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Conclusion

In conclusion, the high prevalence of Salmonella spp. in pig farms and of Salmonella spp. and coliforms in these types of pork products suggested important safety breakdowns along the pig production and pork elaboration processes. An important effort should be done to reach suitable hygienic and sanitary standards for pig and pork production in the province of Córdoba.

Acknowledgements

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References


31. Characterization of Salmonella enterica serovars Typhimurium and 4,5,[12]:−− isolated from pigs and pig environmental-related sources in the NE of Spain

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Abstract

A total of 117 Salmonella Typhimurium and 59 monophagic Salmonella Typhimurium (S. 4,5,[5,12]:−−) strains isolated from pig, wild bird, rodent, and farm environmental samples were characterized by phage typing, antibiotic susceptibility testing and Multiple-Locus Variable-Number Tandem Repeat Analysis (MLVA) in order to evaluate their phenotypic and genetic relatedness. In S. 4,5,[5,12]:−− the most prevalent phage types were U311 (40.7%) and DT195 (22%) which did not correspond with the so-called Spanish clone, and generally showed a different resistant pattern (ASSuT). Antibiotic resistance was found in 85.8% of the isolates, with 94.1% of them displaying multirdrug resistance (MDR). MLVA identified 92 different profiles, six of them shared by both serovars. The Minimum Spanning Tree showed one major cluster that included 95% of the S. 4,5,[12]:−− isolates, which came from different animal sources, geographic locations and time periods, suggesting high clonality among those Salmonella strains and the ability to spread among pig farms. Phenotypically, isolates of S. 4,5,[12]:−− were closer to European strains than to the well-characterized Spanish clone. The spread of these new strains of S. 4,5,[12]:−− would have been likely favoured by the important pig trade between this Spanish region and other European countries.

Introduction

Salmonella Typhimurium is the second of all reported Salmonella serovars in human-confirmed cases in the EU (Anon., 2015) and the first in Spain (Anon., 2014a). Its monophasic variant, S. 4,5,[5,12]:−−, was the third most common reported serotype among humans, pigs and pork isolates in the EU in the same year (Anon., 2015). This serovar was reported in Spain in 1997 (Echeita et al., 1999) and since then it has been responsible of foodborne outbreaks in Europe. Salmonella Typhimurium and S. 4,5,[5,12]:−− are present in more than 50% of the Salmonella-infected pigs in Spain (Vico et al., 2011), and pig meat is considered an important food vehicle for their transmission to humans (Hauser et al., 2010).

The aim of this study was to gain further insight into the epidemiology, phenotypic and genetic diversity and relatedness of these two main serovars in an area of high pig Salmonella prevalence. For this purpose a collection of S. Typhimurium and S. 4,5,[5,12]:−− isolates obtained from pigs and, wild birds, rodents and pig farm environments were analyzed by phage typing, antibiotic susceptibility testing and Multiple-Locus Variable-number Tandem Repeat Analysis (MLVA).

Material and Methods

A total of 117 isolates of serovar S. Typhimurium and 59 isolates of S. 4,5,[5,12]:−− were used. Ninety-three of these isolates originated from pig mesenteric lymph nodes (MLN), 41 from pig faeces, 22 from wild bird faeces, 5 from rodents and 15 from pig farm environmental samples. The isolates were obtained between February 2008 and September 2012 from a total of 121 pig fattening farms.

Salmonella culture was performed according to the EN ISO 6579:2002/A1:2007 and confirmed isolates serotyped at the National Reference Laboratory for Animal Salmonellosis (Madrid, Spain). A duplex PCR assay

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(Tennant identified, being the most frequent U311 and DT195. observed U311, DT193, DT104b and DT195. Regarding (Anon., 2011). The VNTR loci selected and primers used were those of Lindstedt antimicrobial classes were defined as multidrug resistant (MDR).

MLVA was based on the protocol described by the European Centre for Disease Prevention and Control (Anon., 2011). The VNTR loci selected and primers used were those of Lindstedt et al. (2004). A Minimum Spanning Tree (MST), based on the allelic diversity of the five VNTR loci, was created in BioNumerics (version 6.6.11; Applied Maths, Sint-Martens-Latem, Belgium).

Results

Among the 117 S. Typhimurium strains 16 different phage types were identified, being the most commonly observed U311, DT193, DT104b and DT195. Regarding S. 4,[5],12:: strains, 7 different phage types were identified, being the most frequent U311 and DT195.

