BirEx JOURNAL
Budapest International Research in Exact Sciences
Medical, Biological, Argiculture, Engineering
Science and other related areas
e-ISSN : 2655-7827
p-ISSN : 2655-7835
http://www.bircu-journal.com/index.php/birex

Microbiological Quality of Broiler Meat Sold on the Streets of Fianarantsoa

Rajaonarision J. L.[1,3,4,6], Rabemananjara V. A.[2,4], Rakotoarimanana R.[1,3,4], Ramahatradraibe N. J. A.[3,4], Razafindrazanakolona A.D.[1,4,5,6], Randrianarivelo R.[1,4,7], Robijaona Rahelivololoniaina B.[8,9], Rafanomezantsoa R. M.[1,4,6]

[1]*Faculty of Sciences;*
[2]*Faculty of Law, Economics and Management and Social Development Sciences*
[3]*Higher Institute of Science and Technology;*
[4]*University of Fianarantsoa;*
[5]*Institute of Sciences and Techniques of the Environment, University of Fianarantsoa, Madagascar*
[6]*Doctoral School Geochimed University of Fianarantsoa, Madagascar*
[7]*Technological Research Department, FOFIFA-Ambatobe, Antananarivo*
[8]*Doctoral School of Industrial, Agricultural and Food Process and Systems Engineering, University of Antananarivo, Madagascar*
rjacqlive@gmail.com

Abstract: *The aim of this article is to study the microbiological quality of broiler meat sold in the city of Fianarantsoa. A series of observations among producers showed that there are slaughterhouses set up in deplorably hygienic conditions throughout the districts. Microbiological analyses have shown the presence of indicators of faecal contamination accompanied by dangerous germs. All stages of the process were studied, from the slaughterhouse exit to the butcher's stalls and through to the most commonly consumed foods. All indicators far exceed the recommended health numbers. The accumulation of undesirable and pathogenic microorganisms has been distinguished. Enterobacteria indicative of fecal contamination, particularly fecal coliforms, were particularly noteworthy. In raw meats, laboratory analyses revealed Enterobacter cloacae (1,5. 102 CFU/g to >106CFU/g), Enterococcus sp (104 CFU/g), Escherichia coli (4. 103CFU/g) including E. coli -glucuronidase positive at 2,3.102CFU/g, les Staphylococcus ssp (102CFU/g), St. saprophyticus (2.104CFU/g), St. aureus (≈102/g CFU/g) and small colony visibilities of Salmonella ssp (≈1CFU/g to ≈2CFU/g). In grilled meats, Enterococcus feacalis (102CFU/g), Enterobacter cloaceae (4.102CFU/g) Enterococcus sp (3.102 CFU/g), E. coli (2.103CFU/g), St. saprophyticus (1,5.103CFU/g) and some levures Candida sp (>102CFU/g), were spotted. In fried foods, Enterococcus feacalis (103UFC/g), Enterococcus sp (3.105UFC/g), Escherichia coli (≈106UFC/g), Staphylococcus ssp (103UFC/g) and Candida sp yeasts (>102UFC/g) were identified. As for snacks, atypical forms were discovered by the presence of Enterococcus sp and Streptococcus sp (from 102 to 104UFC/g), E. coli (4.104/g), St. haemoliticus (104 UFC/g), St. aureus (2.102UFC/g) and Candida sp yeasts (>104UFC/g) fied. This article concludes that the sanitary and microbiological quality of broiler meat sold on the streets of Fianarantsoa is very critical, and requires appropriate measures and controls.*
Keywords: *broiler, slaughter, microbiology, fecal contamination, pathogenic bacteria, Fianarantsoa*

I. Introduction

The poultry farming is one of the major families of short-cycle livestock production, one of the most widely practiced in urban and peri-urban areas over the past few decades (Skarp et al., 2016; Hantanirina, 2010). Since its popularization in Madagascar, many urban and peri-urban households have become involved and invested in it (Rakotondrabe, 2013). Potential producers and consumers of broiler meat have now been identified in

Fianarantsoa's neighborhoods, and this meat is available in the form of grilled meats, appetizers and other menus in garrottes, and all over the streets and public squares.

However, a number of research studies have already shown that street foods are potentially "invisible" risk factors for food safety. They can damage the health of uninformed consumers (Baba-Moussa, 2006; Simon & Argenti, 2009). Consumption of broiler meat is one of the vectors of undesirable and pathogenic microorganisms.

