathogenic *Y. enterocolitica* that remain on a belly cut after derinding was set at 25% and 75% to represent less and more removal during cutting and removal (U11 and U12). The lower and upper limits of the uncertainty about the weight of a batch of minced meat ($W_b$), the proportion of bellies that is used (%bellies), the weight of a belly cut ($W_{bdr}$), the temperature ($Temp_{rg}$) and the time during storage at retail ($Time_{rg}$) were considered reasonable by the authors (U13-U22). The uncertainty regarding the growth of pathogenic *Y. enterocolitica* in minced meat was studied by reducing the maximum growth rate by half (U23).

## 3. Results

Using the baseline scenario, the prevalence of pathogenic *Y. enterocolitica* in 0.5-kg minced meat packages was estimated at 15.4% (≥1 CFU/package). Only a small percentage of packages (1.4%, i.e. 9.2% of the contaminated packages) contained more than $10^3$ pathogenic *Y. enterocolitica* at the end of storage. The distribution of pathogenic *Y. enterocolitica* in positive minced meat packages at the end of storage (just before consumption or preparation) in the baseline scenario is shown in Fig. 2.

### 3.1. Initial contamination of carcasses before chilling

The effect of initial carcass contamination on pathogenic *Y. enterocolitica* contaminated minced meat packages was evaluated varying the initial prevalence and concentration of pathogenic *Y. enterocolitica* on carcasses ($P_{initial}$ and $C_{initial}$) to represent minced meat that is produced using carcasses from slaughterhouses with either low or high contamination with pathogenic *Y. enterocolitica*. Lowering the prevalence of pathogenic *Y. enterocolitica* on carcasses from 16.39% to 7.5% reduced the proportion of highly contaminated meat packages by half (Fig. 3). A similar reduction was seen when the average initial concentration on pork carcasses is reduced by 0.5 $\log_{10}$ CFU/cm$^2$. The combined effect of reducing the prevalence and the concentration resulted in the highest effect, with a more than 5-fold decrease in the number of highly contaminated packages before consumption. A similar but opposite effect was seen for a higher prevalence and/or higher concentration (Fig. 3).

### 3.2. Effect of decontamination

The results of different scenarios to evaluate the effect of decontamination methods for carcasses at slaughterhouse level are shown in Fig. 4. The use of solely conventional chilling resulted in twice as many pathogenic *Y. enterocolitica* contaminated minced meat packages compared to when it's combined with blast chilling, during which the carcass surface is frozen. Steam condensation had a larger effect on the final outcome estimates as it would reduce the number of contaminated and highly contaminated pathogenic *Y. enterocolitica* packages 95 to 158 times. The use of 2% lactic acid sprays would also reduce the proportion of pathogenic *Y. enterocolitica* contaminated minced meat packages, resulting in a larger effect in combination with blast chilling than with conventional air chilling. Using carcasses that are chilled for 68 h resulted in more than 10 times as many pathogenic *Y. enterocolitica* contaminated 0.5-kg minced packages compared to minced meat that is produced using 20 h-chilled carcasses (Fig. 4).

### 3.3. Addition of head meat and tonsillar tissue

The additional use of 1%–50% head meat for the production of minced meat increased the proportion of pathogenic *Y. enterocolitica* positive minced meat packages 2 to 6 times compared to the baseline scenario that only assumed pork bellies as a source of pathogenic *Y. enterocolitica* contamination (Fig. 5). The impact of adding head meat was larger for highly contaminated packages than for the prevalence of pathogenic *Y. enterocolitica* positive minced meat packages. The use of 10% head meat in minced meat resulted in almost 20 times as many highly contaminated minced meat packages at time of consumption (Fig. 5).

The addition of 1 g tonsillar tissue to a 900-kg minced meat batch resulted in a 7-fold increase of the number of minced meat packages containing >3 log pathogenic *Y. enterocolitica* at time of consumption (Figs. 5 and 2). The addition of one tonsil of 10 g resulted in a similar but slightly higher increase. The addition of 1-g tonsil pieces of 10 different pigs resulted in over 35 times as many highly contaminated minced meat packages at time of consumption (Fig. 5).

Changing the batch size ($W_b$) from 900 kg to 140 kg or 1500 kg had very little effect on the endpoint estimates (data not shown).

