

Epidemiological investigation of *Salmonella enterica* subsp. *enterica* all along the food chain poultry production in tropical area: example of Reunion Island

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Introduction

Reunion Island is a French island in the south-west Indian Ocean, between Madagascar (700 km) and Mauritius (300 km). Reunion Island has a tropical climate, and possesses immense biological diversity. It is also a European territory with modern infrastructures and services for professional activities.

Pig and poultry production is one of the main activities in Reunion Island as in European countries where the main animal product is pork (48.7%) followed by poultry (23.6%). So, meat and poultry products have to be shielded from contamination to avoid public health risks to consumers and financial problems for Reunion's poultry companies. The first industrial chicken farm on Reunion Island was constructed 60 years ago and the main slaughter house was built in the 1990s.

Reunion's poultry meat industry produced around 10 tons in 2009, which represented an average of 5 800 000 birds with a typical weight per chicken of 1.762kg. Broilers were slaughtered at around 45 days old. The industry aims to increase production by 3% per year. To attain this objective, the whole poultry production industry has improved its efficiency in procedures and costs. At the present time, chicken meat production is locally consumed (66% local production and 33% imported frozen from France). Contamination of chicken with *Salmonella* is a public health and an economic problem particularly because the Reunion island population consumes a lot of chicken (more than 35 kg per person per year) and also eats 100% chicken sausages.

In Reunion Island, except some bacteriological controls carried out in farms, no epidemiological study has yet been undertaken. Nevertheless, poultry producers consider *Salmonella* to be a major problem for broiler production. This study aims (i) to define *Salmonella* prevalence on broiler flocks at the farm and at the slaughterhouse, (ii) to identify risk factors of broiler infection, (iii) to identify any cross contamination from the farm to the slaughterhouse.

Materials and Methods

Sample collection

The study was carried out from May 2007 to February 2009 and involved 60 broiler farms randomly selected among those affiliated with Production Company in Reunion Island. The location and the day of placing chicks were given by the poultry company.

Data collection

A total of around 1,800 samples were collected. Each chicken farm was visited three times. The first visit was made before slaughtering at 43 days old to define the previous flock's *Salmonella* status. The second visit was made just 48 hours before the settle of chicks to assess the efficiency of cleaning and disinfection procedures. The third visit occurred at the end of the rearing period of the study flock between day 43 and day 45 before slaughtering. On broiler farms, samples were collected from 60 flocks at the end of the rearing period (faeces, litter, changing room, wall and equipment, outdoors). Data were collected from a questionnaire administered to each farmer concerning poultry house characteristics, practices and treatment on day-old chicks, management of dead birds, control of rodents and other domestic animals, watering practices, farm staff, cleaning and disinfection. This questionnaire was always submitted by the same person and it was pre-tested in a preliminary study. The final questionnaire was closed-ended questions. At slaughterhouse, samples were collected all along the process, from the transport crates, before defeathering, after defeathering, before clamping plan, after clamping plan, at the evisceration steps, from scalding water, from caeca, neck skin and carcasses; at the cutting plan on utensils and working plan, and from sausages. Samples from pigs, from poultry and other chicken were also collected from previous course. For humans, isolates were collected from hospital (GHSR Reunion), private laboratories and from Pasteur Institute (Paris, France).

Statistical analysis

The flock was the unit of observation. A flock was infected by *S. enterica* subsp. *enterica* if at least one pooled sample taken from the poultry house collected at the end of the rearing period tested positive. Thus, the outcome variable was dichotomous (infected versus non-infected). All variables were categorical with a number of

categories per variable limited. All frequencies of categories were > 10%. To assess the relationship between explanatory variables and *Salmonella* status of the flock, two stage procedures were used. Factors associated (Pearson χ^2 test, $P < 0.25$) with *Salmonella* infection of the flock were input a full model on R software for multivariable analysis. The second step included a logistic multiple regression model (1). Contribution of each factor to the model was tested with a likelihood-ratio χ^2 through a stepwise procedure.

Pulsed field gel electrophoresis (PFGE), Multiple loci variable number tandem repeats and antimicrobial susceptibility

The following harmonized protocol for PFGE was used for the study as described by Kerouanton (2). The method of MLVA was according to Lindstedt (3). Antimicrobial susceptibility was tested by the disk diffusion method following the CLSI guidelines (Clinical and Laboratory Standards Institute, 2008)

Results

In our study, 22% of chicken broiler flocks were infected by *Salmonella enterica* subsp. *enterica* at the end of the rearing period. The main serovar identified was *S. Virchow* (38%), then *S. Blockley* and *S. Livingstone* (15%) and *S. Typhimurium*, *S. Hadar* and *S. Senftenberg* (8%). At the slaughterhouse, 68% of batches were positive to *Salmonella enterica* subsp. *enterica*. The main risk factors identified at the end of the rearing period are described in table 1.