However, the broiler production chain is one of the ways in which rural and urban farms in Madagascar's southern regions, including Haute Matsiatra, are being revitalized. Deficiencies in the sanitary quality of production affect several areas, including family farms, agri-food processing and the regional economy.

Given the disparities between sellers and consumers, and the inadequacy of functional control systems, the aim of this article is to study the microbiological qualities of broiler meat sold in the city of Fianarantsoa. The presence of indicator germs of fecal contamination and pathogenic germs was particularly identified. This work will contribute to the promotion of the sector and the consumption of protein-rich foods, helping to solve one of the public health problems in the Urban Commune of Fianarantsoa.

II. Materials and Method

2.1 Delimitation of the Study Area

This study was conducted in fifty (50) Fokontany or Neighborhoods of the City of Fianarantsoa. The city is located at latitude 21°27′09″ South and longitude 47°05′08″ East. It covers an area of 138.69 km2, divided into seven arrondissements and some fifty Fokontany or Quartiers (PRD, 2015).

2.2 Data Collection

First, street foods prepared with broiler meat and their respective consumers were identified. Street food vendors were located and inventoried by observers who were equipped with pre-tested observation sheets and a sketch of the locations of the sales outlets. Considering the preliminary study's results, the passive observation method was chosen. (Chauvin, 2010; Boutinot, 2014; Rana, 2016).

Secondly, this study identified and observed broiler slaughter sites with the consent of the owners. Each observer was also provided with pre-tested observation sheets and a sketch of the sales outlet locations.

2.3 Sampling

A quantity of approximately 25g of meat was taken at random for each sample. Sample repeatability rules were followed (Berche et al., 2000; García-Sánchez et al., 2017). For cooked foods, sampling was scheduled during peak consumption times.

The manipulator was equipped with a pair of single-use sterile gloves, sterile sample tubes, and a temperature-stabilized cooler at $T \leq 4°C$. Each tube was coded. Finally, the day's series of samples were delivered to the laboratory within $t \leq 3h$ maximum. The results of preliminary and similar studies have demonstrated the possible beginnings of the multiplication of most pathogenic microorganisms beyond these conditions. (Minvielle, 1999). Upon arrival at the laboratory, each sample was immediately reweighed and processed.

2.4 Laboratory Analysis

All sample preparation and processing in the laboratory was strictly carried out under a laminar flow hood and around a sterile circular space created by a constantly powered Bunsen burner. In compliance with NF V 08 guidelines, each sample was first introduced into a solution of peptone water, homogenized and aliquoted at a rate of 1.10-1, 1.10-2, 1.10-3 and 1.10-3 (Association française de Normalization, 2014).

Next, each aliquot was inoculated onto appropriate ready-to-use media, followed by incubation in a 37°C isothermal oven for 24h, 48h and 72h. Finally, light microscopy was used to count, identify and classify cocci and bacilli. The GRAM staining technique using the GRAM-Nicolle RAL kit was adopted (Randrianomenjanahary, 2006; Association française de Normalisation, 2014).

Ainsi:

- Total coliforms, faecal coliforms and faecal streptococci were enumerated on VRBL medium (Bio-Rad®), in which Violet Red Bile Lactose was adopted, in accordance with NF ISO 4832 guidelines.
- Escherichia coli β-glucuronidase positive was identified on UriSelect chromogenic culture medium (Bio-Rad®). Confirmation of a suspect colony was achieved by pouring Kovacs reagent onto the isolated agar.
- Coagulase-positive Staphylococcus spp was detected on Chapman's mannitol agar (Bio-Rad®) in accordance with NF V 08-014 ISO 6888 and V08-057-2 standards.
- And Salmonella ssp bacteria were identified on the Hektoën medium using the guidelines of standard V 08-052.

The microbiological criteria proposed by European Commission regulation (EC) no. 02073/2005 - applicable to foodstuffs "ready-made meals at the retail stage sold hot or cooked on the spot" - were used as references when interpreting the results (RÈGLEMENT (CE) N°2073/2005 DE LA COMMISSION).

2.5 Results Processing

Chi-2 and ANOVA comparison tests were adopted according to the hypotheses and objectives previously formulated for this study (Gatellier et al., 2012). Statistical processing was carried out using R 4.3.0 software (Ngoma Kouandzi et al., 2019).

