

Prevalence and risk factors susceptibility of *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. isolated from pork and poultry sausages, in Reunion Island, France

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Abstract

Zoonoses are a public health burden in France. The most predominant pathogens incriminated in TIAC are *Salmonella* spp., *Campylobacter* spp., and *Listeria* spp. The population of Reunion Island eats a lot of pork and poultry meats. One of the local cooking characteristic is the sausage 100% poultry or 100% pig. Actually, no similar study has been conducted in Reunion Island. We aimed to determine the health risk related to the consumption of this kind of products, through a transversal analysis, by identification and quantification of bacteria in pork and poultry sausages. Meat characteristics and sale practices associated with these three bacteria in outlets of Reunion Island were identified by a binomial regression. The microbiological culture was carried out according to AFNOR methods.

Two hundred and three pork or poultry sausages were sampled randomly from 67 local distributors in Reunion Island. *Salmonella enterica* was detected in 11.87% (95% confidence interval: [7.80-17.32]) of sample and in 26.87% (CI 95% [17.11-39.2]) of the outlets, with a most probable number count ranging from 6.00 bacteria per gram to 380 bacteria per gram. *Salmonella* serotypes isolated from pork or poultry sausage were S. Typhimurium (45.83 %), S. London (20.83 %), S. Derby (16.67 %), S. Newport (8.33 %), S. Blockley (4.17 %) and S. Weltevreden (4.17 %). We found that *Salmonella* spp. infection was positively associated with two things. The first one is the packaging for sausages (plastic or paper) and the second one is the fact that there is no fight against rodents. High surface areas of sale (> 250 m²) decrease the risk.

Only 4.48% of the outlets studied and 1.48% of sausages were contaminated with *Campylobacter* spp. Risk factors couldn't be determined because of the low prevalence.

Listeria spp. was isolated in 64.18% of the outlets studied and in 30.00% of sausages. Dirty clothes of restaurant employees increased risks of *Listeria* spp. contamination whereas the use of a disinfectant to clean the refrigerated displays decreased risks of contamination.

Introduction

Poultry and pigs breeding are very important in Reunion Island. Pork and poultry products should be protected from any bacteriological contamination to avoid risks for human infection . Contamination of pigs and poultry production by *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. is a major public health burden and also an economic problem. It should be noted that *Campylobacter* spp. and *Salmonella* spp. are the main bacteria responsible for collective food-borne infections in industrialized countries. (EFSA., 2012).

One of the notable features of the local cooking remains 100% pork and 100% poultry sausages which are composed of lean, fat, but also skin. This last feature is not without consequence on the health risks associated with the consumption of this type of product since the skin is the headquarters of *Salmonella* spp., *Campylobacter* spp and *Listeria* spp and is being used for the manufacture of sausages (Chamber of Trades and Crafts, 2012). In Reunion Island, except bacteriological controls conducted on farms and in slaughterhouses, there is not enough available information about the impact of this bacterium in this type of food preparation on the consumers.

This study aimed to determine the health risk related to the consumption of this kind of products, through a transversal analysis. We determine the presence *Salmonella spp.*, *Campylobacter spp.* and *Listeria spp.* and the quantification of *Salmonella spp.* in pork and poultry sausages. Products' characteristics and risk factors for sale practices associated with the *Salmonella spp.*, *Campylobacter spp.*, and *Listeria spp.* seroprevalence in outlets in Reunion Island were identified.

Material and Methods

Study sample

To obtain a representative and randomly sample, a random drawing on an exhaustive list was done. Our study included 67 outlets (supermarkets, grocery trades and butcher shops) in Reunion Island.

Data collection

In each outlet, we took 1 to 5 samples of different categories of sausages following availability (fresh, frozen or packed sausages). For each outlet, data on manufacturing practices and conservation were collected from a questionnaire on the following topics: general characteristics, cleaning and disinfection procedures, staff hygiene, presence of rodents and other domestic animals and waste management.

Microbiological analysis

On samples, we determined the presence and identification of *Salmonella spp.*, *Campylobacter spp.* and *Listeria spp.* After identification of *Salmonella spp.*, the most probable number (MPN) technique was used to estimate microbial populations. For *Salmonella spp.* this whole procedure referred to the French AFNOR (French Agency of Standardization) procedure NF EN ISO 6579 which is the reference method. For *Campylobacter spp.* isolation and identification, procedure used the reference method NF EN ISO 11272-1. For *Listeria spp.* isolation and identification, procedure used the reference method NF EN ISO 11290-1.

Data analysis

One outlet is declared infected by *S. enterica* subsp. *enterica*, *Campylobacter spp.*, and *Listeria spp.* if at least one sausage sample is tested positive. Binomial regression was used to assess the relationship between explanatory variables and *Salmonella spp.*, *Campylobacter spp.* and *Listeria spp.* status of the outlet. The contribution of each factor to the model was tested with a likelihood-ratio χ^2 through a backward stepwise procedure. At the same time, the best parsimonious model was compared to the full model by the Akaike information criterion.

Results

Sample description. In total, we investigated 67 outlets including 15 butchers shops, 4 butchers shops & grocery trades, 28 supermarkets and 20 grocery trades.

Salmonella spp. The global prevalence of pork sausage was 11.82% (CI 95% [7.80-17.32]). 26.87% (CI 95% [17.11-39.24]) of the outlets studied. The most prevalent serotypes isolated were *Salmonella* Typhimurium and *Salmonella* London. Pork sausages, smoked sausages, fresh sausages and butcher shops were more significant infected.

Average of quantity of *Salmonella spp.* per sample infected is 72.90 bacteria per gram (Min: 6.00 bacteria per gram; Max: 380.00 bacteria per gram). Butcher shops associated with grocery trade shows a higher probable number than the others outlets.

