Food Control 75 (2017) 116–125

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

A risk-based approach for evaluation of hygiene performance at pig slaughter

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ARTICLE INFO

Article history:
Received 4 October 2016
Received in revised form
14 December 2016
Accepted 21 December 2016
Available online 22 December 2016

Keywords:
Hygiene indicator
Salmonella
E. coli
Risk modeling
Pig slaughterhouse

ABSTRACT

In Denmark, the pig slaughterhouses have a daily input of pigs infected and/or contaminated with *Salmonella*, and the slaughter hygiene has major influence on the level of *Salmonella* contamination on the meat leaving the slaughterhouse. However, the relationship between the effect of improved hygiene performance and the consequential reduction of human health risk has not been estimated so far. In this study, swab samples from 2702 pig carcasses were collected, originally for other purposes, from five large Danish slaughterhouses in a period from 2005 to 2007, covering all seasons of the year. The samples were analysed quantitatively for *E. coli* and semi-quantitatively for *Salmonella*. A positive association between the number of *E. coli* on carcasses and the prevalence of *Salmonella* positive carcasses was shown. For carcasses positive for *Salmonella*, a positive association was also shown between the number of *E. coli* and the number of *Salmonella* on the carcass. As no biological association has been reported between faecal shedding of *E. coli* and presence of *Salmonella*, the relationship was considered to be associated with the level of faecal contamination. The positive association between *E. coli* and *Salmonella* was used as basis for developing a quantitative risk assessment model for *Salmonella*, using the level *E. coli* as model input. The model output associated the hygiene performance with a relative risk estimate of human salmonellosis. The overall objective was to develop a decision support tool that can be used to support risk-based hygiene interventions in pig slaughterhouses.

1. Introduction

Salmonella is a food-borne zoonotic pathogen, which annually gives rise to more than 100,000 human cases of salmonellosis in the European Union (EFSA & ECDC, 2015). In Denmark, the total number of human registered cases in 2014 were 1,122, equal to 19.9 cases per 100,000 inhabitants, with domestic pork as the largest source, being responsible for 15.4% of all cases (Anonymous, 2015).

In 1993, the incidence of human salmonellosis in Denmark, associated to consumption of domestic pork, rose to 22 cases per 100,000 inhabitants (Anonymous, 2009) and around the same time 22% of the Danish pig farms were estimated to be *Salmonella* positive (Christensen, Baggesen, Nielsen, & Stryhn, 2002). In 1995 this situation led to implementation of the Danish surveillance-and-control program for *Salmonella*. The program covered the primary production and the slaughter industry and was initiated with the aim to reduce consumer risk through a reduction in carcasses contaminated with *Salmonella*. Elements of the program are still in place today. Based on serological testing of meat juice samples from individual pigs taken at slaughter, the herds are divided into three serological levels. Level I and II herds are subjected to normal slaughter. Pigs from level III herds, classified as having unacceptable *Salmonella* problems, are subjected to sanitary slaughter, which include hot water treatment of carcasses prior to cooling (Alban & Sørensen, 2010; Alban, Stege, & Dahl, 2002). Around 1% of Danish herds are classified as level III herds and goes through this intervention at a special slaughterhouse.

Despite the Danish surveillance-and-control program for *Salmonella*, an increase in prevalence of pigs carrying *Salmonella* in caecum at slaughter has been observed. The prevalence has increased from 6.2% in 1993 to 24.2% in 2013 (Anonymous, 2014; Wingstrand & Sørensen, 2012). The prevalence of *Salmonella* infected pig herds in Denmark was estimated to 60% in 2011 (Dahl, 2014). As carcasses from only 1% of herds are subjected to sanitary slaughter, all slaughterhouses have a daily input of *Salmonella* positive pigs. Pigs can show no symptoms of disease and still be subclinically infected with *Salmonella* and excrete varying amounts of *Salmonella* during slaughter as e.g. observed for *Salmonella* Typhimurium (Rostagno & Callaway, 2012). Additionally, pigs can

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http://dx.doi.org/10.1016/j.foodcont.2016.12.034
0956-7135/

become contaminated from other pigs and thus, a substantial part of the *Salmonella* burden in pigs at slaughter needs to be controlled by hygiene measures.

In order to protect consumers, it is important to be able to control the *Salmonella* output from the slaughterhouses. Despite a fourfold increase in the input of *Salmonella* positive pigs to slaughter, during the last two decades, the Danish slaughter industry has managed to keep the number of *Salmonella* positive carcasses at a level around 1.3% for more than a decade (Anonymous, 2015). Closures of malfunctioning slaughterhouses and hygiene improvements of slaughter processes are thought to be the main reasons for this. The role of malfunctioning equipment should not be underestimated, as studies have pointed out cross contamination from equipment, in particular, to be main responsible factors for the *Salmonella* carcass contamination (Hald, Wingstrand, Swanenburg, von Altrock, & Thorberg, 2003; Nauta et al., 2013).

