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Enterococci as indicator of potential growth of *Salmonella* in fresh minced meat at retail

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ABSTRACT

The present study had the purpose of demonstrating a positive correlation between enterococci and *Salmonella* in minced pork and beef. Data from 2001 to 2002 from retail minced pork and beef in Denmark were used and the association between concentration of enterococci and prevalence and concentration of *Salmonella* was examined. A total of 2187 and 2747 samples of minced pork and beef, respectively, were collected from butcher shops and supermarkets throughout the country. In pork, 2.1% of all samples were positive for *Salmonella* whereas 1.5% of beef samples were positive. Among samples with ≥100 CFU/g of enterococci, prevalence of *Salmonella* positive samples was 3.4%, which was significantly higher than 1.2% observed in minced meat with less than 100 CFU/g of enterococci ($P < 0.001$). A positive association between occurrence of enterococci and presence of *Salmonella* in retail minced meat was supported as both prevalence and concentration of *Salmonella* in positive samples increased with increasing concentrations of enterococci in minced meat. From our data, we suggest that minced meat containing more than 500 enterococci per gram is suspected of having been exposed to temperatures allowing growth of *Salmonella*. This is to our knowledge the first report, which links presence of an indicator to potential growth of *Salmonella*.

1. Introduction

Salmonella is an important foodborne zoonotic bacterium that is spread from production animals via contaminated meat to the consumer, and pork and beef are substantial sources of human *Salmonella* infections (Anonymous, 2009b). In Denmark, pork and beef have been estimated to account for approx. 10% of all annual *Salmonella* cases from 2006 to 2009 (Anon, 2006, 2007, 2008, 2009a). The prevalence of *Salmonella* positive carcasses in Danish slaughterhouses has reached an almost steady level in recent years and was in 2006 around 1% for pigs and 0.5% for cattle, respectively (Anonymous, 2006). *Salmonella* is, thus, continuously spread from slaughter into the subsequent parts of the meat chain and retail investigations in Denmark in 2006 suggested that *Salmonella* had spread and multiplied through cutting to retail as prevalence in retail pork cuttings was observed to be as high as 4.2% (Hansen et al., 2010).

Mincing of meat at retail is a particular critical process, as *Salmonella* will be physically distributed onto a much larger meat surface. As a result of poor hygiene and inadequate temperature control during handling and mincing, this process may lead to spread and growth of *Salmonella* leading to higher consumer risk. Despite this, prevalence as well as concentration of *Salmonella* is still expected to be relatively low (Hansen et al., 2010). Both the presence of *Salmonella* and too high temperatures in the meat chain can be very challenging, or practically impossible, to reveal. Therefore, it may be relevant to analyse for indicator organisms such as *Escherichia coli* or *Enterobacteriaceae*.

E. coli and *Enterobacteriaceae* are used worldwide as indicators of faecal contamination. They are expected to be more prevalent than the pathogen of concern and also to be present in higher concentrations. Both *E. coli* and *Enterobacteriaceae* have been used as indicators for potential presence of *Salmonella* in fresh meat (Delhalle et al., 2009) and meat handling environments (Prendergast et al., 2008). Especially, *E. coli* seems to work well when meat is close to the slaughterline (Ghafir et al., 2008). However, as the present study was carried out at retail level, indicator organisms that are relatively resistant to the stressful

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conditions, such as desiccation, were sought. Therefore, enterococci were chosen in preference to *E. coli*. Enterococci, which also inhabit the intestinal tract of pigs and cattle, has been shown to survive adverse environmental conditions better than coliforms (Raj et al., 1961; Turantaş, 2002) and can be found in rooms, where meat products are handled (Knudtson and Hartman, 1993). Moreover, enterococci have a long history of being used as indicators for many purposes using a well-established method (Anon, 1992, 2011).

The present study aimed to investigate for a positive correlation between enterococci and *Salmonella* in minced pork and beef, which could imply an application of enterococci as indicators for potential growth of *Salmonella* at retail. Recently we have shown that enterococci and *Salmonella* share similar growth characteristics in minced meat and meat cuts (Møller et al., 2013; unpublished data) and this is, to our knowledge, the first time the level of enterococci in meat is suggested to indicate degree of growth support of *Salmonella*. In the study, a distinguishing between type of meat (pork, beef), type of packaging (atmospheric air, modified atmosphere (MAP)) and type of food business operator (butcher shop, supermarket) was sought.

2. Materials and methods

2.1. Sample collection

Meat samples were collected during official food control inspections throughout the country during 2001 and 2002. Minced pork and beef samples of at least 300 g were obtained from butcher shops and supermarkets. Samples were transported at temperatures not exceeding 5 °C in closed containers to the laboratories where they were kept at 2 °C ± 1 °C. Microbiological analysis was started within 24 h after sampling.

Each meat sample was accompanied by information including (*i*) the type of meat, (*ii*) the country of origin, (*iii*) the type of packaging used for the meat, and (*iv*) the type of food business operator.

