

Salmonella in Irish pig farms; prevalence, antibiotic resistance and molecular epidemiology

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Abstract

The objective was to examine the prevalence of Salmonella in manure from 30 Irish pig farms and to characterize any recovered isolates in order to assess potential risks and epidemiological relationships. Salmonella was detected in the manure from finisher pigs in 50% of the herds investigated. *S. Typhimurium* was the predominant serotype recovered and the most common phage types were DT104 and DT104b. Nineteen of the 29 Salmonella isolates recovered were resistant to one or more antibiotics and 15 of these (all Typhimurium) were multi-resistant. Molecular analysis revealed 19 PFGE types and facilitated tracking of isolates across farms. Overall, Salmonella prevalence correlated well with Irish findings from an EU-wide study of pig production holdings conducted in 2008 (47.7%). The high level of antibiotic resistance observed among the porcine isolates is a concern, but not uncommon in *S. Typhimurium*.

Introduction

Pigs are well recognized as carriers of Salmonella and transmission of the pathogen to humans via carcass contamination at slaughter is a major food safety concern (Boyen et al., 2008). A number of baseline surveys have been conducted to determine the prevalence of Salmonella in pigs. In 2008, a European Food Safety Authority (EFSA) study found that 33.3% of pig production holdings in the EU were positive for Salmonella (EFSA, 2009). However, Ireland had a much higher prevalence (47.7%); in fact it was the third highest in the EU.

Because of the high prevalence of Salmonella in pigs, a number of countries have introduced Salmonella surveillance and control programmes. In Ireland, a monitoring and control programme was first established in 2002. Monitoring is based on determining the Salmonella status of pig herds by serological testing of meat juice at slaughter and not bacteriological testing. In the past, if less than 10% of samples were positive the herd was classed as Category 1; if 11-49% of samples were positive it was categorized as 2 and if greater than 50% of samples were positive it was a category 3 herd. However, a revised national pig Salmonella control programme was implemented in Ireland in January 2010. Monitoring is still based on serological testing of meat juice samples but there are now only two categories; 1 (< 50% prevalence) and 2 (> 50% prevalence).

While some studies have investigated Salmonella carriage in Irish pig herds, none to date have performed molecular as well as phenotypic analysis on the Salmonella recovered. The objectives of this study were (1) to determine the prevalence of Salmonella in finisher pigs from a sample of Irish pig farms representing different Salmonella categories and (2) to characterize any Salmonella isolates recovered, both phenotypically and genotypically in order to identify predominant isolates and thereby assess potential risks as well as epidemiological relationships.

Materials and Methods

Pig manure sampling

A total of 30 manure samples were collected from the finisher houses of commercial pig farms in Ireland between January 2009 and March 2010. Farms were chosen based on their categorization within the initial (pre-2010) Irish National Salmonella Control Programme. It should therefore be noted that any reference to farm categories in this study refers to this historical categorization system (as outlined above). Ten farms were sampled from each of Categories 1, 2 and 3. Samples (~100 ml) were obtained from manure storage tanks situated directly underneath finisher houses at a depth of

1 m below the crust or from a sluice, if present. They were collected into sterile containers and transported on ice to the laboratory where they were stored at 4°C until analysis (within 24 hr).

Microbiological analysis of manure samples

The presence/absence of Salmonella in 25 g samples of manure was determined according to standard procedures (ISO, 2007) with modified brilliant green agar (Merck, Darmstadt, Germany) used for additional selective plating. Based on results of biochemical tests, presumptive Salmonella isolates were tested using a Salmonella latex agglutination kit (Oxoid, Basingstoke, Hampshire, UK). Isolates confirmed as Salmonella were grown in brain heart infusion (BHI) broth overnight at 37 °C and stored at -20 °C in BHI containing 40 % glycerol. Two Salmonella isolates per sample were sero-typed based on O- and H-group antigens according to the White Kaufmann Leminor scheme. Antimicrobial susceptibility testing was performed according to the broth dilution method of the Clinical and Laboratory Standards Institute (formerly NCCLS) (NCCLS, 1999). Salmonella Typhimurium isolates were phage typed by the National Salmonella Reference Laboratory at Galway University Hospital, Ireland. Molecular typing of Salmonella isolates was performed by pulsed field gel electrophoresis (PFGE) using XbaI (New England Biolabs, Hitchin, Herts, UK) according to the standardized protocol of PulseNet (CDC, 2002). PFGE was performed on a CHEF-DRII system (Bio-Rad Laboratories, Hercules, California) using a mid-range II PFGE marker (New England Biolabs) with the following parameters; run time of 19h, initial switch time of 2.2 s, final switch time of 63.8s, 6 V, 14 °C. Gel images were visualized under UV light and saved as TIFF files which were analyzed using BioNumerics software (v3.5, Applied Maths, Sint-Martens-Latem, Belgium). Clustering analysis was performed using Pearson correlations. The similarity coefficient was used to create a dendrogram using the unweighted pair group for arithmetic means (UPGMA).