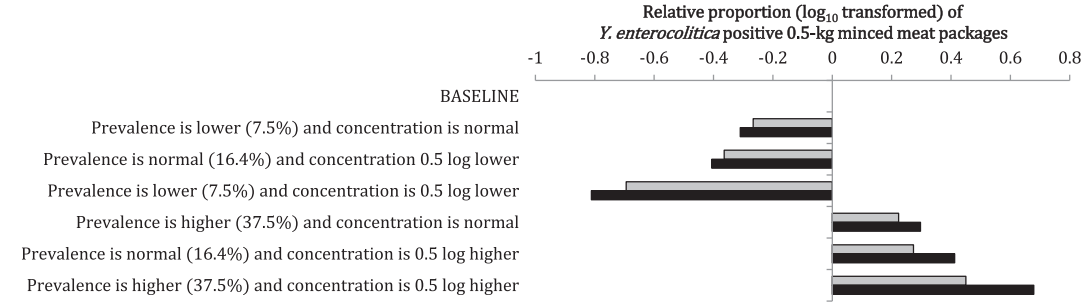


**Fig. 3. Effect of initial pig carcass contamination in slaughterhouses on *Y. enterocolitica* contamination of minced meat packages just before consumption.** The proportion of *Y. enterocolitica* positive minced meat packages of the alternative scenarios are expressed relative to the proportion of minced meat packages in the baseline model. Relative proportions are log transformed, so the baseline gets a value zero, and −1 and 1 represent a tenfold reduction and increase of the proportion, respectively. The baseline model used a prevalence ($P_{initial}$) of 16.4% and a mean concentration ($C_{initial}$) of −2.565 $\log_{10}$ *Y. enterocolitica*/cm$^2$. Alternative scenarios were simulated using a lower/higher prevalence ($P_{initial}$ of 7.5% or 37.5%, respectively) and/or a lower/higher concentration (mean $C_{initial}$ of 0.5 $\log_{10}$ lower or higher compared to the baseline value, respectively). The grey bars represent the outcome of the proportion of *Y. enterocolitica* positive 0.5-kg minced meat packages. The black bars represent the results for 0.5-kg minced meat packages that contain more than 3 $\log_{10}$ *Y. enterocolitica* at time of consumption or preparation.

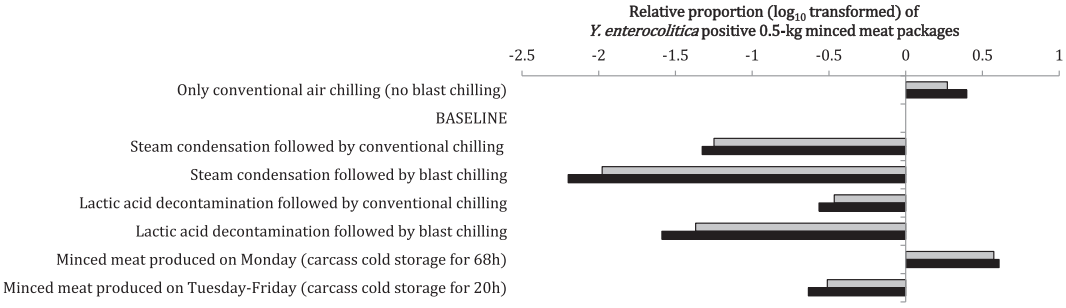


**Fig. 4. Effect of cooling and carcass decontamination steps on *Y. enterocolitica* contaminated minced meat packages**. The proportion of *Y. enterocolitica* positive minced meat packages of the alternative scenarios are expressed relative to the proportion of minced meat packages in the baseline model. The baseline model assumed a 0.6 log reduction of *Y. enterocolitica* during blast chilling. The storage time of carcasses in the baseline model was 20 h (for carcasses of pigs slaughtered on Monday-Thursday) or 68 h (for carcasses of pigs slaughtered on Friday). The grey bars represent the outcome of the proportion of *Y. enterocolitica* positive 0.5-kg minced meat packages. The black bars represent the proportion of 0.5-kg minced meat packages that contain more than 3 $\log_{10}$ *Y. enterocolitica* at time of consumption or preparation.

