SPATIAL VARIABILITY OF AIRBORNE BACTERIA IN THE MUNICIPAL SLAUGHTERHOUSE OF CONSTANTINE - ALGERIA

Amir Agabou [*], *Mouhamed-Cherif Abdeldjalil, Abderahmane Bensegueni, Soumia Semouma*

Address(es): Dr. AGABOU Amir
[1]PDESCA Laboratory, Institute of Veterinary Sciences, University Mentouri of Constantine 1., Algeria.

*Corresponding author: amirveto@gmail.com

ABSTRACT

The spatial distribution of airborne bacterial contamination (with Total aerobic bacteria, Gram-negative bacteria, Coliforms, Staphylococci and Streptococci) was studied within the municipal abattoir of Constantine (North-East of Algeria).

Open plate air samples were collected at twenty sampling locations weekly during two months. At the same chosen locations, temperature and humidity of the air were measured. The spatial distribution of bacterial contamination was studied using a Geographic Information System.

The viscera processing section recorded the highest bacterial concentration and poor correlations had been recorded between bacterial counts and the environmental conditions within the abattoir. The high air bacterial loads recorded all over the slaughterhouse were consequence of the execution of all processing operations within the same section (for both bovine and ovine carcasses) associated to the absence of structural barriers, spatial separation between processing operations and uncontrolled airflow.

This study highlights the poor hygienic level of the municipal slaughterhouse of Constantine. The implementation of serious measures is needed to reduce the risk of contamination and proliferation of pathogens in meat during slaughtering processes.

Keywords: Airborne contamination, spatial distribution, slaughterhouse, North-East of Algeria

INTRODUCTION

Meat is an important dietary source of high biological value proteins, amino-acids, fat and fatty acids, B complex, D and E vitamins, bioavailable minerals and trace elements. This composition makes meat a good medium for the development and the spread of a great number of food-borne microorganisms, especially bacteria such as: drug resistant *E. coli* (**Sheikh *et al.*, 2012**), *Salmonella spp* (**Van *et al.*, 2012**), *Listeria monocytogenes* (**Zhu *et al.*, 2012**), *Staphylococcus aureus* (**Kelman *et al.*, 2011**) and other consumers' health threats and spoiling organisms.

Meat contamination has often a fecal origin resulting of slaughter operations (bleeding, dressing, evisceration and washing) which expose sterile muscle to microbiological contaminants present on hide, in the digestive tract, and in the environment (**Gill *et al.*, 1999; Gill *et al.*, 2003; Serraino *et al.*, 2012**). It has also been demonstrated that workers and their instruments can participate to that contamination (**Dickson *et al.*, 1992; Guyon *et al.*, 2001; Podpečan *et al.*, 2007**).

The importance of air as a source of contamination in food processing plants (dairy, chicken, quail and beef) has been widely investigated (**Rahkio *et al.*, 1997; Salustiano *et al.*, 2003; Northcutt *et al.*, 2004; Heber *et al.*, 2006**). It is supposed that air acts only as a vector and the major sources of airborne microorganisms are humans, animals, vegetation, sewage, dust and aerosols (**Al-Dagal et al., 1990**). According to **Jericho *et al.* (2000)** the majority of processes in cattle slaughterhouses are associated with the creation of bioaerosols.

In Algeria, limited information is available on abattoirs hygiene status and fewer are the studies of airborne contamination within those plants.

This study was carried out during two months (Mai and June 2011) with the following objectives:

- To monitor the bacteriological quality of the air within Constantine municipal abattoir (North-East of Algeria);

- To study the spatial distribution of several airborne bacteria groups during the slaughtering operations to identify sections of high meat airborne contamination risk;

- To assess any existing relationships between those airborne contamination levels and air temperature and humidity within the abattoir.

MATERIAL AND METHODS

The slaughterhouse

Constantine slaughterhouse is a craft type establishment with an average monthly production of 700 tons of cattle and sheep meat. It is divided into three sections (as presented in figure 1) without any drying room. Separations between sections are assured either by ¾ limited height walls or complete walls with openings not equipped by doors. The whole structure is ventilated through a great number of opened windows unsecured against flying insects. All the slaughtering operations are achieved in the same section where carcasses are hanged after inspection for variable periods (few minutes to few hours).

