Food Research International 45 (2012) 871–879

Contents lists available at ScienceDirect

Food Research International

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

The role of transport, lairage and slaughter processes in the dissemination of *Salmonella* spp. in pigs in Ireland

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ARTICLE INFO

Article history:
Received 22 November 2010
Accepted 8 February 2011

Keywords:
Salmonella spp.
PFGE
Phage typing
Abattoir
Pigs
Ireland

ABSTRACT

Salmonella is an important foodborne pathogen worldwide and is commonly isolated from pigs and pig products in Ireland. Pigs, reared in an environment free of *Salmonella* spp. or with low levels of infection, may acquire infection or become contaminated during transport, lairage or post-slaughter. The main objective of this study was to determine the role of the abattoir as a potential factor that contributes to the dissemination of *Salmonella* spp. in slaughter pigs from herds with a low *Salmonella* seroprevalence (≤10%). A total of 128 pigs from eight herds were monitored from farm through the slaughter process in three separate abattoirs. The prevalence of *Salmonella* spp. was determined in samples collected from trucks, lairage pens and the slaughterline before pigs entered, from pigs after slaughter (caecal contents and ileocaecal lymph nodes) and carcass surfaces post-evisceration. Isolates were characterised by serotype, phage type and pulsed-field gel electrophoresis (PFGE) patterns. Of the swabs taken from the trucks, lairage and slaughterline, before the pigs entered, 4.3% (3/70), 80% (64/80) and 16.7% (4/27) were positive for *Salmonella* spp., respectively. The proportion of pigs showing serological evidence of infection was 3.1% (4/128). *Salmonella* spp. were isolated from the ileocaecal lymph nodes and caecal contents of 14.8% (19/128) and 11.7% (15/128) of pigs, respectively, and 13/128 (10.2%), 5/128 (3.9%), 2/111 (1.8%) and 8/111 (7.2%) carcass swabs pre wash, post wash, post chill and belly-strip samples, respectively, were *Salmonella*-positive. There was only slight agreement between serological and bacteriological data at the pig level. *Salmonella* isolates from 45% of all positive pig samples and 82% of positive carcass samples were indistinguishable, based on PFGE patterns, from salmonellae isolated from the lairage and slaughterline. Based on these results it is concluded that the lairage and the slaughterline provide a substantial source for *Salmonella* contamination of pigs and carcasses.

1. Introduction

Salmonella is the one of the most common causes of foodborne bacterial infections in the EU. In Ireland 10.6 cases per 100,000 persons were reported in 2008 and *S.* Typhimurium was the dominant serovar isolated (Anonymous, 2008b). *Salmonella* spp. is commonly recovered from pigs in EU Member States (Anonymous, 2008a) and pork is regarded as a common food vehicle for human exposure (Berends, Van Knapen, Mossel, Burt, & Snijders, 1998). It is estimated that approximately 10–20% of human cases of salmonellosis in EU Member States (MS) may be attributed to the consumption of pig meat (Anonymous, 2010).

Recent data from the EU baseline survey of *Salmonella* spp. in slaughter pigs showed a mean prevalence of 10.3% in lymph node samples (ranging from 0.0 to 29.0% in the 25 participating MS) and a mean prevalence of 8.3% in carcass swabs (ranging from 0.0 to 20.0% in the 13 participating MS) (Anonymous, 2008a). A report on the incidence of *Salmonella* spp. on pork cuts in 2007 in Ireland showed 2.6% tested positive for *Salmonella* spp. (Prendergast et al., 2009) and Jordan et al. (2006) found 53% of *Salmonella*-positive porcine raw meat and meat products were *S.* Typhimurium, mostly DT104 and DT104b. As *S.* Typhimurium is commonly isolated from pigs, and with the majority of high-risk herds infected with this serotype (Rowe et al., 2003), a National *Salmonella* Control Programme was introduced in Ireland in 2002 with an overall aim of reducing *Salmonella* prevalence both in pigs and pork. The *Salmonella* control programme was based on that developed some years earlier in Denmark (Alban, Stege, & Dahl, 2002) in which herds are categorised based on a risk of exposure to *Salmonella* spp. Herds are assigned a category of 1, 2 or 3 based on a serological prevalence of ≤10%, >10 to 50% or >50%, respectively, following a meat-juice ELISA antibody test with a positive cut-off of 40%.