III. Results and Discussion

3.1 Meat Production Processes

This study observed ten (10) sites carrying out 15 to 25 felling operations per session. It was found that the equipment and workspaces adopted by the producers are simple. Slaughtering and meat processing are carried out under artisanal conditions. The meat production stages are summarized in **Figure 1**.

According to **Figure 1**, the preparation of broiler meat in the study area consisted of 7 main stages.

a. Preparation of space and equipment. 75% of the sites are set up outside or in the owner's own yard. The workspace at the sites observed consisted of simple, ordinary wooden tables (65%) or directly on wooden planks flush with the ground. In general, the equipment consists of two or three well-lit charcoal stoves, two (02) or three (03) pots filled with boiling water, bowls, buckets, cups, sharp knives, a scale (electronic or Roberval) and a notebook.

b. Reception of the animals: the animals were taken one by one by hand. Over 45% of poultry legs were found to be visibly unsanitary.

c. Slaughtering involves cutting the animal's throat directly with a knife, one by one. In this way, the bird is drained of as much blood as possible. Some operators in this sector use cones. A certain amount of blood has been recovered.

d. De-feathering involves removing all the feathers in boiling water. Depending on the number of chickens to be slaughtered, two or three large kettles are placed on the hot charcoal hearths. The water in these pots remains unchanged during the operation. De-feathering was done by hand. Cleaning the buckets in cold water completed the plucking. Feathers and used water were immediately disposed of in the wild.

e. The cutting involved separating the animal's body from its feet. These were then placed in a bucket, ready for sale.

f. Evisceration, which involves removing the chicken's contents by hand and knife. The liver, heart and gizzard were cleaned and placed in a separate bucket. The intestines were immediately discarded.

g. And finally, the cleaned, gutted broiler bodies were weighed and registered.

The finished products for sale include broiler bodies, feet, liver, heart and gizzard. Deliveries start very early in the morning.

The total duration of operations varies from site to site. According to site managers, the duration is relative and depends on the number of people involved (manpower) and the number of birds to be treated. Staff are accustomed to the task and carry it out almost "mechanically". None has undergone any special training. Staff numbers vary from 5 to 10. No significant correlation was found between staff numbers and poultry numbers (p=0,833).

Waste, including blood, feathers, intestines, water, etc., significantly invades and piles up the workspace, the floor and the personnel. The latter has no personal protective equipment. 80% of sites do not have appropriate waste recovery systems.

All operations are carried out during the night to ensure very early morning deliveries. This study could not assess the conditions under which meat is transported or moved, the times and routes taken by meat from slaughterhouses to shelves, or the various stages of handling and preservation.

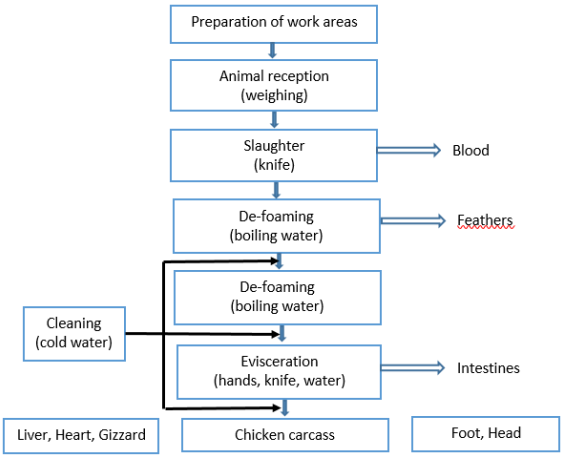

***Figure 1**. Broiler meat production steps*

3.2 Microorganisms in Raw Meats

Considering the preliminary results, three (03) collection points were selected for microbiological analysis of the raw meats: at the slaughterhouse exit, at the end of transport, and about 30 minutes on the shelf. Overall, the laboratory analyses enabled us to study some 50 samples from each collection point (n= 10 sites x 5 samples). **Table 1** summarizes the results obtained.