One hundred and fifty-one (85.8%) of the isolates were resistant to at least one antimicrobial class (79.5% of the S. Typhimurium and 98.3% of the S. 4,[5],12:: strains). Seventeen different AR patterns were observed (12 in S. Typhimurium and 7 in S. 4,[5],12::). The most frequent resistance phenotypes (R-type) (81%) were ASSuT and ACCSSuT with/without additional resistance to nalidixic acid. Majority of the S. Typhimurium resistant isolates (96.8%) displayed MDR, with the most frequent R-types ACCSSuT and ASSuT with/without additional resistance to nalidixic acid. Among S. 4,[5],12:: resistant isolates 89.6% showed MDR. The most frequent AR pattern was ASSuT; however, ACCSSuT was much less frequent than in S. Typhimurium isolates. Only one isolate was pan-susceptible.

A total of 92 different MLVA profiles were observed, 29 (31.5%) of which were shared by ≥ 2 isolates, and 6 by both serovars. MLVA typing showed 74 different profiles among the S. Typhimurium strains. Fifty-four profiles (73%) were unique to single isolates. The most frequent profiles were 2-11-03-NA-112 (n=8), 3-14-11-NA-211 (n=8) and 4-09-14-10-211 (n=7).

Twenty-four different MLVA profiles were identified for the 59 S. Typhimurium strains and appear to be closer to European isolates. Two main phage types U311 and DT195 were prevalent, both showing the tetra-resistant pattern ASSuT. These S. 4,[5],12:: strains may have evolved from S. Typhimurium strains and appear to be closer to European isolates than to the previously described Spanish clone. The spread of these new strains of S. 4,[5],12:: would have been likely favoured by the important pig trade between this Spanish region and other European countries.

Discussion

Two main clonal lines have been usually considered in the EU: the “Spanish clone” defined as a U302 phage type with plasmid-mediated MDR, and a second one that emerged in several European countries since 2000 and expresses chromosomally-encoded resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (ASSuT R-type) involving DT193 and DT120 phage types (Hopkins et al., 2010). In this study, we found 7 different phage types for S. 4,[5],12::, being the most prevalent U311 and DT195, and U302 one of the less frequent. The fact that the most common phage types for S. Typhimurium and S. 4,[5],12:: (i.e. U311 and DT195) mostly shared same R-type profile, and could be grouped mostly within MLVA cluster I (Figure 1), suggested that these new strains of the monophasic type were more closely related to S. Typhimurium strains than to previous S. 4,[5],12:: strains.

Only a single strain of S. 4,[5],12:: was considered pan-susceptible to the antimicrobials tested, highlighting the high level of resistance among strains within this serotype. Most of these isolates were associated with the R-type ASSuT, which is also the most frequently AR profile from humans and fattening pigs in the UE (Anon., 2010b), but different from the so-called “Spanish clone”. This R-type ASSuT was also one of the AR profiles more commonly seen in S. Typhimurium as well, but far less common than for the monophasic variant. Thus, this observation would add further support to the hypothesis that these monophasic isolates may have likely evolved from European strains of S. Typhimurium. In 86% of the isolates some type of AR was observed, with 94.1% of them displaying MDR. This figure is higher than those reported by previous studies carried on Spain (Astorga et al., 2007).

MLVA patterns found among the 59 S. 4,[5],12:: isolates did not match with the major MLVA patterns observed in the recent study on the Spanish clone (García et al., 2013), but three of the most prevalent MLVA patterns in this study, i.e. 3-13-11-NA-211, 3-13-10-NA-211, and 3-12-10-NA-211, were commonly found in other European countries (Hopkins et al., 2010; Argüello et al., 2014). In addition, the most prevalent phage type found within the monophasic isolates (i.e. U311) has been reported in UK, Italy, and France in recent years (Hopkins et al., 2010). Overall, it seems that the S. 4,[5],12:: isolates identified in this region are different than the so-called Spanish clone but would be genetically closer to other European isolates. These S. 4,[5],12:: strains were isolated from samples from different animal sources, geographic locations or time periods, and were grouped predominantly within cluster I (Figure 1), which may be considered as an indication of the ability to spread of this clone.

Conclusion

This work suggests the emergence of new S. 4,[5],12:: strains among animal isolates in this area. Two main phage types U311 and DT195 would be prevalent, both showing the tetra-resistant pattern ASSuT. These S. 4,[5],12:: strains may have evolved from S. Typhimurium strains and appear to be closer to European isolates than to the previously described Spanish clone. The spread of these new strains of S. 4,[5],12:: would have been likely favoured by the important pig trade between this Spanish region and other European countries.

Acknowledgements

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(Tennant et al., 2010) was used to differentiate between serovar Typhimurium and S. 4,[5],12:i:-. Bacteriophage typing was carried out at the National Reference Laboratory for Human Salmonellosis (Madrid, Spain).

