

## Airborne *Listeria* spp. in the Red Meat Processing Industry

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### Abstract

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The aim of this study was to determine the potential presence of the airborne *Listeria* spp. and its correlation with the aerobic mesophilic bacteria and *Listeria* carcass contamination in three red meat slaughtering and three processing plants. Airborne *L. seeligeri* and *L. innocua* were determined using 8 (5.06%,  $n = 158$ ) air samples taken on the locations characteristic for aerosol generating and in a chilly environment. The positive airborne samples of *Listeria* spp. were in an insignificant ( $P > 0.05$ ) relation with the highest airborne bacteria counts. On the carcass, only 1 positive case (0.69%,  $n = 144$ ) of *L. innocua* was determined, presumably owing to the low airborne *Listeria* counts and its unpredictable settling rates. In addition, insignificant ( $P > 0.05$ ) influences of air moisture and airflow on the airborne *Listeria* were found. Nevertheless, the methods currently used to determine the airborne *Listeria* and its relationships to aerosol viable mesophilic bacteria and carcass contamination need to be reconsidered in future investigations.

**Keywords:** airborne *Listeria* spp.; aerobic mesophilic bacteria; carcass; slaughterhouse; meat processing plant

*Listeria monocytogenes* and other *Listeria* species are widely spread in the environment. Due to the fact that ten people in Austria and Germany died in 2009 and 2010 after eating the deadly cheese and several more were taken ill owing to *Listeria* contamination, the routes of *Listeria* spreading in the food processing plants should be investigated more intensely in the future. The risk of contamination with *Listeria* in the red meat processing industry has to be considered as rather probable. Possible *Listeria* cross-contamination by employees, equipment, and environment surfaces, animal skin, food additives, packing material and many other sources has been reported (MARINŠEK & GREBENC 2002; GRIFFITHS 2003), thus effective sanitation programmes in the slaughtering and meat processing plants are strongly recommended (FRANK *et al.* 2003; DOYLE *et al.* 2004; HEIR 2004). The carcasses and their products may be contaminated during slaughtering

and meat processing, thus they can be recognised as feasible transmission routes of *Listeria* to humans (NESBAKKEN *et al.* 1996; EFSA 2006).

However, relatively small attention is given to the air acting as a potential vector of contaminants of carcasses and equipment (KANG & FRANK 1990; DE ROIN *et al.* 2003; PEARCE *et al.* 2006). *Listeria* can potentially become airborne owing to the sanitation maintenance and meat processing, especially within solid particles suspended into the air, as single organisms or in droplets in the form of aerosols created by the use of water sprayers (SPURLOCK & ZOTTOLA 1991). Therefore, it could be potentially transmitted by air and colonise various surfaces including raw and ready-to-eat meat products (BURFOOT *et al.* 2003). Furthermore, recent investigations in aerosol studies have made this theory even more credible since McEVoy *et al.* (1999) reported the possibility of the airborne

*Listeria* contamination of carcasses during meat processing. Nevertheless, there is still insufficient information available about the environmental conditions, routes, and sources, and on how *Listeria* can become airborne. Consequently, the possibilities of airborne *Listeria* contamination of the air in food-processing facilities have to be considered as an important prediction of the potentially anticipated route of the meat and meat products contamination (KANG & FRANK 1990; ZHANG *et al.* 2007). Therefore the main objectives of this test were to examine the potential presence of *Listeria* spp. in the air and on the carcass surfaces in relation to the aerobic mesophilic bacteria in the red meat processing industry (BYRNE *et al.* 2008). In this study, mainly the survey of airborne *Listeria* spp. in slaughterhouses and processing plants is intended; attention is also paid to whether or not any correlation can be established between the number of the airborne mesophilic bacteria and *Listeria* carcass contamination.

## MATERIALS AND METHODS

**Air sampling.** Three large commercial slaughterhouses with maximum slaughtering capacity per year: No. 1: 9007 beef cattle and 7318 pigs, No 2: 20 000 beef cattle and 30 000 pigs, and No. 3: 12 000 beef cattle and 90 000 pigs, and three small meat processing plants with processing capacity per month: No. 1: 120 t raw meat and 44 t of meat products, No. 2: 38 t raw meat and 10 t of meat products, and No. 3: 20 t raw meat and 4,5 t of meat products were selected for the experiment. The plants were equipped and regulated according to EU standards relating to pig and beef cattle slaughtering and meat processing, providing the operating and environmental conditions necessary for proper meat manufacturing.

