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UNIVERSITE DE LIEGE – GEMBLOUX AGRO-BIO TECH

Risk assessment and control measures for *Salmonella* contamination in the Rwandan meat chain

Eugène NIYONZIMA

Essai présenté en vue de l'obtention du grade de Docteur
en sciences agronomiques et ingénierie biologique

Promoteurs : Prof. Marianne SINDIC
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Eugène Niyonzima (2017). Appréciation du risque et étude des mesures de maitrise pour *Salmonella* dans la filière viande au Rwanda. Ph.D thesis. Université de Liège – Gembloux Agro-Bio Tech. 164 p., 29 tableaux, 5 figures.

ABSTRACT

Salmonellosis constitutes one of the major food borne diseases worldwide. In Rwanda, as in other countries with developing economy, diarrheal diseases including salmonellosis are recognized as the major cause of morbidity and mortality in the population, after malaria. One of the sources of *Salmonella* infection in humans is the consumption of contaminated meat based-meals. In order to efficiently control the salmonellosis attributable to the consumption of meat based meals in Rwanda, a Quantitative Microbial Risk Assessment (QMRA) was undertaken. This exercise required the collection of data on factors associated to the risk of meat contamination by *Salmonella* at different stages of the chain, from the slaughter to the consumption, and conducted to the construction of a modular risk assessment model representing as much as possible the Rwandan meat chain. The objective of the model was to determine concrete options to mitigate the risk of *Salmonella* illness associated to the consumption of meat-based meals in Kigali (Rwanda).

Based on the collected data in animal slaughtering establishments, meat cutting and retail points, as well as the households and collective catering establishments in Kigali; a Quantitative Microbial Risk Assessment model was built up. The model considered three main risk exposure pathways namely the consumption of bovine meat within the household, the consumption of bovine meat outside the home, as well as the consumption of goat meat (grilled goat brochettes) in snack-bars and restaurants of Kigali.

According to the model, the risk of *Salmonella* illness associated to the consumption of meat based meals in Kigali city inhabitants appeared to be relatively low (ranging from 1.7 to 3.4% depending of the risk exposure pathway) and was found to be increasing with the meat consumption level in different socio-economical category of the consumers. The risk exposure level of female and young-adult consumers appeared to be significantly low. The analysis of risk mitigation scenario revealed that an efficient control of *Salmonella* illness associated to the consumption of meat based meals can be achieved through a simultaneous application of control measures at different levels of the meat chain, in particular at the preparation stage within households and collective catering establishments.

Key Words

Quantitative Microbial Risk Assessment, meat, *Salmonella*, risk factor, bacterial contamination, food, Rwanda

Eugène Niyonzima (2017). Appréciation du risque et étude des mesures de maîtrise pour *Salmonella* dans la filière viande au Rwanda. Thèse de doctorat. Université de Liège – Gembloux Agro-Bio Tech. 164 p., 29 tableaux, 5 figures.

RÉSUMÉ

La salmonellose est l'une des principales maladies d'origine alimentaire dans le monde. Au Rwanda, comme dans la plupart des pays en voie de développement, les infections alimentaires, dont les salmonelloses, constituent la principale cause de morbidité et de mortalité dans la population. L'une des sources de salmonellose humaine est la consommation des plats à base de viandes contaminées. Dans le but de lutter efficacement contre la salmonellose associée à la consommation des plats à base de viande au Rwanda, une appréciation quantitative du risque a été effectuée le long de la filière de production de viande. Cet exercice a requis une collecte de données sur les facteurs du risque de contamination de la viande par *Salmonella* à différentes étapes de la chaîne de production de viandes, de l'abattoir à la consommation; et a abouti à la construction d'un modèle d'appréciation quantitative du risque. L'objectif final du modèle était de déterminer les mesures concrètes afin de réduire le risque de salmonellose humaine associée à la consommation des plats à base de viande à Kigali (Rwanda).

Sur base des données collectées au niveau des établissements d'abattage, des ateliers de découpe et de vente au détail de viandes ainsi que dans les ménages et les établissements de restauration collective de Kigali, un modèle d'appréciation quantitative des risques a été construit. Celui-ci a considéré trois voies d'exposition au risque à savoir : les plats à base de viande bovine consommés au sein et en dehors du ménage ainsi que les plats à base de viande de chèvre (brochettes grillées) consommés dans les snack-bars et restaurants de Kigali.

Les résultats du modèle ont révélé que le risque de salmonellose en lien avec à la consommation des plats à base de viande dans la population de Kigali était relativement faible (1.7 à 3.4% en fonction de la voie d'exposition) et que le niveau d'exposition au risque était associé au niveau de consommation de viandes dans différentes catégories sociales des consommateurs. Les femmes de même que les jeunes-adultes étaient moins exposés au risque. L'analyse des scenarios d'atténuation du risque a montré qu'une lutte contre les salmonelloses humaines associées à la consommation de plats à base de viande devrait passer par une application simultanée d'une série de mesures à différents niveaux de la chaîne de production de viandes et en particulier à l'étape de préparation des repas dans les ménages et les établissements de restauration collective.

Mots clés

Appréciation quantitative des risques microbiologiques, viande, *Salmonella*, facteurs de risque, contamination bactérienne, aliment, Rwanda

DEDICATION

It is with great pleasure that I dedicate this work to:

My Professors who supervised me,

My parents who encouraged me

My beloved spouse Marie Claire,

My friends in Rwanda as well as in Belgium

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Eugène NIYONZIMA

Gembloux, 15st November 2017

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2. **Niyonzima, E**., Ongol, M. P., Brostaux, Y., Koulagenko, N. K., Daube, G., Kimonyo, A. and Sindic, M. (2016) 'Daily intake and bacteriological quality of meat consumed in the households of Kigali, Rwanda', *Food Control*, 69, pp. 108–114. doi: 10.1007/978-1-4419-7988-9.
3. **Niyonzima, E**., Ongol, M. P., Brostaux, Y., Korsak, N., Daube, G., Kimonyo, A. and Sindic, M. (2017) 'Consumption patterns , bacteriological quality and risk factors for Salmonella contamination in meat-based meals consumed outside the home in Kigali , Rwanda', *Food Control*, 73, pp. 546–554. doi: 10.1016/j.foodcont.2016.09.004.
4. **Niyonzima, E**., Ongol, M. P., Brostaux, Y., Koulagenko, N. K., Daube, G., Kimonyo, A. and Sindic, M. (2017) 'Meat retail conditions within the establishments of Kigali city (Rwanda): bacteriological quality and risk factors for Salmonella occurrence', *Tropical Animal Health and Production*, (in press). https://doi.org/10.1007/s11250-017-1466-6.

Poster communications

1. **Niyonzima, E**., Kimonyo, A. and Sindic, M. (2013) 'Analyse du risque de Salmonella et Escherichia coli dans la filière viande bovine au Rwanda' in *Conseil du département de chimie et bio-industries, Université de Liège-Gembloux Agro-Bio Tech*, Gembloux–Belgium, 2 July 2013. http://hdl.handle.net/2268/187076.
2. **Niyonzima, E**., Ongol, M. P., Kimonyo, A. and Sindic, M. (2015) 'Daily Intake and Bacteriological Quality of Meat Consumed In the Households of Kigali City (Rwanda)', in *First CAVM Research Exhition*, Kigali – Rwanda. http://hdl.handle.net/2268/180723.
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INTRODUCTION

INTRODUCTION

Meat is worldwidely known to be a nutrient-rich food. It provides important amount of proteins, vitamins (retinol and vitamin B12) as well as minerals (iron, zinc and selenium) with greater bioavailability than other food sources (McAfee et al., 2010). The meat consumption modes vary from one region to another depending to a number of factors such as the volume of livestock production, religious and cultural considerations as well as the socio-economic status of consumers; but the wealth was identified as the main determinant of meat consumption in both developed and developing countries (Mann, 2000; Speedy, 2003). The population meat intake is therefore expected to increase as the consumers wealth grows (Heinz & Hautzinger, 2007; Ma et al., 2006).

Meat constitutes a favorable medium for the proliferation of spoilage and pathogenic microorganisms. The high meat protein content provides nutrients for microbial proliferation whereas its neutral pH and high water activity are favorable conditions for the growth of a wide range of microorganisms. Thus, meat constitutes a potential vehicle for microbial pathogens and was reportedly associated to various bacterial food borne diseases in humans (Erickson & Doyle, 2007; Greig & Ravel, 2009). Salmonellosis is recognized as one of the major food borne infections in humans (CDC, 2013; EFSA & ECDC, 2015). Although meat is known to be one of the major vehicles for human *Salmonella* infection, the proportion of human salmonellosis associated to the consumption of contaminated meat-based meals is difficult to estimate accurately. This is mainly due to the limited number of illness cases that are officially reported to competent authorities; and the fact that even within the reported cases, a very small proportion allows the identification of the food vehicle (Scallan et al., 2011).

As in many developing countries, data on food borne infections particularly *Salmonella* are very scarce in Rwanda mainly because of an under-reporting of illness cases. Previous studies have reported the isolation of *Salmonella* species in stool samples from patients with gastroenteritis in Rwanda indicating the occurrence of *Salmonella* illness in the Rwandan population (Kabayiza et al., 2014; Lévy et al., 1986; Nzabahimana et al., 2014). However, little is known on the possible contribution of the consumption of meat-based meals to the occurrence of human salmonellosis in Rwanda. No published study, to the best of our knowledge, has yet assessed the occurrence of *Salmonella* in meat-based meals in Rwanda, though their consumption is continuously growing particularly in highly populated cities such as Kigali.

The aim of this study was to determine the prevalence of *Salmonella* species in meat along the meat production chain (from the slaughterhouse to the consumption), address the factors associated to the risk of *Salmonella* occurrence and finally, assess the risk of human salmonellosis attributable to the consumption of meat-based meals in Kigali city inhabitants through a modular microbiological risk assessment model representing as much as possible the Rwandan meat chain. The choice of Kigali city as the study area was justified by the fact that it is the most populated city in Rwanda. Available statistics indicate that Kigali city

counts for more than 10% of the Rwandan national population (National Institute of Statistics of Rwanda, 2012).

The present research is divided in seven chapters and counts five experimental studies. The first chapter deals with the review of the literature. It addressed the current knowledge on the risk factors and control measures for bacterial contaminations along the meat chain. The review concerned particularly the bovine meat as cattle are of great socio-cultural importance in the Rwandan society (Adekunle, 2007), and the focus was put on *Salmonella* and pathogenic *E. coli* as they are known to be the bacterial pathogens mostly associated to meat borne infections in humans (Greig & Ravel, 2009).

The chapters two and three present the findings from the first and second experimental studies. These studies addressed respectively the microbiological quality and safety of carcasses as well as meat cuts under retail; and elucidated the factors associated to the risk of meat contamination by *Salmonella* during the slaughtering process and retail within the establishments of Kigali city. The studies N°3 and 4 investigated the consumption patterns of meat-based meals in Kigali city inhabitants and assessed the microbiological quality as well as the safety of the consumed meals. The findings from these studies are presented in the fourth and fifth chapters respectively.

The chapter six addresses the risk assessment for *Salmonella* illness associated to the consumption of meat-based meals in Kigali city inhabitants as well as the analysis of scenarios aimed at reducing that risk (Study N°5); whereas the chapter seven is allocated to the general discussion of research findings, recommendations and future perspectives.

CHAPTER ONE

Risk factors and control measures for bacterial contaminations in the bovine meat chain – A review on *Salmonella* and pathogenic *E. coli*

Drafted from:

Niyonzima, E.,Ongol, M. P., Kimonyo, A., & Sindic, M. (2015). Risk Factors and Control Measures for Bacterial Contamination in the Bovine Meat Chain: A Review on *Salmonella* and Pathogenic *E. coli*. *Journal of Food Research*, 4(5), 98 –121. https://doi.org/10.5539/jfr.v4n5p98

CHAPTER 1. Literature review: Risk factors and control measures for bacterial contaminations in the bovine meat chain – A review on *Salmonella* and pathogenic *E. coli*

Abstract

Salmonella and pathogenic *Escherichia coli* are known to be the major bacterial agents responsible for human foodborne infections attributable to meat. A review of the specialized literature was carried out to identify the risk factors for bovine meat contamination by these pathogens from the cattle farm to meat consumption. Animal stress during transport to the slaughterhouse and the duration of the lairage period were identified as the key factors influencing the faecal excretion of *Salmonella* and pathogenic *E. coli* as well as cattle contamination prior to slaughter. At the abattoir level, hides and visceral contents appeared to be the main sources of pathogenic bacteria that contaminate carcasses along the meat production chain. Finally, temperature abuses during distribution and meat contamination by infected handlers were found to be important contributors to the post-slaughter contamination of bovine meat. The findings of this study indicate that efficient management of human food borne infections attributable to bovine meat requires an integrated application of control measures involving all actors along the meat chain, namely slaughterhouses, meat processing plants, distributors and consumers.

Keywords: bovine meat, *Salmonella*, pathogenic *E. coli*, safety, risk factors

1.1. Introduction

Meat is consumed in different parts of the world as a source of animal proteins (Food and Agriculture Organization, 2013) and its chemical composition is favourable for the proliferation of a wide range of microbial populations which makes raw meat to be one of the vehicles of foodborne infections in humans (Doulgeraki et al. 2012; Scallan et al., 2011). The actual number of foodborne infections attributable to meat is difficult to assess accurately, principally because only a small proportion of illness cases is officially reported especially in developing countries. On the other hand, even within the reported cases, only a limited number allow identification of the food vehicle. Data from outbreaks constitute an interesting source of information to associate foodborne illness cases to their respective food vehicles and causal agents (Scallan et al., 2011). Greig and Ravel (2009), by using outbreak data published internationally from 1996 to 2005, noted that 12.7 % of reported foodborne outbreaks were attributable to bovine meat while 10.5 and 4.6 % were associated with chicken and pork, respectively. According to the same authors, *Salmonella* and pathogenic *Escherichia coli*, respectively, were identified as the causal agents in 32.9 and 34.6 % of food borne outbreaks of bacterial origin attributable to beef.

Several studies have addressed the sources and potential control measures of bovine meat contamination by *Salmonella* and pathogenic *E. coli* at different stages of the meat chain i.e. primary production (Barkocy-

Gallagher et al., 2003; Millemann, 2008), animal transportation to the slaughterhouse (Arthur et al., 2007; Barham et al., 2002) ; slaughtering operations (Antic et al., 2010); further processing (Carney et al., 2006; Scanga et al., 2000), distribution (Haileselassie, Taddele, Adhana, Kalayou, & Tadesse, 2013); cooking (Juneja et al., 2001); however literature on bovine meat contamination and possible control measures considering the entire meat chain is still limited, probably because of the length and the complexity of the chain. The contamination of meat by microbial pathogens can occur at any stage of the meat chain (Duffy et al., 2006; Rhoades et al., 2009). Furthermore, the prevention or control of meat contaminations can be carried out at a stage of the chain different from the stages at which the contamination has occurred (Chen et al., 2012). Therefore, the food chain approach constitutes an efficient method to control bacterial contaminations of meat at consumption. The objective of this study was to review the existing knowledge on sources and risk factors for bovine meat bacterial contamination and provide an up to date view on control measures of the same by using a meat chain approach. The focus was put on *Salmonella* and pathogenic *E. coli*, as they are reported to be the leading causes of foodborne bacterial infections attributable to bovine meat (Greig & Ravel, 2009).