Air samples collection

Bacterial air contamination was examined ones a week during eight weeks (At the same period of the day: between 8 and 10 am). Twenty sampling locations have been defined *(Fig 1)*.
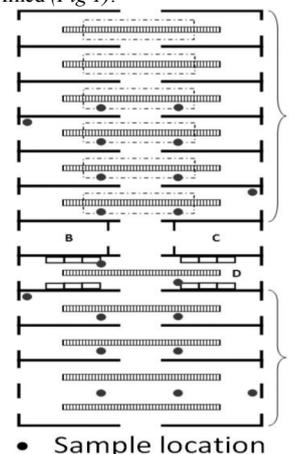

Figure 1 Slaughterhouse plan and sampling locations. [**A**: Ovine slaughtering section; **B**: Hide processing section; **C**: Incinerator; **D**: viscera processing (emptying and cleaning) section; **E:** Bovine slaughtering section.]

At each location four Petri dishes were put 70 cm above the floor and maintained uncovered for 15 min. They contain the following growth media: tryptic soy agar (for total aerobic flora counts), MacConkey`s agar (for enterobacteria and total coliforms counts), Baird-Parker agar (for Staphylococci+micrococci and *Staphylococcus aureus* counts) and Bile Esculine Azide agar (for Streptococci counts). The Petri dishes were conveyed quickly to the laboratory for incubation at 37°C for 48 hours, after what counts were performed and the bacterial loads are expressed as CFU cm^{-2} h^{-1}.

Environmental data registration

At the same chosen locations, temperature and humidity of the air were measured using a digital hygro-thermometer (Oregon Scientific, model ETHG913R).

Spatial analysis

To determine the spatial distribution of bacterial groups' counts, the x, y coordinates of the sample location and the corresponding numbers of colonies were entered in Surfer 9 **(Golden Surfer 9 User's manual, 2009)** for contour analysis. This software posted the bacterial loads to the appropriate coordinates on a plan of the slaughterhouse, which had been entered as a base map, and then created a denser grid by interpolation, using the kriging algorithm, a statistical linear method estimating the best value based on weighted linear combinations of neighboring values while aiming to minimize the error variance between samples. Loads were then grouped into ten levels per group of bacteria.

Statistical analysis

The statistical software GraphPad Instat prism 5 **(GraphPad Instat prism 5 User's manual, 2007)** was used to perform the following tests:

* ANOVA tests: to compare the aerial data (for the different bacterial groups) from the different sections (A, D and E).

* Pearson correlation coefficients: to determine correlations between bacterial loads (Log_{10} CFU+1 cm^{-2} h^{-1}), temperature and humidity of the air.

Statistical significance was set at a P value <05%.

RESULTS AND DISCUSSION

Table 1 shows mean air temperature and humidity during the sampling period. These two parameters were higher in the bovine slaughtering section than the ovine one.

Table 1 Air temperature and humidity at the different processing section during the study period.

	Section A	Section D	Section E
Temperature °C	23.04 ± 0.15	23.06 ± 0.43	23.16 ± 0.26
Humidity %	64.04 ± 0.84	64.25 ± 2.66	65.48 ± 1.25

Table 2 shows the relationship between temperature, humidity and bacterial loads within the different sections of the slaughterhouse. No significant correlations were found between temperature, humidity, coliforms, Staphylococci+Micrococci, *Staphylococcus aureus* and streptococci contamination levels at any section of the slaughterhouse. Total Aerobic Flora counts were negatively correlated at a significant level (*r*: -0,403) to air temperature at section A and to its humidity at section E (*r*: 0,359). Air contamination with Gram negative bacteria and temperature levels were positively correlated at section D (*r*: 0,578). All the other correlations were not significant.

Table 2 Pearson correlation coefficients (*r*) for environmental temperature and Log_{10} bacterial loads (CFU cm^{-2} h^{-1}) within the different processing sections

Section	Environmental data	Total aerobic flora	Gram-negative bacteria	Coliforms	Staphylococci+M icrococci	*Staphylococcus aureus*	Streptococci
A	Temperature	-0,403[+]	0,021	0,034	0,058	-0,006	-0,109
	Humidity	0,001	-0,121	0,010	-0,144	-0,165	-0,076
D	Temperature	0,098	0,578[+]	0,298	0,185	-0,368	0,106
	Humidity	0,258	-0,373	-0,052	-0,492	-0,249	-0,248
E	Temperature	-0,217	0,196	0,100	-0,070	-0,114	-0,038
	Humidity	0,359[+]	0,117	0,057	0,023	0,095	0,097

Figures 2, 3, 4, 5 and 7 show that the spatial distribution of the mean airborne contamination with the different bacteria groups is of an aggregated type. Among all of the slaughterhouse sections, the viscera processing section (D) recorded the highest bacterial concentration (Total Aerobic Flora, Enterobacteria, Coliforms, Staphylococci+Micrococci and streptococci).