The risk of outlet infection with *Salmonella spp.* was increased when sausages are sold in plastic bags (OR = 26.63; CI 95% [11.85 -752.55]) or in paper bags (OR = 9.00; CI 95% [1.59-171.89]). As well, no rodents control increased the risk of *Salmonella spp.* infection (OR = 5.42; CI 95% [1.48-20.43]). However, high surface area sale (> 250 m²) decreased the risk (OR = 0.99; CI 95% [0.98-0.99]).

Campylobacter spp. Only 4.48% of the outlets studied and 1.48% of sausages were contaminated with *Campylobacter spp.* Risk factors couldn't be determined because of the low prevalence. The results can be explained by the sensitivity of this bacterium to desiccation and cold treatment.

Listeria spp. *Listeria spp.* was isolated in 64.18% of the outlets studied and in 30.08% of sausages. Dirty clothes for restaurant employees (OR=1.96 ; CI 95% [1.01-3.79]) increased risks of *Listeria* contamination whereas the use of a disinfectant for cleaning of the refrigerated displays (OR=0.13 ; CI 95% [0.01-0.64]) decreased the risk of contamination.

Discussion

Salmonella spp. This study is the first one achieved in Reunion Island. The most prevalent *Salmonella* serotype among outlets was *Salmonella* Typhimurium. This is the first serotype detected on humans (CNRSS., 2009). This *Salmonella* serotype was also isolated frequently from pigs (Cardinale et al., 2010) rearing in Reunion Island. Quantities of *Salmonella* identified using MPN method doesn't exceed 1 100 bacteria per gram, which minimum concentration to cause infection non-typhoid *Salmonella* (Spricigo et al., 2008).

The fight against rodents is a major problem in Reunion Island. These rodents are known to be carriers of *Salmonella* spp. (Meerburg and Kijlstra, 2007; Meerburg, 2006) and represent a significant risk of transmission in outlets and in environment. In addition, paper or plastic packaging already contaminated, a cross-contaminated by a lack of personal hygiene (Norrung, 2000) or raw products sold in the refrigerated display next to the sausages are all possible hypotheses for the risk of contamination.

A bigger retail store could be related to the practical cleaning and disinfection. In addition, a bigger retail store limits the possibilities of cross-contamination because there is more space between products.

Campylobacter spp. The prevalence of *Campylobacter* spp. in outlets of Reunion Island is very low (1.48 %). This result is surprising since contamination of pork and poultry production is important (Henry et al., 2011).

Listeria spp. In Reunion Island, prevalence of *Listeria* spp. is high (30.00 %), which can be explained by a good ability of *Listeria* spp. to withstand cold because it's a psychrophilic bacteria (Rosset et al., 2002). The personal hygiene practices are very important because they can be the source of cross-contamination (Kahraman et al., 2010). Using a detergent to clean refrigerated displays can limit contamination or persistence of *Listeria* spp. in pork and poultry sausages.

Conclusion

In view of the results of our study, there may be a health risk to the consumer, considering that some people consume these products slightly cooked. Hygiene practices are necessary and essential in the fight against *Salmonella* spp., *Campylobacter* spp., and *Listeria* spp. in each sector of pork and poultry. The Chamber of Trades and Crafts carried important work by proposing guide to good practice, HACCP and to conduct an assessment of risks in the butchers. However, butchers and grocers are not sensitive to all these arguments and many efforts are still needed.

Acknowledgements

Thanks to Martine Denis for her help in organization of bacteriological analysis.

References

- Cardinale, E., Maeder, S., Porphyre, V., Debin, M., 2010. *Salmonella* in fattening pigs in Reunion Island: Herd prevalence and risk factors for infection. Preventive Veterinary Medicine 96,, 281-285.
- Chamber of Trades and Crafts 2012 (Saint Denis, Reunion island).
- CNRSS. 2009. Rapport d'activité annuel (Paris, Laboratoire des Bactéries Pathogènes Entériques Institut Pasteur.), 71.
- EFSA. 2012. The European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks in 2010 (European Food Safety Authority), 378.
- Henry, I., Reichardt, J., Denis, M., Cardinale, E., 2011. Prevalence and risk factors for *Campylobacter* spp. in chicken broiler flocks in Reunion Island (Indian Ocean). Preventive Veterinary Medicine 100, 64-70.
- Kahraman, T., Cetin, O., Dumen, E., Buyukunal, S.K., 2010. Incidence of *Salmonella* spp. and *Listeria monocytogenes* on equipment surfaces and personnel hands in meat plants. Revue Méd. Vét. 161, 108-113.
- Meerburg, B.G., Kijlstra, A., 2007. Role of rodents in transmission of *Salmonella* and *Campylobacter*. Journal of the Science of Food and Agriculture 87, 2774-2781.
- Meerburg, J.-R.W., Wagenaar JA, Kijlstra A., 2006. Presence of *Salmonella* and *Campylobacter* spp. in wild small mammals on organic farms. Appl Environ Microbiol 72, 960-962.
- Norrung, B., 2000. Microbiological criteria for *Listeria monocytogenes* in foods under special consideration of risk assessment approaches. International Journal of Food Microbiology 62,, 217-221.
- Rosset, P., Beaufort, A., Cornu, M., Poumeyrol, G., 2002. La chaîne du froid en agroalimentaire. Cahier de Nutrition et de Diététique 37, 124-130.
- Spricigo, D.A., Matsumoto, S.R., Espindola, M.L., Ferraz, S.M., 2008. Prevalence, quantification, and antimicrobial drug resistance of *Salmonella* serotypes isolated from fresh pork sausage. Ciencia E Tecnologia De Alimentos 28, 779-785.