The literature search was undertaken first by reviewing literature in databases of peer-reviewed scientific publications, namely Scopus, PubMed and Google Scholar, using the following key words: cattle, bovine, beef, meat, safety, abattoir, slaughter, slaughterhouse, *Salmonella*, salmonellosis, *Escherichia coli*, microbial (bacterial) contamination, hygiene, risk factors and distribution. Only articles in English or French were retained. On the other hand, books and other official publications dealing with the subject were consulted.

In this paper, an overview of the prevalence of *Salmonella* and pathogenic *E. coli* in bovine meat was carried out before tackling their risk factors along the bovine meat chain and discussing their respective control measures.

1.2. *Salmonella* and pathogenic *E. coli* in bovine meat

Contaminated bovine meat is considered to be one of the sources of foodborne *Salmonella* and pathogenic *E. coli* infections in humans. The reported prevalence of *Salmonella* and pathogenic *E. coli* in bovine meat and products thereof varies from one product to another, but wide variability is also observed amongst different countries (Tables 1 and 2).

The prevalences are globally lower in bovine carcasses at the slaughterhouse level and higher in meat cuts and minced beef at retail (EFSA and ECDC, 2013b; Stevens et al., 2006). This could be associated with bacterial contamination of meat that can occur during the transport of bovine carcasses from the slaughterhouse to the meat processing units, during cutting and mincing operations within meat processing plants and/or during the marketing of bovine meat in retail outlets.

Table 1. The prevalence of *Salmonella* in fresh bovine meat

Product	% of positive samples	Number of tested samples	Country	References
Beef carcasses	42.8	236	Senegal	(Stevens et al., 2006)
	0.2	1275	Australia	(Phillips et al., 2001)
	6	250	Mexico	(Narvaez-Bravo et al., 2013)
	0	53	Poland	(EFSA and ECDC, 2014)
Butcher shop beef	20	25	Egypt	(Hassanein et al., 2011)
	9.9	354	Botswana	(Gashe & Mpuchane, 2000)
	2.4	370	Nigeria	(Tafida et al., 2013)
	1.02	2885	USA	(Vipham et al., 2012)
	0.8	274	France	(EFSA and ECDC, 2014)
	0.3	747	Germany	(EFSA and ECDC, 2014)
	1.1	117	Hungry	(EFSA and ECDC, 2014)
	0	26	Italy	(EFSA and ECDC, 2014)
	0.9	649	Netherland	(EFSA and ECDC, 2014)
Ground beef	20	25	Botswana	(Gashe & Mpuchane, 2000)
	11	88	Mexico	(Heredia et al., 2001)
	4.2	4136	USA	(Bosilevac et al., 2009)

Table 2. The prevalence of pathogenic *E. coli* in fresh bovine meat

Product	% of positive samples	Number of tested samples	Country	References
Beef carcasses	0.4	250	Mexico	(Narvaez-Bravo et al., 2013)
	0.9	453	Belgium	(EFSA and ECDC, 2014)
	1.3	622	Czech Rep.	(EFSA and ECDC, 2014)
	5.7	315	Germany	(EFSA and ECDC, 2014)
	0	203	Romania	(EFSA and ECDC, 2014)
Butcher shop meat	10	20	Turkey	(Temellİ, Eyİgör, & Anar, 2012)
	11.1	27	Egypt	(Mohammed et al., 2014)
	5.22	134	Botswana	(Magwira et al., 2005)
	1.8	492	Germany	(EFSA and ECDC, 2013b)
	0	45	Spain	(EFSA and ECDC, 2013b)
	3.2	555	Netherland	(EFSA and ECDC, 2014)
Ground beef	3.76	133	Botswana	(Magwira et al., 2005)
	3.85	52	Turkey	(Temellİ et al., 2012)
	16.7	30	Egypt	(Mohammed et al., 2014)
	3.8	479	Germany	(EFSA and ECDC, 2013b)

Niyonzima et al., (2013) reported a 2.2 log cfu increase in *E. coli* load between the slaughtering and marketing of beef at a commercial abattoir in Kigali city (Rwanda). Similarly, an increase in the prevalence and concentration of *Salmonella* and *E. coli* during the cutting and mincing of bovine meat is generally reported in meat processing plants (Hassanein et al., 2011; Rhoades et al., 2009; Scanga et al., 2000). The variations in *Salmonella* and pathogenic *E. coli* prevalence amongst different countries could be attributed to a number of factors (including the farming systems and practices, slaughtering practices and post-slaughter handling of meat as well as the general hygiene at different stages of the meat chain); which differ from one country to another. Higher prevalences are principally observed in developing countries, where poor hygienic conditions during slaughtering and meat handling are generally reported (Gashe & Mpuchane, 2000; Hassanein et al., 2011; Magwira et al., 2005; Stevens et al., 2006), whereas lower prevalence are mostly observed in developed countries where good hygienic practices are reported to be strictly followed and monitored along the meat chain (EFSA and ECDC, 2013; Vipham et al., 2012; Bosilevac et al., 2009).

The reported prevalence in different countries would be, however, not comparable because of differences in the sampling strategy and the analytical methods used. In some studies the number of analyzed samples amounted to thousands (Bosilevac et al., 2009; EFSA and ECDC, 2013b) , whereas in others only a very limited number of samples was analysed (Gashe & Mpuchane, 2000; Temellİ et al., 2012). Differences were also observed in sampling methodology, where the surface swabbed on bovine carcasses to detect pathogens or the weight of the meat samples analysed varied between different studies. In the studies conducted in European Union countries for example, the surface area covered by a carcass swab was reported to vary from 100 to 600 cm^2, while the weight of the meat sample analysed varied from 1 to 25 g (EFSA and ECDC, 2013b, 2014). Additionally, the analytical methods used to detect *Salmonella* and pathogenic *E. coli* in meat and meat products differed from one study to another. For *Salmonella,* a culturing method including a pre-enrichment phase in buffered peptone water, a selective enrichment and isolation followed by biochemical confirmation of isolates was the predominant method used (Bosilevac et al., 2009; Tafida et al., 2013). However, in other studies other detection methods such as PCR were used alone or in combination with a culturing method (Hassanein et al., 2011; Vipham et al., 2012). The same trend was observed in the methodology used to detect verotoxinogenic *E. coli* in meat and meat products (Temellİ et al., 2012). The prevalence of *Salmonella* or pathogenic *E. coli* in faeces, on hides or on bovine carcasses was reported to be higher when a PCR-based method was used than when the pathogen was detected by conventional culturing methods (Barkocy-Gallagher et al., 2003; Mainil & Daube, 2005). This would be due to the fact that PCR methods consider the bacterial DNA and take into account all the bacterial cells, whether living or dead; whereas the culture method only consider living bacterial cells (Johansson et al., 2000).

Even if differences in the sampling strategy and analytical methods used in different studies do not allow an accurate comparison of the prevalence of pathogenic *E. coli* and *Salmonella* in meat amongst different countries, it appears that these two pathogens are detectable worldwide in significant proportions in meat in general, and particularly in bovine meat. According to the EFSA and ECDC report (2014) on zoonoses, data collected in 2012 from nine European Union member states showed prevalences of 1.3 and 0.1%, respectively, for verocytotoxigenic *E. coli* (VTEC) and VTEC O157 in fresh bovine meat. The prevalence of VTEC in meat from animal species other than bovines in the EU was not estimated, probably because of the non-representativeness of the data available. However, the prevalence of VTEC in different Member States in 2011 was reported to be higher in bovine meat compared to meat from other animal species.This could be probably due to the fact that the enteric carriage of pathogenic *E. coli* is mostly observed in cattle than in other animal species (Mainil &Daube, 2005). In Ireland, VTEC was detected in 1% of 291 bovine carcass samples, while no positive finding was reported from 134 sheep carcass samples (EFSA and ECDC, 2013b).

A comparable observation was reported in the Czech Republic, where 0.3% of 1159 bovine carcasses were reported to be positive for VTEC while not a single positive sample was found in 1395 pig carcasses (EFSA and ECDC, 2013b). At the retail level, the Netherlands reported 0.3% of 702 bovine meat samples were positive for VTEC while no positive sample was found from 86 sheep meat samples (EFSA and ECDC, 2013b). In contrast, a higher prevalence of VTEC was reported in Spain, where 2.9% of 34 poultry samples were found to be positive for VTEC against a prevalence of 0.0% (n=45) in bovine meat (EFSA and ECDC, 2013b).

The prevalence of *Salmonella* in bovine meat has been found to be low compared to meat from other animal species. In the European Union, during 2012, the prevalence of *Salmonella* in bovine meat and products thereof was reported to be 0.2% whereas in pig and broiler meat it was estimated to be 0.7 and 4.1%, respectively (EFSA and ECDC, 2014).

The highest *Salmonella* prevalence observed in poultry meat could be attributed to the colonization of the reproductive tract of infected subjects by the pathogen that may increase the probability of *Salmonella* dissemination on carcasses under preparation through cross contamination (Gast, Guraya, Guard-Bouldin, Holt, & Moore, 2007). Although the prevalence of *Salmonella* in bovine meat seems to be relatively low, contaminated bovine meat remains a significant risk for *Salmonella* infection in humans, particularly for people consuming more beef than meat from other animal species. Additionally, the high protein and fat content of foods such as meat was reported to protect the bacterium against the gastric acidity (Birk et al., 2012; Blaser & Newman, 1982; Kothary & Babu, 2001). This suggests that the consumption of contaminated meat, even with a limited number of pathogens, would present a significant risk of infection and/or intestinal colonization in humans.

As for other bacterial pathogens, the minimum number of *Salmonella* capable of causing illness, is difficult to determine as it depends on a number of factors including (but not limited to) the food matrix, the host susceptibility and the virulence factors of the pathogen (McEntire et al., 2014). However, recent studies using outbreak data indicate that doses as low as 36 colony forming units can cause illness in humans (Teunis et al., 2010a). This infective dose would be qualified as "low "comparatively to foodborne pathogens such as *Vibrio cholerae* that require doses as high as 10^4-10^8 cells to cause infection in humans (Kothary & Babu, 2001). The infective dose for pathogenic *E coli* is also known to be "low". Coia (1998) reported contamination levels as low as 2 organisms per 25 grams in food and environmental samples incriminated in VTEC O157 outbreaks. Because of the low infective dose, the contamination limit for these pathogens has been fixed to the absence in 25g of meat preparations intended to be eaten raw (European Commission, 2005).

It is assumed that the level of microbial contamination of meat at the end consumer stage is function of contaminations acquired during different stages of meat preparation. Therefore, reducing the prevalence of foodborne infections such as *Salmonella* and verotoxinogenic *E. coli* attributable to bovine meat in humans requires integrated control measures involving all actors in the bovine meat chain from primary production to the final consumer.

1.3. Bacterial contamination of bovine meat along the production chain

1.3.1. Preslaughter contamination of live cattle

Salmonella infection is commonly reported in different animal species. Considering their adaptation to hosts, *Salmonella* serotypes are grouped in three categories: namely serotypes only pathogenic for humans like *S.* Typhi and *S.* Paratyphi; serotypes adapted to animal species such as *S.* Gallinarum, *S.* Dublin, *S.* Abortusequi, *S.* Abortusovis and *S.* Choleraesuis which are pathogenic for poultry, cattle, horses, sheep, and pigs respectively; and finally ubiquitous serovars like *S.* Typhimurium and *S.* Enteritidis adapted to humans and other animal species (Jay et al., 2005). In cattle, *Salmonella* infection can be clinically manifested by a wide range of symptoms including diarrhoea and possible dysentery, joint infections, pneumonia as well as abortions (Millemann, 2008). However, bovines may also carry *Salmonella* in their gastro-intestinal tract without any clinical symptom of the disease. In the latter case bovines are called asymptomatic carriers. In both infected and asymptomatic carriers, *Salmonella* can be excreted through the faeces for a relatively long period. Gopinath et al. (2012) reported that the faecal shedding of *Salmonella* in cattle may last up to 400 days.

As with *Salmonella*, asymptomatic carriage and faecal shedding of pathogenic *E. coli* are common in bovines of all ages; but clinical manifestations of the disease are mainly observed in young calves with 2 weeks to 2 months of age with diarrhoea as the main symptom (Alexa, Konstantinova, & Sramkova-Zajakova, 2011; Millemann,

2008). The duration of faecal shedding in cattle can last up to 19 weeks (Khaitsa et al., 2003). On a clinical basis, pathogenic *E. coli* strains are grouped in 3 classes namely those rarely associated to diseases either in animals or in humans (i.e VTEC-2), strains associated to disease in both animals and humans (i.e EHEC-2) and finally strains such as EHEC-1 and VTEC-1 reported to be highly infectious for humans but rarely in animals (Mainil & Daube, 2005).

The faecal shedding of *Salmonella* and pathogenic *E. coli* constitutes an important factor of cattle contamination. In fact, pathogens excreted in the faeces may contaminate the environment through which other cattle can acquire contamination and carry the bacteria in their digestive tract and/or on their hides (Rhoades et al., 2009). The contamination of live cattle destined for slaughter may occur at the farm level, during the transportation of bovines to the slaughterhouse or during the lairage period in the abattoir.

At the farm level, contaminated feed and water have been reported to be the main sources of *Salmonella* and pathogenic *E. coli* infections in cattle (Millemann, 2008). However, dissemination of the infection within the herd is mainly attributable to faecal excretion of the pathogens. The prevalence of pathogenic *E. coli* and *Salmonella* is generally reported to be higher on cattle hides than in the faeces. This is due to the fact that a single animal shedding the pathogen in its faeces may contaminate the hides of many other animals in the herd, either directly or via the ground and lairage fixtures (Small et al., 2002). In a study conducted on 200 steers and heifers in a large feed yard, Barham et al. (2002) reported an *E. coli* O157 prevalence of 18% on hides while its prevalence in faeces was as low as 9.5%. A similar relationship was reported by Barkocy-Gallagher et al. (2003), who detected *E. coli* O157:H7 on 60.6% of cattle presented for slaughter, while the faecal prevalence was 5.9%. As with VTEC, *Salmonella* prevalence was reported to be higher on cattle hides than in faeces. Barkocy-Gallagher et al. (2003) reported a *Salmonella* prevalence of 71% on the hides of feedlot cattle while a prevalence of only 4.3% was recorded from faecal samples of the same group.