Staphylococcus aureus contamination concentrated mainly in bovine slaughtering section and secondly in the ovine one near the doors (Figure 6).
Bovine slaughtering section was more contaminated than the ovine one with Total Aerobic Flora, Enterobacteria and Coliforms.
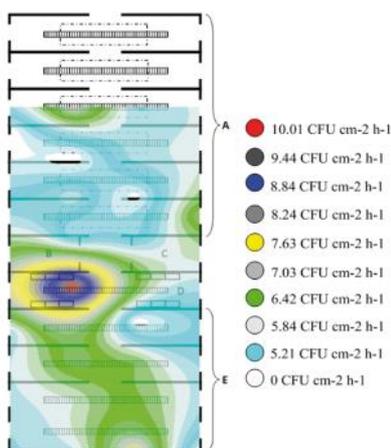

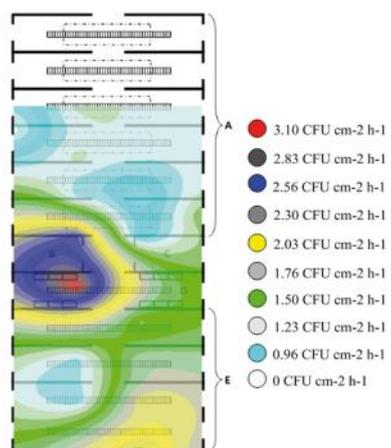**Figure 2** Spatial distribution of air contamination with Total Aerobic Flora.

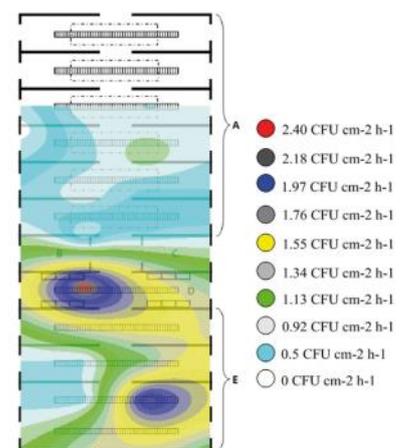**Figure 3** Spatial distribution of air contamination with Enterobacteria.

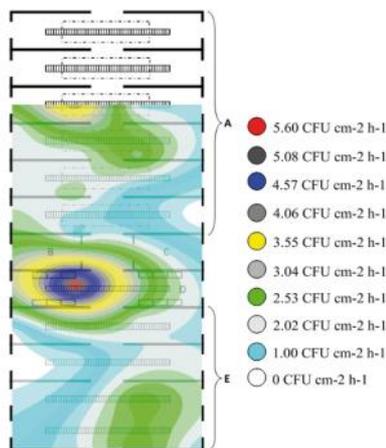**Figure 4** Spatial distribution of air contamination with Coliforms.

Figure 5 Spatial distribution of air contamination with Staphylococci+Micrococci.
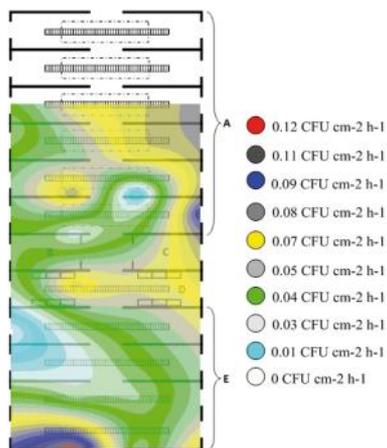

Figure 6 Spatial distribution of air contamination with *Staphylococcus aureus*.
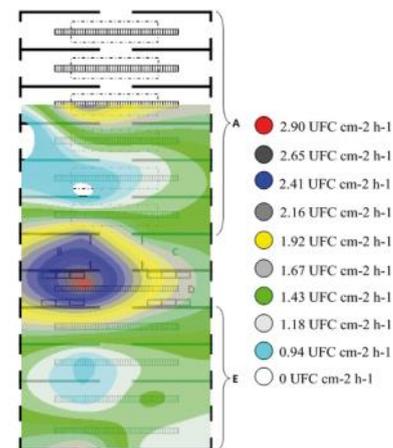

Figure 7 Spatial distribution of air contamination with Streptococci.

