Abstract

The study of the prevalence of Salmonella spp. in the pig and pork production chain is important to reduce the risk of spreading this pathogen into the human population. The aim of this study was to estimate the prevalence of Salmonella spp. in pigs and pork products from Córdoba. Mesenteric lymph nodes samples from 580 finishing pigs from different pig farms, and 420 samples (83 batches) of fresh pork products (the so-called “chorizo fresco de cerdo”) from different retailers were analyzed. ISO 6579:2002 standard protocol was used for Salmonella isolation. Prevalence of Salmonella spp. in finishing pigs in Córdoba was 41.5% (95%CI 37.6%-45.6%). According to pig’s origin the province was split in North, Central and South region. The prevalence observed in each region was 49.5%, 43.0% and 36.0% respectively. The significantly higher prevalence observed in the North may be associated with the precarious conditions of the pig production sector in that area, compared to the better pig producing practices in the southern region. Prevalence of Salmonella spp. in fresh pork products was 17.4% (95%CI 15.8%-23.4%). Based on the Argentine Food Codex criteria, 34/83 (40.9%) of the analyzed batches should be rejected given the presence Salmonella spp. positive. In addition, total coliforms were also analyzed on these pork samples following national legislation and 42 (63.0%) of the batches should have been rejected according to coliform total count. Overall, only 13% of the batches fulfilled both criteria for acceptance. In conclusion, the high prevalence of Salmonella spp. in pig farms and of Salmonella spp. and coliforms in these type 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.

Introduction

Salmonella spp. is recognized as major zoonotic pathogens of economic significance in animals and humans; the infection caused by these bacteria is one of the most frequently reported foodborne diseases worldwide. Pigs farming is increasing in Argentine in the last decade, and Córdoba it is the second major pig producing region in the country. The study of the prevalence of Salmonella spp. in the pig and pork production chain is important to reduce the risk of spreading this pathogen into the human population. The aim of this study was to estimate the prevalence of Salmonellas pp. in pigs and pork products from Córdoba.

Material and Methods

Study design and sampling collection. Pig sampling was carried out on six abattoirs between February 2014 and February 2015. A total of 580 pigs from 20 different pig herds (an average of 30 animals per herd) were analyzed. In addition, between July 2014 and July 2015, 420 retail pork products, the so-called “chorizo fresco porcino”, were obtained from different retail stores (batcher shops or supermarkets) in Córdoba. These latter samples were collected in batches of 5 samples following Argentine regulation on Food safety (ANMAT, 2013).

Determination of the presence of Salmonella spp. Bacteriology was performed on MLN and pork samples, following the standard International Organization for Standardization Method 6579:2002.
Enterobacteria (coliforms) count. An aliquot was obtained and serial dilutions were made for total Enterobacteria (coliforms) counting. From each dilution 100 μl was spread in petri dishes by duplicate containing violet red glucose agar (Britania®). Plates were incubated at 37 ºC for 24hs. Plates containing < 250 typical Enterobacteria colonies (pink or red, with or without precipitation) were counted. Microbiological results for Salmonella spp. and total count of Enterobacteria were compared to Argentine Microbiologic criteria (ANMAT, 2013) for food safety.

Statistical analysis. Basic descriptive statistics were performed. Individual and herd/batch prevalence with their corresponding 95% confidence intervals (95% CI) were estimated for pigs and pork samples. Enterobacteria counts were transformed to log 10 CFU/g prior to statistical analysis.

To study the possible association between Enterobacteria numbers and some factors (being a Salmonella positive/negative sample, type of retail store, etc.) a Student t-test was used. Data analysis was carried out using InfoStat versión 2015 free version. (Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. URL http://www.infostat.com.ar)

### Results

The prevalence of Salmonella in MLN was 41.5% (95%CI: 37.6%-45.6%). The province was divided in the North, Central and South regions, and the prevalence observed was high for the three of them, although slightly lower in the South (49.5%, 43.0% and 36.0% respectively). The overall prevalence of Salmonella spp. in fresh pork products was 17.4% (95%CI: 15.8%-23.4%). Butcher shops showed a higher proportion of Salmonella positive samples (Table 1).