In the slaughterhouses, the samples for *Listeria* and aerobic mesophilic bacteria examination were taken both in winter and in summer times from the air before and during the slaughtering processes and on the surfaces of carcasses. In the meat processing plants the samples were taken during meat products manufacturing. Air samples, each of the volume of 1500 litres ( $n = 158$  (237.0 m<sup>3</sup> of the air) for *Listeria* examination,  $n = 141$  (211.5 m<sup>3</sup> of the air) for aerobic mesophilic bacteria examination – CFU/m<sup>3</sup>), were taken with an impaction air sampler (MAS 100 Microbial Air Monitoring Systems®) operating

at a constant air flow rate (real time) of 100 l/min according to EN ISO 14698-1/2, 1 h before and at subsequent 3 h intervals during the slaughter operations (PRENDERGAST *et al.* 2004); they were also taken randomly during the meat chilling, meat processing, and packing processes.

A gravity sedimentation method, such as the open Petri dish method using the exposure to the open air, combining both gravitational and inertial processes, was used for the determination of the number of mesophilic aerobic bacteria (CFU/plate) only in the slaughterhouses (RECK *et al.* 2002; KORNACKI 2006). The samples were taken using Petri plates covered with 56.72 cm<sup>2</sup> of Tryptic glucose yeast agar (Biolife Italiana, Milan, Italy). The exposure time of the agar plates ( $n = 43$ ) was 30 min; they were placed at the same locations as were those for the air sampler sampling along the slaughtering lines.

Air samples ( $n = 151$ ) were taken in the slaughterhouses from nine locations along the slaughtering line from the stunning to the weighing areas at the heights of 0.5 m, 1.0 m to 2 m from the floor at the distance of 1 m to 2 m from the carcasses, thus on: (a) two locations at stunning and skinning ( $n = 24$ ), (b) four locations near exsanguination ( $n = 18$ ), evisceration ( $n = 39$ ), near the viscera conveyor ( $n = 18$ ), and carcass storage entrance ( $n = 3$ ), (c) one location between/behind carcass splitting and washing ( $n = 36$ ), and (d) at two locations in front of carcass dressing ( $n = 6$ ) and in the centre of the floor in the refrigerating rooms ( $n = 7$ ).

In the processing plants, the air samples ( $n = 7$ ) were taken on the floor surfaces from three locations, thus in the: (a) refrigerating chambers, (b) packing, and (c) processing rooms.

The method of surface smearing was used for *Listeria* examination on the beef carcass. The samples ( $n = 144$ ) were collected from three locations only in the slaughtering plants and at the time of weighing: (a) on the thighs, (b) on the ribs, and (c) on the shoulders. The samples were taken using sterile cotton swabs soaked in distilled water. Each sample was taken on a surface area of 20 cm<sup>2</sup>.

**Microbial analysis.** Following the airborne *Listeria* spp. determination, the air stream was focused using the air sampler onto the Petri plate bottom covered with a thin piece of polycarbonate membrane, soaked with 2 ml of primary enrichment medium F1 (Half Fraser broth with half concentration of antibiotics, Oxoid, Basingstoke, UK). The moistened filter material served as a

trap for the dust particles, aerosol, and possible bacterial cells. The samples were transported to the laboratory within a few hours. Before incubation, an additional amount of 8 ml of half Fraser broth was added into each dish and the contents were shaken gently. The cultures were incubated at 30°C for 24 hours. Later on, 0.1 ml of the culture from F1 was transferred into 10 ml of the second enrichment broth F2 (Fraser broth, with full concentration of antibiotics, Oxoid). From the primary enrichment, one loop was also taken for either of the selective plating media: ALOA agar (Biolife Italiana, Milan, Italy) and Palcam agar (Oxoid, Basingstoke, UK), with subsequent incubation at 37°C for 24–48 hours. The same procedure was repeated with a culture obtained on the secondary enrichment medium after 48 h of incubation. Up to five typical colonies of *Listeria* spp. grown on ALOA and Palcam agar were transferred onto the blood agar for pure culture to determine the haemolytic activity. Final identification was performed with the commercial biochemical kit API *Listeria* (Bio Merieux, Craponne, France) following the producer's instructions.