The control of pathogenic *E. coli* and *Salmonella* infections on cattle farms includes the treatment of all carriers and infected subjects but also limiting the spread and severity of the disease. When the infection is identified early in the herd and few animals are affected, their isolation is an important measure to consider. Furthermore, faecal dejections from infected animals should be managed in a manner to avoid contamination of feed, water or livestock equipments. Antibiotic therapy, especially in subjects affected by salmonellosis, should be used cautiously as the emergence of *Salmonella* strains resistant to antibiotics commonly used in veterinary medicine is reported to be increasing (EFSA and ECDC, 2013a; Stevens et al., 2006). The treatment of *E. coli* and *Salmonella* infections in cattle herds has been thoroughly reviewed by Millemann (2008) and is not further developed in this paper.

The faecal shedding of pathogens from asymptomatic carriers constitutes a serious obstacle on the control of *Salmonella* and pathogenic *E. coli* infections in cattle; as shedders are not clinically identifiable and consequently, in most of times, not subjected to treatment. Traditionally, asymptomatic carriers can be detected through the culture of multiple faecal samples collected from suspected shedders during a relatively long period (Gopinath et al., 2012; Guy, Tremblay, Beausoleil, Harel, & Champagne, 2014). However, this approach presents a disadvantage of being logistically difficult to conduct and inefficient especially in carriers where the faecal shedding of *Salmonella* or pathogenic *E. coli* is intermittent (Edrington et al., 2004; Fitzgerald et al., 2003). As an alternative to the cultural methods, serological methods that consist in the detection of antibodies specifically directed against some antigens expressed by the pathogen exist. An example is the measurement of immunoglobulins directed against O-antigens from *Salmonella* Dublin in the blood that was reported to be used as an indicator of *Salmonella* infection in cattle (Robertsson, 1984). However, further studies indicate that serological tests are indicative on the current and/or previous infection status of the subjects but not on their shedding status (Olopoenia and King, 2000). Therefore, considering the importance of the detection of shedders in the control of *Salmonella* and pathogenic *E. coli* infections in cattle farms and the weaknesses of the existing methods, it is recommended to develop more sensitive methods to detect shedding animals in the herd. Meanwhile, one should consider an approach consisting of serological screening followed up by a faecal culture of all seropositive animals to detect active carriers (Nielsen, 2013).

Animal stress is known to induce high levels of secretion of *Salmonella* and pathogenic *E. coli* in cattle faeces and increase the probability of contaminating healthy animals (Gopinath et al., 2012; Mainil & Daube, 2005). During their transport to the slaughterhouse cattle may be subjected to a number of stresses, including high stocking densities, long transport duration, abnormal temperatures, noise pollution and changes in the general environment that can significantly increase the number of shedders. Cattle can also be infected by pathogenic microorganisms from a contaminated truck that has not been properly cleaned and disinfected or by direct contact with infected animals transported in the same truck. Similarly, contaminated transport trucks can be a source of infection for slaughterhouses and farms initially free of *Salmonella* or pathogenic *E. coli*. At the slaughterhouse level, cattle are kept in lairage before killing them. In Europe and the United States, cattle are generally slaughtered on the day of their arrival to the abattoir, while in other countries they are usually slaughtered the day after. In the latter case, the period of lairage allows animals to rest, rehydrate and recover from the stress of transport (Ferguson & Warner, 2008). During the period of lairage, cattle can be subjected to these same stress factors that increase the risk of contamination. On the other hand, in most cases the lairage is only cleaned at the end of the day and is therefore a potential source of contamination for cattle that can acquire an infection from contaminated animals or a soiled environment (Beach et al., 2002). Different authors have reported significant increases in pathogen prevalences on cattle hides during their transport and in the lairage period in the slaughterhouse. In a study

conducted on 286 cattle, Arthur et al. (2007) reported that the prevalence of *E. coli* O157:H7 on hides increased from 50.3 to 94.4% between the time the cattle were loaded onto tractor-trailers at the feedlot and the time their hides were removed in the slaughterhouse. Similarly, Barham et al. (2002) reported an increase in *Salmonella* prevalence on cattle hides (from 6 to 89%) during the transport and lairage of 200 cattle, whereas the prevalence of *Salmonella* in the faeces of the same group increased from 18 to 46%. A number of measures could contribute significantly to reducing the risk of bacterial contamination of cattle destined for slaughter in the preslaughter environment. At the farm level, cattle destined for slaughter should be clean and dry with no visible dirt on their hides (Antic et al., 2010). Any practice that can generate animal stress during transport, such as mixing cattle from different farms and over loading trucks should be avoided (Small & Buncic, 2009). Likewise, trucks should be cleaned and disinfected after each transport of cattle (Swanson & Morrow-Tesch, 2001). At the abattoir, the lairage period should be kept to a strict minimum. Heavily contaminated animals must be separated from the others and the lairage pens must be cleaned and disinfected at the end of each slaughtering day and monitored by visual and bacteriological control (Wong et al., 2002).

1.3.2. Contamination during the slaughtering process

In the abattoir, the cattle slaughtering process includes successive steps, namely: stunning, sticking, skinning, evisceration, carcass splitting, refrigeration and eventually cutting and deboning (Figure 1) that can contribute significantly to the overall microbial load of bovine carcasses and meat cuts. In this section, sources, risk factors and control measures for meat contamination by *Salmonella* and pathogenic *E. coli* throughout the cattle slaughtering process are reviewed and discussed.

1.3.2.1. Cattle stunning

Stunning is an operation that aims to render animals destined for slaughter unconscious prior to sticking and bleeding. It allows suffering by the animals to be minimised during the slaughtering process, especially the sticking. Beside animal welfare considerations, stunning also makes the sticking and bleeding operations less hazardous for the operator (FAO, 2006). Although chemical and electrical stunning methods are allowed in domestic ungulates, mechanical stunning is the most commonly used stunning method in cattle (Gregory et al., 2000). The devices used for mechanical stunning can be of a penetrating or non-penetrating type. A number of studies have been conducted to address possible microbial contamination of meat during the stunning process. In one experimental study, Buncic et al. (2002) demonstrated that the use of a penetrating captive bolt (PCB) in sheep presents a risk of microbial contamination for stunned animals through the stun wound.

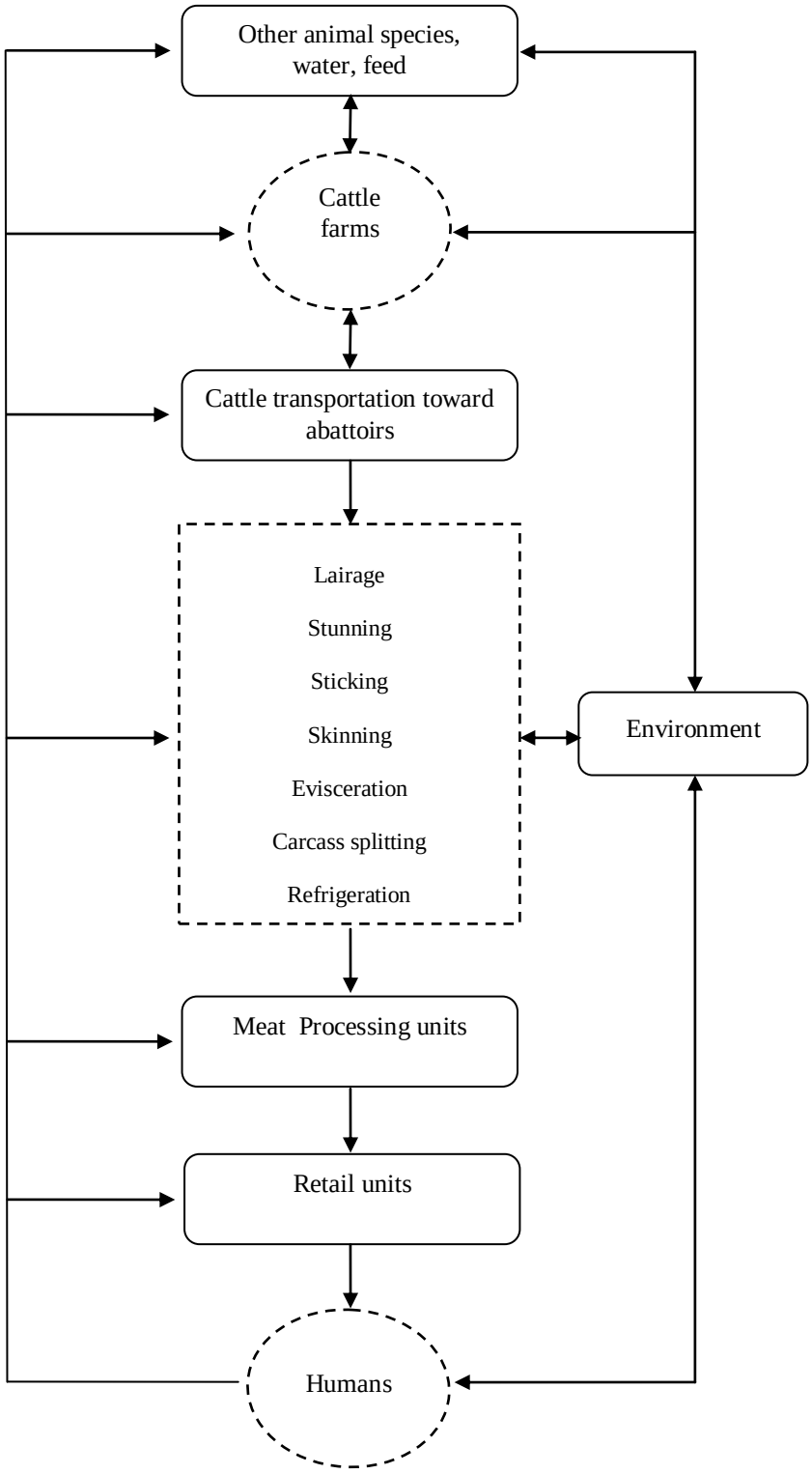

Figure 1.Potential sources and pathways for microbial contamination of bovine meat. (Adapted from : FAO, 2006 and Millemann, 2008)

Marked organisms (*E. coli* K12 or *Ps. fluorescens*) were inoculated into the brains of sheep through the stun wound immediately after stunning by a cartridge-operated, penetrative captive bolt pistol. The marked organisms were found in blood, liver, lungs, spleen and lymph nodes and on the surface of inoculated animals. When the same pistol was then used to stun subsequent healthy sheep, marked organisms were found in the blood of 30% to 40% of the animal carcasses. Similar findings were reported by Daly et al. (2002) after inoculation of a marker strain of *Ps. fluorescens* into the central nervous system of cattle. Prendergast et al. (2003) reported dispersion of central nervous system tissues when a PCB was used for animal stunning.

Although the contamination of bovine carcasses by microorganisms introduced into the central nervous system during the stunning process by penetrating devices has been demonstrated experimentally, further studies are needed to assess the risk of such contaminations under commercial conditions. During the mentioned studies (Buncic et al., 2002; Daly et al., 2002), the levels of bacteria inoculated experimentally into the brain were relatively higher comparatively to the levels of bacteria commonly reported in slaughterhouses; suggesting that the risk of transmitting pathogens through the stun wound would be much lower under commercial conditions. However, as it known that *Salmonella* and pathogenic *E. coli* require low infective doses (Blaser & Newman, 1982; Coia, 1998) the risk should be considered as significant. Beside the possible contamination of cattle via the contaminated stunning gun, different authors reported regular cross contamination of hides in the stun box between stunned animals consecutively fallen in the same box via contaminated surfaces (Small et al., 2002; Small & Buncic, 2009) highlighting the need of a proper sanitation of the stun box.

As a control measure for food safety issues associated with the use of penetrating stunning devices in cattle, alternate stunning methods should be considered. The use of non-penetrating guns appears to be a good alternative. Nevertheless, potential problems associated with this type of gun, such as the frequent recovery before sticking, need to be resolved (EFSA, 2004). The use of electrical stunning seems to be another safer option (Anil et al., 2001). This method is used in different countries, namely New Zealand, Australia and the United Kingdom (Wotton et al., 2000); however, its high cost and some doubts about animal welfare associated with the ineffective use of this method need to be addressed (Heim et al., 2007). Furthermore, the possibility of cleaning and disinfecting the stun box after stunning each animal should be considered to avoid cross contamination of hides from faecally soiled surfaces during the stunning process.

1.3.2.2. Cattle sticking

Sticking is an operation that consists of severing the major blood vessels of the animal in order to extract the maximum amount of circulating blood during bleeding. Two sticking methods are generally used in cattle: thoracic and cervical sticking. Thoracic sticking includes a section of major blood vessels from the heart and

allows rapid and complete bleeding, whereas during cervical sticking only vessels in the neck (carotid arteries and jugular veins) are cut and bleeding out is slower (FAO, 2006). The stick wound constitutes a channel that can allow the introduction of microbial contaminants into the carcass. The main source of contamination is the sticking knife, which can contaminate carcasses by direct transfer of bacteria from the transpierced skin but also by cross contamination if the knife is not sterilized between successive sticking operations.

In an experimental study (Mackey & Derrick, 1979), it was demonstrated that microbial contamination of bovine carcasses can occur during sticking. Marked strains of *E. coli*, *Cl. perfringens* and *Bacillus thuringiensis* were placed on a sticking knife before use. After the sticking operations, marked organisms were isolated from the internal organs, namely the heart, lung, spleen, liver and kidneys and from muscles. However, even if the potential for meat contamination from the sticking knife has been shown under laboratory conditions, the risk of such transfers, especially for pathogens like *Salmonella* and pathogenic *E. coli*, under commercial conditions seems to be quite low. Mackey & Derrick (1979) reported that in order to induce contamination of the deep tissues of a carcass a large inoculum of 10^{10} to 10^{12} bacteria was required, whereas the actual level of contamination generally encountered in slaughterhouses is many orders of magnitude less. In a study conducted on bovine hides at a beef slaughter plant in Ireland, hide contamination by *E. coli* O 157 was reported to be as low as 100 cfu per 100 cm^2 or less in 90.8% of 109 cattle (O'Brien et al., 2005). Comparable findings were reported in the USA, where 62.7% of 124 cattle were found to carry fewer than 100 cfu/100 cm^2 of *E. coli* O 157 (Rhoades et al., 2009). The concentration of *Salmonella* on cattle hides is also known to be relatively low. In a study conducted on 100 cattle at slaughter, Fegan et al., (2005) reported a prevalence of 68% with the highest concentration being 4.8 MPN per cm^2. Nevertheless, contaminated knives remain an important source of localised microbial contamination of the sticking wound (Rheault et al., 1999). Additionally, the sticking wound can be contaminated by microorganisms from the environment, especially when exsanguination is performed on animals lying on the ground.

