Identification of Salmonella clonal groups and enterobacteria quantification in different risk areas of manufacturing process in four Brazilian feed mills

Cardoso M.[1]*
Pellegrini, D. C. P.[1]; Paim, D.[1]; Lima, G. J. M. M.[2]; Kich, J.[2], Coldebella, A.[2]

[1]Departamento de Medicina Veterinária Preventiva, Universidade Federal do Rio Grande do Sul, Brazil
[2]Embrapa Suínos e Aves, Concórdia, Brazil.

Faculdade de Veterinária – UFRGS. Av. Bento Gonçalves 9090, 90450-000 Porto Alegre, RS, Brazil.
mcardoso@ufrgs.br.

Abstract

Identification of critical points for contamination of feed and spread of Salmonella may contribute to the development of control plans. A cross-sectional study was conducted to identify Salmonella clonal groups in feed mill facilities. A total of 1,322 samples were collected in four swine feed manufacturing facilities. Samples were taken from ingredients and from dust deposited on the floor and on the inner surface of storage bins, bucket elevators, mills, mixers, scales, pelleting chamber and cooler. Besides, all samples were submitted to enumeration of enterobacteria. Salmonella was isolated from a total of 66 (5.0%) samples; most of positive samples were taken from transportation equipment (bucket elevator and conveyor belt). In two facilities, Salmonella was detected in the end product. Serovars Montevideo, Infantis, Orion, Senftenberg, Agona, Worthington and Tennessee were found in more than one step of the manufacturing process, and they were submitted to molecular typing by pulsed-field gel electrophoresis (PFGE). Results from XbaI and BlnI-digestions revealed from one to nine PFGE-profiles. Pulsotypes analyses indicated that dust deposited on the inner surface of equipments and on the floor is responsible for spreading and persistency of Salmonella in feed mills over time. The highest enterobacteria counts were found in the dosage step in all sampled feed mills, indicating lack of cleanliness in this area. Salmonella was isolated in 8.75% (28/320) of the samples presenting enterobacteria counts >100 cfu.g-1. Dust accumulation on the floor and surface of equipment and a high production flow were identified in all feed mills; these factors may have contributed to the spread of Salmonella clonal groups.

Introduction

High quality feed manufacturing and delivery is essential to success of swine production. Contaminated feed can be the introduction vehicle of Salmonella in farms. Therefore, Salmonella has been considered an important microbiological hazard in animal feed (EFSA, 2006). Among the most important sources of feed contamination are the ingredients from both animal and vegetal origin (Davies & Hinton, 2000; Coma, 2003). Moreover, the spread of Salmonella in the feed mills facilities has been associated to factors such as cross-contamination by dust, presence of vectors and poor hygiene condictions (EFSA, 2008). The flow of ingredients and feed through the machinery also contributes to the level of contamination. Thus, the identification of critical points for contamination of feed and spread of Salmonella clonal groups may contribute to the development of control plans. This study was carried out to determine the frequency of Salmonella isolation and to identify Salmonella clonal groups in different areas of feed mill facilities.

Material and Methods

A cross-sectional study was conducted in four swine feed manufacturing facilities. The production flowchart of each facility was studied and sampling spots were defined. Each facility was visited six times. Feed ingredients and dust deposited on the floor and on the inner surface of storage bins, bucket elevators, mills, mixers, scales, pelleting chamber and cooler were sampled. Five to ten samples (100 g) were aseptically collected from each sampling spot and pooled before the analysis. This sampling method has been proposed to increase the probality of Salmonella detection (Richardson, 2008). A total of 1,322 samples were collected. Salmonella isolation was performed according to a protocol consisted of non-selective pre-enrichment, selective enrichment, and plating onto selective solid medium Xylose Lysine Tergitol 4 agar (XLT4, Merck) and Brillian-green Phenol-Red Lactose Sucrose agar (BPLS, Merck) (Michael et al., 2003). Typical colonies were submitted to biochemical and serological confirmation. Salmonella isolates were serotyped at Fundação Oswaldo Cruz (FIOCRUZ) following the Kauffmann-White scheme. Salmonella isolates were analysed by Pulsed-Field Gel Electro-

phoresis (PFGE) to identify clonal relationships between strains of a same Salmonella serovar. The Pulsenet protocol for molecular subtyping by PFGE was used (Ribot et al., 2006). PFGE was performed on the CHEF DR II system. Gels were stained with ethidium bromide (10mg.ml-1) and photographed under UV illumination with the Kodak 2200 system. Restriction profiles were visually analyzed, and band position was determined. Enterobacteria enumeration was performed in all pooled samples tested for Salmonella. Aliquots (1 mL) from serial dilutions (10-1 to 10-4) were transferred to selective medium for enterobacteria (Violet Red Bile Agar-VRBA, Merck). Typical colonies were counted and the results expressed as colony forming units per gram (cfu.g-1). Enterobacteria enumeration results were categorized into above or below 100 cfu.g-1. Association of enterobacteria count level and Salmonella isolation was tested by chi-square analysis. P values <0.05 were considered significant.