Table 3 shows the mean air contamination levels of the different sections. The highest levels were recorded at the viscera processing section followed successively by bovine and ovine slaughtering sections.

Table 3 Mean contamination levels of the air with the different bacteria groups during the sampling period (Log CFU cm^{-2} h^{-1})

Section	Total aerobic flora	Enterobacteria	Coliforms	Staphylococci+ Micrococci	*Staphylococcus aureus*	Streptococci
A	5.53 ± 0.39 [0.69 – 21.47]	1.30 ± 0.17 [0 - 13.06]	0.82 ± 0.06 [0 – 3.26]	2.13 ± 0.33 [0.12 – 21.4]	0.06 ± 0.01 [0 – 0.62]	1.47 ± 0.20 [0 – 10.35]
D	7.37 ± 1.71 [0.62 – 27.60]	2.29 ± 0.59 [0.06 – 8.47]	1.39 ± 0.41 [0.12 – 7.03]	3.90 ± 1.37 [0.43 – 22.75]	0.06 ± 0.02 [0 – 0.31]	2.26 ± 0.56 [0.12 – 7.85]
E	6.15 ± 0.36 [1 – 17.57]	1.69 ± 0.17 [0.31 – 7.27]	1.29 ± 0.13 [0.12 – 6.22]	1.92 ± 0.22 [0.18 – 9.35]	0.05 ± 0.01 [0 – 031]	1.35 ± 0.11 0.25 – 5.45]

The results recorded in this study are in accordance with those reported by other studies.

Fournaud *et al.* (1981) found mean counts ranging from 22.9 to 76.8 CFU cm^{-2} h^{-1} in the dressing area, from 5.7 to 45.0 CFU cm^{-2} h^{-1} in the evisceration area, and from 11.1 to 125.6 CFU cm^{-2} h^{-1} in the spraying area.

The high air bacterial loads recorded in our study were due to the execution of all the processing operations within the same section (for both bovine and ovine carcasses). It has been confirmed that lairage, exsanguination, and dehiding lead to more air contamination than evisceration and splitting **(Prendergast *et al.*, 2004)**. **Jericho *et al.* (2000)** found the air within the hide removal area to be more contaminated than the dressing area with total viable counts. In lamb slaughterhouses, the highest counts were found at the fleece puller **(Burfoot *et al.*, 2006)**. This is in contradiction with our findings, where viscera processing section concentrated more air contaminants because of the aerosolizitation of water mixed to viscera contents during viscera emptying and cleaning. According to **Yokoyama *et al.* (1988)**, Enterobacteria concentration within rumen and colon content may reach respectively 10^{10} to 10^{11} bacteria.g^{-1} and 10^{10} to 10^{11} bacteria.g^{-1}.

Air contamination levels are strongly affected by the slaughterhouse and the line layout design. **Worfel *et al.* (1996)** found plants with a wall separating the clean and unclean areas had significantly lower bacterial counts in the clean areas compared with the plant with no areas separation. They concluded that separation between plants contributes to the reduction of air contamination. The same conclusions are made by **Whyte *et al.* (2001)** in a poultry slaughterhouse.

In our slaughterhouse, absence of structural barriers and spatial separation between processing operations, associated to an uncontrolled airflow, participated to the spread of contaminants from a section to the whole abattoir atmosphere. Taking into consideration that airflow depends on staff movements and openings' locations.

In a previous study on carcasses surface contamination conducted in this same abattoir, **El Hadef-El Okki *et al.* (2005)** recorded an average contamination levels of 5.34 log$_{10}$ CFU cm^{-2} for bovine carcasses and 5,42 log$_{10}$ CFU cm^{-2} for ovine ones. They concluded that those levels of contamination were greater than those found in similar studies in France, Morocco, and Tunisia. This may be explained by the poor hygienic procedure in this slaughter house and the high airborne contamination levels revealed by our present study.

Rahkio *et al.*, (1997) found a strong association between carcass and air contamination. The same observation was made by **Sirami (1989)** and **Pearce *et al.* (2006)**. This is why it should be considered in a HACCP scheme **(Mothershaw *et al.*, 2008)**. However, **Prendergast *et al.* (2004)** reported a poor correlation between air and surface counts and they suggested that it is difficult to define such a relationship. **Roberts (1980)** and **Burfoot *et al.* (2006)** concluded that the airborne route is less important than carcasses contacts with contaminated materials.