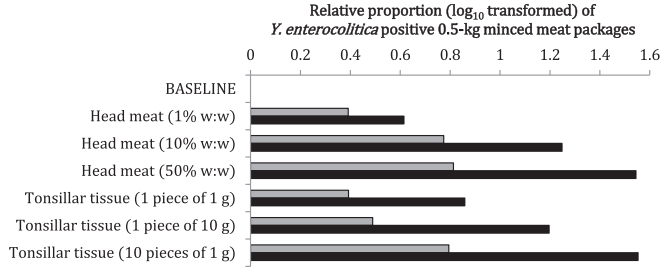


**Fig. 5. Evaluation of the addition of head meat and tonsillar tissue to a 900-kg batch of minced meat on *Y. enterocolitica* contaminated minced meat packages just before consumption**. The proportion of *Y. enterocolitica* positive minced meat packages of the alternative scenarios are expressed relative to the proportion of minced meat packages in the baseline model. The baseline model only assumed pork bellies as a source of *Y. enterocolitica* contamination. The grey bars represent the outcome of the proportion of *Y. enterocolitica* positive 0.5-kg minced meat packages. The black bars represent the proportion of 0.5-kg minced meat packages that contain more than 3 $\log_{10}$ *Y. enterocolitica* at time of consumption or preparation.

### 3.4. Consumer storage

When storage of minced meat at consumer level would always be at 4 °C, the proportion of highly contaminated packages would be reduced with more than a 1000-fold compared to the baseline scenario (Fig. 6). If minced meat would always be consumed or prepared within one day after purchase, a reduction of the endpoint estimate was observed, whereas a constant storage time of two or more days increased the proportion of highly contaminated packages compared to the baseline scenario. For each of the scenarios, storage at ambient atmosphere resulted in a higher proportion of highly contaminated packages than storage in MAP (Fig. 6). Storage of minced meat until the use-by date was simulated using a storage time at consumer level of 7 days or storage at retail for 9 days (to simulate purchase and consumption at the end of shelf life). Both scenarios estimated that nearly all pathogenic *Y. enterocolitica* positive packages after packaging (15%) would contain $>10^3$ pathogenic *Y. enterocolitica* at the end of the 9-day storage period. The endpoint estimate was higher when packages were stored until the use-by date in MAP, as compared to storage at ambient atmosphere for two days or less (Fig. 6).

### 3.5. Uncertainty

The results for the uncertainty analyses are shown in Fig. 7. A reduced growth rate during storage at retail and consumer level had the highest impact on the proportion of highly contaminated minced meat packages. Uncertainty regarding the standard deviation of pathogenic *Y. enterocolitica* numbers on carcasses before chilling ($C_{initial}$), reduction during blast chilling, and growth during carcass cold storage had a large effect on both endpoint estimates. For all variables that were evaluated, the uncertainty had a larger effect on the proportion of highly contaminated packages than on the prevalence of pathogenic *Y. enterocolitica* in minced meat packages. The uncertainty during minced meat production regarding the exact weight of a minced meat batch, the proportion of bellies and the weight of a pork belly had only a minor effect on the endpoint estimates.

## 4. Discussion

### 4.1. Modelling approach

The consumption of raw minced pork has been shown to be the main risk factor for yersiniosis infections in Germany (Rosner et al., 2012) and the knowledge of consumers regarding the correct handling of raw minced meat seems to be limited (Bremer et al., 2005). Therefore, the effect of different control measures during the production of minced meat on pathogenic *Y. enterocolitica* contaminated and highly contaminated minced meat packages were evaluated in this study. The modelling approach used was based on the Modular Process Risk Model approach (Nauta, 2008) that has frequently been applied to model the transmission of microbial pathogens through food chains for quantitative microbiological risk assessment (e.g. Nauta et al., 2007; Daelman et al., 2013; Møller et al., 2015). A full risk assessment, ending at an estimation of the risk of illness, was not feasible as only few reports are available estimating the numbers of *Yersinia* spp. in food products that are related to yersiniosis cases (Pärn et al., 2015; Todd et al., 2008) and, to our knowledge, no dose-response model is available for pathogenic *Y. enterocolitica*. Moreover, due to a lack on consumption data of raw minced pork and uncertainty about preparation styles, it was decided to end the analysis at the end of storage, just before consumption of raw minced pork or preparation. Using a similar approach as Nauta et al. (2003), and acknowledging that all microbial dose response models show an increasing probability of illness with an increasing dose, it was assumed that every contaminated package may pose a health risk and that the risk of yersiniosis is higher for highly contaminated packages. The choice of the critical level $10^3$ was arbitrary, balancing the need for a high level with the need for a level that occurs regularly, as to get robust results with a feasible number of model iterations. When comparing two scenarios, it is assumed that the relative proportion of highly contaminated packages can be