2.2. Salmonella analysis

One-hundred gram of minced meat were prepared for analysis according to established bacteriological practice as described in International Standard – ISO 6579: 2002(E) (Anonymous, 2002), with convenient modifications. In short, the sample was mixed with 100 ml Buffered Peptone Water (BPW), and homogenized for 2 min using a stomacher. From this sample suspension, 150 g homogenate was placed in a sealed container and kept refrigerated at 2 °C ± 1 °C for later semi quantitative detection of *Salmonella* if the qualitative detection was positive. The remaining 50 g suspension, containing 25 g of meat, was added 200 ml BPW and pre-enriched at 37 °C for 16–24 h. For selective culturing of *Salmonella*, 0.1 ml BPW culture was applied to Modified Semisolid Rappaport-Vassiliadis medium (MSRV) (Oxoid CM910 or equivalent) and incubated at 41–42 °C for 18–24 h. From the edge of the swarming zone, colony material was streaked onto the indicative medium Xylose-Lysin-Desoxycholate agar (XLD) followed by incubation at 37 °C for 18–24 h. At least two typical colonies were selected for genus verification.

For *Salmonella* positive samples, the sample suspension was recovered from the refrigerator for the semi quantitative analysis, mixed for 30 s in a stomacher and subsequently, 6 g was suspended in 54 ml BPW (1:9) and mixed thoroughly. For the 10-fold dilution row, aliquots of 50, 5, 0.5, 0.05, and 0.005 ml corresponding to 2.5, 0.25, 0.025, 0.0025 and 0.00025 g meat, respectively, were prepared. For the aliquots of 5 ml or below, BPW was added to obtain a total volume of 10 ml. Each aliquot was analysed for *Salmonella* according to the principles described above.

2.3. Enumeration of enterococci

For quantitative analysis for enterococci, 10 g minced meat were taken aseptically according to the bacteriological practice NMKL No 91 (Anonymous, 1988). Subsequently, the analysis for enterococci was performed using the standard method of NMKL No 68, second edition (Anonymous, 1992), which prescribed serially 10-fold dilutions in BPW, followed by surface plating of 0.1 ml onto Slanetz and Bartley medium (Oxoid CM377) or equivalent, and incubated at 44 °C for 2 d. Pink to dark red colonies were counted as enterococci.

2.4. Data analysis

The prevalence of *Salmonella* positive samples was reported as the proportion of the 25-g-samples that tested positive, whereas the prevalence of enterococci positive samples was reported as the proportion of samples where enterococci was detected using a detection limit of 100 CFU/g. Likelihood Ratio Tests compared differences in prevalence between types of meat, types of packaging and types of retailer. In cases with less than five observations, Fisher's Exact Test was used. All statistically significant differences were reported at the $P < 0.05$ level. Exact 95% confidence intervals (95% C.I.) were calculated based on standard methods for binomial data (Armitage and Berry, 1999).

The point-biserial correlation, r_{pb}, was used to determine a correlation between prevalence of *Salmonella* positive samples and enterococci concentration found for the same sample. Furthermore, correlation between concentrations of *Salmonella* and enterococci in samples were tested by categorizing samples into six groups according to their *Salmonella* concentration and comparing enterococci counts in these groups by analysis of variance. To allow statistical testing, the concentration of enterococci was set to 1.7 $\log_{10}$ CFU/g corresponding to 50% of the detection limit in samples, where enterococci were not detected. For comparisons of the mean values in the groups, 95% confidence intervals were computed.

3. Results

3.1. Salmonella

A total of 2187 and 2747 samples of minced pork and beef, respectively, were collected and analysed for presence of *Salmonella* (Table 1). The overall prevalence of *Salmonella* in minced meat at retail in Denmark in 2001/2002 was 2.1% for pork and 1.5% for beef. As shown in Table 1, the overall prevalence of *Salmonella* was 1.2% for samples packaged in modified atmosphere (MAP), which was significantly lower than 2.0% found for samples packaged in atmospheric air ($P = 0.048$). Six percentage (296 out of 4934) of the minced meat samples originated from imported meat (Table 1). The prevalence of *Salmonella* in imported meat was significantly higher than minced meat of Danish origin ($P = 0.003$). The fraction of imported meat from pork and beef was 0.8% (18 out of 2187) and 10% (278 out of 2747), respectively. In samples from supermarkets and butcher shops, *Salmonella* was detected in 1.6% and 2.4%, respectively (Table 1).

The concentration of *Salmonella* in positive samples varied between 0.04 and > 400 CFU/g as shown in Fig. 1. There was no statistically significant ($P = 0.799$) difference between concentrations found in minced pork and beef, respectively. Pooling the findings resulted in 44 samples (54.3%) containing 0.04–0.4 *Salmonella* per gram, 14 samples (17.3%) containing 0.4–4 *Salmonella* per gram, 18 samples (22.2%) containing 4–40 *Salmonella* per gram, four sample (5.0%) containing 40–400 *Salmonella* per gram and a single sample