In order to avoid/prevent microbial contamination of bovine carcasses through the sticking wound, cattle should be bled out in a suspended position to prevent contamination from the slaughtering environment (FAO, 2006); two separate knives should be used for sticking (one for the skin and another for muscles) and they should be decontaminated in hot water at 82°C or by another method with equivalent effect after being used (Eustace et al., 2007) ; and finally, the sticking site should be trimmed if any microbial contamination is suspected (Rheault et al., 1999).

1.3.2.3. Hide decontamination treatments

Cattle hides constitute one of the main sources of carcass contamination by bacterial pathogens such as *Salmonella* and pathogenic *E. coli,* whereas the contamination of hides is generally acquired from faeces of colonised animals or indirectly from the soiled environment (Arthur et al., 2010). The contamination of carcasses from soiled hides occurs during the skinning process. A number of intervention strategies to reduce the bacterial load on cattle hides and consequently reduce the risk of carcass contamination during skinning operations, have been addressed by various authors. These include physical, chemical and biological treatments applied alone or in combination. In this section major hide decontamination treatments are reviewed and their effect on reducing the bacteriological load on cattle hides is discussed.

The reported physical decontamination treatments include hide washing with cold or hot water, steam sprayings and cattle dehairing. Washing cattle hides with water has been found to remove dirt from the hides but seemed to have a minimal effect on the bacterial load of treated hides. The study by Mies et al. (2004) showed that washing cattle with cold water for 2 minutes did not permit significant reductions in aerobic bacteria, coliforms and *E. coli* on the hides. However, raising the water temperature from 15 to 60 °C reduced the load of aerobic bacteria by 0.5 logarithmic units (Bosilevac et al., 2005).

The effect of steam sprayings in decontaminating cattle hides was studied under laboratory conditions by McEvoy et al. (2003). These authors, by treating cattle hide pieces with steam at subatmospheric pressure during 1 to 20 seconds, reported reductions in total viable bacteria on treated hides of 2.9 to 3.9 logarithmic units after a treatment at 80°C, while similar treatments at 75°C reduced total viable bacteria counts by only 1.9 to 2.6 log units. In another study, reductions of inoculated *E. coli* O157 by 4.2 to 6.0 log units were reported after spaying cattle hides by steam at 80°C during 10 to 20 seconds (McEvoy et al., 2001).

Dehairing cattle can be carried out by clipping the hide or using chemicals. The study by Small et al.(2005) showed that dehairing cattle hides with a clipper does not reduce the aerobic bacterial load on the hides, probably because of dust generation and subsequent dispersal of the bacteria. However, treating previously clipped hides with other physical or chemical hide decontamination methods was found to afford bacterial load reductions significantly higher than these obtained on unclipped hides (Baird et al., 2006). The use of chemical dehairing has been studied by Castillo et al. (1998). These authors, using a solution of sodium sulphide, water rinses, and hydrogen peroxide under laboratory conditions, achieved significant reductions in *E. coli* O157:H7 and *S.* Typhimurium previously inoculated on bovine hides (more than 4 logarithmic units). However, in a study conducted on 240 cattle in a commercial beef processing plant, Nou et al. (2003), using a similar method on cattle

immediately after stunning, reported a reduction in *E. coli* O157 prevalence on the treated cattle hides from 88 to 67% without any significant reduction in aerobic bacteria or *Enterobacteriacea* populations.

A wide range of chemical antimicrobials have reportedly been used in hide decontamination treatments. These include organic acids (Mies et al., 2004), commercial detergents and disinfectants (Baird et al., 2006; Small et al., 2005), ozonated and electrolysed water (Bosilevac et al., 2005), and combinations of different chemicals (Carlson et al., 2008). However, their efficacy in reducing the bacterial load on cattle hides has been found to be dependent on a number of experimental factors such as the mode of application, the product concentration and temperature, the duration of exposure and the target microbial species. Limited studies have addressed the effect of chemical antimicrobials on pathogens such as *Salmonella* and *E. coli* present on cattle hides destined to slaughter. Nevertheless, organic acids appear to be the most studied group of chemical (Loretz et al., 2011). The effect of organic acid sprays in reducing *Salmonella* load on cattle hides was studied by Mies et al. (2004). These authors sprayed experimentally cattle hides with different concentrations (2 to 6%) of acetic and lactic acids and noted reductions in *Salmonella* Typhimurium previously inoculated on the hides of 2.4 to 4.8 and 1.3 to 5.1 logarithmic units, respectively. However, treating live cattle with a lactic acid solution (0.5%) during 1 minute did not reduce the proportion of *Salmonella*-positive hide samples. This could be attributed to the used low concentration of lactic acid in respect to the animal welfare. In another study, a reduction average of 2 log units in *Salmonella* and *E. coli* O157 loads was reported on previously inoculated cattle hides using lactic and acetic acid (10 %, 55°C) sprays (Carlson et al., 2008).

Although a variety of biological treatments are reported to be used in carcass decontamination, bacteriophages constitute the only biological treatment reported to be used in hide decontamination (Bolder, 1997; Chen et al., 2012). Some bacteriophages targeted to bacteriological pathogens namely *Salmonella* and *E. coli* O157:H7 have been already approved in United States for cattle hide decontamination, however the possibility of their utilization under commercial conditions is still being investigated (Loretz et al., 2011).

Apart from reducing the bacterial load on hides and possibly improving carcass microbiological quality, some hide decontamination interventions were found to present some disadvantages. It has been reported that treatments with water or steam increase the humidity on the surface of the treated hides (Loretz et al., 2011). This makes the skinning operations more difficult for the operator and may increase the risk of carcass contamination from the hide, especially when hide removal is carried out manually. Antic et al. (2010) reported that microbial contamination of bovine carcases during skinning was more likely to occur when the animal hide was wet. Steam treatments were also found to deteriorate the commercial quality of hides (McEvoy et al., 2003). Furthermore, animal and operator welfare problems, namely eye and skin irritation as well as corrosion of slaughtering

equipment, have reportedly been associated with the use of chemical antimicrobials, particularly organic acids (Chen et al., 2012; Mies et al., 2004).

Hide decontamination, treatments appear to be an important strategy that can significantly reduce the risk of carcass contamination from soiled hides during the skinning process. However, considering existing data, it is difficult to accurately appraise their effect under normal slaughtering conditions as most of available informations derive from experimental studies. Additionally, very limited number of studied treatments concerned bacterial pathogens such as *Salmonella* or pathogenic *E. coli*. It is therefore imperative to conduct further studies to assess the effects of these interventions on major bacterial pathogens under practical slaughtering conditions. Another issue is to identify the optimal moment in the slaughtering process at which the hide decontamination treatment should be carried out under commercial conditions.

The moment between stunning and sticking would be appropriate provided that the animal's unconsciousness lasts until the hide decontamination process ends. Alternatively, the moment after sticking but before hide removal would be used. In the latter case, appropriate measures should be taken so as not to contaminate the sticking wound during the hide decontamination process.

1.3.2.4. Cattle skinning

The skinning stage is one of the slaughtering steps where microbial contamination of bovine carcasses is most likely to occur. This is due to the fact that the hide is, in most cases, heavily populated by a wide range of microorganisms that can be transferred to carcasses during skinning operations (Loretz et al., 2011). Bacterial pathogens such as *Salmonella* and *E. coli* O157 are also commonly isolated from hides of cattle destined to slaughter (Barham et al. 2002; Barkocy-Gallagher et al. 2003). During the skinning process, carcass contamination may occur through direct contact between the carcass and the hide or indirectly through equipment or operators contaminated by hides. Carcass contamination by airborne transfer is also possible (Antic et al., 2010).

Cattle hide removal can be carried out either manually or mechanically by means of a hide puller. The advantage of manual hide removal relies mainly in its low financial investment in equipment, but it has been found to present several disadvantages in terms of slaughter productivity and meat hygiene (FAO, 2006). These include the requirement for a very high skill level for effective hide removal without damaging both hide and carcass; the difficulty of the task and the time consumed even for a skilled operator; and a high risk of contaminating the carcass with microorganisms from the hide during the skinning process. On the other side, mechanical skinning by means of a hide puller seems to require less manual contact with the hide and consequently minimizes the risk

of carcass contamination by microorganisms from operators and slaughtering equipments. Additionally, it increases the productivity of the slaughterhouse and improves the value of the hides by damaging them less. The disadvantages of mechanical skinning include the high cost of the equipment and fractures of the spinal column sometimes associated with the use of a downward hide puller.

Peer-reviewed studies addressing the quantification of microorganisms transferred from hides to carcasses during the skinning indicate that, under commercial conditions, dressed bovine carcasses carry a very small proportion (ranging from 1.6 to 0.003%) of the hide microflora (Arthur et al., 2004; Bacon et al., 2000). Another study showed that only 0.5 to 0.00002% of the hide microflora is transferred to dressed bovine carcass via direct contact (Antic et al., 2010) highlighting the importance of other transmission pathways such as indirect contamination via knives and/or hands or airborne transfers. Nevertheless, even if the reported hide-to-meat microbial transmission rates appear to be relatively low, it should be noted that the risk associated to these transmissions is still significant. In fact, carcass contamination from hides occurs regularly under commercial slaughtering conditions and the reported bacterial loads on hides are so high that proportions as low as less than 1% would constitute levels of many logarithmic units (Loretz et al., 2011). By summarizing data from numerous studies published internationally, Antic et al., (2010) reported bacterial contamination levels of 6 – 10 log cfu/cm^2 and 4.5–8 log cfu/cm^2 respectively on visually dirty and clean hides from cattle destined to slaughter.

The control of carcass bacterial contamination from hides during skinning operations consists basically in preventing hide-to-meat contaminations through process hygiene means and/or the elimination microbial contaminants from hides before skinning operations by adequate treatments. Concerning the process hygiene, several studies have reported an association between the hide cleanliness and the microbiological status of dressed carcasses (McEvoy et al. 2000; McCleery et al. 2008). Thus, in many countries (including but not limited to Australia, Ireland, Finland, Norway and United Kingdom) Good Hygienic Practice programs in cattle dressing are based on the cleanliness of cattle hides. In these countries, only cattle with clean hides are slaughtered under normal conditions whereas dirty animals are either cleaned (and allowed to dry before slaughtering) or are slaughtered separately under special conditions as they are considered to present a high risk for cross contaminations (McEvoy et al., 2000). A recent study conducted in Norwegian abattoirs (Hauge et al., 2012) confirmed that, under commercial conditions, carcasses from clean animals present levels of hygiene indicator bacteria (total aerobic bacteria and *E. coli* counts) significantly lower than these from dirty animals. Although the cleanliness of cattle hides prior to skinning presents considerable beneficial effects on the bacteriological status of dressed carcasses, it should be noted however, that these effects are not absolute. In fact, it is known that pathogenic bacteria such as *E. coli* O157 are commonly isolated from visually clean hides (Nastasijevic et al., 2008). Therefore, the selection of cattle with clean hides for slaughter should be combined with other good

hygienic practices including hygiene for staff and skinning equipments as well as good manufacturing practices particularly an immediate carcass trimming when any carcass contamination is suspected (Kiermeier et al., 2006; Sheridan, 1998). The elimination of bacterial contaminants from hides prior to skinning constitutes a promising alternative to consider. However, as presented in previous sections of the present paper, most of the existing informations on the effects of hide decontamination treatments derive from experimental studies. Further studies are therefore still needed to accurately appraise the effects of these treatments under commercial slaughtering conditions.

1.3.2.5. Evisceration

As the skinning step, evisceration constitutes a critical slaughtering stage where microbial contamination of carcasses is most likely to occur. The gastro-intestinal tract of cattle is naturally colonised by microorganisms that may be transferred to carcasses during the evisceration process (McEvoy et al., 2000). Additionally, bacterial pathogens such as *Salmonella* and *E. coli* are also frequently isolated in faeces of cattle destined to slaughter highlighting their probable presence in the digestive tract of the same animals (Rhoades et al., 2009). During the evisceration process, carcass contamination occurs by direct contact between the carcass and the gastro-intestinal contents or indirectly through soiled slaughtering equipments and staff. Contaminations may also occur during the removal of pharynx, tonsil and tongue as they are reported to be heavily contaminated by various microbial contaminants (Sheridan, 1998; Wheatley et al., 2014).

Several peer-reviewed studies indicate a significant increase of bacterial loads on carcasses during the evisceration process; however the degree of increase varies from one study to another. The observed variation could be attributed to a number of factors including the differences in experimental designs and the process hygiene that differ from one slaughterhouse to another. For example, an average increase of 0.7 logcfu/cm^2 in *Enterobacteriaceae* counts was reported during the evisceration of lamb carcasses in 4 Irish abattoirs (Sierra et al., 1997); whereas in Rwanda increases of 3 and 1.3 log cfu/g were respectively observed in total aerobic bacteria and *E. coli* counts during the evisceration of cattle at a commercial abattoir (Niyonzima et al., 2013). Another Irish study reported an increase of 2-4 log in *Enterobacteriaceae* populations during the evisceration of pork carcasses (Wheatley et al., 2014).

The control of carcass bacterial contaminations during the evisceration process relies mainly on Good Slaughtering Practices. The techniques mostly used include the “bunging” and the “rodding”. The bunging or bung tying consists in sealing the rectum and covering it with a plastic bag in order to reduce the spread of faecal material from the rectum to the carcass; whereas the rodding corresponds to sealing the oesophagus to avoid the spread of its content onto the carcass (McEvoy et al., 2004). These techniques are effective in reducing the risk of

bacterial transfers during the evisceration. Nesbakken et al. (1994) reported that bunging reduced significantly the occurrence of *Yersinia enrerocolitica* on pig carcasses. Furthermore, the introduction of that technique in Norwegian pork abattoirs resulted in decreasing the incidence of Yersiniosis by 25% in the population (Sheridan, 1998). Similarly, special attention must also be paid to the training of staff on Good Hygienic Practices as well as on the sanitation of slaughtering equipments particularly knives to minimize the risk of cross contaminations. Bolton et al. (2002) recommend sanitizing knives by a two-knife system that consists in the utilization of one knife while the other is being sanitized in hot water at 82°C or above.

Despite the reported increases in bacterial load on carcasses during their evisceration, some authors indicate that the existing measures including rectum and oesophagus sealing, intact removal of visceral contents and an appropriate training of staff in Good Hygienic Practices could reduce the risk of carcass contamination from viscera to the point where they do not contribute significantly to the overall contamination of the carcass (McEvoy et al., 2000; Wheatley et al., 2014).

1.3.2.6. Carcass splitting

The carcass splitting stage is not generally considered as a major source of contamination (Wong et al., 2002). However, the splitting saw as well as other slaughtering equipment can be contaminated with pathogenic bacteria such as *Salmonella* and *E. coli* and may contribute to their spread to several carcasses.

