Epidemiology and control of hazards in pork production chain – SAFEPORK
One health approach under a concept of farm to fork

Conclusion
The carcasses contamination was not statistically different between slaughterhouses removing the tongue on the slaughter line compared to the ones leaving the tongue intact inside the head.

This, despite the experimental design, we were not able to confirm that the removal of the tongue on the slaughter line had a significant impact on the carcass contamination with Yersinia enterocolitica. However, these results confirmed that the carcasses contamination is linked to the initial contamination of the corresponding tonsils. Cross contaminations appeared to be low but existed and good hygiene practices remain necessary to limit the transfer of Y. enterocolitica from the tonsils, or the feces, to the carcasses.

Acknowledgements
This study received financial support from FranceAgriMer.

References

04. Biofilm formation by Salmonella enterica strains isolated from feed mills
Lavivini, V.1,2, Lopes, G. V.3,4, Pellegrini, D. P.5,6

Abstract
Feed supplied to pigs is considered an important vehicle for Salmonella enterica subsp. enterica introduction on farm. Salmonella can be able to form biofilm on several abiotic surfaces, which may contribute to environmental persistence. This study aimed to evaluate the biofilm formation capacity in Salmonella strains isolated from four Brazilian feed mills. The biofilm formation was assessed in 54 Salmonella isolates belonging to different serovars by phenotypic assays: i. expression of curli fimbriae and cellulose in Luria-Bertani agar supplemented with Congo red, Coomassie brilliant blue and calcofluor; ii. adhesion on 96-well polystyrene microtiter plates. The results showed that all isolates presented the rdar morphotype (read, dry and rough colonies) on agar incubated at 28° C. From the total of isolates displaying rdar-morphotype, 14.8% (8/54) showed to be weakly adherent on polystyrene microtiter plates, and were thus considered presumptively biofilm producers. These strains were originated from ingredients and equipment samples, and were distributed among the following serovars: Montevideo (n=2), Senftenberg (n=2), Tennessee (n=1), Orion (n=1), Morehead (n=1), and S. enterica O: 16 (n=1). In this sense, biofilm formation might have played a role in Salmonella colonization of equipment in feed mills, and should be further investigated.

Introduction
Salmonella can be able to form complex communities attached to surfaces, and this biofilms may contribute to its persistence in the environment (Steenackers et al., 2012). The formation of biofilms has been assessed in vitro mainly by phenotypic methods, such as colony morphology and adhesion to abiotic surfaces (Stepanovic et al., 2000; Malcova et al., 2008). Several studies demonstrated that bacterial isolates, which are originated from residual contamination of food handling environments, often display positive results in biofilm formation phenotypic tests (Stepanovic et al., 2004; Vestby et al., 2009).

The feed supplied to the animals is considered an important vehicle for the introduction of Salmonella in pig farms (Binter et al., 2011). Salmonella contamination during feed manufacturing process has been related to the use of contaminated ingredients or the persistence of strains in the environment (Wierup & Häggblom, 2010; Pellegrini et al., 2015). In this sense, biofilm formation by Salmonella strains on the equipment surface may play an important role in the persistence of certain strains reported in feed mills (Pellegrini et al., 2015). Thus, the aim of this study was to assess the biofilm formation ability of Salmonella strains isolated at various stages of feed processing.