Aerobic mesophilic bacteria were collected by means of the air sampler and gravity sedimentation method onto Petri plates, containing Tryptic glucose yeast agar (Biolife Italiana, Milan, Italy). The plates with the cultures were incubated at 30°C for 72 h and the colonies on the plates were counted. The counting was accurate up to 100 colonies per plate, while higher counts were only estimated.

The swabs from the carcasses examined for the presence of *Listeria* were suspended in 50 ml of half Fraser broth (Oxoid) and treated using the same procedure as was that for the air samples. All the samples were transported to the laboratory within 8 h after collection, and processed immediately.

**Microclimate measurements.** The microclimate conditions of the slaughterhouses and processing plants, such as the air temperature, relative humidity, and airflow, were measured (Testo® 350-M/XL testo 454 Control Unit) using a 3-function probe for simultaneous measurements of temperature, humidity, and velocity, with plug-in head: meas. range –20°C to +70°C, 0–100% RH, air flow 0–10 m/s at the time of sampling on each sampling location.

**Statistical analysis.** *Listeria* and mesophilic bacterial values were analysed using the analysis of variance by ANOVA, *t*-test, correlations, and means using SPSS (Statistical Package for the Social Sciences) 17.0 statistics programme.

## RESULTS AND DISCUSSION

Notwithstanding the weak possibility of determining the airborne *Listeria* in the air in the tested slaughtering and processing plants, which is in agreement with the report by BYRNE *et al.* (2008) who did not detect *Listeria* in the air in the pork processing plant, the determination of *Listeria* spp. in the air in the tested plants in this study strongly suggests that *Listerias* can be airborne and consequently transmitted by single colonies, solid particles, or aerosols (ZHANG *et al.* 2007). None of the air samples taken by the air sampler within any of the 3 tested slaughterhouses and 3 meat processing plants were positive for the pathogen *L. monocytogenes*, however, *L. seeligeri* and *L. innocua* were isolated from 8 air samples (5.06%,  $n = 158$ ) of the entire sample (237.0 m<sup>3</sup> of the air) taken in all 6 investigated plants (Table 1).

Although the measurements of aerosols and other airborne particles were not performed during this study, it was found that the highest airborne *Listeria* and the highest aerobic mesophilic bacteria counts (CFU/m<sup>3</sup>, CFU/plate) in the slaughterhouses were detected near the locations where a strong likelihood existed of the occurrence of aerosol and other airborne particles owing to the working routines, e.g. carcass splitting, washing, evisceration, meat processing, and viscera transport (DOYLE *et al.* 2004; PRENDERGAST *et al.* 2004). This was the case especially in slaughterhouse No. 1, where the highest counts of the airborne positive air samples of *L. seeligeri* (3 positive air samples,  $n = 63$ ) and the highest number of aerobic mesophilic bacteria (CFU/m<sup>3</sup>) were determined on the location near the carcass evisceration at the sampling levels of 0.5 m, 1.0 m and 2 m over the floor (Table 1, Figure 1). At this location, the eviscerated entrails have to drop at least 0.5 m from the carcass and are splashed onto a trolley standing on the floor, which is lifted to the viscera conveyor into which the entrails are thrown. We believe there is a strong likelihood that these actions are accompanied by high amounts of waste water sprinkling, which consequently leads to *Listeria* and bacteria contaminated aerosol generation, as we also assumed in other slaughterhouses tested. In addition, at this location, in comparison to other locations in slaughterhouse No. 1 the highest mean number was determined of aerobic mesophilic bacteria taken by the air sampler (CFU/m<sup>3</sup>) and the gravity sedimentation method (CFU/plate) (Figure 1). However, the bacterial counts (CFU/m<sup>3</sup>)

Table 1. Positive samples of airborne *Listeria* spp. in Slaughterhouses (No. 1–3) and Processing plants (No. 1–3)