Table 1: Levels of pathogenic microorganisms in raw meats

	IDENTIFIED MICROORGANISMS (UCF/g)				
	Total coliforms	Faecal coliforms	*Escherichia coli*	*Staphylococcus spp*	*Salmonella ssp*
Health standards	10^1	10^1	10^1	10^1	Abs
Slaughterhouse exit	$5\ 10^3$	$2\ 10^5$	$3\ 10^2$	Abs	Abs
End of transport	10^4	$2.5\ 10^5$	$1.5\ 10^4$	$1.8\ 10^2$	1
30 min on display	$1.5\ 10^5$	$5\ 10^6$	$4\ 10^4$	$3\ 10^2$	2

According to **Table 1**, here has been a very significant deterioration in the microbiological quality of meats over time and space. All indicators far exceed the recommended health standards; from when the meat leaves the slaughterhouse to when it is displayed on the vendor's shelves. The accumulation of enterobacteria as indicators of fecal contamination is particularly remarkable, especially in the fecal coliform groups. In practice, fecal coliforms are the indicators of recent fecal contamination.

It was found that each time the meat was moved, there was a significant increase in the microorganism load of the raw meat. By way of illustration, a duplication of the number of microorganisms was recorded from the time the meat left the slaughterhouse until the end of the transport. These were indicators of fecal contamination *of Staphylococcus* and *Salmonella*. Microorganism loads multiplied by 15 to 30 times from the end of transport to display, except for *Salmonella*.

In addition to a few atypical forms, this study revealed the following enterobacteria: *Enterobacter cloacae* (ranging from 1,5. 10^2 UFC/g to $>10^6$UFC/g), and *Enterococcus sp* (10^4 UFC/g). *Escherichia coli* (4. 10^3UFC/g), including *Escherichia coli*-glucuronidase positive 2,3.10^2UFC/g, were also well distinguished.

The progressive appearances of *Staphylococcus ssp*, des *Staphylococcus saprophyticus* (2.10^4UFC/g) and *Staphylococcus aureus* ($\approx 10^2$/g UFC/g) were remarkable. This study found small colony visibilities of *Salmonella ssp* ≈1UFC/g jusqu'à ≈2UFC/g.

3.3 Microorganisms in Cooked Meats

After processing the results, this study retained three types (03) of samples of the street items most consumed during the day (3,821 consumers). These were grilled broiler meat (9.8% of consumers), fried broiler meat (14.18% of consumers), and snacks prepared with broiler meat (76.03% of consumers). Grilled meats were identified by night sellers and accompanied by alcoholic beverages. Les fritures ont été identifiées chez les vendeurs sur des places fixes et ambulants. Fried meats were identified by vendors in fixed places and on the move. And snacks were found all over Fianarantsoa's neighborhoods.

Meat used by street food vendors observed and collected during this part of the study is not necessarily from the batches of the 10 slaughtering sites previously. The choice of street food outlets is independent of the choice of the 10 slaughtering sites.

Identical to the approach adopted for raw meats, the laboratory analyses as a whole made it possible to study some 50 samples from each stage. **Table 2** summarizes the numbers of consumers and microorganisms identified in street foods.

Table 1. Levels of microorganisms in street food

	MICROORGANISMES IDENTIFIÉS (UFC/g)				
	Coliformes totaux	Coliformes fécaux	*Escherichia coli*	*Staphylococcus spp*	*Salmonella ssp*
Normes sanitaire	**10^1**	**10^1**	**10^1**	**10^1**	**abs**
Grillade de poulets	±2,7.10^2	≈10^3	±2.10^3	≈10^2/g	abs
Friture de poulet	±3,5.10^5	±2.10^2	≈4 .10^3	≈10^3/g	*1*
Amuses gueules	+4.10^3	≈2,5.10^2	±10^5	≈10^6/g	abs

According to **Table 2**, all the foodstuffs observed had contamination levels well in excess of the health recommendation. For grilled meats, the following enterobacteria were identified. *Enterococcus feacalis* (10^2UFC/g), *Enterobacter cloacae* (4.10^2UFC/g) *Enterococcus sp* (3.10^2 UFC/g) and some *Candida sp* yeasts (>10^2UFC/g). The study also found *Escherichia coli* (2.10^3UFC/g) and *Staphylococcus saprophyticus* ($1,5.10^3$UFC/g). *Salmonella* was recorded as absent in the samples studied.

In fried foods, *Enterococcus feacalis* (10^3UFC/g), and *Enterococcus sp* (3.10^5UFC/g), yeasts from g. *Candida sp* (>10^2UFC/g), *Escherichia coli* (≈10^6UFC/g) and *Staphylococcus ssp* were estimated at a load of around 10^3UFC/g. Identical to grilled meats, Salmonella was also recorded as absent in the samples studied.