In a study conducted in 4 European countries, Hald et al. (2003) reported that 9.4% of 384 carcass splitter machines were contaminated with *Salmonella* during the slaughtering process. In addition, Warriner et al. (2002) demonstrated that *E. coli* and potential enteric pathogens can be transferred between pork carcasses through the splitting saw. Therefore, cleaning and disinfection of the splitting saw should be carried out after splitting each carcass in order to reduce the risk of cross contaminations. The European regulations recommend disinfecting the splitting saw after splitting each animal using water at 82°C or above or using another method with an equivalent effect (European Commission, 2004).

Although adherence to Good Hygiene Practices in abattoirs improves the microbiological quality of the meat significantly, it is generally recognized that contamination of meat is unavoidable during the cattle slaughtering process (McCann et al., 2006). Therefore, carcass decontamination before refrigeration appears as a corrective measure to restore the bacterial load of carcasses to the acceptable range.

1.3.2.7. Carcass decontamination treatments

Various treatments including physical, chemical and biological methods applied alone or in combination have been identified to reduce the levels of bacterial load on carcasses. In this section majors carcass decontamination treatments as well as their respective effects on the bacterial load of carcasses are discussed.

The physical decontamination treatments mostly reported for carcasses include hot water washes and application of steam. These treatments are generally carried out in special cabinets where carcasses are sprayed with water or steam at controlled pressure and temperature. Carcass sprays with hot water were found to lower significantly the bacterial load on treated carcasses. However, the reduction rates reported were found to be dependent of experimental factors such as the temperature, pressure and the duration of the treatment (Loretz et al., 2011). The effectiveness of carcass decontamination by hot water was demonstrated by Bosilevac et al. (2006). These authors conducted a study in a commercial abattoir and reported 2.7 log reductions in both aerobic plate counts and *Enterobacteriaceae* counts on pre-evisceration bovine carcasses washed in a cabinet with water at 74°C for 5.5 seconds. The prevalence of *E. coli* O157 was also reduced by 81% in treated carcasses. The decontamination of carcasses with steam was reported to yield bacterial reductions comparable to these obtained with hot water spays. However, the treatments with steam presents an advantage of reaching cavities and crevices of carcasses that are generally inaccessible to hot water (Hugas & Tsigarida, 2008). One of the side effects reportedly associated with carcass decontamination treatments by steam or hot water is the change in the carcass colour after a prolonged treatment. McCann et al. (2006) reported a cooked appearance on the surface of carcasses having undergone a steam decontamination treatment of 10 seconds or longer. Furthermore, weight gain resulting from water absorption by treated carcasses generally reported in hot water decontamination treatments may be perceived as a fraud by meat consumers (EFSA, 2010).

Organic acid sprays, namely acetic, citric and lactic acids are the most-reported chemical decontamination methods used on beef carcasses. They are known to reduce the number and prevalence of food borne pathogens and the microbial load on meat carcasses (Huffman, 2002), but their efficacy depends on the type of meat tissue, the type and load of initial microbial contamination, as well as the pH, concentration and temperature of the organic acid solution (Hugas & Tsigarida, 2008). Various studies conducted under laboratory conditions showed that spraying inoculated bovine carcasses with acetic or citric acid yielded bacterial reductions varying between 0.7 and 4.9 logs for aerobic bacteria, non pathogenic *E. coli*, *E. coli* O157:H7 and *Salmonella* (Loretz et al., 2011). However, lower reductions are generally reported in studies conducted under commercial conditions. This could be due to lower acid concentrations used in respect to meat quality and staff welfare considerations (Chen et al., 2012). By spraying acetic acid (2.5%) to bovine carcasses prior to chilling Algino et al. (2007) reported reductions of coliforms, *Enterobacteiaceae* and *E. coli* levels ranging from 0.6 to 1.4 logs. In a study by Barboza

de Martinez et al. (2002) spraying carcasses at the end of slaughter by lactic acid (1.5%) yielded reductions of 0.5, 1.8 and 0.6 logs, respectively, for aerobic bacteria, coliforms and *E. coli*. In another study, lactic acid (2%; 42°C) spraying of pre-eviscerated bovine carcasses was reported to reduce the prevalence of *E. coli* O157:H7 by 35% as well as aerobic bacteria and *Enterobacteriaceae* counts by respectively 1.6 and 1.0 logs (Bosilevac et al., 2006). Other chemicals such as chlorine, trisodium phosphate, acidified sodium chlorite and peroxyacids are also used for meat decontamination but to a lesser extent. Generally, the use of these substances leads to 1–1.5 log reductions in foodborne pathogens such as *Salmonella* and *E. coli* O157 (Hugas & Tsigarida, 2008). A number of drawbacks have however been reportedly associated to the chemical decontamination of carcases especially by organic acids. These include staff welfare problems such as eye or skin irritations and the corrosion of slaughtering equipments (Chen et al., 2012; Mies et al., 2004).

Reported biological treatments for meat decontamination include the use of bacteriocins and bacteriophages. Bacteriocins are anti-microbial proteinaceous compounds produced by some bacteria. The most widely known bacteriocin is nisin, which is produced by *Lactobacillus lactis* subsp. *lactis* and is effective against Gram-positive bacteria. Nisin is used as a preservative agent in foods like cheese but its use in carcass decontamination has been limited by a number of factors, namely its deficient inhibitory effect on Gram-negative bacteria, low level of production *in vivo* and likely inactivation of its effect due to interactions with other food components (Bolder, 1997; Chen et al., 2012). Nevertheless, combinations of nisin and other treatments have been reported to reduce microbial contamination on carcasses. Barboza de Martinez et al. (2002) reported that a combination of nisin and lactic acid sprays under commercial conditions reduced aerobic bacteria, coliforms and *E. coli* populations on carcasses by 2.0, 2.2, and more than 1.0 log, respectively, whereas treatment with nisin alone reduced bacterial levels by less than 0.2 log. The use of bacteriophages has also been reported to present a number of benefits as an alternative biocontrol method. These include their high host specificity and lack of effect on the organoleptic qualities of the food as well as their ability to survive under commercial processing procedures (Hugas & Tsigarida, 2008). However, their use in food decontamination is still limited by factors such as the potential development of resistance in targeted bacteria (Chen et al., 2012). The use of *E. coli* O157:H7 and *Salmonella* targeted bacteriophages for cattle hide decontamination has already been approved in the USA. However, further investigations to address their efficacy under long-term commercial conditions and their possible utilization for carcass decontamination are still required (Loretz et al., 2011).

Although obtaining bovine carcasses free of pathogenic bacteria and with low microbial contamination appears to be a shared goal of all countries, carcass decontamination policies vary from one country to another. In the USA, for example, a number of carcass decontamination treatments are allowed and commonly used in cattle slaughterhouses. These include physical interventions such as hot water or steam spraying and chemical treatment

with organic acids, namely lactic and acetic acids (Chen et al., 2012). Contrary to this, in Europe important efforts have been put into the application of Good Manufacturing Practices throughout the entire meat production line, and for many years carcass decontamination treatments in the European Union were limited to the use of clean or potable water. The current European regulation (European Commission, 2004) allows the use of substances other than water for the removal of surface bacterial contamination from meat; however, the European Food Safety Authority (EFSA) must provide a chemical and microbiological risk assessment before the European commission authorizes the use of such substances (Hugas & Tsigarida, 2008). Currently, the use of lactic acid has been approved for the decontamination of beef carcasses within the European Union (European Commission, 2013).

Carcass decontamination treatments constitute a potential control measure to reduce the levels of bacterial and pathogen loads on carcasses. However, available informations indicate a wide variability in bacterial reduction yields as most of data results from studies conducted under different conditions. Thus, studies to compare the effectiveness of different treatments under the same conditions would be of valuable importance to identify the cost-effective interventions to be used in cattle slaughterhouses. Furthermore, the risk, in some abattoir, to rely only on the carcass decontamination step and abandon existing good hygienic and manufacturing practices in previous slaughtering stages need to be considered before the adoption of such interventions.

1.3.2.8. Carcass refrigeration

Apart from meat maturation purposes, refrigeration of carcasses after the slaughtering process is performed to inhibit the growth of spoilage and/or pathogenic bacteria that could still be on the carcasses and consequently increase their shelf-life (Dave & Ghaly, 2011). Carcass refrigeration is generally carried in two phases including the rapid chilling phase consisting in rapidly reducing the carcass temperature and a second phase of cold storage intended to maintain the low temperature of carcasses. Different methods of carcass chilling were thoughtfully reviewed by Savell et al. (2005) and are not further developed in this paper. In commercial slaughterhouses, carcasses are generally chilled for 48-72 hours before their transfer in the boning hall. Nevertheless, the duration of carcass chilling may be extended beyond 72 hours to improve the quality of meat. This process is referred to as aging (EFSA, 2014). The inhibition of bacterial growth on carcasses at refrigeration temperatures is a consequence of low-temperature stress undergone by microorganism. In fact, as the temperature decreases, the bacterial lag phase extends whereas the growth rate decreases and the ultimate cell numbers may decrease (Beales, 2004; Russell et al., 1995).

Although bacterial growth on carcasses is known to be inhibited at refrigeration temperatures (Korsak et al., 2004; Russell, 2002), several published studies indicate increases in levels of bacterial loads on refrigerated carcasses. In a study conducted in a commercial abattoir, Bolton et al. (2002) reported an increase in total viable bacterial

counts from 3.8 to 4.5 log cfu/cm^2 on carcasses at the refrigeration stage. Another study conducted on poultry carcasses showed that after 9 days of storage, *Salmonella* loads were slightly reduced (by less than 1 log unit) on carcasses refrigerated at 2 and 6°C whereas in carcasses refrigerated at 8°C *Salmonella* number increased by 1.5 log units (Jiménez et al., 2009).

Microbial growth on carcasses under refrigeration is mainly attributable to the temperatures of chilling equipments that are not sufficiently low to inhibit the microbial growth and/or to intermittent rupture of the cold chain. In fact, bacterial pathogens such as *Salmonella* may multiply to hazardous levels during periods of temperature abuse (Delhalle et al., 2009; Wong et al., 2002). Thus, slaughterhouses should be equipped with chilling equipments capable to rapidly decrease and maintain low the temperature of carcasses during the entire refrigeration period. In European Union countries for example, the carcass temperature must be decreased to maximum 7°C in the first 24 hours of refrigeration (European Commission, 2004). Additionally, trough cross-contaminations, microorganisms present on carcasses under refrigeration or on chilling equipments may get disseminated to other carcasses and proliferate when the environment become favourable to their growth (for example during temperature abuses). Various studies indicate that bacterial pathogens can survive on surfaces of refrigerators (Jackson et al., 2007) or on chilling evaporators (Evans et al., 2004) and pose a cross-contamination risk to the refrigerated foods. This highlights the need for a regular cleaning and disinfection of chilling rooms and/or equipments in the slaughterhouse.

The refrigeration of carcasses constitutes one of the determinant slaughtering stages influencing the final bacterial load on carcasses. However, despite the exiting control measures (including adequate chilling equipments, the regular monitoring of the temperature of carcasses and the mastery of cross-contaminations through an effective appliance to Good Hygienic Practices) that have proven their effectiveness in significantly reducing the risk of bacterial/pathogen growth on carcasses under refrigeration (Delhalle et al., 2009); in some slaughterhouses, bacterial growth is still being reported on refrigerated carcasses probably due to the failure in applying adequate control measures. It is thus imperative for slaughterhouses to deploy all material, technical and financial means required to control the bacterial growth on carcasses at this critical stage of slaughter.

1.3.3. Post slaughter contamination of bovine meat

The post slaughter section of the meat chain comprises a series of sub-stages (including cutting/deboning, transportation of carcasses or meat cuts, meat storage, manufacture of meat products, retail, and eventually cooking) at which contamination of meat may occur. In this section majors sources of post slaughter meat contamination by pathogenic bacteria and their respective control measures are reviewed.

At the end of the slaughtering process carcasses are generally cut in special meat pieces (cutting) and separated from bones (deboning) for industrial and commercial utilisations. The cutting and deboning of carcasses may take place in the slaughterhouse or in specialised plants. The cutting and deboning operations are generally performed on refrigerated carcasses however deboning of non refrigerated carcasses (hot deboning) is also possible (Røtterud et al., 2006). Even if hot deboning presents a number of advantages including a reduced cost and fewer requirements in chilling equipments and space (Pinto-Neto et al., 2013) it is rarely used in European countries. This is mainly due to a possible proliferation of pathogenic bacteria on processed meat and a reduced shelf life of subsequent vacuum packed meat (Yang et al., 2011). A recent report from the European Food Safety Authority indicate that the surface temperature of boned beef cuts from chilled carcasses decreases to 8°C in few hours whereas the temperature of hot boned and vacuum packed meat pieces may remain at 25°C for many hours; creating favourable conditions for the proliferation of spoilage and pathogenic microorganisms (EFSA, 2014). Data from published studies indicate that bacterial/pathogen loads on carcasses may significantly increase during the cutting/deboning operations even in slaughterhouses where cold deboning is practiced. In a study conducted in an Irish beef abattoir, McEvoy et al. (2004) reported increases of 2.3 and 2.1 logcfu/cm^2 respectively for total viable bacteria and *Enterobacteriaceae* counts on the inside round of carcasses during the cutting/deboning operations. Similar increases were also reported in *E. coli* numbers during the deboning of beef carcasses (Gill et al., 2001). Increases in bacterial/pathogen numbers following the cutting and deboning operations could be associated to cross-contaminations. During these operations, carcasses or meat pieces of various origins and different contamination levels are handled in close proximity, creating numerous opportunities for cross-contamination or spread of pathogenic bacteria (Lo Fo Wong et al., 2002). Various origins of microbial contamination during cutting/deboning were reported in literature. These include carcasses or meat pieces to be processed (McEvoy et al., 2004); meat cutting/deboning equipments such as knives, meat conveyors or cutting boards (Gill et al., 1999; Gill et al., 2001; Jiménez et al., 2009) and soiled surfaces or operators (Sheridan et al., 1992).

One of the measures to prevent cross contaminations resulting from contaminated carcasses or meat pieces would be to identify the most contaminated raw materials and to process them separately preferably at the end of the production (Koohmaraie et al., 2012). Contaminations from equipments can be mastered through a regular cleaning and disinfection of equipments and surfaces whereas effective training of staff on Good Hygienic Practices could help in preventing contaminations from personnel (Delhalle et al., 2009). The working temperature constitutes another factor influencing bacterial growth on meat during the cutting/deboning operations. In fact, during these operations the temperature of processed meat increases and this would favour the growth of existing microbial populations. It is therefore recommended to carry out deboning operations under

refrigerated conditions. In some commercial abattoirs, the deboning hall is refrigerated at 10-12°C (EFSA, 2014b; McEvoy et al., 2004).