Slaughterhouse	Near evisceration	Near viscera conveyor	Between/behind carcass splitting, washing	Processing plant	Processing room floor
No. 1 ( $\Sigma n = 63$ )	$n = 12$	$n = 0$	$n = 15$	No. 1 ( $n = 5$ )	$n = 3$
<i>L. monocytogenes</i>	0	/	0	<i>L. monocytogenes</i>	0
<i>L. innocua</i>	0	/	0	<i>L. innocua</i>	1
<i>L. seeligeri</i>	3	/	0	<i>L. seeligeri</i>	0
<i>L. welsheimeri</i>	0	/	0	<i>L. welsheimeri</i>	0
No. 2 ( $\Sigma n = 64$ )	$n = 27$	$n = 18$	$n = 15$	No. 2 ( $\Sigma n = 1$ )	$n = 0$
<i>L. monocytogenes</i>	0	0	0	<i>L. monocytogenes</i>	/
<i>L. innocua</i>	0	1	2	<i>L. innocua</i>	/
<i>L. seeligeri</i>	0	0	0	<i>L. seeligeri</i>	/
<i>L. welsheimeri</i>	0	0	0	<i>L. welsheimeri</i>	/
No. 3 ( $\Sigma n = 24$ )	$n = 0$	$n = 0$	$n = 6$	No. 3 ( $\Sigma n = 1$ )	$n = 0$
<i>L. monocytogenes</i>	/	/	0	<i>L. monocytogenes</i>	/
<i>L. innocua</i>	/	/	1	<i>L. innocua</i>	/
<i>L. seeligeri</i>	/	/	0	<i>L. seeligeri</i>	/
<i>L. welsheimeri</i>	/	/	0	<i>L. welsheimeri</i>	/
Total ( $\Sigma n = 151$ )	$\Sigma n = 39$	$\Sigma n = 18$	$\Sigma n = 36$	Total ( $\Sigma n = 7$ )	$\Sigma n = 3$
<i>L. monocytogenes</i>	0	0	0	<i>L. monocytogenes</i>	0
<i>L. innocua</i>	0	1	3	<i>L. innocua</i>	1
<i>L. seeligeri</i>	3	0	0	<i>L. seeligeri</i>	0
<i>L. welsheimeri</i>	0	0	0	<i>L. welsheimeri</i>	0
Isolates 7 (4.6%)	3 (7.7%)	1 (5.5%)	3 (8.3%)	Isolates 1 (14.2%)	1 (33.3%)

$\Sigma n$  = number of total taken samples;  $n$  = number of taken samples on subscribed sampling location

determined were the highest even in comparison to other slaughterhouses, most presumably due to the small size of slaughterhouse No. 1, lack of room, and very narrow space around the slaughtering line, so the “dirty” areas of the slaughtering line were in proximity to the “clean” areas, although PRENDERGAST *et al.* (2004) observed no significant differences in bacterial counts between these areas.

In slaughterhouse No. 2, 2 samples ( $n = 64$ ) were positive for airborne *L. innocua* at the location of the carcass splitting and washing (at the heights of 0.5 m and 2.0 m), where the aerobic mesophilic bacteria (CFU/plate) were the highest (Table 1, Figure 1). In our opinion, on account of the carcass splitting and washing at both locations, the likelihood that the air was saturated with aerosol in higher concentrations than at other locations along the slaughtering line of slaughterhouse No. 2 was very strong, which was presumably the same at the location near the viscera conveyor at the height of 2 m, where one of the air samples ( $n = 64$ ) was positive for *L. innocua* as well (Table 1, Figure 1).

Irrespective of the aerosol saturation, the mean counts of aerobic mesophilic bacteria (CFU/m<sup>3</sup>)

were obviously the lowest in slaughterhouse No. 2 in comparison to other slaughterhouses, probably because it had the largest available working space in comparison with the other slaughterhouses tested, although the differences were not significant (Figure 1).

A similar situation was manifested in slaughterhouse No. 3 at the location behind the carcass splitting, situated at the height of 1 m over the floor, where 1 of the air samples ( $n = 24$ ) was *L. innocua* positive (Table 1, Figure 1), and where the highest mean count of aerobic mesophilic bacteria (CFU/m<sup>3</sup>, CFU/plate) was determined once again. On account of the most intensive slaughtering process (especially during the pig slaughtering), the highest mean counts of CFU/plate and the second highest number of CFU/m<sup>3</sup> were determined in slaughterhouse No. 3 in comparison with the other slaughterhouses tested (Figure 1).