Material and methods
Fifty-four Salmonella strains isolated during a cross-sectional study conducted in four feed mills were tested. The strains were originated from ingredient or environmental samples and belonged to sixteen serovars: Agona (n = 5), Anatum (n = 4), Cerro (n = 1), Infantis (n = 2), Mbandaka (n = 1), Montevideo (n = 18), Morehead (n = 1), Newport (n = 2), Orion (n = 3), Salmonelea enterica O:3,10 (n = 2), Salmonella enterica O:16:– (n = 1), Schwarzenburg (n = 1), Senftenberg (n = 6), Tennessee (n = 4), Typhimurium (n = 1) and Worthington (n = 2).
Biofilm formation was assessed by two phenotypic assays: i. morphology of colonies formed on Luria-Bertani (LB) agar; ii. adhesion on polystyrene microtiter plates. *Salmonella* strains were streaked on Luria Bertani agar with low salt content (LB low salt; Sigma-Aldrich, St. Louis, USA) supplemented with 40 µg/mL of Congo Red (Sigma-Aldrich) and 20 µg/mL of Coomassie brilliant blue (Sigma-Aldrich). Cellulose production was determined on LB agar supplemented with 50 µM Calcofluor (Fluorescent brightener 28) (Sigma-Aldrich). The plates were incubated at 37°C for 24 hours or at 28°C for 96 hours. After incubation, colony morphology was classified as: rdar (presence of curli fimbriae and cellulose), pdar (presence of cellulose), bdar (presence of curli fimbriae), saw (absence of curli fimbriae and cellulose). For cellulose detection, colony fluorescence was evaluated under UV light at 366 nm (Römling et al., 2003). All assays were performed in duplicate and repeated three times in different days. The adherence test was conducted in flat bottomed 96 well polystyrene plates (TPP® Techno Plastic Products AG, Switzerland) containing 20 µL of bacterial suspension (approximately 5 x 10^8 CFU/mL) and 230 µL of Tryptic Soy Broth without glucose (TSB; Bacto®, New Jersey, USA). After incubation at 37°C for 24 hours, the microplates were processed according to the protocol described by Stepanovic et al. (2004), and evaluated by spectrophotometry at 570 nm. The optical density cut-off (ODc) was defined as the mean OD of the negative control (culture medium) and the isolates were classified as follows: non-adherent (ODc o 0.1 ODc); weak adherent (0.1 ODc < 2 x ODc); moderate adherent (2 x ODc < 4 x ODc); and strong adherent (ODc > 4 x ODc). *Staphylococcus epidermidis* ATCC 35984 and *Salmonella Typhimurium* ATCC14028 were included in each plate as positive controls. All tests were performed in triplicate and repeated in three different days.

**Results**

Among the 54 *Salmonella* isolates tested, all presented the rdar morphotype at 28°C and saw morphotype at 37°C (Figure 1). Among them, 14.8% (8/54) showed to be weak adherent on polystyrene microtiter plates, and were considered as presumptive biofilm producers. These isolates belonged to six serovars [Montevideo (n=2), Serftenberg (n=2), Tennessee (n=1), Orion (n=1), Morehead (n=1), and *S. enterica* sub. *enterica* O: 16 (n=1)], and were originated from equipment (n=5) as well as ingredients delivered at the feed mills (n=3).

**Discussion**

In this study, we evaluated the phenotypic characteristics associated with biofilm formation capability in *Salmonella* isolates originated from feed mills. All isolates expressed rdar morphotype at 28°C, which is characterized by the production of an adhesive extracellular matrix consisting of curli fimbriae and cellulose (Römling et al., 2003). The curli fimbriae are considered to be expressed in response to nutrient limitation, while cellulose is an extracellular component produced for mechanical and chemical protection of bacterial cells (Jain & Chen, 2010). Both components are important for survival under challenging conditions, which may be encountered by bacteria in the environment. Moreover, the expression of curli fimbriae and cellulose, leading to rdar morphotype, was temperature dependent, indicating that both components may play a much more important role at room temperature. The adherence test to abiotic surfaces revealed that 14.8% (8/54) of these isolates were weakly adherent to polystyrene plates, and were thus considered as presumptive biofilm producers. These strains were originated from ingredient delivered at the feed mills or were isolated from dust or debris samples taken from the equipment surfaces. The results indicate that *Salmonella* isolates able to produce biofilm may be introduced in feed mills through contaminated ingredients. The formation of biofilm in turn is influenced by several environmental factors (temperature, surfaces, nutrients and pH), which regulate the expression of genes responsible for biofilm formation (Linou & Koutsoumanis, 2012, Nguyen & Yuk, 2013, Simm et al., 2014). In this sense, the environmental conditions found inside feed mills, such as high moisture and temperature, may influence the formation of biofilm by certain *Salmonella* isolates, allowing their residual colonization of surfaces. The influence of these factors in the formation of biofilms needs to be further investigated.

**Conclusion**

Strains isolated from feed mills can be able to form biofilm. This fact might play a role in *Salmonella* residual colonization of equipment, and should be further investigated.

**Acknowledgements**

Laviriki V. is supported by scholarship of the Higher Education Personnel Improvement Coordination (CAPES, Brazil). Lopes G. V. is engaged in the Post-doctoral program of the Higher Education Personnel Improvement Coordination (PNPD-CAPES, Brazil).