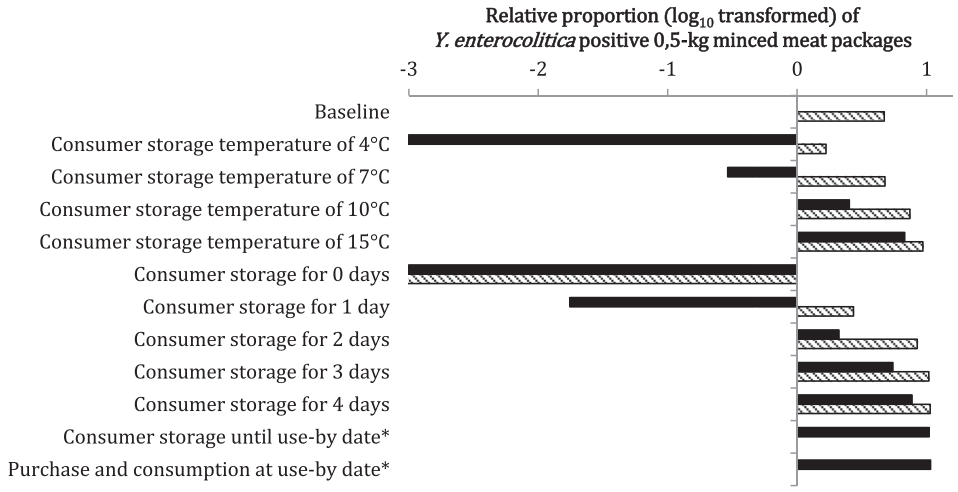


**Fig. 6. Evaluation of consumer practices on *Y. enterocolitica* contaminated minced meat packages just before consumption**. The proportion of highly contaminated (>3 $\log_{10}$) *Y. enterocolitica* 0.5-kg minced meat packages of the alternative scenarios are expressed relative to the proportion of highly contaminated 0.5-kg minced meat packages in the baseline model (= stored in modified atmosphere packages (MAP), 30% $CO_2$). The black bars represent minced meat packages stored in MAP. The bars with diagonal stripes represent storage at ambient atmosphere. * Storage until use-by date was only simulated for MAP minced meat.