The genotyping of isolates showed a similarity with stable patterns. For example, for *S. Typhimurium*, the observed similarities were considerable for 10 patterns with more than 90% similarity between isolates from chicken, poultry, environment and humans (figure 1). The main resistances were observed for ampicillin, streptomycine, sulfonamides and tetracycline. The main resistance pattern was A,S,SuT, A,Su,SXT, T and S,Su, T.

Table 1 Risk factors identified at the end of the rearing period

Variables	Logistic regression model	
	OR	90% CI
Proximity of production to sugar cane	7.92	1.1, 90.05
<i>Salmonella</i> status of previous flock	6.89	1.3, 36.45
Age of poultry house (more than 15 Years)	5.36	1.2, 29.52
Use of antibiotics at Day1	4.9	1.1, 30.95
Thorough Cleaning and disinfection of poultry house and surroundings	0,05	0.001, 0.79

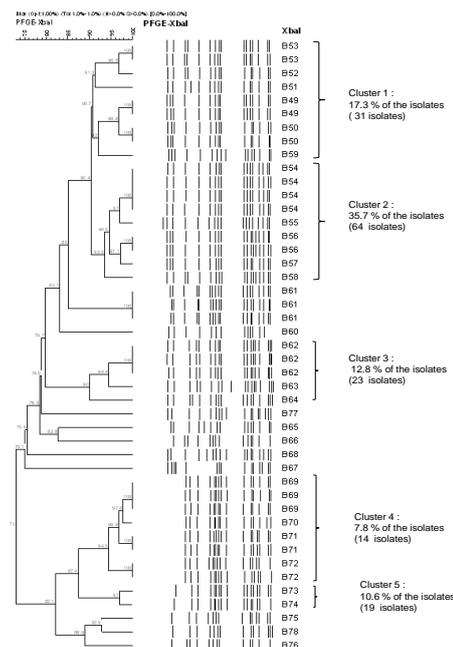


Figure 1 Dendrogram showing the cluster analysis of PFGE XbaI patterns of *Salmonella* Typhimurium generated by bionumerics software using the UPGMA method

Discussion

In our study, 22% of the broiler flocks were infected with *Salmonella*. This result is in conforms to 23.7% prevalence generally observed in European Union. Most of risk factors identified in this study have been reported in the literature (4) but some are specific and related to conditions in Reunion Island.

The original risk factor identified was the presence of sugar cane and plant fields next to poultry houses. Rodents are a legitimate problem because most of the territory is covered with sugarcane fields, natural habitats of rodents that can be very close to poultry farms. Rodents have been recognized as a vehicle of *Salmonella* and are often implicated in the infection of poultry (5). The age of the poultry house (> 15 years old, often built with tobacco drier) was often correlated with presence of porous walls that could contribute to the persistence of *Salmonella* because these walls are difficult to clean and because crevices could constitute recesses full of faeces contributing to development of bacteria. The administration of antibiotic drugs to one day-old chicks often used as prophylactic drugs against stress and mortality during the first day of life might reduce the number of colonized and shed bacteria (6).

The common PFGE pattern (B54, B62,B64 and B69) observed with *S. Typhimurium* for poultry and human isolates underlines a possible contamination of humans

with chicken as previously described by Nogrady et al. (7). The MLVA retained the high co-clustering with PFGE typing.

Furthermore, strong similarity was observed between isolates among clusters from chicken, environment, pig and human isolates. Homology found from humans, chicken and pigs' isolates highlighted a possible human contamination by infected chicken or pork. Cross contamination was also underlined at the slaughterhouse and positive flocks detected at the farms have been recovered at the slaughterhouse. Analyses using serotyping and more specifically macrorestriction profiling by PFGE with XbaI showed that no clonal relationship existed between PFGE and antibiotic resistance profiles. The antimicrobial resistance characteristics could have been acquired by selective pressure of drugs or by horizontal transfer. So it is necessary to investigate pig production to understand differences of veterinary practices with poultry industry. Furthermore, diversity but proximity of *S. Typhimurium* and S.I. 4,5,12:i:- was observed suggesting importation of this serovar into Reunion Island. So *Salmonella* Typhimurium and S.I. 4,5,12:i:- colonized Reunion Island and widespread in many hosts and vectors. Thus, many sources of infection of *S. Typhimurium* exist and it is not always possible to determine specific ways of transmission.

Most of *Salmonella* isolates were susceptible to all the tested antibiotic drugs by contrast with results observed in continental France. Most of the isolates from Reunion Island showed resistance to ampicilin, streptomycin, sulfonamides and tetracycline as shown previously demonstrated (8). These antibiotics have been the most commonly used antibiotics for animal production which explained the frequent occurrence of resistance for these antimicrobial agents (9).

The results of this study indicate that poultry is a potential vector for *Salmonella* in Reunion Island.

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