Environmental conditions such as humidity, density and temperature have a great effect on the type of population and amount of microorganisms in the air. According to **Handley *et al.* (1993, 1995)** temperatures above 24°C decrease airborne Gram positive and negative bacteria survival. Coliform bacteria are known to be sensitive to temperature. Positive correlations were observed between temperature and total coliform and *Escherichia coli* counts in a swine nursery **(Sirami, 1989)**. This bacterium exhibits rapid death at low humidity (<50%) and temperatures between 15°C and 30°C **(Yao *et al.*, 2010)**. **Wathes *et al.* (1984)** reported that bacterial numbers in the air were positively correlated with absolute humidity in veal calf units. The poor correlations recorded in our study may be explained by the movements of air from and into the slaughterhouse and between the different sections within it.

CONCLUSION

This study highlights the poor hygienic level of the municipal slaughterhouse of Constantine revealed by the high level of airborne bacterial contamination. The risk of contamination could be reduced by building effective structural separations between clean and unclean areas; controlling airflow by an effective system of ventilation and personnel training on good hygiene practices. These measures will guarantee a safe meat quality and prevent contamination and proliferation of pathogens in meat during slaughtering processes.

REFERENCES

AL-DAGAL, M., FUNG, D. 1990. Aeromicrobiology: a review. *Critical Reviews in Food Science and Nutrition,* 29(5), 333-340.
BURFOOT, D., WHYTE, R., TINKER, D, MCINTOSH, J. 2006. Importance of airborne contamination during dressing of beef and lamb carcasses. *Journal of Food Protection,* 69(12), 2828-2836.
DICKSON, J. ANDERSON, M. 1992. Microbiological decontamination of food animal carcasses by washing and sanitizing systems. *Journal of Food Protection,* 55, 133-140.
EL HADEF-El OKKI, S., EL GROUD, R., KENANA H., QUESSY, S. 2005. Évaluation de la contamination superficielle des carcasses bovines et ovines provenant de l'abattoir municipal de Constantine en Algérie. *Canadian Veterinary Journal,* 46(7), 638-640.

FOURNAUD, J., BERTAUD, M. 1981. Contamination de l'air à l'abattoir et qualité bactériologique des carcasses de bovins. *Siebenundzwanzig* (proceeding of the 27th Europaischer Fleischforscher Kongress) Vienna: Austria, 1, 188-191.

GILL, C., JONES, T. 1999. The microbiological effects of breaking operation on hanging beef carcass sides. *Food Research International*, 32, 453-459.

GILL, C., BRYANT J., LANDERS, C. 2003. Identification of critical control points for control of microbiological contamination in processes leading to the production of ground beef at a packing plant. *Food Microbiology*, 20(6), 641-650.

GOLDEN Surfer 9, Golden, CO, April 2009: User's manual.

GRAPHPAD Instat prism 5: *GraphPad Software, Inc., San Diego, CA, 2007*: User's manual.

GUYON, R., DOREY, F., MALAS J., LECLERCQ, A. 2001. Hazard Analysis of *Escherichia coli* O157:H7 Contamination during Beef Slaughtering in Calvados, France. *Journal of Food Protection*, 64(9), 1341-1345.

HANDLEY, B., WEBSTER A. 1993. Some factors affecting airborne survival of *Pseudomonas fluorescens* indoors. *Journal of Applied Bacteriology,* 75(1), 35-42.

HANDLEY, B., WEBSTER A. 1995. Some factors affecting the airborne survival of bacteria outdoors. *Journal of Applied Bacteriology,* 79, 368-378.

HEBER, A., PEUGH, M., LUTGRING, K., ZIMMERMAN N., LINTON, R. 2006. Poultry Slaughtering Plants: Concentrations of Microbial Aerosols in Poultry Slaughtering and Processing Plants. *ASHRAE Transactions*, 112(2), 644-655.

JERICHO, K., HO, J., KOZUB, G. 2000. Aerobiology of a High–Line Speed Cattle Abattoir. *Journal of Food Protection*, 63, 2000, 1523-1528.

KELMAN, A., SOONG, Y., DUPUY, N., SHAFER, D., RICHBOURG, W., JOHNSON, K., BROWN, T., KESTLER, E., Li, Y., ZHENG, J., MCDERMOTT, P., MENG, J. 2011. Antimicrobial susceptibility of *Staphylococcus aureus* from retail ground meats. *Journal of Food Protection,* 74(10), 1625-1629.