Susceptibility to antimicrobial agents was determined using the Kirby-Bauer disc diffusion method and according to the guidelines of the Clinical and Laboratory Standards Institute (Anon., 2012). Antibioc-resistant (AR) profiles were considered after antimicrobial agents were classified within the following classes: aminopenicillins (A), phenicols (C), aminoglycosides (S), sulphonamides and di-hydrofolate reductase inhibitors (Su), tetracyclines (T), cephalosporins (Cf), and quinolones (Na). Isolates displaying resistance to three or more antimicrobial classes were defined as multiresistant (MDR).

MLVA was based on the protocol described by the European Centre for Disease Prevention and Control (Anon., 2011). The VNTR loci selected and primers used were those of Lindstedt et al. (2004). A Minimum Spanning Tree (MST), based on the allelic diversity of the five VNTR loci, was created in Bionumerics (version 6.6.11; Applied Maths, sint-Martens-Latem, Belgium).

Results

Among the 117 S. Typhimurium strains 16 different phage types were identified, being the most commonly observed U311, DT193, DT104b and DT195. Regarding S. 4,[5],12:i:-, 7 different phage types were identified, being the most frequent U311 and DT195.

One hundred and fifty-one (85.8%) of the isolates were resistant to at least one antimicrobial class (79.5% of the S. Typhimurium and 98.3% of the S. 4,[5],12:i:-); and 14 different AR patterns were observed (12 in S. Typhimurium and 7 in S. 4,[5],12:i:-). The most frequent resistance phenotypes (R-type) (81%) were ASSuT and ACSSuT with/without additional resistance to nalidixic acid. Majority of the S. Typhimurium resistant isolates (96.8%) displayed MDR, with the most frequent R-types ACSSuT and ASSuT with/without additional resistance to nalidixic acid. Among S. 4,[5],12:i:- resistant isolates 89.6% showed MDR. The most frequent AR pattern was ASSuT; however, ACSSuT was much less frequent than in S. Typhimurium isolates. Only one isolate was pan-susceptible.

A total of 92 different MLVA profiles were observed, 29 (31.5%) of which were shared by ≥ 2 isolates, and 6 by both serovars. MLVA typing showed 74 different profiles among the S. Typhimurium strains. Fifty-four profiles (73%) were unique to single isolates. The most frequent profiles were 2-11-03-NA-112 (n=8), 3-14-11-NA-211 (n=8) and 4-09-14-10-211 (n=7).

Twenty-four different MLVA profiles were identified for the 59 S. 4,[5],12:i:- isolates analyzed in this study. The most four common, accounting for 46% of strains, were 3-13-11-NA-211 (11 isolates); 3-12-10-NA-211 (6 isolates); 3-11-05-NA-211 and 3-12-10-NA-211 with 5 isolates each.

The MST (Figure 1) showed one major cluster (I) and 10 minor ones (II-XI). Cluster I included 35 distinct MLVA profiles, representing 89 isolates from all origins. Ninety-five per cent (56/59) of S. 4,[5],12:i:- isolates were grouped in cluster I. There was an apparent association between resistance types with MLVA clusters as 93% (62/67) of the isolates with R-type ASSuT belonged to cluster I. Likewise, 100% of isolates from clusters II, IV and VII were ACSSuT (with or without nalidixic acid resistance) while in the other clusters just a few isolates showed that R-type. Clusters III and VI were characterized by including most of the pan-susceptible isolates. Fifteen MLVA profiles grouped isolates from different sources.

Discussion

Two main clonal lines have been usually considered in the EU: the “Spanish clone” defined as a U302 phage type with plasmid-mediated MDR, and a second one that emerged in several European countries since 2000 and expresses chromosomally-encoded resistance to ampicillin, streptomycin, sulphonamides and tetracyclines (ASSuT R-type) involving DT193 and DT120 phage types (Hopkins et al., 2010). In this study, we found 7 different phage types for S. 4,[5],12:i:-, being the most prevalent U311 and DT195, and U302 one of the less frequent. The fact that the most common phage types for S. Typhimurium and S. 4,[5],12:i:- (i.e. U311 and DT195) mostly shared same R-type profile, and could be grouped mostly within MLVA cluster I (Figure 1), suggested that these new strains of the monophasic type were more closely related to S. Typhimurium strains than to previous S. 4,[5],12:i:- strains.

Only a single strain of S. 4,[5],12:i:- was considered pan-susceptible to the antimicrobials tested, highlighting the high level of resistance among strains within this serotype. Most of these isolates were associated with the R-type ASSuT, which is also the most frequently AR profile from humans and fattening pigs in the UE (Anon., 2010b), but different from the so-called “Spanish-clone”. This R-type ASSuT was also one of the AR profiles more commonly seen in S. Typhimurium as well, but far less common than for the monophasic variant. Thus, this observation would add further support to the hypothesis that these monophasic isolates may have likely evolved from European strains of S. Typhimurium. In 86% of the isolates some type of AR was observed, with 94.1% of them displaying MDR. This figure is higher than those reported by previous studies carried on Spain (Astorga et al., 2007).

