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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.

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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 (λ_{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 λ_{ccg} was higher than $Time_{ccg}$, no growth was allowed, so N_{ccg}

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