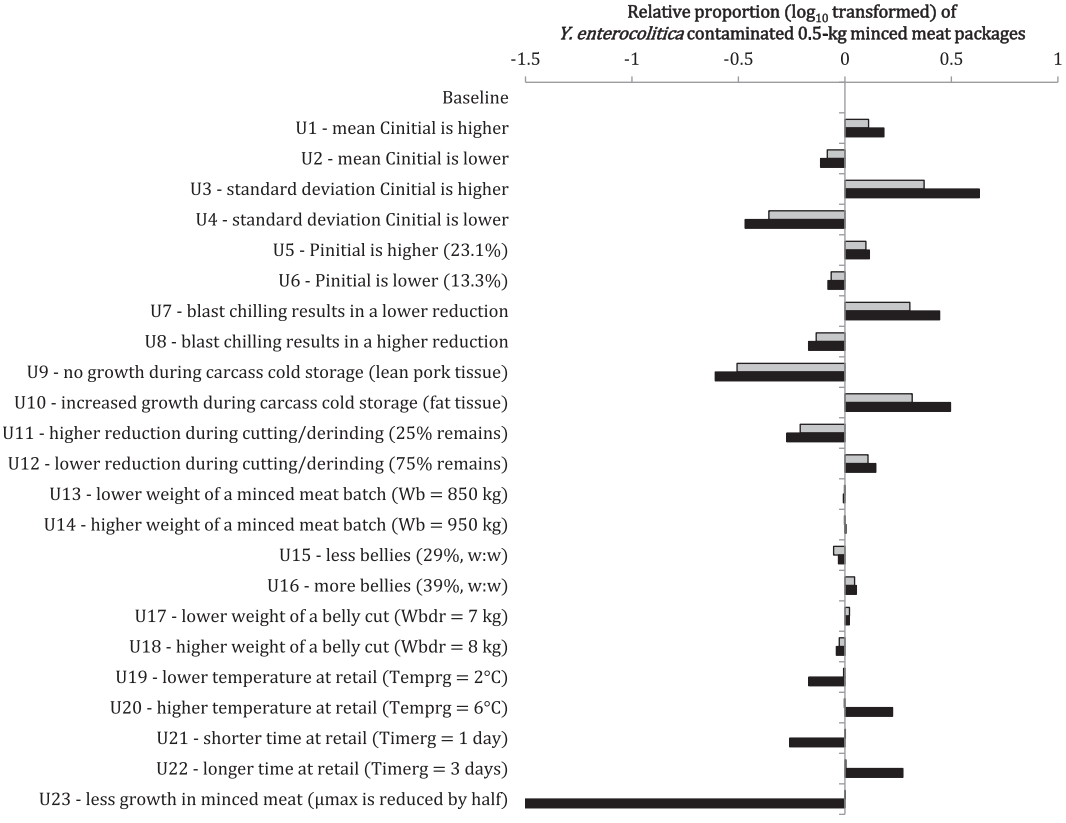


**Fig. 7. Results of the uncertainty analyses of the baseline model**. The grey bars represent the outcome of the proportion of *Y. enterocolitica* positive 0.5-kg minced meat packages. The black bars represent the proportion of 0.5-kg minced meat packages that contain more than 3 $\log_{10}$ *Y. enterocolitica* at time of consumption or preparation. The relative proportion for U23 (reduced growth in minced meat) was truncated at −1.5.

considered a reasonable surrogate for the relative risk as applied elsewhere (e.g. Møller et al., 2015).

### 4.2. Uncertainties of the model and relevant data gaps

The present model used pathogenic *Y. enterocolitica* numbers that are found on the sternal region of carcasses as input variables to represent contamination of the belly area, and assumed that pork bellies were the sole source of contamination of minced meat. Laukkanen-Ninios et al. (2014b) quantified plasmid-carrying *Yersinia* in meat cuts in Finland that were intended to be used in minced meat and found *Yersinia* in 39% of pork cuts, varying between 0.1 and 1.6 MPN/g (average 0.41 MPN/g) using nested PCR. Nevertheless, as pathogenic *Y. enterocolitica* were isolated from one

pork cut only (0.6%) (Laukkanen-Ninios et al., 2014b), the contamination level of meat cuts for the production of minced meat seems very low. Nevertheless, since contamination from shoulder cuts and cross contamination between belly cuts were not included in the present model, the contamination of meat cuts with pathogenic *Y. enterocolitica* before grinding is probably underestimated. Moreover, the uncertainty analysis showed that the standard deviation of the initial concentration on carcasses had a large effect on the final prevalence of contaminated packages and especially for the proportion of highly contaminated packages. This importance of the standard deviation of concentrations has been found previously (Duarte et al., 2016). Clearly, more accurate estimations on the numbers of pathogenic *Y. enterocolitica* on bellies and other pork cuts that are used for minced meat production, including the variation between carcasses and slaughterhouses, could improve the estimations of the model.

The level of growth and inactivation of *Y. enterocolitica* has been shown to differ according to the tissue. As such, Greer and Dilts (1995) observed immediate growth of pathogenic *Y. enterocolitica* at 4 °C after artificial inoculation of fat tissue whereas no growth was observed on lean tissue for several days after inoculation. The authors also found that pathogenic *Y. enterocolitica* on lean tissue were more resistant to lactic acid than those on fat tissue (Greer and Dilts, 1995). Moreover, larger reductions of *Y. enterocolitica* have been observed on pig skin compared to muscle tissue when evaluating steam-ultrasound decontamination (Morild et al., 2011) or water spraying followed by steam decontamination (Smulders et al., 2012). Nevertheless, the effect of lactic acid treatment has been shown to vary between studies. As such, van Netten et al. (1997) found a 4.7 log immediate death of *Y. enterocolitica* serotype O:3 on pork skin after dipping in 2% lactic acid at 37 °C for 120s. Such reductions would reduce the proportion of highly contaminated packages with more than a 1000-fold (data not shown), though this is likely an overestimation of the reduction as such conditions may not be accomplished under field conditions. Besides the immediate effect of lactic acid, the present model assumed a reduced growth of *Y. enterocolitica* during carcass cold storage after the application of 2% lactic acid, which are based on data using pork skin (van Netten et al., 1997). Nevertheless, Greer and Dilts (1995) observed a persistent reduction of *Y. enterocolitica* in the next seven days following a 3% lactic acid treatment of pig lean and fat tissue stored at 4 °C. Therefore, studies quantifying the immediate and long-term effect of lactic acid on carcasses under field conditions are necessary to improve the predictions for lactic acid decontamination. As the attachment, inactivation, and growth of pathogenic *Y. enterocolitica* may differ according to the surface type (Greer and Dilts, 1995; Morild et al., 2011), the inclusion of these differences would be a more realistic approach to model *Y. enterocolitica* on carcasses, but this would considerably increase the complexity of the model. Moreover, this would require comprehensive data on the distribution, growth and inactivation of the pathogens on each of the different tissues on carcasses, which are currently not available. Nevertheless, as the level of growth and inactivation of *Y. enterocolitica* during cold storage may have a large influence on the outcome variables, more accurate studies on the level of reduction of pathogenic *Y. enterocolitica* on carcasses under different chilling and cold storage conditions - including the biological and strain variation - should be performed to obtain more accurate endpoint estimates.