Optimally, targets for hygiene performance at slaughter should be set according to the corresponding *Salmonella* contamination it causes. However, due to the relatively low prevalence of *Salmonella* positive carcasses, monitoring *Salmonella* on carcasses at slaughter is not considered an operational parameter for risk-based hygiene interventions on day-to-day basis. For many years, *E. coli* have been used as indicator of faecal contamination and possible presence of enteric pathogens (Ghafir, China, Dierick, De Zutter, & Daube, 2008; Ray, 2004). Presence of *E. coli* on e.g. pig carcasses is an indication of direct or indirect contamination with faecal material (Ray, 2004). Therefore, *E. coli* is also used as an indicator of slaughter-hygiene performance. If it is possible to link bacterial counts of slaughter-hygiene indicators, such as *E. coli* or *Enterobacteriaceae*, to the possible occurrence and level of *Salmonella* on carcasses, a principle for risk-based hygiene intervention, based on indicator bacteria, can be developed. In contrast to *Salmonella*, *E. coli* is always present in the intestinal tract of pigs, which makes it an advantage if *E. coli* can be linked to potential presence of *Salmonella* together with the potential contamination level. It is also a practical advantage that *E. coli* is more easily detected and quantified than *Salmonella*. Previous studies have already shown an increased level of *E. coli* to correlate to an increased probability of simultaneous detection of *Salmonella* on pig carcasses and pork cuts (Delhalle et al., 2008; Ghafir et al., 2008). However, an association between the quantitative level of *E. coli* contamination on pig carcasses and the potential presence and numbers of *Salmonella* on the carcasses, has not been reported so far.

The aim of this study was first to investigate if increasing counts of *E. coli* on pig carcasses correlated to increased potential of *Salmonella* carcass contamination and increasing levels of *Salmonella*, when present. Secondly, based on the risk model published by Duarte et al. (Duarte, Nauta, & Aabo, 2016), the aim was to develop an extended risk model that estimate the relative risk of human salmonellosis associated to certain hygiene performance levels, as measured by the *E. coli* contamination on the carcasses at pig slaughter. A similar approach was recently published by Bollerslev, Nauta, Hansen, and Aabo (2016) who found a positive association, based on simultaneous growth potential, between the level of enterococci and the concentration of *Salmonella* in fresh pork. This association enabled the construction of a quantitative human salmonellosis risk model, based on enterococci counts from pork cuttings.

2. Materials and methods

2.1. Sampling

In the period between May 2005 and May 2007, a total of 2822 pigs were sampled at five Danish pig slaughterhouses. Based on an evaluation of the data by Nauta et al. (2013), the data set was narrowed down to samples from a total of 2702 pigs. From a subset of 1826 pigs, samples from both carcass and faeces were analysed for *E. coli* and a subset of 1522 pigs, comprised paired data on both *E. coli* and *Salmonella* from carcass and faeces. Two slaughterhouses were sampled six times, two were sampled five times and one slaughterhouse was sampled once. Generally, samples were collected evenly distributed over the two-year period in order to avoid bias related to seasonal variation. On each sampling day 80, 105, 119 and most often (83% of the visits) 120 pigs were sampled. Carcass swabs were taken immediately prior to cooling by use of sterile gauze swabs (16 ply, 10 × 10 cm, KRUUSE), pre-moistened with approximately 12.5 ml buffered peptone water (BPW; Oxoid) in a sterile stomacher bag. The swabbing area covered 2800 cm^2 (1400 cm^2 on each side) from the forepart via the brisket through the mid-section line to the pelvic region as described by Nauta et al. (2013). All samples were stored at 5 °C until further analyses were performed. After evisceration, a faecal sample of around 40 g was collected from the colon just caudal to the colon helix.

2.2. Sample preparation and bacteriological analyses

2.2.1. E.coli analysis

The procedures for the microbiological analyses are described elsewhere (Nauta et al., 2013). In short, swab samples from 1836 carcasses were analysed quantitatively for *E. coli*. To stomacher bags with the carcass swabs were added 75 ml of peptone water (1 g Bacto Peptone (BD211677; Becton, Dickinson and Company, Sparks, MD, USA) and 8.5 g NaCl per litre). Bags were stomached for 60 s followed by preparation of a ten-fold dilution series in peptone water. From appropriate dilutions, 1 ml was spread on Select *E. coli* Count Plate Petrifilm (3M Microbiology, St. Paul, MN, USA). All Petrifilm plates were incubated at 41.5 °C for 23–25 h according to the instructions of the supplier. Colony counts were determined automatically by use of a Petrifilm plate reader MI649 9 (3M Microbiology, St. Paul, MN, USA). All readings were verified by manual check-up of the colonies counted.

Faecal samples were ten-fold diluted in peptone water (1 g Bacto Peptone (BD211677; Becton, Dickinson and Company, Sparks, MD, USA) and 8.5 g NaCl per litre) and homogenised in a stomacher. After additional ten-fold dilutions, 1 ml of appropriate dilutions were plated on Select *E. coli* Count Plate Petrifilm (3M Microbiology, St. Paul, MN, USA) and incubated as described above.

2.2.2. Salmonella analysis

Carcass swabs from all animals (2,822) and faecal samples from approximately every second animal were analysed semi-quantitatively for *Salmonella*. The analysis was first carried out qualitatively for both faeces and swab samples according to the Modified Semisolid Rappaport-Vassiliadis (MSRV) method (Anonymous, 2002b, 2006, p 40). From faecal samples, 15 g were placed in a beaker and added 135 ml BPW. From swab samples, 11 ml of the stomachat (see Section 2.2.1) were mixed with 4 ml BPW. Both sample types were incubated at 37 °C for 16–20 h followed by adding three drops of the overnight culture, corresponding to 0.1 ml, on MSRV-agar with novobiocin (20 mg/l) with subsequent incubation at 41.5 °C for 18–24 h. Samples showing a grey-white turbid swarming zone around the inoculated drop were characterized as potential *Salmonella* positive and were re-analysed semi-quantitatively. Sample material for the semi-quantitative analyses was stored at 2–5 °C awaiting the result of the qualitative analysis. For *Salmonella* positive faecal samples, 16.5 g faecal material was stomached (30 beats) in 150 ml BPW. A ten-fold serial dilution was made in BPW, followed by incubation of the dilution