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 characteristics 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 $\chi^2$ 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.

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**References**


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