Pigs are often healthy carriers of *Salmonella* spp. and the stresses associated with transport and lairage, may induce these latent carriers to become active shedders (Hansen, Rogers, Emge, & Jacobs, 1964; Williams & Newell, 1970), resulting in the contamination of the

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doi:10.1016/j.foodres.2011.02.001

surrounding environment and the increased risk of *Salmonella*-free pigs becoming infected. Studies have shown that pigs can acquire this pathogen following exposure times of 30 min to 2 h (Boughton, Egan, Kelly, Markey, & Leonard, 2007a; Hurd, Gailey, McKean, & Rostagno, 2001a; Hurd, Gailey, McKean, & Rostagno, 2001b). Attempts to eliminate the resident *Salmonella* flora in holding pens, despite intensive cleaning and disinfection have met with little success. Although Schmidt, O'Connor, McKean, and Hurd (2004) significantly reduced the prevalence of *Salmonella* spp. in the lairage from 20 to 100% to 0 to 15% by applying a vigorous cleaning protocol they failed to reduce the prevalence of *Salmonella* spp. in the associated pigs. Mannion, Egan, Lynch, Fanning, and Leonard (2008) subsequently demonstrated the need for more stringent cleaning of transport trucks so as to further reduce the potential for contamination of pigs. Studies have often reported a disparity not only between serotypes isolated between slaughter and primary production but also between prevalences of infection (Hurd, McKean, Wesley, & Karriker, 2001c; McKinley et al., 1980) which suggests that transport and/or lairage may influence infection rates.

Salmonella-infected pigs are a crucial source of contamination of carcasses in the abattoir. Unpublished observations of Mannion (2007) suggested that for high-risk herds, gastro-intestinal contamination of pigs at slaughter originated on the farm and not at the abattoir, and for low-risk herds, the lairage was a highly important source of contamination. A study by McDowell, Porter, Madden, Cooper, and Neill (2007) showed a significant association between the caecal or serological result and the carcass result of homebred pigs indicating the importance of individual pig status. Other studies have suggested cross-contamination during slaughter as an important source of *Salmonella* contamination for carcasses of sero-negative pig herds (Swanenburg, Van der Wolf, Urlings, Snijders, & Van Knapen, 2001) and that high prevalences of *Salmonella* spp. on the surfaces of carcasses, were indicative of defective equipment or poor hygiene on the slaughterline (Käsbohrer et al., 2000).

Further clarification of the role of transport, lairage and slaughter processes is required. The slaughterhouse remains a critical step along the entire pork production chain which can influence the contamination rate of pigs and carcasses. In the present study pigs from eight low-risk/category 1 herds were tracked through three participating abattoirs in Ireland and sampled for *Salmonella* spp. at selected stages along the slaughter chain. The primary objective of this study was to determine the role of transport, the lairage and the slaughterline as potential factors which contribute to the dissemination of *Salmonella* spp. by comparing serotypes, phage types and PFGE data from the truck and abattoir environments and associated pig samples post slaughter. A secondary objective was to determine if the herd serological status could be correlated with the individual pig bacteriological data.

2. Materials and Methods

2.1. Herd selection

Eight farms with good management practises and a low *Salmonella* seroprevalence (category 1) were selected to participate in this study. Pigs from these farms were processed through one of three abattoirs, two of large and one of medium capacity. From each herd, sixteen finishing pigs were monitored from the farm, to the lairage and along the slaughterline. Sampling commenced in late July 2007 and ran for an eight-month period. All pigs were slaughtered mid-week and mid-morning.

