Salmonella Brandenburg in the pork chain in Italy: Genetic comparison with the human isolates

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Abstract

Salmonella Brandenburg ranked 16th among the serovars responsible for human infections in EU in 2015 and it was found to be associated with swine. In Emilia-Romagna and Lombardy regions of northern Italy, *S.* Brandenburg was isolated from mesenteric lymph nodes, fecal matter, carcasses and conveyor belts at pig slaughterhouses in 2014 and 2015. In the same area, *S.* Brandenburg was detected in pork salami in 2015. In the present study, 12 isolates of *S.* Brandenburg recovered from the pork food-chain were typed by *Xba*I PFGE and their three profiles were compared to all human *S.* Brandenburg isolates processed by the Surveillance System of Emilia-Romagna region from 2012 to 2017 (105 isolates). The most frequent pulsotype of porcine origin (6/12) was the second most frequent in humans (16/105). Of the other two pulsotypes of porcine origine (3/12 each), one was the most frequent in humans (41/105), the other was undetected among human isolates.

Introduction

Salmonella infections in humans are commonly foodborne, with an important source being food of animal origin. The most frequently implicated foodstuffs are table eggs, pig meat, poultry meat and cheese, which were responsible for 21.2%, 13%, 9.2% and 8.7% of the strong-evidence outbreaks notified in the European Union in 2015, respectively (EFSA and ECDC, 2016).

In 2015, the most common *Salmonella* serovars isolated from pork and pork products in the EU countries were *S.* Derby (22.9%), *S.* Typhimurium monophasic variants (22.5%), *S.* Typhimurium (20.6%), *S.* Rissen (5.1%), *S.* Infantis (4.1%), *S.* Brandenburg (1.7%) and *S.* London (1.6%) (EFSA and ECDC, 2016). However, in 2014, the prevalence of *S.* Brandenburg from pig meat and products thereof was 4.9% in the EU reporting countries (EFSA and ECDC, 2015). In 2015, *S.* Brandenburg ranked 16th among salmonellae of animal origin in the EU, with pigs as the unique animal source linked to the serovar. A recent study identified *S.* Brandenburg in 2.5% of the mesenteric lymph nodes and 14% of the fecal samples in pigs at slaughter in Italy. In that survey, *S.* Brandenburg was the third most frequently detected serovar in pig feces after *S.* Derby (51%) and *S.* Typhimurium monophasic variant 4,[5],12:i:- (20%) (Bonardi *et al*., 2016a). *S.* Brandenburg was also isolated from pig carcasses and conveyor belts in 2015 (Bonardi, unpublished data). Since this serovar is among the 10 most common serovars associated with human cases of salmonellosis in the Emilia Romagna region (northern Italy) (Surveillance System of Emilia-Romagna Region, 2016) a comparison of the genetic lineages circulating in pigs and humans of the same area would be informative of the sharing of this agent between the two compartments. The level of sharing is an indicator of the role of pigs as the source of infection for humans and this report may be useful for source attribution studies during foodborne outbreaks.

The aim of our report is to evaluate the sharing of *S.* Brandenburg lineages between pigs and humans in northern Italy to infer the role of pigs as reservoir of human infection.

Materials and Methods

Detection of *S.* Brandenburg from pork salami

During 2015, a total of 100 ready-to-eat pork salami were purchased at retail in the provinces of Parma, Piacenza, Reggio Emilia, Mantua and Modena (Emilia-Romagna and Lombardy regions, northern Italy). The samples were represented by 39 salami Felino (manufactured in Parma province only), 37 salami Milano (manufactured in all provinces), 11 salami Piacentino (manufactured in Piacenza province only), which are characterized by a curing period of 30-40 days. The remaining samples were represented by 13 salami *strolghino*, which are small and short-curing products (13-15 days of curing) manufactured in all provinces.

Pork salami are prepared with ground meat mixed with salt and pepper. Nitrates and nitrites are added to inhibit *Clostridium botulinum* (Hospital *et al*., 2016) and to favour the cured red colour of the meat (Villaverde *et al.*, 2014). Many salami formulations contain starter bacterial cultures to improve safety of the final product and standardize the production process (Lücke, 2000).

