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A risk modelling approach for setting microbiological limits using enterococci as indicator for growth potential of *Salmonella* in pork

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ABSTRACT

Microbiological limits are widely used in food processing as an aid to reduce the exposure to hazardous microorganisms for the consumers. However, in pork, the prevalence and concentrations of *Salmonella* are generally low and microbiological limits are not considered an efficient tool to support hygiene interventions. The objective of the present study was to develop an approach which could make it possible to define potential risk-based microbiological limits for an indicator, enterococci, in order to evaluate the risk from potential growth of *Salmonella*. A positive correlation between the concentration of enterococci and the prevalence and concentration of *Salmonella* was shown for 6640 pork samples taken at Danish cutting plants and retail butchers. The samples were collected in five different studies in 2001, 2002, 2010, 2011 and 2013. The observations that both *Salmonella* and enterococci are carried in the intestinal tract, contaminate pork by the same mechanisms and share similar growth characteristics (lag phase and maximum specific growth rate) at temperatures around 5–10 °C, suggest a potential of enterococci to be used as an indicator of potential growth of *Salmonella* in pork. Elevated temperatures during processing will lead to growth of both enterococci and, if present, also *Salmonella*. By combining the correlation between enterococci and *Salmonella* with risk modelling, it is possible to predict the risk of salmonellosis based on the level of enterococci. The risk model used for this purpose includes the dose–response relationship for *Salmonella* and a reduction factor to account for preparation of the fresh pork. By use of the risk model, it was estimated that the majority of salmonellosis cases, caused by the consumption of pork in Denmark, is caused by the small fraction of pork products that has enterococci concentrations above 5 log CFU/g. This illustrates that our approach can be used to evaluate the potential effect of different microbiological limits and therefore, the perspective of this novel approach is that it can be used for definition of a risk-based microbiological limit for enterococci. The limit for enterococci can then be used for development of a process hygiene criterion in cutting plants and retail butcher shops, with the purpose of reducing the risk of *Salmonella* for the consumer.

1. Introduction

Pork is known to be a major source of foodborne salmonellosis in many European countries (EFSA and ECDC, 2015; Hald et al., 2003). Pigs are often healthy carriers of *Salmonella* in the gastro-intestinal tract, including the oral cavity. Subclinically infected pigs shed the bacteria in faeces, which easily leads to contamination of the skin or oral uptake by other pigs during transport or slaughter (Berends et al., 1996, 1997; Hald et al., 2003).

In 1995, a surveillance-and-control program for *Salmonella* was initiated in Denmark with the aim of reducing the *Salmonella* prevalence in Danish pig herds and pork meat (Alban and Stärk, 2005). The program divides Danish pig herds into three levels according to their serological status regarding occurrence of *Salmonella*. With the aim of lowering the risk of salmonellosis for consumers, pigs from herds with the highest *Salmonella* prevalence, representing approximately 1% of the production, are subjected to decontamination by a hot water wash of 80 °C for 12–15 s (Alban and Sørensen, 2010). *Salmonella* has become widespread in Danish pig production with 24% of the Danish slaughter pigs and 1.3% of the carcasses being positive for *Salmonella* at slaughter in 2013 (Anonymous, 2014). This means, that only a minor part of the pigs contaminated with *Salmonella* is decontaminated by a hot water wash, which leaves a substantial proportion of the *Salmonella* input to the slaughterhouse to be continuously passed on into the pork processing chain.

The consumer exposure to *Salmonella* from pork will depend both on the level of slaughter hygiene and the level of hygiene and temperatures in the subsequent processing steps, i.e. cutting and retail. In cutting plants and at retail, cross-contamination of pork is likely to occur and the handling of meat at temperatures above 5 °C in the process environments can potentially lead to growth of *Salmonella*. A contribution to consumer exposure from the meat processing chain is indicated from a Danish surveillance study conducted in 2010. The study showed that *Salmonella* prevalence on average was 1.3% in pork meat but varied in

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particular among smaller cutting plants from 0 to 22% (Anonymous, 2013). In 2011, a second study was conducted in Danish cutting plants, where the *Salmonella* prevalence was compared between the incoming meat and outgoing cuts. In two of the cutting plants, increases in the *Salmonella* prevalence from input to output from 5 to 18% and from 5 to 8% were observed (Birk et al., 2014). The extensive increase in prevalence at cutting, compared to the average carcass contamination at slaughter, indicates that cross-contamination, and potentially also growth of *Salmonella*, are likely to take place during cutting. Also in retail butcher shops, cross-contamination seems to be of significant importance indicated by observed increases in the level of *Enterobacteriaceae* in the processed meats, compared to the raw materials (Anonymous, 2013). In both cutting plants and retail butchers, insufficient cleaning and disinfection, as well as temperatures above 5 °C in the processing area, have been identified as main *Salmonella* risk factors (Anonymous, 2013; Berends et al., 1998). These observations bring focus on hygiene management as a prerequisite to reduce *Salmonella* levels on the meat and ultimately to reduce the *Salmonella* consumer risk.

Salmonella is often hard to detect in meat due to low prevalence and low numbers. This makes it difficult to design effective hygiene intervention programs based on *Salmonella* monitoring. If a link between the occurrence of *Salmonella* and a common occurring hygiene indicator can be established, such indicator can potentially be used to indicate poor hygiene that also affects *Salmonella*. In that way, when indicator levels are high, potential growth of *Salmonella* can be controlled by improving the hygiene. The optimal indicator organism would be non-pathogenic, be prevalent in meat, possess similar growth and survival characteristics as *Salmonella*, e.g. in terms of multiplication rate, and be easily and quickly detected (Ghafir et al., 2008).