Data regarding the growth of pathogenic *Y. enterocolitica* on pork, and minced meat in particular, are limited. Therefore, the growth rate represented a large uncertainty in the present model. Kleinlein and Untermann (1990) observed growth of pathogenic *Y. enterocolitica* in minced beef stored in MAP (20% $CO_2$, 80% $O_2$), especially at temperatures of 10 °C or higher, whereas Strotmann et al. (2008) observed a reduction of *Y. enterocolitica* bioserotype 4/O:3 during storage at 2 °C, regardless of the $CO_2$ concentration. After 13 days of storage of pig cheeks at 6 °C in 30% $CO_2$ and 70% $O_2$, Fredriksson-Ahomaa et al. (2012) observed *Y. enterocolitica* bioserotype 4/O:3 in numbers varying between 2.3 and 5.4 log CFU/g. Due to the different factors affecting growth and the large impact it has on prevalence and concentrations found in packages after consumer storage, more studies are needed regarding the growth of the pathogen in minced meat at different temperatures, including the variation between strains and varying meat characteristics.

### 4.3. Interventions to control pathogenic Y. enterocolitica

The prevalence of pathogenic *Y. enterocolitica* on carcasses was set at 16.4% for the baseline model, though the proportion of carcasses that are pathogenic *Y. enterocolitica* positive at the sternal region have been shown to vary between slaughterhouses from 7.5 to 37.5% (Van Damme et al., 2015). Comparing minced meat that is produced from carcasses originating from “good” slaughterhouses (that produce carcasses with a low prevalence and low concentration) compared to “bad” slaughterhouses (that produce carcasses with a high prevalence and a high concentration), results in a more than 30-fold increase in the proportion of highly contaminated *Y. enterocolitica* minced meat packages. This finding demonstrates the utility of risk differentiation of slaughterhouses (EFSA, 2011) to control pathogenic *Y. enterocolitica* transmission via minced meat. As the combined effect of reducing the prevalence and concentration of pathogenic *Y. enterocolitica* on carcasses resulted in the greatest reduction of highly contaminated minced meat packages, measures to decrease both the number of positive carcasses and the concentration of pathogenic *Y. enterocolitica* on carcasses would result in the largest benefit. Many different physical and chemical decontamination treatments have been described to reduce bacterial contamination on pig carcasses (Loretz et al., 2011). Besides the effect of (blast) chilling as the most conventional way to reduce bacterial contamination on carcasses, the effect of steam decontamination and lactic acid decontamination were simulated to represent commonly used physical and chemical decontamination procedures of pig carcasses. Although blast chilling before conventional chilling has been shown to result in a larger reduction than conventional air chilling alone for different pathogens (Loretz et al., 2011), blast chilling has been shown not to reduce pathogenic *Y. enterocolitica* recovery from carcasses (Nesbakken et al., 2008). The effect of blast chilling on the outcome estimate also seemed rather limited in the present model. The use of decontamination procedures on carcasses before chilling was estimated to result in higher reductions of the proportion of highly contaminated minced meat packages, and would thus likely reduce the public health risk.