MOTHERSHAW, A., Al RAISI, A. 2008. Air as a source of bacterial contamination in a slaughterhouse prior to implementation of hygienic control systems. *International Journal of Postharvest Technology and Innovation*, 1(3), 337-347.

NORTHCUTT, J., JONES D., MUSGROVE, M. 2004. Airborne Microorganisms During the Commercial Production and Processing of Japanese Quail. *International Journal of Poultry Sciences*, 3(4), 242-247.

PEARCE, R., SHERIDAN, J., BOLTON, D. 2006. Distribution of airborne microorganisms in commercial pork slaughter processes. *International Journal of Food Microbiology*, 107(2), 186-191.

PODPEČAN, B., PENGOV, A., VADNJAL, S. 2007. The source of contamination of ground meat for production of meat products with bacteria *Staphylococcus aureus*. *Slovenian Veterinary Research*, 44(1/2), 25-30.

PRENDERGAST, D., DALY, D., SHERIDAN, J., MCDOWELL, D., BLAIR, I. 2004. The Effect of Abattoir Design on Aerial Contamination Levels and the Relationship between Aerial and Carcass Contamination levels in two Irish Beef Abattoirs. *Food Microbiology*, 21(5), 589-596.

RAHKIO, T., KORKEALA, H. 1997. Airborne Bacteria and Carcass Contamination in Slaughterhouses. *Journal of Food Protection*, 60, 38-42.

ROBERTS, T. 1980. The Effects of Slaughter Practices on the Bacteriology of the Red Meat Carcass. *Journal of the Royal Society of Health*, 100(1), 3-9.

SALUSTIANO, V., ANDRADE, N., BRANDÃO, S., AZEREDO, R., LIMA, S. 2003. Microbiological air quality of processing areas in a dairy plant as evaluated by the sedimentation technique and a one-stage air sampler. *Brazilian Journal of Microbiology*, 34(3), 255-259.

SERRAINO, A., BARDASI, L., RIU, R., PIZZAMIGLIO, V., LIUZZO, G., GALLETTI, G., GIACOMETTI, F., MERIALDI, G. 2012. Visual evaluation of cattle cleanliness and correlation to carcass microbial contamination during slaughtering. *Meat Science*, 90(2), 502-506.

SHEIKH, A., CHECKLEY, S., AVERY, B., CHALMERS, G., BOHAYCHUK, V., BOERLIN, P., REID-SMITH, R., ASLAM, M. 2012. Antimicrobial Resistance and Resistance Genes in *Escherichia coli* Isolated from Retail Meat Purchased in Alberta, Canada. *Foodborne Pathogens and Disease,* 9(7), 625-631.

SIRAMI, J. 1989. Contamination microbiologique de l'air dans le hall d'abattage. Facteur de variation et influence sur la carcasse. *Viandes Produits Carnés*, 10(3), 109-116.

VAN, T., NGUYEN, H., SMOOKER, P., COLOE, P. 2012. The antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* isolated from food-producing animals, retail meat and humans in South East Asia. *International Journal of Food Microbiology*, 154(3), 98-106.

WATHES, C., HOWARD, K., JONES, C., WEBSTER, A. 1984. The balance of airborne bacteria in calf houses. *Journal of Agricultural Engineering Research*, 30, 81-90.

WHYTE, P., COLLINS, J., MCGRILL, K., MONAHAN, C., O'MAHONY, H. 2001. Distribution and prevalence of airborne microorganisms in three commercial. poultry processing plants. *Journal of Food Protection*, 64(3), 388-391.

WORFEL, R., SOFOS, J., SMITH G., SCHMIDT, G. 1996. Airborne Bacterial Contamination in Beef Slaughtering-Dressing Plants with Different Layouts. *Dairy Food Environmental Sanitation*, 16, 440-443.

YAO, H., CHOI, H., LEE, J., SURESH, A., ZHU, K. 2010. Effect of microclimate on particulate matter, airborne bacteria, and odorous compounds in swine nursery houses. *Journal of Animal Science*, 88(11), 3707-3714.

YOKOYAMA, M., JOHNSON, K. 1988. Microbiology of the rumen and intestine. In The ruminant animal, Digestive physiology and nutrition, DC Church edition, 125-144.

ZHU, L., FENG, X., ZHANG, L., ZHU, R., LUO, X. 2012. Prevalence and serotypes of *Listeria monocytogenes* contamination in Chinese beef processing plants. *Foodborne Pathogens and Disease*, 9(6), 556-560.