Molecular epidemiology of Salmonella Typhimurium and Salmonella 4,5,12:i:- isolated from pig farms in Spain.

Vidal, A.,1 Rubio, P.,2 Carvajal, A.2 & Liebana, E.1

1Department of Food and Environmental Safety, Veterinary Laboratories Agency Weybridge, Addlestone, Surrey, UK
2Department of Animal Health, University of Leon, Campus de Vegazana s/n, 24071, Leon, Spain
*corresponding author:a.vidal@vla.defra.gsi.gov.uk

Abstract

The genetic diversity of 194 salmonella isolates belonging to different phagetypes of S. Typhimurium and S. 4,5,12:i:- isolated from both healthy slaughter (157) and diarrhoea-affected (37) pigs was assessed using molecular typing (plasmid profiling and PFGE). The aim of this study was to elucidate the sources of infection, and to follow the spread of specific clones within the infected farms. In spite of the genetic diversity observed amongst the isolates, some clones were more prevalent and widely distributed in the pig population, being detected in several slaughter batches from the same and different farms. This finding suggests the existence of multiple and recurrent infection sources, as well as mechanisms favouring survival, persistence and spreading of certain clones within and between pig farms.

Introduction

Salmonella is an important human pathogen worldwide. The primary route of human infection is via contaminated foods of animal origin. Pigs are important reservoirs for different serotypes of salmonella and the importance of pork as a source of human salmonellosis has been increasingly recognised in the last two decades (Wegener & Baggesen, 1996).

Serovar Typhimurium is the second most frequent type of Salmonella isolated from human, food and animal samples in Spain (Usera et al., 2001). In 1997, the Spanish National Reference Laboratory for Salmonella first reported on the emergence of a new Salmonella serovar with the antigenic formula 4,5,12:i:-, which became the fourth most frequently isolated Salmonella serotype in Spain. This serotype has often been found in pigs and pork products in this country (Usera et al., 2001).

Molecular techniques have been developed and used to investigate the epidemiology and ecology of Salmonella in animal populations. Limited information is available about the genetic diversity of Salmonella within the pig population in Spain. This study was undertaken to determine the distribution and persistence of different Salmonella clones within and between pigs farms.

Material and methods

A total of 194 Salmonella isolates belonging to different phagetypes of Salmonella Typhimurium and Salmonella 4,5,12:i:- were characterized. Most of the isolates (157) were obtained from slaughter pigs from 23 batches and 11 farms located in the North-West region of Spain that belonged to the same pig production company. Additionally, 37 isolates recovered from diarrhoea-affected pigs belonging to 20 epidemiologically unrelated farms were included in the study.

Salmonella cultures were serotyped following a microagglutination method (Poppoff & Le Minor, 2001) and phage typed (Anderson et al., 1977) at the National Reference Laboratory for Salmonella and Shigella in Spain.

Pulsed Field Gel Electrophoresis (PFGE) was performed according to the "One-Day (24-28 h) Standardized Laboratory Protocol for Molecular Subtyping of Non-Typhoidal Salmonella by PFGE"
Plasmid DNA was obtained by the alkaline lysis method according to Kado & Liu (1981). Samples were analysed by electrophoresis in 1X TBE buffer at 150 V on 0.8 % agarose gels with recirculation at 20°C. Plasmids were compared by the use of BioNumerics software.

Results

PFGE. Electrophoresis of DNAs from the 194 isolates digested with either XbaI or BlnI generated thirty-six different macrorestriction profiles. The PFGE types obtained with each of the restriction enzymes did not coincide, and a combination of both profiles (XbaI/BlnI) allowed for a better discrimination. In total, 53 XbaI/BlnI combined types were identified of which 35 were found only in a single isolate from specific farms. In contrast, 6 genomic types accounted for more than 65% of the isolates and were found on different farms and in several batches within the same farm.

Plasmid profile. Thirty-six different plasmid profiles with 0 to 6 plasmids were identified. Thirteen plasmid profiles, containing the serotype-specific plasmid (approximately 90.6 Kb), were frequently found in isolates belonging to S. Typhimurium. The remaining plasmid profiles showed a larger plasmid of about 120 or 140 Kb and predominated in isolates of S. 4,5,12:i:-.

Combination of fingerprinting profiles. The use of various typing methods identified different groups of clones. Therefore, their results were combined to obtain a detailed overall fingerprint type. With the combination of the results described above we were able to identify a total of 79 combined types (CT). The 157 isolates from slaughter pigs were differentiated into 56 CTs, most of them (45 of 56) were represented by one single isolate, while 4 CTs accounted for 60% of the isolates. The vast majority of the CTs were found only in individual batches with the exception of 5 CTs (CT53, CT70, CT55, CT6 and CT23) that were found in several batches from different farms and 4 CTs (TC55, TC23, TC47 and TC14) which were recovered from different batches within the same farm. The 37 isolates belonging to diarrhoea-affected pigs were divided into 25 CT. Only CT18, that was found in 7 S. 4,5,12:i:- isolates, was recovered from more than one farm.

Discussion

Both molecular techniques (PFGE and plasmid profile), individually, discriminated among isolates belonging to different `phagetypes of S. Typhimurium and S. 4,5,12:i:-. However, when used simultaneously a higher discriminatory power was achieved. S. 4,5,12:i:- strains, both from healthy and diarrhoea-affected pigs, formed a genetically homogeneous cluster closely related to contemporary S. Typhimurium isolates.

The heterogeneity of Salmonella isolates between and within pig slaughter batches has been reported previously (Wonderling et al., 2003). In spite of the genetic diversity observed among the isolates from slaughter pigs, some clones were more prevalent and widely distributed among the population being detected in several batches of the same and different farms.

The high percentage of types isolated less frequently during the study period could have resulted from minor alterations in the genetic material of the predominating strains, which may or may not be maintained in the population. It has also been suggested that rarely isolated types could result from rare exposures or introductions of types, which fail to persist. In addition, some strains may be recovered at a different frequency due to differential performance of sampling and isolation techniques. The much higher isolation frequencies of relatively few types are consistent with the description of predominating strains on pig farms and slaughterhouses (Wonderling et al., 2003). Types that were more frequently isolated and shared between several batches of the same or different farms may represent frequent common exposures or may be more apt to survive, be maintained and propagate in the population.
Only one of the combined types isolated from diarrhoea-affected pigs was found in more than one epidemiologically-unrelated farm which suggests a possible pathogenic role of these strains in pigs.

Molecular characterization of *Salmonella* isolates, complemented by conventional epidemiological information is a valuable tool for investigation of the sources of infection and transmission mechanisms of *Salmonella* within pig populations. The information provided is essential for the implementation of efficient control measures within pig production.

**References**


CDC (2002) Standardized Molecular Subtyping of Foodborne Bacterial Pathogens by Pulsed-Field Gel Electrophoresis. Centres for Disease Control and Prevention, Atlanta, Georgia.