Results

Salmonella was detected in the manure from finisher pigs in 50% (15/30) of the herds investigated; 30% (3/10) of Category 1 herds and 60% (6/10) of each of Category 2 and 3 herds. Two isolates from each Salmonella-positive manure sample were characterized (except for Farm 14 where only one isolate was obtained) and both were identified as the same serotype and had the same antimicrobial resistance profile (Fig. 1). In total, 29 isolates, comprising seven serotypes were recovered. *S. Typhimurium* predominated, both overall and within each category. It was isolated from 30% (9/30) of herds and accounted for 58.6% (17/29) of all isolates recovered. Within these, six phage types were identified; DT104 and DT104b were the most common and were isolated from two and three herds, respectively, while U288, DT193, U311 and DT17 were each isolated from one herd. The other serotypes recovered were Manhattan, Goldcoast, Bredeney, Brandenburg, Livingstone and Derby, each from one herd. PFGE revealed 19 banding patterns among the 29 Salmonella isolates (Fig. 1). Where two isolates from one farm were characterised both had the same PFGE fingerprint, except for isolates from Farms 8, 12, 15, 16, 30 and 38. The 29 Salmonella isolates grouped into three clusters (1–3) based on their PFGE patterns (Fig. 1). However, there was no correlation between clustering of isolates and herd categorization. *S. Typhimurium* grouped into clusters 2 (which contained phage types DT104, DT104b, U288, DT193 and DT17) and 3 (containing phage types DT104, D104b, U311, an untypable isolate and two Brandenburg isolates). Within cluster 2, two *S. Typhimurium* DT104b isolates from Farm 8 (WIT 385 and 386) were just less than 80 % similar to a DT104 isolate from Farm 1 (WIT 384) and all had the same antimicrobial resistance profile. Within cluster 3 an untypable Typhimurium isolate from Farm 16 (WIT 412) was highly related (> 90% similar) to a DT104 isolate from Farm 30 (WIT389), although they had slightly different resistance profiles. Furthermore, other isolates with different phage types, albeit from the same farm were highly related e.g. the U288 and DT193 isolates within cluster 2. The other five Salmonella serotypes recovered (Bredeney, Goldcoast, Manhattan, Derby and Livingstone) grouped into cluster 1 (Fig. 1).