Detection of *Salmonella* was performed by real-time PCR followed by microbiological confirmation. Aliquots of 25 g of samples were suspended in 225 mL of Buffered Peptone Water (BPW, Oxoid, Basingstoke, UK) and homogenized for 2 minutes in a Stomacher blender. After 18±2 h at 37±1°C DNA was extracted from 1 mL of the pre-enrichment culture using SureFood PREP *Salmonella* Kit (R-Biopharm, Darmstadt, Germany) and PCR master-mix was prepared with SureFast *Salmonella* ONE Kit (R-Biopharm) for a final volume of 25 µL containing 5 µL of template DNA. PCR reactions were run on a Mx3005P QPCR System (Agilent Technologies, Italy) with the following thermal program: a cycle of DNA polymerase activation of 5 min at 95°C followed by 45 amplification cycles of 15 s at 95°C and 30 s at 60°C (annealing-extension step). Positive samples (cycle threshold value lower than 40) were

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confirmed following ISO 6579:2002. Serotyping of the isolates was performed following the White-Kauffmann-Le Minor scheme by slide agglutination with O and H antigen specific sera (DID, Milan, Italy; Biogenetics, Padua, Italy). Discrimination of *S.* 4,[5],12:i:- from *S.* Typhimurium was done genotypically by PCR (Barco *et al.*, 2011).

Pulsed-field gel electrophoresis of *S.* Brandenburg isolates of porcine and human origin

Pulsed-field gel electrophoresis (PFGE) typing is based on the digestion of chromosomal DNA with a rare-cutting restriction enzyme, followed by electrophoresis. In this study PFGE was performed according to standard methods (PulseNet, 2010) with *Xba*I (Roche Italia, Milan, Italy) restriction of DNA. PFGE patterns were analyzed using Bionumerics Software Package (Applied Maths) and Cluster analysis was performed using the Dice coefficient of similarity and the UPGMA method (tolerance and optimization were set up at 1%).

A total of 12 *S.* Brandenburg isolates of porcine origin were tested to identify different pulsotypes circulating among pigs and pork products in Italy. Three strains were detected from the pork salami and the remaining nine were previously isolated from pig carcasses, meat conveyors, mesenteric lymph nodes (MLN) and fecal samples in northern Italy from 2014 to 2015 (Bonardi *et al*., 2016a; Bonardi, unpublished data). In the previous studies pig carcasses were tested swabbing four different carcass sites of 100 cm^2 each (back, belly and jowl externally and the diaphragmatic area internally), working surfaces were tested swabbing areas of various size (200 cm^2 to 400 cm^2 *ca.*) and fecal samples were collected at lairage swabbing the pens floor for approximatively 1600 cm^2. Swabbing technique was performed using sterile sponges moistened with Buffered Peptone Water. MLNs were aseptically collected at slaughter after carcass evisceration and placed in sterile containers. The obtained PFGE profiles were compared to the profiles of 105 *S.* Brandenburg strains of human origin (Surveillance System of Emilia-Romagna region from 2012 to 2017), to detect shared pulsotypes between porcine and human compartments.

Results

A total of 6/100 (6%) salami was found to be contaminated by *Salmonella*. *S.* Brandenburg was isolated from 3 samples, while *S.* Typhimurium monophasic variant 4,[5],12:i:-, *S.* Rissen and *S.* London were isolated from one sample each, respectively. Overall, three PFGE profiles were identified among the 12 isolates of *S.* Brandenburg recovered from the pork food-chain (pulsotypes SXB_BS.0062; SXB_PR.0657; SXB_PR.1100 - codes used within the Surveillance System of Emilia-Romagna region; Figure 1). Among all human *S.* Brandenburg isolates processed by the Surveillance System of Emilia-Romagna region from 2012 to 2017 (105 isolates), 20 different pulsotype were identified. The most frequent pulsotype in samples from pig carcasses and slaughter environment (SXB_BS.0657, 6/12 isolates) was the second most frequent in humans (16/105 isolates). Of the other two pulsotypes of pig origin (SXB_BS.0062, SXB_PR.1100, 3/12 isolates each), SXB_BS.0062, which was detected in pork salami, was the most frequent in humans (41/105 isolates). On the contrary the other pulsotype, isolated from swine fecal matter, was undetected among human isolates.

Discussion

S. Brandenburg was not considered a common *Salmonella* serovar until recent years and data from literature are poor. It has probably a restricted host range, with the pig and pork meat as the most important non-human source (Mammina *et al.,* 2011). Data shown in this study were collected from different surveys performed on pigs at slaughter (Bonardi *et al.,* 2016a; Bonardi, unpublished data) and from a study on *Salmonella* contamination in pork salami. The detection of *S.* Brandenburg in the pork chain (mesenteric lymph nodes, rectal content, carcasses, slaughter equipment, salami) is of concern because this serovar has been recently responsible for human cases of salmonellosis in Italy (Surveillance System of Emilia-Romagna region, 2016). Moreover, an outbreak involving 27 persons caused by *S.* Brandenburg occurred in Tuscany region (central Italy) in 2008, probably linked to consumption of a meat dishes combination including lamb, pork, beef, rabbit and sausages (Mammina *et al*., 2011).