Results

A total of 66 (5.0%) samples were Salmonella-positive, and most of positive samples were originated from the transportation equipment (bucket elevator and conveyor belt). In two feed mills (A and D), Salmonella was also detected in the end product samples (2/78; 2.5%). Among the 66 Salmonella strains isolated, serovars Montevideo (22.7%), followed by Mbandaka (10.6%), Senftenberg (10.6%) and Agona (9.0%) were the most prevalent. Serovars Montevideo, Senftenberg, Agona, Worthington, Infantis, Orion, and Tennessee were found in more than one step of the manufacturing process, and were submitted to PFGE. From one to nine PFGE-profiles were identified in the aforementioned serovars (Table 1). Strains belonging to a common genotype were identified in serovars Orion, Montevideo, Worthington and Agona. Serovar Montevideo presented the highest number of clonal groups, which were distributed among ingredients, dust collected from the floor and equipment, and feed. The highest frequency of samples presenting enterobacteria counts above 100 cfu.g-1 was found in the dosage area followed by crusts formed on equipment surfaces, milling and mixing steps, while end product and storage bins had a low frequency of enterobacteria counts above this limit (Figure 1). Salmonella isolation was significantly (P<0.001) more frequent in samples with enterobacteria counts above 100 cfu.g-1 (28/320; 8.75%) than in samples with counts below this limit (38/1127; 3.26%).

Discussion

Salmonella was detected in ingredients and dust collected from equipment and floor of all feed mill facilities, as well as in end product samples of two feed mills. These results highlight the importance of feed contamination during manufacturing, since the amount of feed produced daily by a feed mill will suply a high number of farms. Dust deposited on the floor and on the inner surface of the bucket elevators were found to be the most important sources of Salmonella isolation. A higher frequency of Salmonella enterica was found in dust compared to ingredients, and dust was pointed as the main risk factor for cross-contamination of feed (Torres et al., 2011). Dust produced during the feed manufacture process may set down on the surface of the equipment and on the floor. Deposited dust on equipment surfaces may absorb moisture and originate crusts that may foster bacteria.

Salmonella Montevideo and S. Agona have been found to persist in biofilms formed in feed mill equipment for several years (Vestly et al., 2009). In our study, S. Montevideo was the most prevalent serovar and presented the highest number of clonal groups. The analyses of pulsotypes demonstrated the cross-contamination during feed processing as well as the persistence of serovar Montevideo strains over time. Pulsotype Mo4 encompassed strains isolated from ingredient, equipment and feed collected in a same sampling event, while pulsotypes Mo2 and Mo5 were found in samples collected in more than one visit. Thus, ingredients, such as bone meal and soybean meal, may introduce Salmonella in the equipment, while dust and crusts accumulated on their surface may facilitate the persistence of clonal groups. As a consequence, ingredients and feed flowing through the equipment may be cross-contaminated.

Enterobacteria enumeration has been considered an indicator of Salmonella presence in feed (Davies & Hinton, 2000). In line with this result, Salmonella isolation was significantly (P<0.001) more frequent in samples with enterobacteria counts above 100 cfu.g-1 in our study. Moreover, the highest frequency of samples above this limit was found in the dust deposited on the inner surface of equipment, which proved also to be a major hazard for Salmonella feed contamination. High counts of enterobacteria indicate uncleanliness of equipment and facilities. In all sampled feed mills a lack of inspection windows and the presence of dead areas and crevices in the equipment were observed. The poor hygienic design of equipment associated to the high flow of production may hinder proper cleaning operation and contribute to the accumulation of dust, enhancing the hazard of cross-contamination of processed feed.

Conclusion

Salmonella clonal groups can persist in feed mills over time and cross-contaminate the processed feed. Dust and crusts accumulated on surface of equipment are the main site of Salmonella persistence. Thus the improvement of hygienic design of equipment and the avoidance of dust accumulation should be targeted in Salmonella control programs in Brazilian feed mills.

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Table 1: Sources of Salmonella pulsotypes isolation at four Brazilian feed mills.

Feed mill	Visit	Serovar	Pulsotype	Source
A	5	Montevideo	Mo7	Bucket elevators*
	5		Mo8	Bone meal
	5		Mo9	Bone meal
	5	Infantis	In1	Crust
	5		In2	Crust (Drag conveyors)
B	1	Orion	Or1	Wheat bran, scale*
C	1	Montevideo	Mo1	Feed truck
	1, 5		Mo2	Bone meal, floor*, feed
D	1	Montevideo	Mo3	Bucket elevators*
	1		Mo4	Rice meal, milk, bone meal, Bucket elevators*, feed
	1, 2, 3		Mo5	Sorghum, bone meal, floor*
	2		Mo6	Mill*
	1	Worthington	Wo1	Floor*, crust
	2	Tennessee	Te1	Mill*
	2		Te2	Floor*
	3	Senftenberg	Se1	Floor*
	2		Se2	Floor*
	2		Se3	Mixing*
	1		Se4	Soybean meal
	1	Agona	Ag1	Wheat bran
	1		Ag2	Floor*
	2		Ag3	Corn gluten, floor*

**Dust collected from the equipment or floor was analyzed.*

Figure 1: Frequency (%) of samples presenting enterobacteria counts above 100 cfu.g-1 in four Brazilian feed mills.