2.2. Sample collection

The trucks that transported the groups of pigs studied were commercial pig transporters used by the individual farms and only transported pigs originating from a single herd on any particular journey. On the day of slaughter environmental swabs from the trucks were collected immediately before the pigs were loaded and from the lairage before the pigs entered into the pens. At both stages ten environmental samples were taken by vigorous swabbing of 1.0 m^2 surface area using a large gauze surgical swab (Robinson Healthcare, Chesterfield, UK: No. 5345), which had been autoclaved and premoistened with 10 ml of buffered peptone water (BPW, Lab M, Bury, Lancashire, U.K.). In order to prevent external contamination of samples, aseptic measures were taken at all times e.g. sterile gloves worn by the sampler were changed between samples and the sampling procedure progressed from the front to the rear of the truck and lairage pen.

The pigs were identified on the slaughterline by their herd slap number. Identification of individual pigs was facilitated by tagging each pig with a numbered ear tag upon leaving the polisher. Environmental swabs of the gambrelling table, arm and apron of the eviscerator and carcass-splitting saw were collected immediately before the pigs were slaughtered by vigorous swabbing of 200 cm^2 surface area using sterile polyurethane sponges (Ashtown Food Research Centre, Dublin 15, Ireland). Caecal contents, ileocaecal lymph nodes, carcass swabs and belly-strips for *Salmonella* isolation, and muscle samples for meat-juice serology were collected as follows: Gastrointestinal tracts were placed in individual clean plastic trays prior to sample collection. For each pig, the caecum was incised with a sterile scalpel blade and approximately 50 ml of the contents was placed in sterile 100-ml pots. Ileocaecal lymph nodes were collected by blunt dissection and placed in sterile bags. Muscle samples were collected at the veterinary inspection point and comprised a small portion of diaphragm (2 cm^2), which was placed into a specific meat-juice sampling container (Sarstedt, Nümbrecht, Germany).

From each pig carcass swabs were collected at three stages: pre wash, post wash and 24 h post chill. Belly strips were collected from each pig post chill only. Pre and post wash swabs were collected from opposite carcass halves and post chill swabs and belly strip samples were also collected from opposite carcass halves. Carcass swabs were collected by swabbing a 100 cm^2 area at each of the four sampling sites (ham, belly, rump and jowl) by vigorously rubbing the sponge 10 times in the vertical and horizontal directions. Belly strip samples were collected from carcasses in the chill by excising a thin strip of belly skin and muscle (100 cm^2) from the edge of the evisceration opening and placed in sterile bags. Aseptic techniques were used at all times and samples were stored at 4 °C until analysed and all samples were cultured within 72 h of collection.

2.3. Microbiological analysis

Each truck and lairage swab and each 10 g of caecal contents were suspended in 90 ml of BPW and were shaken vigorously in a stomacher before analysis. Each slaughterline swab, carcass swab and belly strip were suspended in 100 ml of BPW and 25-g samples of ileocaecal lymph node were surface de-contaminated, crushed and added to 225 ml of BPW.

Salmonella isolation procedures were performed on 60 ml of all sample suspensions or 210 ml in the case of lymph node suspensions. The remaining 40 ml was stored at 4 °C for quantification. Samples of the suspension (60 ml and 210 ml) were tested following a protocol adapted from BS EN ISO 6579:2002+A1 (Anonymous, 2007). In brief they were incubated for 18 ± 2 h at 37 °C. All samples (0.1 ml) were then selectively enriched in MSRV (Semi-Solid Rappaport Vassiliadis; Lab M, Bury, Lancashire, UK) at 41.5 °C for 24 and 48 h. All samples were plated onto xylose lysine desoxycholate agar (XLD; Lab M), brilliant green agar (BGA; Lab M) and mannitol lysine crystal violet brilliant green agar (MLCB; Lab M) after 24 h and 48 h of selective enrichment and incubated at 37 °C for 18–24 h. Up to five suspect colonies per plate were identified by subculture onto MacConkey agar