Moreover, one of the samples was positive for airborne *L. innocua* ( $n = 5$ ) at the centre of the processing hall floor of the processing plant No. 1, where the main processes with the raw meat manipulation were running with a great opportunity

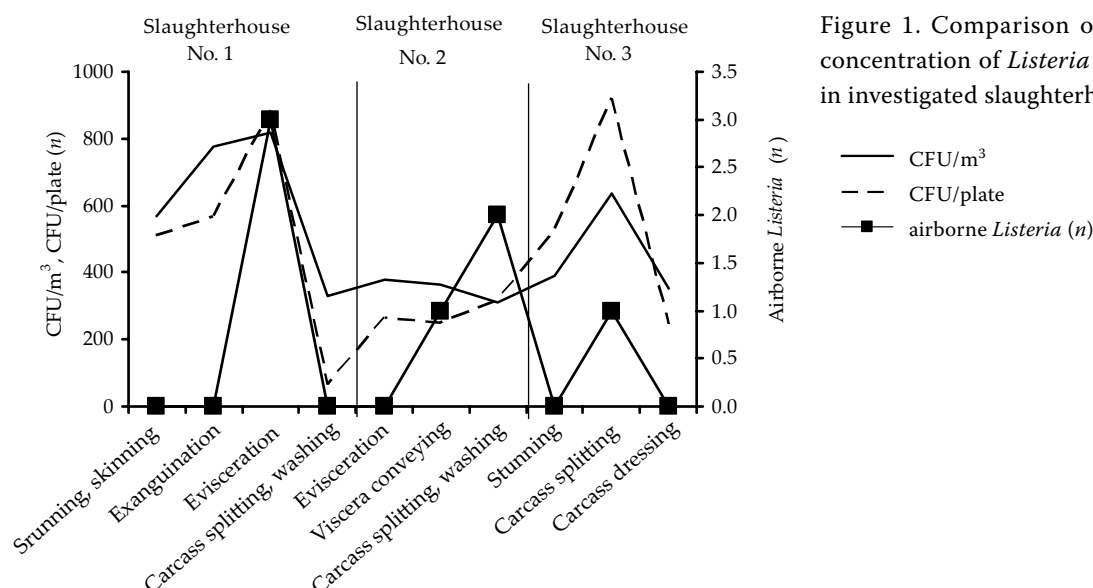


Figure 1. Comparison occurrence and concentration of *Listeria* in some places in investigated slaughterhouses

for aerosol or other particles releasing (Table 1). The results were similar to those by BYRNE *et al.* (2008) who stated the raw area of the pork processing plant to be most at risk of microbial contamination, due to higher levels of microbial sources.

We assumed that the airborne *Listeria* findings in our study were dependent on the air conditions as well. KORNACKI *et al.* (2006) stated that many factors affect the microbial growth, including moisture, pH, temperature, oxidation-reduction potential, consequently, the best places to determine *Listeria* are high moisture environments. Since the measurements in slaughterhouses No. 1 and No. 2 were carried out in the winter period, the average mean temperatures ( $T$ ) along the slaughtering lines were 11.9°C; however, the average temperature in slaughterhouse No. 3 was 21.6°C, because the measurements were carried out in the summer period. The mean values of air relative humidity (RH) were 78%, 73%, and 66%, respectively, in slaughterhouses No. 1, No. 2, and No. 3. However, the irregular and non-constant directions of a relatively weak airflow (mean = 0.12 m/s) between the unclean and clean parts of the slaughter lines were not clearly determined, although the airflow in slaughterhouse No. 3 was slightly stronger than in the other slaughterhouses. The mean microclimate parameters in the packing and processing rooms of the processing plants were  $T = 13.1^{\circ}\text{C}$ ,  $\text{RH} = 73.2\%$ , airflow = 0.06 m/s, while the parameters in the refrigerating chambers indicated  $T = 3.2^{\circ}\text{C}$ ,  $\text{RH} = 74.5\%$ , airflow = 0.02 m/s. The measurements were carried out in winter. Therefore, by comparison of all slaughterhouses

tested, in slaughterhouse No. 3 the combination of the lowest number of the positive samples of airborne *Listeria*, the lowest moisture ( $\text{RH} = 66\%$ ), highest air temperature ( $T = 21.6^{\circ}\text{C}$ ), and strongest airflow (mean = 0.18 m/s) was established, therefore a certain relation ( $P > 0.05$ ) between those parameters was assumed. These results resemble those by DOYLE *et al.* (2004) who revealed that the air humidity at higher rates – 75% RH – has a greater effect on *L. monocytogenes* survival than a lower RH (40%) of the air in the environment however settling rates of *Listeria*. Finally, we found that the carcasses were exposed in the slaughterhouses tested to *Listeria* first of all by the airborne routes, since there was a low possibility for their contact with the surfaces and equipment. According to the skinning location and the location in the vicinity of viscera conveying, both locations were thought to be the main sources of the airborne carcass contamination, yet almost no *Listeria* was determined on the carcasses on this locations. Out of the 144 smear samples, only one sample was positive for *Listeria* spp. (*L. innocua*) – although the sampling was performed before the carcasses were washed. Therefore as expected, no correlations between carcass *Listeria* spp. contamination and the airborne *Listeria* spp. were observed. These findings are similar to other reports where the correlations between the aerial and carcass contaminations in slaughterhouses were poor (PRENDERGAST *et al.* 2004). However, this is contrary to some other reports where a strong association between the carcass and microbiological contaminations of the air was observed