As for snacks, atypical forms included microbial loads of *Enterococcus sp* and *Streptococcus sp* from 10^2UFC/g jusqu'à 10^4UFC/g. Yeasts of the *Candida sp* genus were also found (>10^4UFC/g) were also distinguished. *Escherichia coli* (4.10^4/g), *Staphylococcus haemoliticus* (10^4/g) and S*taphylococcus aureus* (2.10^2/g) were particularly identified. *Salmonella*, on the other hand, was absent.

It appears that the exposure times of cooked foods at markets are positively correlated with the contamination or accumulation of microorganisms on the food (*p = 0,0403*). Grilled food outlets only started selling at around 6 p.m. (67%), while fried food and snacks remained at the markets all day, according to the findings of this study. Secondly, the results in **Table 2** clearly showed that higher levels of total coliforms - indicators of past contamination - were recorded in all the samples studied. However, the levels of pathogenic microorganisms in g. *E. coli* and g. *Staphylococcus* is much higher in snacks than in the other two foods studied. The differences are not significant from one type of food to another (*p=0,0723*), and the nature of the contamination is independent of the raw material used (*p=0,0633*).

3.4 Discussion

The daily consumption of broiler meat can be seen day and night in the 50 districts of the city of Fianarantsoa. The choice of study area and types of food were thus justified. The approaches adopted made it possible to cover all the city's districts.

The methodological approaches adopted made it possible to track the essential levels of microbiological contamination of meats (from slaughterhouses to butchers' stalls) on the one hand, and the vectors of contamination (i.e. the main microorganisms identified) on

the other. The choice of slaughter sites and street food outlets observed are independent of one another. Indeed, the results of this microbiological study of raw and cooked broiler meats are representative of the realities of street foods in the city of Fianarantsoa.

The initial results have contributed to the elucidation of the production process in the reality of the players, including small and medium-sized enterprises. Although legislation requires the formalization of slaughtering and slaughtering operations, the specific characteristics of poultry for local consumption are masked by other factors (Order n°7708-97; Order n°7699/97; Law n° 2006-030; Order nº 9054-07; Law n°2015-014).

Operators involved in slaughtering and meat production are apparently invisible in the sector, at least in the town of Fianarantsoa. They are individual businesses in the informal sector, but located just about everywhere (PRD, 2015). The results of this article will contribute to the recognition of the realities and activities of these deniers. In addition to under-equipment, the negligence of operators, accentuated by the absence of control systems, is likely to contribute to the gradual introduction of significant health risks in the short and medium term (Taulo et *al*., 2008; Lytou et *al*., 2017; Rivera et *al*., 2018).

The results of this study showed that the levels of microorganisms in ready-to-deliver meats far exceeded recommended consumption standards (CE N°2073/2005). The results of a recent survey of sales outlets in the capital, Antananarivo, showed much lower contamination rates (Rajaonarison, 2018). Samples taken at markets in different contexts recorded only fecal contamination indicators of around 10^2UFC/g to 10^4UFC/g and *E. coli* ranging from 10^2UFC/g à 10^4UFC/g. As a result, chicken meat sold at retail outlets in Fianarantsoa (the provincial capital) is more rapidly contaminated than that sold in Madagascar's capital.

Direct or cross-contamination of the working environment, travel and the absence of cold chains are among the probable explanations or causes. In addition, results in the literature have already shown that broiler meat from wet slaughtering and plucking processes is more susceptible to the accumulation of post-mortem microorganisms (Cardinale, 2001; Boubendir, 2019).

As for the comparison of street foods (finished products) with raw meats (raw materials), the figures speak for themselves, showing that the quantities and qualities of microorganisms are similar, albeit with a few differences. While raw meats with indicators of fecal contamination have been noted and noticed, pathogenic bacteria have been observed in street foods. Therefore, the sanitary and microbiological quality of broiler meat sold on the streets of Fianarantsoa is highly critical. Unless decisions are taken in the short and medium term, there is a risk of damaging public health, family farms and businesses, agri-food processing and the regional economy. This sector must set up a standard slaughterhouse or school abattoir, apply appropriate administrative measures and regionalize or set up a food quality control laboratory.

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