**References**

- Jain, S., Chen, J., 2010. Attachment and biofilm formation by various serotypes of *Salmonella* is influenced by cellulose production and thin aggregate fimbriae biosynthesis. J. Food Prot., 70, 2473-2479.

[11]Departamento de Medicina Veterinaria Preventiva, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil
*corresponding author: mcardoso@ufrgs.br
Biofilm formation was assessed by two phenotypic assays: i. morphology of colonies formed on Luria-Bertani (LB) agar; ii. adhesion on polystyrene microtiter plates. *Salmonella* strains were streaked on Luria Bertani agar with low salt content (LB low salt; Sigma-Aldrich, St. Louis, USA) supplemented with 40 µg/mL of Congo Red (Sigma-Aldrich) and 20 µg/mL of Coomassie brilliant blue (Sigma-Aldrich). Cellulose production was determined on LB agar supplemented with 50 µM Calcofluor (Fluorescent brightener 28) (Sigma-Aldrich). The plates were incubated at 37°C for 24 hours or at 28°C for 96 hours. After incubation, colony morphology was classified as: rdar (presence of curli fimbriae and cellulose), pdar (presence of cellulose), bdar (presence of curli fimbriae), saw (absence of curli fimbriae and cellulose). For cellulose detection, colony fluorescence was evaluated under UV light at 366 nm (Römling et al., 2003). All assays were performed in duplicate and repeated three times in different days. The adherence test was conducted in flat bottomed 96 well polystyrene plates (TPP® Techno Plastic Products AG, Switzerland) containing 20 µL of bacterial suspension (approximately 5 x 10^5 CFU/mL) and 230 µL of Tryptic Soy Broth without glucose (TSB; Bacto®, New Jersey, USA). After incubation at 37°C for 24 hours, the microplates were processed according to the protocol described by Stepnanov et al. (2004), and evaluated by spectrophotometry at 570 nm. The optical density cut-off (ODc) was defined as the mean OD of the negative control (culture medium) and the isolates were classified as follows: non-adherent (OD ≤ ODc); weak adherent (ODc < OD ≤ 2xODc); moderate adherent (2x ODc < OD ≤ 4 x ODc); and strong adherent (OD > 4xODc). Staphylococcus epidermidis ATCC 35984 and *Salmonella* Typhimurium ATCC14028 were included in each plate as positive controls. All tests were performed in triplicate and repeated in three different days.

**Results**

Among the 54 *Salmonella* isolates tested, all presented the rdar morphotype at 28°C and saw morphotype at 37°C (Figure 1). Among them, 14.8% (8/54) showed to be weak adherent on polystyrene microtitre plates, and were considered as presumptive biofilm producers. These isolates belonged to six serovars [Montevideo (n=2), Senftenberg (n=2), Tennessee (n=1), Orion (n=1), Morehead (n=1), and *S. enterica* sub. enterica O: 16 (n=1)], and were originated from equipment (n=5) as well as ingredients delivered at the feed mills (n=3).

**Discussion**

In this study, we evaluated the phenotypic characteristics associated with biofilm formation capability in *Salmonella* isolates originated from feed mills. All isolates expressed rdar morphotype at 28°C, which is characterized by the production of an adhesive extracellular matrix consisting of curli fimbriae and cellulose (Römling et al., 2003). The curli fimbriae are considered to be expressed in response to nutrient limitation, while cellulose is an extracellular component produced for mechanical and chemical protection of bacterial cells (Jain & Chen, 2010). Both components are important for survival under challenging conditions, which may be encountered by bacteria in the environment. Moreover, the expression of curli fimbriae and cellulose, leading to rdar morphotype, was temperature dependent, indicating that both components may play a much more important role at room temperature. The adherence test to abiotic surfaces revealed that 14.8% (8/54) of these isolates were weakly adherent to polystyrene plates, and were thus considered as presumptive biofilm producers. These strains were originated from ingredient delivered at the feed mills or were isolated from dust or debris samples taken from the equipment surfaces. The results indicate that *Salmonella* isolates able to produce biofilm may be introduced in feed mills through contaminated ingredients. The formation of biofilm in turn is influenced by several environmental factors (temperature, surfaces, nutrients and pH), which regulate the expression of genes responsible for biofilm formation (Linou & Koutsoumanis, 2012, Nguyen & Yuk, 2013, Simm et al., 2014). In this sense, the environmental conditions found inside feed mills, such as high moisture and temperature, may influence the formation of biofilm by certain *Salmonella* isolates, allowing their residual colonization of surfaces. The influence of these factors in the formation of biofilms needs to be further investigated.