(PEARCE *et al.* 2006; POSH *et al.* 2006). Therefore, the low carcass *Listeria* contamination seems to be explained by the low airborne *Listeria* as well as the unpredictable settling rates of aerosol-borne *Listeria* which depend on the particle size and relative humidity of the environment (McEVOY *et al.* 1999; DOYLE *et al.* 2004; POSH *et al.* 2006); this needs to be investigated in the future.

The mean airborne viable bacterial counts (CFU) as found in this study were higher than or similar to those observed in other investigations (RAHKIO & KORKEALA 1997; KANG & FRANK 1990) in slaughterhouses or meat processing plants. Aerobic mesophilic bacteria (CFU/m<sup>3</sup>) analysis revealed significant negative correlations between CFU counts in slaughterhouses No. 3 and No. 2 ( $r = -0.501$ ,  $P < 0.05$ ). Additionally, these correlations were determined between slaughterhouses No. 2 and No. 1, even though they were not significant ( $P > 0.05$ ) (Figure 1). Furthermore, in all slaughterhouses tested a significant association ( $r = 0.824$ ,  $P < 0.01$ ) between aerobic mesophilic bacteria counts sampled using the air sampler (CFU/m<sup>3</sup>) and gravity sedimentation (CFU/plate) was observed (Figure 1). Unfortunately, the airborne *Listeria* findings cannot be compared to other results due to the lack of research in this field.

In general, we assumed that the air could be considerably saturated with aerosol on the locations where evisceration and rough carcass manipulation was implemented. Mechanical evisceration often leads to intestinal rupture and discharge of the gut contents, however, the processes along the slaughter line can cause microorganisms occurrence after dissipation or evaporation in the air of aerosols, airborne droplets, and solid particles (PRENDERGAST *et al.* 2004; POSH *et al.* 2006). Hence, the vigorous physical activities of slaughter and carcass dressing like carcass splitting and washing can be actually the sources of aerosols due to carcass-saw water cooling and the water stream meat washing, which actively spread potentially contaminated aerosols into the air (KANG & FRANK 1990; BURFOOT *et al.* 2003; PRENDERGAST *et al.* 2004).

According to the results gained in this study, the findings indicate a feasible appearance of airborne *Listeria* spp., particularly at such locations where we assumed that the potentially contaminated aerosol was spread into the air, with the bacterial air contamination increasing and microclimatic properties being suitable. This indicates that the number of airborne *Listeria* could be somehow related to the number of aerobic mesophilic bacte-

ria, although no significant correlations ( $P > 0.05$ ) were established in this study.

Since we did not determine aerosols in this study, we can only anticipate that the working procedures result in the formation of aerosol containing different particles sizes and being contaminated with different numbers of microorganisms. For this reason, we have to pay more attention to studying bio-aerosols in the future. Therefore, it would be necessary to investigate further precise information on all the environmental factors in addition to the possibility of airborne *Listeria* generation (DOYLE *et al.* 2004). Special attention should be focused on the evaporator cooling coils in the food storage refrigeration rooms where the conditions for the bacteria growth are favourable and where the cleaning processes are usually not sufficient to prevent the build up of debris and substrates suitable for the bacterial and presumably *Listeria* growth (KANG & FRANK 1990; EVANS *et al.* 2004). Notwithstanding the regular decontamination and HACCP operations, more attention should be given to preventing the overall *Listeria* distribution (EU Commission 1999; SAMELIS & METAXOPOULOS 1999; HENNING & CUTTER 2001). The efforts to prevent the meat products contamination – especially with zoonotic *L. monocytogenes* – must be made at all levels of the production, particularly due to the fact that *L. monocytogenes* is ubiquitous with the trend towards becoming airborne.

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