Salmonella and enterococci both have a lower growth limit around 5 °C and share comparable growth rates and lag-phases at temperatures between 5 and 10 °C (Hansen et al., in press). Further, enterococcus is a normal part of the pig intestinal flora (Klein, 2003) and it is, therefore, spread into the meat chain by the same routes as *Salmonella*. Thus, enterococcus possesses properties, which make it a potential candidate as an indicator of a temperature history of meat, supporting growth of *Salmonella*. Danish surveillance studies have shown positive correlation between the quantitative level of enterococci and the prevalence and quantitative level of *Salmonella* in pork from both Danish cutting plants (Hansen et al., 2013) and Danish retail butcher shops (Hansen et al., in press). It was also found that pork carrying enterococci was three to six times more likely also to carry *Salmonella* and that presence of *Salmonella* was associated with poorer hygiene as indicated by high levels of enterococci or *Enterobacteriaceae* (Hansen et al., 2013).

Since 2006, it has been mandatory for food business operators to comply with the microbiological criteria set by the European Union (Anonymous, 2005). Process hygiene criteria have been developed to give guidance on the acceptability of hygiene levels or pathogen levels during food processing with the purpose to protect consumers from hazardous foodborne microorganisms. Mainly indicators such as *Enterobacteriaceae* and *E. coli* are used as target microorganism for process hygiene criteria in fresh meat. However, in few cases, *Salmonella* is also used as target microorganism (Anonymous, 2005). Recently, the demand for risk-based microbiological criteria has increased (Caipo et al., 2015; van Schothorst et al., 2009). To our knowledge, so far, no process hygiene criterion has been established by risk modelling principles. In general, the relationship between the hygiene level given by the criterion and the risk for the consumer is not well known. By disclosing this relationship, a stronger connection between the process hygiene criterion and the risk for the consumer can be established and the reduction in consumer risk, from adhering to a certain limit, can be estimated.

The positive correlation found between concentrations of enterococci and *Salmonella* in previous surveillance studies has been suggested to be caused by temperature exposure (Hansen et al., in press). We hypothesize that enterococci can be used for setting limits or as target organism for a process hygiene criterion to identify meat exposed to temperatures, which can support critical growth of *Salmonella* in pork cutting plants and retail butcher shops. The quantitative correlation can be used to make a risk-based estimate of the consumer exposure to *Salmonella*, based on the level of enterococci in pork. By combining this with the dose–response model for *Salmonella*, the risk of illness for consumers can be assessed. The relative *Salmonella* risk associated to different hygiene levels, expressed by the number of enterococci, could then be calculated and the relative risk reduction from adhering to a certain limit can be expressed.

The objective of this study was to develop an approach to define a risk-based microbiological limit for enterococci in pork that associates to the risk of *Salmonella*. The approach should be based on linking the observed positive correlation between *Salmonella* and enterococci levels in pork from Danish cutting plants and retail butcher shops with consumer risk by use of the *Salmonella* risk assessment model described by Duarte et al. (2016). If this is possible, the potential effect of a microbiological limit for enterococci can be expressed in terms of reduced consumer risk of salmonellosis. As Nauta et al. (2012) showed for *Campylobacter* in broiler meat, it will also allow an evaluation of the potential public health benefit against the number of food products that will need to be withdrawn or that will need to undergo treatment to eliminate *Salmonella*. The perspective is to use this risk-based approach as a basis for defining microbiological limits for a 3-class plan to be used as part of a national process hygiene criterion in pork cutting plants and/or retail butcher shops.

2. Material and methods

2.1. Collection of samples

The data used in the present study have been compiled from five different studies conducted in collaboration between the Danish Food and Veterinary Administration (DFVA) and the National Food Institute, Technical University of Denmark (Table 1). In total, 6440 pork samples were collected and analysed by the regional laboratories associated to DFVA. The samples were taken from cutting plants and retail butchers in Denmark during the years 2001, 2002, 2010, 2011 and 2013. All samples were analysed qualitatively for detection of *Salmonella* and quantitatively for concentration of enterococci no later than 24 h after sampling. Furthermore, the samples testing positive for *Salmonella* were analysed semi-quantitatively to obtain a *Salmonella* concentration.

2.2. Bacteriological analyses

Salmonella analyses were carried out according to the International standard ISO 6579:2002 Annex D (Anonymous, 2002a, 2006) and the standard of the Nordic Committee on Food Analysis (NMKL) 187 (Anonymous, 2007). In short, dependent on the specific study, 50–100 g meat was added 100–200 ml Buffered Peptone Water (BPW) and homogenised for 2 min in a stomacher. A subsample of this suspension was kept at 2 °C for semi-quantitative analysis in case of a positive qualitative analysis. The remaining suspension, containing 25 g of meat, was added BPW until a final volume of 250 ml. The suspension was then pre-enriched at 37 ± 1 °C for 18 ± 2 h. Three drops, corresponding to 0.1 ml of the pre-enriched culture, were placed separately on the selective Modified Semisolid Rappaport-Vassiliadis medium (MSRV) and incubated at 41.5 ± 1 °C for 24 ± 3 h. Positive samples showed a white swarming zone with a clear edge, originating from the inoculation spot. From positive samples, colony material from the edge of the swarming zone was streaked onto selective Xylose-Lysine-Desoxycholate agar (XLD) and incubated at 37 ± 1 °C for 24 ± 3 h. Results were expressed as *Salmonella* positive when detected in 25 g meat.

In case of *Salmonella* positive samples, the stored suspension was used for a semi-quantitative analysis. First, the suspension was mixed 30 s in a stomacher. A subsample of 6 g was suspended in 54 ml BPW

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