DNA FINGERPRINTING OF S. TYPHIMURIUM FROM A PIG LONGITUDINAL STUDY

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Summary: A 400 sow farrow-to-finish farm was sampled for 6 consecutive years to determine the persistence of Salmonella Typhimurium (STM) DT104. Pulsed-field gel electrophoresis, plasmid DNA analysis and antibiotic resistance phenotyping was carried out on selected STM strains, isolated from the farm during the time of the study. Clonal persistence as well and introduction of new clones from external sources were proven to be the main mechanisms by which salmonella infection was maintained in the farm.

Keywords: Pulsed-field gel electrophoresis, plasmid profile, antibiotic resistance, persistence.

Introduction: STM is a common cause of salmonellosis among humans and animals in many countries (Davies, 2001). Phage types DT104 and U302 were the most prevalent types in the UK, in both livestock and humans, in 2001. The aim of this study was to determine the persistence of Salmonella Typhimurium DT104 or related phage types in a sow farrow-to-finish pig farm during a 6 year study.

Materials and Methods: Salmonella isolates. 40 STM DT104 or related phage types (DT104, DT104b, DT104c, U302) were selected to represent a wide range of faecal, environmental and wildlife samples from the study farm over time (1996 n = 7, 1997 n = 5, 1998 n = 6, 1999 n = 9, 2000 n = 4, 2001 n = 9). Antibiotic resistance phenotyping: Isolates were screened for susceptibility to a panel of 16 antibiotics on iso-sensitest agar (Oxoid CM471) by a disk diffusion method similar to that previously described (Phillips, 1991). The following disks (Oxoid, Basingstoke, Hampshire) were used: amikacin (10 mg), amoxycillin/clavulanic acid (30 mg), ampicillin (10 mg), apramycin (15 mg), chloramphenicol (10 mg), cefoperazone (30 mg), cefuroxime (30 mg), colistin (25 mg), furazolidone (15 mg), gentamicin (20 mg), nalidixic acid (30 mg), neomycin (10 mg), streptomycin (25 mg), sulphamethoxazole/trimethoprim (25 mg), tetracycline (10 mg) and triple sulphonamide (300 mg). Organisms with a zone diameter of less than 13 mm were classified as resistant. Molecular typing: Plasmid DNA was isolated by the alkaline lysis method and pulsed-field gel electrophoresis (PFGE) with XbaI and BlnI were performed as described before (Liebana et al., 2002). Fingerprinting data was digitalised and analysed using Gelcompar II (Applied Maths).

Results: All STM isolates from the first 2 years of the study had an identical PFGE type, and 5 plasmids (A, C, D, E, F) were identified amongst them (Figure 1). In 1998 the farm was depopulated, cleaned and disinfected, and was left empty for 6 months. Isolates from samples collected prior to and after cleaning and disinfection (C+D) and also from the replacement animals were studied. The same PFGE type, plasmids and resistance phenotypes were found in isolates from pigs pre depopulation and in isolates from resident mice (droppings) and environment after depopulation and C+D. PFGE and plasmid profiles identified in the first year of the study were still present amongst isolates from 2001, therefore, suggesting clonal persistence. Only, in 2001, did some isolates present variants from the XbaI and BlnI PFGE types, these were only different in a single band and could represent on-farm diversification of the predominant clone. Plasmids A, C, D, E & F were found on farm isolates throughout the study, possibly indicating that the major plasmid profile groups have evolved from each other by the uptake or loss of one of these plasmids. The fact that isolates belonging to different plasmid profiles have the same XbaI and BlnI PFGE types supports this hypothesis. Plasmid B was found only in a single isolate from a badger in 2001 that also had different XbaI and BlnI fingerprints.
Figure 1. Dendrogram generated by the Gel Compar II software showing the relationship of 8 representative plasmid types for 40 S. Typhimurium (DT104 and related phage type) isolates. The clustering analysis was performed using the Dice coefficient and unweighted pair group method with arithmetic averages (UPGMA).

The farm was restocked over a period of three months in 1999. A new clone, bearing only the serotype-specific plasmid and distinctive phenotype (resistance to streptomycin and compound sulphonamides), was identified from these replacement animals and remained established in the farm for at least 2 years.

Discussion/Conclusions: We have proven persistence of S. Typhimurium clones over a period of 6 years in a pig production system, even after depopulation and C+D. The isolates showed the same genomic PFGE type (XbaI and BlnI). The plasmid profiles for these isolates were made up of one or more of only 6 plasmids, possibly indicating that the major profile groups have evolved from each other by the uptake and loss of one of these plasmids. We presented definitive proof that wildlife act as a reservoir of S. Typhimurium in the environment, and also that new breeding stock is often an additional source of new clones.

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References:
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