The baseline model assumed pork bellies as the only source of pathogenic *Y. enterocolitica* contamination during the production of minced meat. Meat cuts originating from other parts of the carcass may be contaminated in higher levels and numbers, which would increase the numbers of pathogenic *Y. enterocolitica* in a minced meat batch and the resulting minced meat packages. Pork cheeks and tongues have been shown to be highly contaminated with pathogenic *Y. enterocolitica* (Laukkanen-Ninios et al., 2014b; Messelhausser et al., 2011). As such, the addition of different levels of head meat for the production of minced meat was simulated using qualitative and quantitative data from the mandibular region on pig carcasses before cooling as input data to represent meat from pork cheeks and the throat region. The use of head meat for the production of minced meat increased the proportion of pathogenic *Y. enterocolitica* positive minced meat packages with

increasing amounts of head meat and had a larger effect on highly contaminated minced meat packages. The addition of just 10% head meat in minced meat resulted in almost 20 as many highly contaminated minced meat packages at time of consumption. The addition of pork cheeks and other potentially highly contaminated meat cuts (such as throat meat) should thus be avoided for the production of minced meat that is potentially consumed raw.

Tonsils have been shown to be highly contaminated with human pathogenic *Yersinia* spp. (Bonardi et al., 2016; Van Damme et al., 2010). and represent an important risk for carcass contamination. Tonsils should be removed hygienically after *post mortem* inspection according to EU regulation (EC) No. 853/2004, though parts may remain in the head and result in contamination further down the pork production line (Fredriksson-Ahomaa et al., 2004). The addition of minimal amounts of tonsillar tissue in minced meat resulted in a large effect in the proportion of highly contaminated minced meat packages before consumption, so special care should be taken to remove all remaining tonsillar tissue from the carcass.

Minced meat produced on Monday resulted in a higher proportion of highly contaminated packages than minced meat produced on Tuesday to Friday. Industrially produced minced meat is usually made from carcasses that are slaughtered the previous day, though carcasses from pigs that are slaughtered on Friday are stored during the weekend for processing on Monday, resulting in a longer cold storage. After storage of pork bellies during 4 and 8 days at 4°C, van Netten et al. (1997) observed more than 1 and 4 $\log_{10}$ increase of cold and acid adapted *Y. enterocolitica* serotype O:3. Therefore, minced meat that is produced from carcasses that have been stored for several days may represent a larger risk for public health than freshly slaughtered pig carcasses. This implies that the shelf life for minced meat may be adapted depending on the cold storage time of carcasses to reduce the proportion of minced meat packages that are (highly) contaminated with pathogenic *Y. enterocolitica*.

Consumer practices were shown to have a large effect on the proportion of minced meat packages with high numbers of pathogenic *Y. enterocolitica* at time of consumption. When all consumers would store minced meat at 4 °C, a 1000-fold reduction in the number of highly contaminated packages could be expected. A similar reduction was seen if consumers would consume the minced meat at the day of purchase. Storage of minced meat in ambient atmosphere leads to higher maximum growth rates for *Y. enterocolitica* compared to packaging with 30% $CO_2$, resulting in higher estimates of highly contaminated packages at the end of storage. Nevertheless, the storage time at ambient atmosphere is presumably shorter compared to minced meat stored under MAP conditions due to the shorter shelf life (Strotmann et al., 2008). Limbo et al. (2010) calculated that the mean shelf life of MAP minced beef was 9 days at the recommended storage temperature of about 4 °C. The proportion of highly contaminated packages in the present study was higher when all MAP would be stored until the use-before date compared to the storage of packages at ambient atmosphere for two days or less. Although MAP is introduced to reduce bacterial growth and prolong shelf-life of products, the longer shelf-life could potentially increase the risk of yersiniosis due to the growth of pathogenic *Y. enterocolitica* during prolonged storage at refrigerated conditions.

## 5. Conclusions

Meat producers should focus on reducing the number of pathogenic *Y. enterocolitica* contaminated minced meat packages, which can be achieved by using meat cuts that are less contaminated with pathogenic *Y. enterocolitica*. As such, belly cuts should be preferred over head meat. Moreover, meat produced from carcasses of slaughterhouses with lower contamination results in less pathogenic *Y. enterocolitica* contaminated minced meat packages. Finally, it's important that the tonsils are completely removed in the slaughterhouse as the (accidental) addition of minimal amounts of tonsillar tissue has a large effect on the proportion of highly contaminated minced meat packages. Nevertheless, the number of packages that contain high numbers of pathogenic *Y. enterocolitica*, which are expected to cause the highest risk of yersiniosis, is primarily influenced by consumer storage practices. A reduced storage time (under one day) or a storage temperature (below 4 °C) would largely reduce the proportion of packages containing high numbers of pathogenic *Y. enterocolitica*.

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