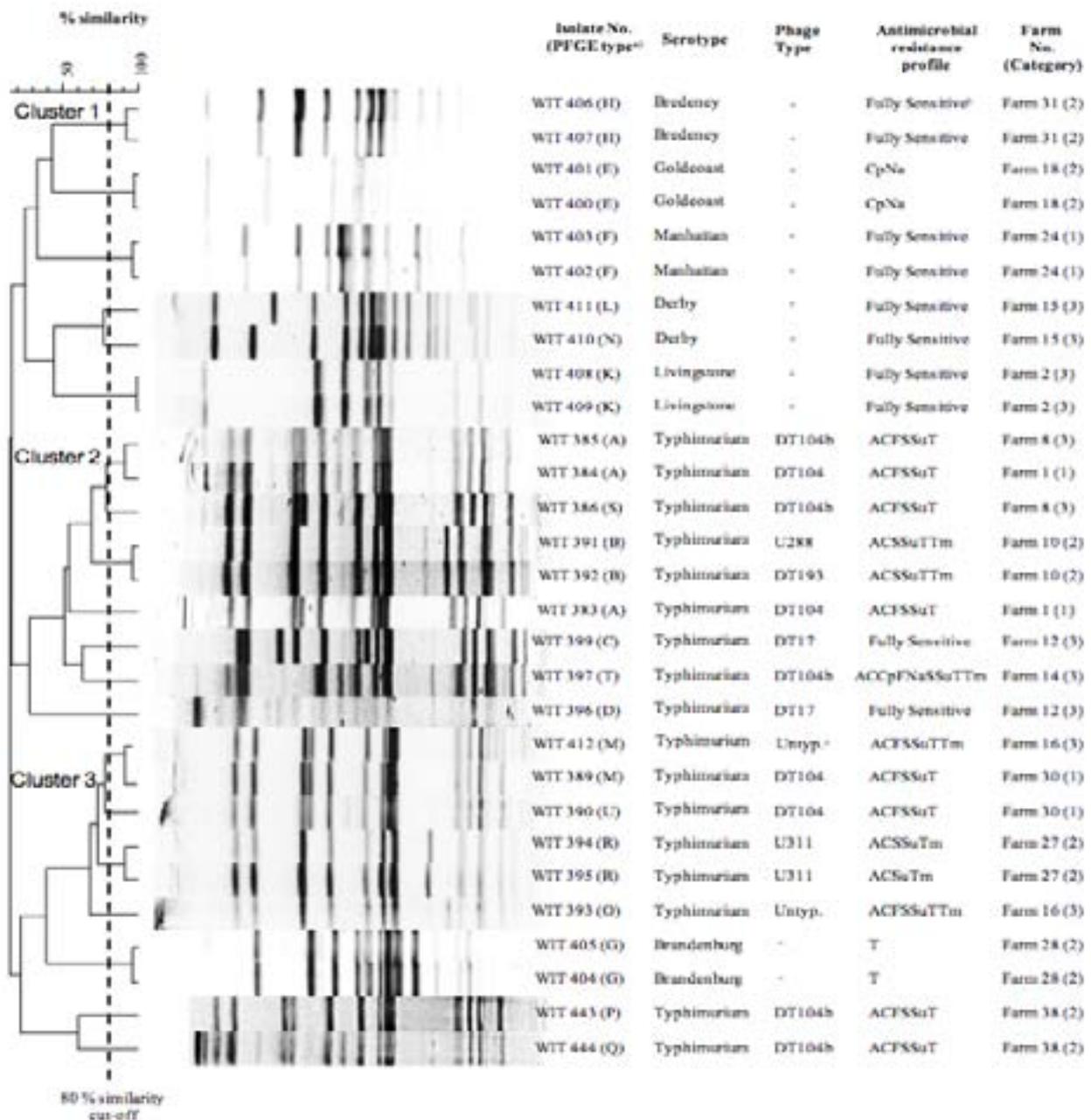


Fig. 1. PFGE patterns (obtained using Xball) of *Salmonella* isolates recovered from pig manure samples. Isolates showing > 80% similarity were assigned the same letter and can be considered highly related. The serotype, phage type, antimicrobial resistance profile and farm of origin are also shown. ^bFully sensitive to all 13 antibiotics tested. ^cA, ampicillin; C, chloramphenicol; Cp, Ciprofloxacin; F, florfenicol; Na, Nalidixic acid; S, streptomycin; Su, sulfamethoxazole; T, tetracycline; Tm, Trimethoprim. ^dUntypable by phage typing

Within this cluster, both Bredeney isolates were highly related (> 90% similar), as were the other isolates of the same serotype, except the two Derby isolates which were only 75% similar. Nineteen of the 29 *Salmonella* isolates recovered were resistant to one or more antibiotics and 15 of these (i.e. all of the Typhimurium isolates) were multi-drug resistant (resistant to 3 antibiotics from different classes). The most common resistance observed was to tetracycline (89% of isolates), followed by ampicillin, sulfamethoxazole and chloramphenicol (79% each), streptomycin (74%), florfenicol (58%), trimethoprim (37%), ciprofloxacin and nalidixic acid (16% each). Thirteen *S. Typhimurium* isolates displayed the typical ACSSuT penta-resistance pattern of *S. Typhimurium* DT104, although they were not all DT104 and they were also resistant to at least one other antibiotic. In fact, one DT104b isolate was resistant to nine antibiotics.

Discussion

Salmonella was detected in manure from finisher pigs on 50% of Irish farms sampled. A lower prevalence was observed in Category 1 herds, demonstrating some correlation between serological and bacteriological data. Seven serotypes were identified; *S. Typhimurium* predominated and DT104 or DT104b were the most common phage types recovered. Although only a small number of farms were sampled, data from the present study correlates well with Irish findings from an EU-wide study of pooled fecal samples from all production stages. This showed that 47.7% of pig production holdings were Salmonella-positive, with *S. Typhimurium* the second most commonly isolated serotype (EFSA, 2009). Our findings are also in agreement with those of Rowe et al. (2003) who found that 51% of faecal samples from at least one production stage of Irish pig farms were Salmonella-positive, with *S. Typhimurium* the most common serotype. However, prevalence in finisher pigs was only 23%. In a Northern Irish survey of slaughter pigs, 31% harbored Salmonella in the cecum, with *S. Typhimurium* again accounting for the majority of isolates (Mc Dowell et al., (2007).

Increasing antimicrobial resistance has been observed in *Salmonella* spp. worldwide. In the present study, antimicrobial resistance was most common amongst isolates of *S. Typhimurium*, in agreement with previous findings (Boyen et al., 2008). Many of these isolates were DT104 or DT104b, which are a common cause of foodborne disease, notoriously multi-drug resistant and frequently isolated from pigs (Boyen et al., 2008). One *S. Typhimurium* DT104b isolate from the present study was resistant to nine antibiotics, including two fluoroquinolones (nalidixic acid and ciprofloxacin), which is worrying, as these are the drugs of choice used to treat human infections. In addition, molecular typing with PFGE facilitated tracking of isolates across farms and to our knowledge this has not been performed to date on Irish pig farms. This analysis revealed that highly related *S. Typhimurium* isolates originated on farms in different geographical locations. However, some *S. Typhimurium* isolates with different phage types and antimicrobial resistance profiles were indistinguishable by PFGE, which is not uncommon.

Conclusion

In this small-scale study of Irish pig farms, Salmonella was detected in the manure from finisher pigs on 50% of farms sampled. In agreement with previous studies *S. Typhimurium* predominated and the DT104 and DT104b phage types were commonly recovered. A number of multi-resistant *S. Typhimurium* isolates were recovered, which is a concern, but not uncommon. PFGE analysis revealed the presence of highly related isolates on different farms. However, a more discriminatory method, such as multi-locus variable number tandem repeat analysis is needed to further differentiate *S. Typhimurium* isolates.

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