Although temperature abuse is reportedly the main factor associated to bacterial load increases on carcasses or meat pieces during transportation and storage (Delhalle et al., 2009; Wong et al., 2002), cross-contaminations originating from chilling equipments or personnel are also significant contributors to the ultimate bacterial load of meat (Evans et al., 2004; Jackson et al., 2007; Sheridan et al., 1992). Thus, it is imperative to prevent cross contamination through an effective and regular sanitization of chilling rooms and meat transportation vehicles. Additionally, personnel involved in the loading of meat transportation vehicles should be educated in good hygiene practices. On the other side, the regular monitoring of temperatures in chilling rooms and meat transportation vehicles could contribute significantly in reducing the risk of temperature abuses occurring during meat storage and transportation (Savell et al., 2005). Recent published studies indicate that meat transportation vehicles with a chilling capacity comparable to the one of conventional chilling rooms are commercially available. Most of these vehicles are designed for longer distances and can decrease the core temperature of bovine carcasses from approximately 20°C at the loading time to 7°C or lower after 48 hours. Additionally, these vehicles are equipped with apparatus to continuously monitor the temperature of meat during the transportation (EFSA, 2014).

As in previous stages of the meat chain, cross-contamination from equipments, personnel or the working environment are likely to occur during the manufacture of meat products if appropriate control measures are not effectively applied (Lo Fo Wong et al., 2002; Roels et al., 1997). Nevertheless, microbial contaminants from incorporated non-meat ingredients as well as contaminations associated to the manufacture method used, appear to be specific to this particular stage of the meat chain. The grinding of meat for example, would result in the dissemination of microorganisms previously localised at the surface of meat pieces in the entire batch of minced meat (Gould et al., 2011). As the meat temperature increases during grinding operations due to friction movements, these microorganisms may proliferate in relatively short period resulting in bacterial number increases in minced meat (Heinz & Hautzinger, 2007). A number of preservative treatments (including thermal interventions, smoking, curing etc.) are commonly used in meat processing to enhance the bacteriological stability of meat products and consequently increase their shelf-life. These interventions, generally based on the control of the temperature, pH, water activity, microbial competition/interaction and oxido-reduction potential, were found to significantly reduce the bacterial/pathogen load in meat (Chen et al., 2012; Hugas & Tsigarida, 2008; Loretz et al., 2011). However, as their preservative effects depend also on the initial bacterial numbers in meat; it is crucial for meat processors to assure that the used raw materials are of good microbiological quality (Lo Fo Wong et al., 2002). Another important measure would be to decide the fate of raw materials according to their microbiological

quality. In some commercial meat processing plants, heavily contaminated raw materials are generally reserved for the manufacture of meat products destined to undergo a heat treatment (Koohmaraie et al., 2012; McCleery et al., 2008; McEvoy et al., 2000; Nastasijevic et al., 2008).

At the retail level, temperature conditions are reportedly an important factor influencing the final microbiological quality and safety of meat products (Delhalle et al., 2009). Several published studies indicate a wide variation in meat product temperatures within retail cabinets but most of the data are simply indicative of the product temperature at the time and place of the study and do not address conditions that would dynamically influence the temperature changes (Nychas et al., 2008). Nevertheless, it is recognised that displayed meat products in retail cabinet must be at temperatures sufficiently low (generally below 4°C) to inhibit the growth of spoilage or pathogenic microorganisms (Lo Fo Wong et al., 2002). It is therefore imperative to regularly monitor the temperature of display cases to prevent temperature abuses during meat storage. Furthermore, appropriate control measures must be applied to prevent cross contaminations from equipments, personnel or working environment in retail establishment where meat processing activities such as cutting or grinding are carried out (Gould et al., 2011). The retail level represents an important stage of the meat chain in regard with the final quality and safety of meat products as it constitutes the last "check point" where contaminated products can be identified before their consumption particularly for ready-to-eat meat products (Lo Fo Wong et al., 2002).

The last section of the meat chain related to the transportation, storage and cooking by the consumer appears to be less studied although it is the most important in regards with the food safety aspects of meat products. This is due to the difficulties in collecting data concerning the mode and the duration of meat transportation toward the consumer's household, temperature conditions in domestic refrigerator and freezers, durations of storage before consumption as well as consumer's cooking habits (Nychas et al., 2008). However, it is recognised that the cooking stage is the last line of defence of consumers against *Salmonella* and pathogenic *E. coli* infections attributable to bovine meat (Korsak et al., 2004; Mainil & Daube, 2005). These pathogens are generally destroyed in foods at conventional pasteurisation temperatures. In beef, *Salmonella* is reported to have a decimal reduction time (D-value) of 0.53 minutes (z=5°C) at 65°C (Juneja et al., 2001; Korsak et al., 2004) whereas *E. coli* O157:O7 presents a D-value of 0.39 minutes (z=6°C) at the same temperature (Juneja et al., 1997). It is therefore recommended to cook meat until the internal temperature reaches a minimal temperature of 70°C to assure a thermal destruction of these pathogens in meat as most of the reported *Salmonella* and *E. coli* O157:H7 outbreaks attributable to meat were found to be associated to the ingestion of raw or undercooked meat products (Abong'o & Momba, 2009; Greig & Ravel, 2009; Roels et al., 1997).

1.4. Conclusion

Despite a large number of control measures along the meat chain, meat contaminations by *Salmonella* and pathogenic *E-coli* remain a serious public health problem in humans. In the pre-slaughter stages of the meat chain, difficulties in identifying asymptomatic shedders constitute the main obstacle to the control of the infection spread in live animals. Further studies are therefore needed to identify cost-effective techniques and approaches to diagnose asymptomatic carriers in cattle herd before animal transportation to abattoirs. During the slaughtering process, the skinning and evisceration operations appears to be the most critical stages for carcass contamination. Thus Good Manufacturing Practices in accordance with HACCP principles must be strictly applied in commercial slaughterhouses to reduce the risk of carcass contamination at those specific stages.

The decontamination of carcasses has also shown a potential in reducing pathogen numbers on carcasses prior to chilling, even if its utilisation in some countries is still limited by a number of factors including the cost of installations, the commercial quality of treated carcasses as well as the risk of relying only on the carcass decontamination step and reduce efforts devoted to Good Hygiene and Manufacturing Practices in previous slaughtering stages.

Along post-slaughter stages of the chain, handling, time and temperature are the main factors influencing the microbial contamination of meat. Therefore application of appropriate GMP and GHP by meat processing plants is of great importance to prevent cross-contaminations during cutting/deboning, processing, transportation and retail of meat products. Similarly, the cold chain must be respected at all stages of meat distribution. Although all stages of the bovine meat chain are of significant relevance in regard to the ultimate bacterial contamination of meat, the cooking step constitutes the most important stage to assure the safety of beef at consumption. In fact, the cooking step is the last stage of the meat chain at which *Salmonella* and pathogenic *E. coli* can be completely destroyed.

CHAPTER TWO

Microbiological quality and safety of carcasses processed within the slaughtering establishments of Kigali city

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CHAPTER 2. Microbiological quality and safety of goat and bovine carcasses processed within the slaughtering establishments of Kigali city

Abstract

The microbial contamination of meat carcasses during the slaughtering operations constitutes one of the major determinants of the final microbiological quality and safety of processed meat products. The present study was conducted to assess the microbiological quality of goat and bovine carcasses processed within three slaughtering establishments of Kigali (Rwanda). A survey to characterize the animal slaughtering conditions was carried out in the studied establishments by using a structured questionnaire, whereas 300 goat and bovine carcasses were analyzed for the enumeration of hygiene indicator bacteria (total mesophilic bacteria and *E. coli*) as well as the qualitative detection of *Salmonella* by using culture methods. The results showed that the load of total mesophilic bacteria in bovine and goat carcasses varied from 2.9 to 5.7 and from 3.9 to 5.2 log cfu/cm^2, whereas the mean *E. coli* counts were found to be 1.9 and 1.7 log cfu/cm^2 respectively. *Salmonella* was detected in 21.3% of bovine carcasses and in 8.0% of goat carcasses. The prevalence of *Salmonella* as well as the levels of hygiene indicator bacteria were found to be significantly higher in goat and bovine carcasses processed in small scale abattoirs in comparison to these processed within large establishments. The findings from this study suggest the need for improvements in hygienic slaughtering practices particularly in small scale establishments of Kigali.

Key words: goat, beef, microbiological safety, abattoir, *Salmonella*, Rwanda

2.1. Introduction

The chemical composition of meat makes that it is one of the most perishable foods as it is favorable to the proliferation of a wide range of spoilage microorganisms. Furthermore, beside the spoilage microorganisms, meat can also get contaminated by pathogenic microorganisms and is currently recognized as one of the major vehicles for microbial food borne infections in humans (Doulgeraki et al., 2012; Greig & Ravel, 2009; ICMSF, 1998).

Although the microbiological contamination of meat may occur at any stage of the production chain, the slaughtering constitutes a crucial step in regards with the ultimate quality and safety of meat as it represents the first stage of the chain where meat contamination by spoilage and/or pathogenic microorganisms can occur. Several authors have highlighted that the microbiological quality and safety of different meat products was directly associated to the microbiological quality of their respective raw materials in occurrence meat carcasses and/or primary meat cuts (Corbellini et al., 2017; Signorini & Tarabla, 2010; Smith et al., 2013). Therefore the microbiological contamination of carcasses during the slaughtering operations represents not only a risk of spoilage in processed meat products but also an important food safety concern for consumers.

In Rwanda, previous studies have showed that goat and beef were the types of meat consumed the most within and outside the households (Niyonzima et al., 2016a, 2017a). However no study, to the best of our knowledge, has yet assessed the occurrence of bacterial pathogens such as *Salmonella* in goat and bovine carcasses processed within the slaughtering establishments of Rwanda. One study conducted in the principal slaughterhouse of Kigali city showed that bovine carcasses processed in that particular slaughterhouse presented higher levels of hygiene indicator bacteria (Niyonzima et al., 2013) and this might suggest the possible contamination of processed carcasses by enteric bacterial pathogens such as *Salmonella*, that are generally associated to poor hygienic slaughtering practices (Buncic & Sofos, 2012; Ivanovic et al., 2015).

The purpose of the present study was to assess the slaughtering conditions for goat and cattle within the establishments of Kigali city as well as the determination of the bacteriological quality and safety of the processed carcasses. Data collected in this study would have helpful applications in monitoring the risk of meat contamination by bacterial pathogens such as *Salmonella* in Rwanda.

2.2. Material and methods

2.2.1. Animal slaughtering conditions

A survey was conducted to characterize the animal slaughtering conditions in the abattoirs of Kigali city. In this study, three animal slaughtering establishments were studied including one large scale (establishment B) and two small scale (establishments A and C) abattoirs. Each establishment presented separate slaughtering compartments for both goat and cattle. The survey was carried out through an interview with the manager of the establishment and information regarding the slaughtering procedures, equipment and staff hygiene, cleaning and sanitation procedures as well as pest control measures within the establishments was collected through a structured questionnaire. The responses from the interview were verified on the site and when necessary corrections were made.

In the studied establishments, the survey was completed by the measurement of other parameters such as the temperature of knives sterilization temperature and the duration of different slaughtering operations. The temperature was recorded by using a probe digital thermometer (VWR, Belgium) and the durations of were measured by using a laboratory chronometer (Fisher Scientific, Belgium).

2.2.2. Microbiological analyses

2.2.2.1. Sample collection

In each slaughtering facility, microbiological samples were collected on carcasses at the end of the slaughtering process but before chilling and five goat and bovine carcasses were randomly sampled once a week and during 10

weeks, from June to December 2016. The sampling was carried out through swabbing carcasses at four different zones namely the rump, the flank, the brisket and the neck by using sterile dry sponges (3M, Belgium). An area of 50cm^2 in goat carcasses and 100 cm^2 in bovine carcasses was swabbed in each sampling zone by using the same sponge as described by Hutchison et al. (2005). After the carcass swabbing process, the sponge used for each carcass was aseptically put in a sterile stomacher bag and transported to the laboratory in a cold box with freeze packs. The duration of sample transportation was approximately 1 hour. Within the laboratory, samples were stored in a refrigerator at 4°C and were analyzed within the following 24 hours.

2.2.2.2. Analytical methods

The collected samples were analyzed for the enumeration of hygiene indicator bacteria namely total mesophilic bacteria and *E. coli,* as well as the qualitative detection of *Salmonella;* and their preparation was performed by following the ISO 7218:2007 Standard procedures (International Organization for Standardization, 2007). The determination of total mesophilic bacteria counts (TMC) was carried out on plate count agar (VWR, Belgium) by using the poor plate method as described in the ISO 4833:2003 Standard protocol (International Organization for Standardization, 2003), whereas *E. coli* counts (ECC) was performed on tryptone bile X-glucuronide agar (Bio-Rad, France) by following the ISO 16649-2:2001 standard guidelines (International Organization for Standardization, 2001).

The qualitative detection of *Salmonella* was carried out by following the ISO 6579:2002 standard procedures (International Organization for Standardization, 2002) with a non selective enrichment stage in buffered peptone water (VWR, Belgium), a selective enrichment in Rappaport Vassiliadis Soya (Sigma-Aldrich, Belgium) and Muller-Kaufmann tetrathionate novobiocin (Sigma-Aldrich, Belgium) broths as well as an isolation on xylose-lysine-desoxycholate agar (Sigma-Aldrich, Belgium) and brilliant green agar (Bio-Rad, France). After the isolation stage, the biochemical confirmation of characteristic or suspected colonies of *Salmonella* was performed by using the API20E gallery (bioMérieux, France).

2.2.3. Statistical analyses

One way analysis of variance was used to assess the variability for normally distributed variables, whereas Kruskal-Wallis H (KWH) and Mann-Whitney U (MWU) tests were used for non-normally distributed variables. The normality of the variable distributions was assessed by using the Shapiro–Wilk's test, whereas the Pearson Chi-Square (PCS) test was used to compare the proportions in different variable categories. The results from bacterial counts namely TMC and ECC were transformed into log cfu scale base 10 before subsequent calculations. The statistical analyses were performed by using SPSS 16.0 Software (IBM, USA).

2.3. Results

2.3.1. Animal slaughtering conditions

In the cattle compartment of the studied abattoirs, the slaughtering procedures were found to be varying from one establishment to another. A technical description of the slaughtering processes for cattle in the studied abattoirs is provided in Table 3.