Recent EU studies reported the detection of *S.* Brandenburg in pigs at slaughter. In Belgian slaughterhouses *S.* Brandenburg was isolated from pig carcasses after split-

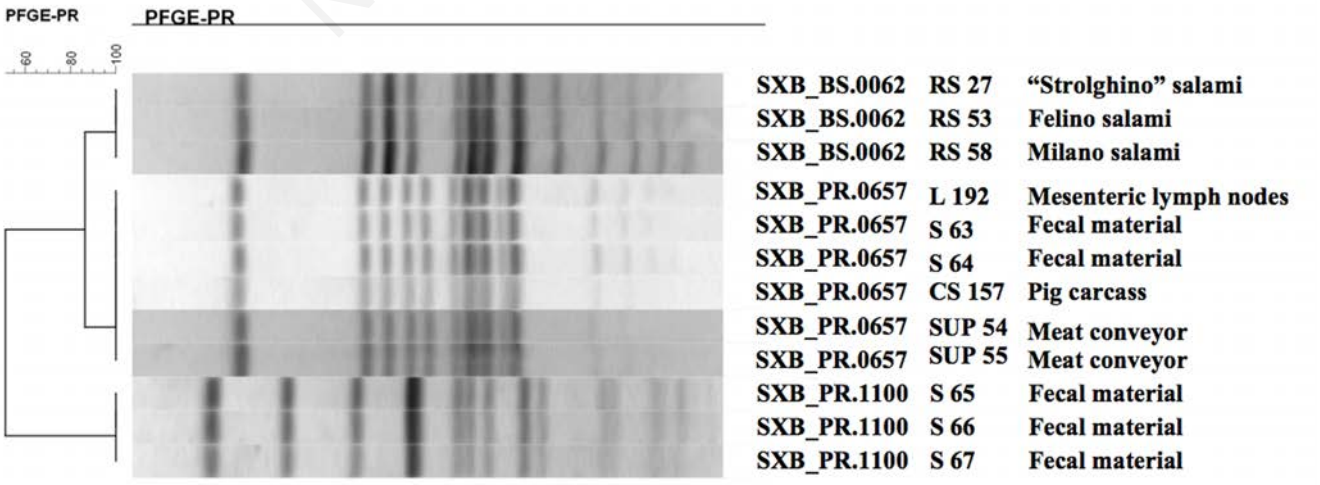

Figure 1. Dendrogram and XbaI PFGE profiles of *S.* Brandenburg of porcine origin.

ting and overshoes in the lairage area. The most common serovars were Typhimurium (58.7%), Rissen (17.4%), Derby (8.3%) and Brandenburg (5.8%) (De Busser *et al.,* 2011). In a Dutch slaughterhouse *S.* Brandenburg was isolated from the pig carcasses after exsanguination and their rectal swabs, with a prevalence of 18%. The other most prevalent serovars of the study were Derby (38%) and Typhimurium (36%) (van Hoek *et al*., 2012).

Contamination of carcasses during the slaughter processing may occur in two ways, *i.e.* i) direct contamination from skin, intestinal content and lymph nodes of pigs; ii) indirect contamination *via* equipment and workers (the so called *cross-contamination*) (Bonardi *et al.,* 2016b; Castelijn *et al.*, 2013; van Hoek *et al.,* 2012). Contaminated equipment may play an important role especially when the microorganisms are able to form biofilm, which enhance their survival and persistence in the slaughterhouse environment. A recent study revealed that *S.* Brandenburg has a greater biofilm forming capacity than *S.* Typhimurium and *S.* Derby and longer survival than *S.* Typhimurium (Castelijn *et al.,* 2013). Furthermore, disinfectants are usually less effective for biofilms than planktonic cells, especially if organic material is present. Since organic material is often present in the pig slaughtering environment even during disinfection treatments, survival of biofilm forming microorganisms is enhanced (Castelijn *et al.,* 2013). The biofilm formation may therefore contribute to the persistence of *S.* Brandenburg in the pork processing environment, thus causing cross-contamination of fresh pork and pork products.

Conclusions

The observed partial sharing of genetic lineages of *S.* Brandenburg between the swine and human compartments, as determined through *Xba*I PFGE (two out of three pulsotypes of porcine origin were detected among human isolates; Figure 1) is indicative of a likely role of pigs as reservoir of infection for humans in northern Italy. Nevertheless, the absence of one of the swine pulsotype among human isolates could be suggestive of a differential ability of different pulsotypes to infect humans or the presence of alternative infection sources. Unfortunately, the lack of information concerning the infection vehicles responsible for the human cases of salmonellosis obstacles their attribution to other possible sources. However, it must not be ruled out that other animal species, as well as environmental sources, can contribute to human infections by *S.* Brandendurg.

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