**Conclusion**

Strains isolated from feed mills can be able to form biofilm. This fact might play a role in *Salmonella* residual colonization of equipment, and should be further investigated.

**Acknowledgements**

Lavirki V. is supported by scholarship of the Higher Education Personnel Improvement Coordination (CAPES, Brazil). Lopes G. V. is engaged in the Post-doctoral program of the Higher Education Personnel Improvement Coordination (PNPD-CAPES, Brazil).

**References**

- Jain, S., Chen, J., 2010. Attachment and biofilm formation by various serotypes of *Salmonella* is influenced by cellulose production and thin aggregate fimbriae biosynthesis. J. Food Prot., 70, 2473-2479.

11Departamento de Medicina Veterinária Preventiva, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil

* correspondiing author: mcardoso@ufrgs.br
Figure 1. Morphotypes of Salmonella isolates. (A) saw morphotype after a 24-hour incubation at 37°C on Congo Red agar. (B) rdar morphotype after a 96-hour incubation at 28°C on Congo Red agar.

05. Effect of protective cultures and different modified atmosphere packaging on Listeria innocua growth and on sensory properties in sliced cured-smoked pork loin
Vaz Velho, M.* 1, Casquete, R. 2,3, Silva, J. 2, Castro, S.M. 2, Pinto, R. 1, Jácome, S. 1, Fonseca, S. 1, Pinheiro, R. 1, Teixeira, P. 2

Abstract
This study aims to evaluate the antimicrobial effect of two protective cultures combined with different modified atmosphere packaging (MAP) systems on Listeria innocua growth in sliced ready-to-eat pork loin, a Portuguese traditional cured-smoked product (Lombo). Two protective lactic acid (LAB) cultures - Lactobacillus sakei ST153 and BLC35 (CHR Hansen) were tested for their ability against L. innocua 20130c growth (as a surrogate for L. monocytogenes) in sliced “lombo” packed in two MAP conditions, (20%CO2/80%N2 and 40%CO2/60%N2) and stored at 5°C. The influence of MAP and protective cultures in the sensory characteristics of the product was also evaluated by semi-trained panel of fifteen judges. The MAP affected the growth of L. innocua, the Listeria population decreasing 3 log CFU/g after 120 days of storage at 5°C. In samples containing protective cultures a reduction of 1–2 log CFU/g in counts of L. innocua was observed after 12 hours. At the end of storage results indicated that L. sakei ST 153 was more efficient than BLC35 culture on inhibiting L. innocua growth and this inhibition was enhanced by MA (40%CO2/60%N2). Results of sensory evaluation showed that oiliness, hardness, succulence, and characteristic taste attributes of “lombo” decreased during storage whereas the bitter taste increased in both LAB applications and no significant differences between LAB cultures or MAP conditions were found.

Introduction
Listeria monocytogenes is one of the most important psychrotrophic foodborne pathogens related to anaerobically packed lightly cooked or cured-smoked meat products because of its ability to survive and multiply at refrigerated temperature. Slicing of such products can lead to further contamination with pathogens. Therefore, the prevalence of pathogens in commercial ready-to-eat fermented meats products requires improvements in packaging and preservation methods maintaining the freshness, quality and safety of foods Lactobacillus species represent the dominant LAB strains currently found in meat starter cultures (Chaillou et al., 2005). One of the most efficient technologies used for product preservation is Modified Atmosphere Packaging (MAP) combined with refrigeration. Several studies have shown that MAP of many types of meat products interferes with the survival and growth of L. monocytogenes (Hudson et al., 1994; Hugas et al., 1998). Research on antimicrobial substances, mainly bacteriocins, produced by LAB, has led to consideration of their use as natural preservatives in meat products (Castellano, et al., 2008; Fadda et al., 2010). Lactic acid bacteria naturally dominate the microflora of meat products that are stored under vacuum or in an environment enriched with CO2, and their use as protective cultures has been studied as an alternative to chemical additives for assuring food safety (Holzapfel et al., 1995).

The aims of this study were to evaluate the antimicrobial effect of two protective cultures combined with different MAP conditions enriched in CO2 on survival of Listeria innocua 2030c (as a surrogate for L. monocytogenes), and on the sensory properties of sliced ready-to-eat “lombo”, a Portuguese cured-smoked pork loin.