Contrary to cattle, the slaughtering process for goats in the studied abattoirs was found to be comparable. In all visited establishments, goats were held in the lairage park 24 hours prior to slaughter and during the lairage period, animals were not fed but were provided with a free access to water. The stunning was not practiced in all visited abattoirs and the sticking was performed on animals lying on the ground. Animals were then after suspended by the hind limb on hooks where manual dressing and evisceration processes were performed. The pelt and viscera were collected in separate stainless steel basins and prepared in another compartment of the slaughterhouse. In the Slaughterhouse B, the slaughtering platform was equipped with a knife sanitization cabinet with hot water (85.2±3.9°C) whereas in other establishments knives were washed with high pressure cold water. The daily slaughtering capacity was estimated at 40 to 60 animals in slaughterhouse A, 130 to 150 animals in slaughterhouse B and 35 to 50 animals in slaughterhouse C.

2.3.2. Professional training and hygiene of workers

All visited slaughterhouses have reported to have trained personnel in hygienic meat handling practices. The wearing of clean coats and hair covers by meat workers in the production area was observed in all slaughtering establishments; however, masks and gloves were regularly worn by meat handling staff only in the slaughterhouse B. All establishments reported to perform a medical checkup (every 6 months in slaughterhouses A and C, and 3 months in slaughterhouse B) for staff in regular direct contact with meat carcasses to determine whether they do not carry microbial pathogens susceptible to contaminate meat they are handling.

2.3.3. Hygiene of the establishment

In all studied slaughtering establishments, the walls and the floor of the production area were found to be covered with an easy to clean and sanitize material (in most cases porcelain tiles) and were equipped with hand washing units for working personnel with potable running water. Though all establishments reported to be cleaning the production area at the end of each production day, the disinfection was only reported in the slaughterhouse B where it was practiced once a week. Furthermore, all establishments were found to be applying pest control measures by using insect traps and gratings on the openings of the establishment.

Table 3. Technical summary of the slaughtering process for cattle in the studied abattoirs

Activity	Bovine slaughterhouses		
	A	B	C
Slaughtering capacity of the abattoir (animals/ day)	35-40	90-120	30-40
Transportation conditions of cattle to the slaughterhouse	x[(a)]	x[(a)]	x[(a)]
Duration of animal holding in the lairage park (hour)	48	24	24
Cattle handling practices in the lairage park	x[(b,c, d)]	x[(b,c, d)]	x[(b,c, d)]
Washing of cattle prior the slaughtering		x[(e)]	
Stunning of cattle prior the slaughtering		x[(f)]	
Exsanguination of cattle	x[(g)]	x[(h)]	x[(g)]
Blood collection practices	x[(i)]	x[(j)]	x[(i)]
Cleaning of the exsanguination knives	x[(k)]	x[(l)]	x[(k)]
Mean temperature of knife disinfection at the exsanguination post (°C)		89.2	
Time between exsanguination and skinning (seconds)	180	60	120
Cattle skinning practices	x[(m,k)]	x[(n,l)]	x[(m,k)]
Mean temperature of knife disinfection at the skinning post (°C)		84.7	
Cattle evisceration practices	x[(k)]	x[(o,l)]	x[(k)]
Mean temperature of knife disinfection at the evisceration post (°C)		82.6	
Handling of accidently contaminated carcasses	x[(q)]	x[(p)]	x[(q)]
Carcass splitting and washing practices	x[(r)]	x[(s,t)]	x[(r)]
Carcass refrigeration practices	x[(u)]	x[(u)]	x[(u)]

Table 3. Technical summary of the slaughtering process for cattle in the studied abattoirs (legend)

[(a)] Animal transportation tracks

[(b)] Animals not fed but have a free access to water

[(c)] Non-cemented floor, collection of faeces once a day.

[(d)] Systematic ante-mortem inspection

[(e)] Presence of a footbath with disinfectant agent at the entrance of the slaughtering block

[(f)] Traumatic stunning with non penetrative captive bolt gun

[(g)] Exsanguination performed on animals lying down

[(h)] Exsanguination performed on suspended animals

[(i)] Blood collection through the floor gutters

[(j)] Blood collection in stainless steel basins

[(k)] Sanitation of knives by using high pressure cold water

[(l)] Use of 2 knives. Sanitation by soaking one knife in a disinfection cabinet with hot water while using the other one.

[(m)] Manual skinning on suspended carcasses

[(n)] Mechanical skinning by using an up-ward hide puller

[(o)] Ligature of the esophagus and rectum prior the removal of abdominal viscera

[(p)] Washing carcasses with high pressure cold water on a special slaughtering line

[(q)] Washing carcasses with high pressure cold water on the same slaughtering line

[(r)] Manual splitting of carcasses by using knives and/or manually operated saw

[(s)] Mechanical splitting of carcasses by using a carcass splitting machine

[(t)] Washing carcasses with high pressure cold water

[(u)] Carcass chilling at the customer's request

2.3.4. Microbiological quality of carcasses

Tables 4 and Table 5 describe respectively the levels of hygiene indicator bacteria namely total mesophilic bacteria (TMC) and *E. coli* (ECC) as well as the occurrence of *Salmonella* in goat and bovine carcasses prepared within three slaughtering establishments of Kigali (Rwanda).

In cattle slaughtering establishments, the mean total mesophilic bacteria load in bovine carcasses was 4.6 log cfu/cm^2. Carcasses from the establishment B were found to be with TMC levels significantly low in comparison with that recorded on the carcasses from the establishments A and C. However, no significant difference was observed between the mean TMCs of carcasses prepared in the establishments A and C (establishment A versus B: MWU test=156.0, df=1, p=0.000; establishment A versus C: MWU test=1038.5, df=1, p=0.145; establishment B versus C: MWU test=89.5, df=1, p=0.000). *E. coli* counts on carcasses were varying between 1.1 and 2.4 log cfu/cm^2 and showed a comparable trend to the total mesophilic bacteria counts. *Salmonella* was isolated in 21.3% of bovine carcasses and its prevalence was not found to vary significantly in different slaughtering establishments. (Establishment A versus B: PCS test=1.1, df=1, p=0.298; Establishment A versus C: PCS test=0.5, df=1, p=0.488; Establishment B versus C: PCS test=3.0, df=1, p=0.086).

Table 4. Microbiological quality of bovine carcasses. Hygiene indicator bacteria: values are means (±standard deviation) of bacteriological counts in log cfu/cm^2. *Salmonella* occurrence: values are numbers and percentages of *Salmonella* positive samples. In the same column, different superscript letters indicate a significant difference ($p \leq 0.05$).

	N	Hygiene indicator bacteria(*)		*Salmonella* occurrence (*)	
		TMCs (**)	ECCs (***)	Positive samples (n)	Positive samples (%)
Establishment A	50	5.2 ± 0.9^a	2.3 ± 1.0^a	11.0	22.0^a
Establishment B	50	2.9 ± 0.9^b	1.1 ± 0.2^b	7.0	14.0^a
Establishment C	50	5.7 ± 1.4^a	2.4 ± 1.0^a	14.0	28.0^a
Total	150	4.6 ±1.6	1.9 ±1.0	32.0	21.3

(*)Total swabbed surface on the carcass: 400 cm$^{2;}$ (**) TMCs: Total Mesophilic Counts; (**) ECCs: *E. coli* counts.

The mean load of total mesophilic bacteria in goat carcasses varied between 3.9 and 5.2 log cfu/cm^2 and was significantly higher in the establishments A and C comparatively to the establishment B (Establishment A versus B: MWU test=596.5, df=1, p=0.000; Establishment A versus C: MWU test=1117.5, df=1, p=0.361; Establishment B versus C: MWU test=561.5, df=1, p=0.000). Nevertheless, the mean ECCs did not vary significantly from one slaughterhouse to another (KWH test=3.6, df=2, p=0.164).

As for bovine carcasses, the prevalence of *Salmonella* in goat carcasses appeared to be statistically equivalent in all slaughtering establishments (Establishment A versus B: PCS test=0.7, df=1, p=0.401; Establishment A versus

C: PCS test=0.3, df=1, p=0.617; Establishment B versus C: PCS test=1.8, df=1, p=0.183). In both bovine and goat carcasses, the Spearman's rank correlation between TMC and ECC was found to be significant and positive (bovine carcasses: r_s=0.684, n=150, p<0.01; goat carcasses: r_s=0.635, n=150, p<0.01), as well as the point biserial correlation between *E. coli* counts and the presence of *Salmonella* (bovine carcasses: r_{pb}=0.249, n=150, p<0.01; goat carcasses: r_{pb}=0.386, n=150, p<0.01).

Table 5. Microbiological quality of goat carcasses. Hygiene indicator bacteria: values are means (±standard deviation) of bacteriological counts in log cfu/cm^2. *Salmonella* occurrence: values are numbers and percentages of *Salmonella* positive samples. In the same column, different superscript letters indicate a significant difference ($p \leq 0.05$).

	N	Hygiene indicator bacteria(*)		*Salmonella* occurrence (*)	
		TMCs(**)	ECCs(***)	Positive samples (n)	Positive samples (%)
Establishment A	50	4.9 ± 1.0^a	1.6 ± 0.8^a	4.0	8.0^a
Establishment B	50	3.9 ± 1.0^b	1.6 ± 1.0^a	3.0	6.0^a
Establishment C	50	5.2 ± 1.4^a	1.8 ± 0.8^a	5.0	10.0^a
Total	150	4.7 ± 1.3	1.7 ± 0.9	12.0	8.0

(*)Total swabbed surface on the carcass: 400 cm$^{2;}$ (**) TMCs: Total Mesophilic Counts; (**) ECCs: *E. coli* counts.

2.4. Discussion

The microbiological analyses of goat and bovine carcass samples revealed a wide variability in regards to the hygiene and safety of meat carcasses prepared in the studied slaughtering establishments. This could be attributed to the animal slaughtering procedures, meat handling practices as well as the hygiene of both meat handling personnel and production premises that varied from one establishment to another. Several authors have reportedly associated the ultimate microbiological quality of meat to the hygiene of meat handlers and the processing environment (Abdalla et al., 2009; Çalicioğlu et al., 2010; Wheatley et al., 2014).

The findings from this study indicate that the levels of total mesophilic bacteria and *E. coli* were lower in bovine and goat carcasses processed in the slaughterhouse B comparatively to these from the slaughterhouses A and C. This would suggest lower hygienic meat handling practices in slaughterhouses A and C, as TMC and ECC are respectively recognized as indicators of general hygiene and fecal contamination in food processing establishments (Delhalle et al., 2008; Ghafir & Daube, 2007). Although various factors including animal handling conditions prior to slaughter could influence the ultimate microbial contamination of meat carcasses at the end of the slaughtering process, a number practices observed in slaughterhouses A and C could have contributed to the greater bacterial loads recorded on carcasses prepared in these establishments. These include the sticking and

exsanguination of animals lying on the ground, the manual skinning of slaughtered animals particularly cattle and the sanitation of slaughtering equipments with cold running water.

Contrary to the slaughterhouse B where the sticking and exsangunation were performed on suspended cattle, the exsanguination was found to be performed on animals lying on the ground in the slaughterhouses A and C. This practice appears to favor the microbial contamination of the sticking wound as well as the fouling of the hide as the slaughtered animal is lying in its own blood during the bleeding period. The microbial contamination of the sticking wound was found to occur most likely when the exsanguination was performed of animals lying on the ground comparatively to animal slaughtered in a suspended position (Niyonzima et al., 2015). Furthermore, several authors have reported significantly higher microbial loads on bovine carcasses from animals whose the hide was dirty comparatively to these whose the hide was relatively clean (McEvoy et al., 2000; Serraino et al., 2012).

Animal hides and visceral contents are recognized to be the major sources of microbial contaminations of meat carcasses and these contaminations occur mainly during the hide removal and evisceration processes (Bell, 1997; Sheridan, 1998). Several studies have indicated that the load of bacteria transferred from hide to carcass when the skinning of animals is performed manually was significantly greater in comparison to that recorded when animals were mechanically skinned by using a hide puller (Antic et al., 2010; Hauge et al., 2012; Niyonzima et al., 2015). Therefore, the utilization of a hide puller for cattle skinning in slaughterhouse B could have significantly contributed to the lower bacterial loads recorded in bovine carcasses prepared in that particular establishment. Moreover, the fact that the ligature of esophagus and rectum during the evisceration process was not practiced in slaughterhouses A and C might have also contributed to the recorded high microbial contamination of the processed carcasses. These practices were reportedly associated to a decreased risk of microbial contamination of carcasses during the evisceration (ICMSF, 1998; McEvoy et al., 2000).

Another explanation to the higher bacterial loads recorded on meat carcasses prepared within the slaughterhouses A and C would be the possibility of cross contamination associated to the use of non sanitized knives. Within these establishments, slaughtering knives were found to be washed with cold water comparatively to the slaughterhouse B where knives were sanitized in specialized cabinets with hot water. In a study conducted to assess the efficacy of sanitation procedures for slaughtering equipments, Taormina et al. (2007) reported a reduction of the total mesophilic bacteria and *Salmonella* DT104 loads on knives by 3.1 and 2.3 logarithmic units respectively, when they were dipped in hot water at 82.2°C for 15 seconds; whereas in a comparable study, de Jong et al. (2008) indicated that cold water rinses did not reduce significantly the load of *C. jejuni* on meat cutting boards. Number of authors recommends the use of a two-knife system to prevent microbial cross-contaminations

that might be associated to the utilization of contaminated knives during the slaughtering operations. This system consists in using one knife in slaughtering operations while the other one is being sanitized in hot water at 82°C and above or by another knife sanitation method with equivalent effect (Bolton et al., 2002; Goulter et al.,2008; Leps et al., 2013). Additionally, the slaughterhouses A and C were found to be lacking special slaughtering lines that could be utilized for processing accidently contaminated carcasses and this might have also favored the occurrence of microbial cross contaminations on carcasses prepared in these establishments.

Although number of measures applied to prevent microbial contamination of carcasses from the processing environment and the working personnel were comparable in all visited slaughtering establishments of Kigali; some such as the disinfection of the slaughtering equipments as well as the regular wearing of mask and gloves by meat handlers were solely recorded in the slaughterhouse B, and might have contributed to the lower microbial loads recorded on goat and bovine carcasses processed in that establishment. Previous studies have reportedly associated the disinfection of food processing equipments to a decreased risk of microbial contaminations in the processed products (Holah, 2014; Møretrø et al., 2012), whereas bare hands as well as uncovered nasal and oral cavities of meat handlers were identified as probable sources of meat microbial contaminations (Bassis et al., 2014; Heinz & Hautzinger, 2007; Nel et al., 2004).