#### Table 1. Prevalence of Salmonella samples by type of retail store.

<table>
<thead>
<tr>
<th>Type of retail store</th>
<th>Positive Salmonella samples</th>
<th>%</th>
<th>95%CI</th>
</tr>
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<tbody>
<tr>
<td>Butcher shop</td>
<td>35/130</td>
<td>26.92</td>
<td>20.01-35.16</td>
</tr>
<tr>
<td>Supermarket</td>
<td>38/290</td>
<td>13.10</td>
<td>9.66-17.5</td>
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A total of 83 batches were considered and 41.0% were positive to Salmonella. The mean of Enterobacteria counts was 3.35 log 10 UFC/g (range 1.30 – 4.51 log 10 CFU/g). Figure 1 showed the results after applying national food safety microbiological criteria for Enterobacteria and Salmonella on the 83 batches. A total of 52 batches exceeded the Enterobacteria total count allowed for national food safety criteria.

Discussion

Salmonella prevalence results in pigs samples were high in Córdoba. Those results were in line with previous studies that found high seroprevalence/prevalence of infection in pig populations from the region (Vigo et al., 2009; Ibar et al., 2009). Although no clear significant association was found between pig Salmonella infection and regions, the higher prevalence observed in the North may be associated with the precarious conditions of the pig production sector in that area, compared to the better pig producing practices in the southern region, as it was described in a technical report on the pig production chain in the region of Córdoba (INTA, 2012). In addition, farms situated at North had longer transport times from the farm to the abattoir as slaughterhouses are located in the Central and South regions and this could be a risk factor (Vico et al., 2014)

Figure 1. Percentage of acceptance or rejection of pork’s batches according to National Microbiological criteria

This is the first study made with samples obtained at retail markets. Due to the absence of official data, we compared Salmonella prevalence in pork’s products with other countries. Salmonella prevalence in pork’s products was higher in Córdoba compared to Ireland (2.6%; Prendergast et al., 2009), United Kingdom (3.9%; Little et al., 2008), Spain (4.5%; Domenech et al., 2015). However Salmonella prevalence was somewhat lower when compared to countries within the same region, such as Brazil (24.6%; Murmann et al., 2008).

The significant differences observed on the proportion of Salmonella positive samples when butcher shops and supermarkets were compared could be explained in part by the lower hygienic standards when manufacturing pork products by the former. Based on the Argentine Food Codex regulations, an important percentage of batches should be rejected given the presence Salmonella spp. positive.

Enterobacteria counts are used as indicators of good manufacturing practices and hygienic conditions during manufacturing of pork. According to national microbiological criteria for food safety, batches with Enterobacteria counts higher than 2.6 log 10 UFC/g are considered as non-viable for human consumption. Thus, in this survey, a large proportion of the batches should have been also rejected. Overall, only 22/83 (26.5%) of the batches fulfilled both criteria for acceptance.

Despite some studies suggest a positive correlation between Enterobacteria total counts and Salmonella presence, and therefore the possibility of some modeling predictions (Ruby and Ingham, 2009), we did not find any association between these two variables.
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One health approach under a concept of farm to fork

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

Andrés-Barranco, S.1, Vico, J.P1, Marin, C.M.1, Herrera-León, S.1, Mainar-Jaime, R.C.1*

Abstract

A total of 117 Salmonella Typhimurium and 59 monophasic Salmonella Typhimurium (S. 4,5,[5,12]:i:-) 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,[12]:i:-: 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 multidrug 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]:i:- 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]:i:- were closer to European strains than to the well-characterized 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.

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,[12]:i:-; 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,[12]:i:- 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]:i:- 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,[12]:i:- 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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