MLVA patterns found among the 59 S. 4,[5],12:i:- isolates did not match with the major MLVA patterns observed in the recent study on the Spanish clone (García et al., 2013), but three of the most prevalent MLVA patterns in this study, i.e. 3-13-11-NA-211, 3-13-10-NA-211, and 3-12-10-NA-211, were commonly found in other European countries (Hopkins et al., 2010; Argüello et al., 2014). In addition, the most prevalent phage type found within the monophasic isolates (i.e. U311) has been reported in UK, Italy, and France in recent years (Hopkins et al., 2010). Overall, it seems that the S. 4,[5],12:i:- isolates identified in this region are different than the so-called Spanish clone but would be genetically closer to other European isolates. These S. 4,[5],12:i:- strains were isolated from samples from different animal sources, geographic locations or time periods, and were grouped predominantly within cluster I (Figure 1), which may be considered as an indication of the ability to spread of this clone.

Conclusion

This work suggests the emergence of new S. 4,[5],12:i:- strains among animal isolates in this area. Two main phage types U311 and DT195 would be prevalent, both showing the tetra-resistant pattern ASSuT. These S. 4,[5],12:i:- strains may have evolved from S. Typhimurium strains and appear to be closer to European isolates than to the previously described Spanish clone. The spread of these new strains of S. 4,[5],12:i:- would have been likely favoured by the important pig trade between this Spanish region and other European countries.

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Abstract

The objective of this study was to investigate the association of single nucleotide polymorphisms (SNPs) in innate immune response genes with Salmonella colonization in nursery pigs. Eight swine farms (7 farrow-to-finish farms and one farrow-feeder farm) in southern Ontario, Canada were included in this study. On each farm, 21 pigs were selected from seven sows at weaning. Fecal samples were collected from selected pigs and cultured for Salmonella, and the isolates were serotyped. DNA was extracted from liver samples and used to genotype pigs for single nucleotide polymorphisms (SNPs) in 21 different innate immune response genes. In total, 15 (9.3%) pigs tested positive for Salmonella; the isolates from six pigs from four different litters on one farm were serotyped as Salmonella Infantis and from nine pigs from six different litters on another farm as Salmonella Hertzing. SNP analysis showed an association of Salmonella shedding with a SNP in the genes encoding mannann-binding lectin (MBL)-associated serine protease-2 (MASP-2) and Toll-like receptor-1 (TLR-1) (P < 0.05). These findings suggest that Salmonella shedding in pigs is controlled by genetic elements and these genetic variants could possibly be used to breed pigs that are more resistant to Salmonella colonization and Salmonella shedding.

Introduction

Salmonella is estimated to cause more than 1.2 million illnesses each year in the United States, with more than 23,000 hospitalizations (the leading cause of hospitalization due to foodborne illnesses), and 450 deaths (Scallan et al., 2011). In Canada, it is estimated that there are 88,000 human cases of salmonellosis annually (Thomass et al., 2011). Painter et al. (2013) have recently estimated that 46 % and 22 % of annual gastroenteritis cases associated with foodborne outbreaks in the United States are attributed to produce (fruits, nuts, and vegetable) and meat (beef, pork, and poultry) commodities, respectively. Asymptomatic pigs play a significant role in the spread and transmission of Salmonella from farm to fork. In fact, a major food safety concern is that Salmonella can be transmitted from pig farms to pork products at slaughterhouses, as well as to ground water and produce if the pig manure is used as land fertilizer. Despite a variety of suggested control measures for Salmonella, asymptomatic carrier pigs are very difficult to eliminate. As such, genetic selection for resistance against a Salmonella carrier-state could be utilized as an effective method to control Salmonella in pigs. However, the genetic markers causing the variation in response to Salmonella colonization and shedding, as part of the innate, cell-mediated and/or antibody mediated responses among pigs are still unknown. The objective of this study is to identify if single nucleotide polymorphisms in genes involved in pattern recognition and innate immunity are associated with altered Salmonella colonization and carrier-state status.

Material and Methods

Eight swine farms (7 farrow-to-finish farms and one farrow-feeder farm) in southern Ontario, Canada were included in this study. On each farm, 21 pigs were selected from 6-7 sows at weaning; in total 168 pigs (21 piglets per farm) were selected from 55 sows. Fecal samples were collected for Salmonella culturing at weaning. The piglets were euthanized at 5 weeks post-weaning and tissue samples collected from...