In both goat and bovine carcasses, a significant and positive correlation was noted between the total mesophilic bacteria and *E. coli* loads as well as between ECC and the occurrence of *Salmonella*. This would suggest that in the visited slaughterhouses, the prevalence of *Salmonella* increased with the loads of hygiene indicator bacteria on goat and bovine carcasses. The prevalence of *Salmonella* in bovine carcasses recorded in our study (21.3%) appeared to be relatively low in comparison with the prevalence observed in countries such as Ethiopia (Jajere et al., 2015), Senegal (Stevens et al., 2006) and Australia (Fegan et al., 2005), where prevalences as high as 61.1% (n=54), 42.8% (n=236), and 26.0% (n=606) in bovine carcasses were respectively reported. However lower prevalence were recorded by Oueslati et al. (2016) in Tunisia (5.7%, n=300), Martínez-Chávez et al. (2015) in Mexico (18.3%, n=142) and by Nouichi et al. (2009) in Algeria (7.0%, n=70). Similarly, in goat carcasses, the prevalence recorded in our study was found to be low in comparison with the prevalence of 24.2% (n=66) reported in Ethiopia (Jajere et al., 2015) and that of 32.2% (n=121) observed in Australian goat carcasses (Duffy et al., 2009). Nevertheless, our prevalence appears to be greater than that of 1.1% recorded in 90 ovine carcasses prepared in an Algerian Slaughterhouse (Nouichi & Hamdi, 2009).

2.5. Conclusion

The findings from this study indicate that the microbiological quality and safety of goat and bovine meat was greater in the slaughterhouse B comparatively to that of carcasses prepared in other slaughtering establishments of

Kigali city. This highlight the need for improvements in regards of slaughtering practices particularly at the bleeding, skinning and evisceration stages as well as the sanitation procedures for slaughtering knives in the slaughterhouses A and C. The positive and significant correlation observed between hygiene indicator bacteria and the occurrence of *Salmonella* in carcasses would suggest that the safety of goat and bovine carcasses can be significantly increased through hygiene improvements in the studied slaughtering establishments.

CHAPTER THREE

Microbiological quality and safety of meat cuts commercialized in retail establishments of Kigali city.

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CHAPTER 3. Microbiological quality and safety of meat cuts commercialized in retail establishments of Kigali city.

Abstract

Meat constitutes one of the major vehicles for human foodborne infections. This study aimed to assess the retail conditions and to determine the microbiological quality and safety of meat retailed within the establishments of Kigali (Rwanda). A questionnaire survey was carried out in 150 retail outlets to characterise meat retail conditions. Additionally, 270 retail meat samples were analysed for the enumeration of hygiene indicator bacteria (total mesophilic bacteria and *Escherichia coli*) and for the qualitative detection of *Salmonella*, using conventional culture methods. The results revealed that beef was the predominant meat sold within the retail premises of Kigali city, while meat from non-bovine animal species was mainly sold in large establishments. *Salmonella* was detected in 19.6% of all the retailed meat samples evaluated, whereas the mean loads for total mesophilic bacteria and *E. coli* were 7.3 and 3.5 log cfu/g, respectively. Three factors, namely the temperature conditions of the meat under retail, the cleanability of the used meat cutting boards, and the training of personnel in hygienic meat handling practices, were found to be significantly ($p \leq 0.05$) associated with the risk of *Salmonella* occurrence in the retailed meat. The findings from this study highlight the need for improvements in hygienic meat handling practices, particularly, in small and medium meat retail establishments in Kigali.

Keywords: meat, retail conditions, *Salmonella*, hygiene indicator bacteria, risk factors, Rwanda.

3.1. Introduction

Meat is known to be an important source of valuable proteins and nutrients for human nutrition. However, its chemical composition favours the proliferation of a wide range of microorganisms. (de Carvalho et al. 2014; McAfee et al., 2010; Sans & Combris, 2015). Consequently, meat constitutes one of the major vehicles for microbial pathogens, responsible for foodborne infections in humans (Doulgeraki et al., 2012; Nychas et al., 2008).

Human salmonellosis, constitutes one of the leading foodborne diseases (CDC, 2013; EFSA & ECDC, 2015) and the consumption of contaminated meat is reported to be one of the major pathways of *Salmonella* transmission. In a study conducted in the United States (US) and European countries, Greig and Ravel (2009) reported that 20.7, 11.5 and 7.2% of *Salmonella* outbreaks with an identified vehicle, were respectively associated with the consumption of poultry, beef and pork products. Nevertheless, it should be noted that the proportion of human salmonellosis linked to the consumption of meat is difficult to estimate accurately, principally because only a

limited number of illness cases are officially reported to competent authorities; and even within the declared cases, very few allow the identification of the food vehicle (Newell et al., 2010; Scallan et al., 2011; Stevens et al., 2006).

The contamination of meat by microbial pathogens, such as *Salmonella*, can occur at any stage along the meat chain, from the farm to consumption (Niyonzima et al., 2015). However, the retail level, constitutes an important stage, regarding the ultimate quality and safety of meat, as it represents the last check-point where contaminated products can be identified (Wong et al., 2002). Therefore, the microbiological quality of meat, at the retail stage, constitutes a notable food safety concern for consumers.

In Rwanda, the microbiological quality of meat consumed within the households, as well as meat-based meals consumed outside the home, were thoroughly investigated by Niyonzima et al. (2016, 2017). However, to the best of our knowledge, no study has yet assessed the occurrence of bacterial pathogens such as *Salmonella*, in earlier stages of the meat chain, particularly at the retail level within Kigali city. In a baseline study conducted in the markets of Kigali, the levels of hygiene indicator bacteria (namely, the total mesophilic bacteria and *Escherichia coli* counts) in the retailed meat, were found to lie outside the European microbiological standards acceptable range (Niyonzima et al., 2013). This would suggest the possible contamination of retailed meat by bacterial pathogens such as *Salmonella*, that are generally associated with poor hygienic practices in meat processing units (Carrasco et al., 2012; Rhoades et al., 2009).

The present study aimed to assess the meat retail conditions in Kigali city, as well as to determine the bacteriological quality and safety of retailed meat in Kigali. Data gathered in this study will be helpful in designing a microbiological risk assessment model for *Salmonella* in the Rwandan meat chain.

3.2. Material and methods

3.2.1. Survey on meat retail conditions

The survey was conducted to characterise the retail conditions within the establishments selling meat in Kigali city. From the list of registered establishments in different districts of Kigali city provided to us by the district authorities, 300 establishments were first selected and approached, to request their willingness and consent to participate in the present study. From those who accepted to participate in the study (n = 272), a second selection was carried out to retain only 150 retail establishments, in which the survey and the collection of meat samples were performed. The selection of establishments was performed by using the random selection function of Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA).

For the survey, each selected establishment was visited once. Through the interview with the manager of the establishment, information regarding the type of meat used, meat handling and storage conditions, equipment and staff hygiene, cleaning and disinfection procedures, as well as pest control measures within the establishment was collected through a structured questionnaire. After the interview, the responses were verified on the site and corrections were made when necessary.

In the present study, retail establishments were classified into three categories, depending on their meat production capacity. Establishments with a daily production below 100 kg were considered as small establishments, whereas those with a production between 100 and 200 kg/day, and beyond 200 kg, were considered as medium and large respectively.

3.2.2. Microbiological analyses

3.2.2.1. Collection of meat samples

Meat samples were collected from 75 establishments, selected randomly from those selling beef; and in the totality of establishments selling meat from animal species other than bovines (24 for goat/mutton, 18 for chicken, 12 for pork and 6 for rabbit). At each establishment, two samples (portions of approximately 50 g), were aseptically collected for each meat type and placed in separate sterile stomacher bags (VWR, Belgium). A total of 270 samples were collected. The meat samples were transported to the laboratory, within approximately 2 h, in a cold box with freeze packs. At the laboratory, samples were stored frozen at -30˚C and microbiologically analysed within 24 h.

3.2.2.2. Analytical methods

Meat samples were analysed for the enumeration of hygiene indicator bacteria (namely total mesophilic bacteria and *Escherichia coli*) and for the qualitative detection of *Salmonella*. The International Organisation for Standardisation protocol ISO 7218:2007 (ISO, 2007) was followed for the preparation of meat samples. The enumeration of total mesophilic bacteria (TMC) was performed by using the pour plate method on plate count agar (VWR, Belgium), as described in the ISO 4833:2003 standard protocol (ISO, 2003); whereas the *E. coli* counts (ECC) were determined on tryptone bile X-glucuronide agar (Bio-Rad, France), as defined by the ISO 16649-2:2001 standard protocol (ISO, 2001). In the case the levels of TMC or ECC were found to be below the bacterial enumeration threshold, subsequent calculations were done with the value of the detection limit of the used analytical method. The ISO 7218:2007 standard, indicates that the enumeration of total mesophilic bacteria and *E. coli* in solid samples, by using ISO 4833:2003 and ISO 16649-2:2001 standard protocols respectively, has a detection limit of 1 log cfu/g (ISO, 2007).

Salmonella was qualitatively detected in the meat samples by following the ISO 6579:2002 standard protocol (ISO, 2002). The non-selective enrichment step, was performed in buffered peptone water (VWR, Belgium), whereas Rappaport–Vassiliadis with soya (Sigma-Aldrich, Belgium) and Müller–Kauffmann tetrathionate-novobiocin (Sigma-Aldrich, Belgium) broths were used for the selective enrichment phase. Xylose-lysine-deoxycholate (Sigma-Aldrich, Belgium) and brilliant green (Bio-Rad, France) agars, were used for the isolation of characteristic or suspected colonies of *Salmonella*, whereas the API 20E gallery (bioMérieux, France) was used for their biochemical confirmation.

3.2.3. Statistical analyses

Statistical analyses were performed using SPSS 16.0 software (IBM, USA). One-way analysis of variance (ANOVA) was used to test the equality of means for normally distributed variables, whereas the Kruskal–Wallis H (KWH) and Mann–Whitney U (MWU) tests, were used for non-normally distributed variables. The normality of the distributions was assessed by the Shapiro–Wilk test, while Pearson's chi-square (PCS) test was used to compare the proportions of different variables. The results from bacterial counts were transformed into log cfu scale base 10 before subsequent calculations and statistical treatments. Correlation between TMC and ECC (continuous variables, was determined using the Spearman's rank correlation (r_s), whereas the point-biserial correlation (r_{pb}) was used to determine a correlation between ECC and *Salmonella* occurrence (binary variable).

A univariate logistic regression analysis was used to determine the risk factors for *Salmonella* occurrence in retailed meat. The outcome variable was the occurrence of *Salmonella*-positive meat in the retail establishment. Meat sold in a retail establishment was considered contaminated by *Salmonella*, when at least one sample was found to be *Salmonella*-positive. Potential risk factors were first screened, to assess their association with the outcome variable, by PCS test, and only significant factors ($p \leq 0.05$), were considered eligible for the logistic regression analysis. The assessed potential risk or protection factors included the processing of meat from various animal species within the establishment; the origin of the processed meat; the temperature conditions for the transportation, the storage, as well as the exposition of meat within the establishment; the control of meat temperature during transportation, storage and exposition within the establishment; the coating of walls and floor of the establishment with an easy to clean and sanitise material; the use of meat cutting boards that are easy to clean and sanitise; the sanitation of meat cutting equipment; the cleaning and disinfection frequencies within the establishment; the use of insect traps within the establishment; the conduct of a regular medical check-up for personnel to assess whether they are carriers of pathogens susceptible to be transferred to meat they are handling; the wearing of protective clothes by meat handlers; and the training of personnel in hygienic meat handling practices.

3.3. Results

3.3.1. Meat retail conditions in Kigali city establishments

3.3.1.1. Retail marketing of meat from various animal species

Bovine meat was found to be the main type of meat sold in retail establishments of Kigali city. The sale of non-bovine meat was predominantly practiced in large meat retail premises. The level of utilisation of meat derived from various animal species in retail establishments of Kigali city is described in Table 6.

Table 6. Utilisation of meat from various animal species within retail establishments of Kigali city. Values are numbers (percentage) of retail establishments selling meat of a given animal species. In the same column, different superscript letters (a, b, c) indicate a significant ($p \leq 0.05$) difference. Values with the same superscript letters (w, x, y, z) are not significantly different ($p \leq 0.05$).

	n	Animal species				
		Cow	Goat/Mutton	Chicken	Pork	Rabbit
Small-scale	39	39 (100.0^{a})	0 (0.0^{a})	0 (0.0^{a})	0 (0.0^{a})	0 (0.0^{a})
Medium-scale	69	69 (100.0^{a})	6 (8.7^{b})	3 (4.3^{b})	0 (0.0^{a})	0 (0.0^{a})
Large-scale	42	42 (100.0^{a})	18 (42.9^{c})	15 (35.7^{c})	12 (28.6^{b})	6 (14.3^{b})
Total	150	150 (100.0$^{[w]}$)	24 (16.0$^{[x]}$)	18 (12.0$^{[xy]}$)	12 (8.0$^{[yz]}$)	6 (4.0$^{[z]}$)

3.3.1.2. Distribution conditions for meat carcasses

Table 7 describes the procurement places for various types of meat in the retail premises of Kigali city. Public slaughterhouses were found to be the main procurement place for retail establishments, particularly for bovine and small ruminant meat; whereas meat from other animal species was supplied directly from farmers, predominantly located in the peri-urban area of Kigali city.

The transportation of meat carcasses from the slaughtering facility to the retail establishment was found to be mainly carried out under ambient temperature conditions. The average duration of carcass transportation was estimated to 29 min in the establishments where carcasses were transported under ambient temperature conditions and to 46 min in the establishments where the refrigeration of carcasses during the transportation was practiced (Table 8).

Table 7. Meat procurement places for retail establishments in Kigali city. Values are numbers (percentage) of retail establishments supplied in meat from a given procurement place. In the same column, different superscript letters indicate a significant ($p \leq 0.05$) difference.

	Animal species				
	Cow (n = 150)	Goat/Mutton (n = 24)	Chicken (n = 18)	Pork (n = 12)	Rabbit (n = 6)
Slaughterhouse	129 (86.0^{a})	21 (87.5^{a})	0 (0.0^{a})	0 (0.0^{a})	0 (0.0^{a})
Other butcheries	21 (14.0^{b})	0 (0.0^{b})	0 (0.0^{a})	0 (0.0^{a})	0 (0.0^{a})
Farmers	0 (0.0^{c})	0 (0.0^{b})	18 (100.0^{b})	12 (100.0^{b})	6 (100.0^{b})
Slaughtering within the establishment	0 (0.0^{c})	3 (12.5^{c})	0 (0.0^{a})	0 (0.0^{a})	0 (0.0^{a})

Table 8. Transportation conditions for meat within retail establishments of Kigali. Temperature conditions: values are numbers (percentages) of establishments transporting meat under the indicated temperature conditions. Transportation duration: values are means (±standard deviation) of the estimated transportation duration (in minutes) for meat from the procurement place to the retail establishment. In the same column, different superscript letters indicate a significant ($p \leq 0.05$) difference.

	n	Meat transportation under refrigeration conditions		Transport under ambient temperature conditions	
		Number (%) of establishments	Duration of meat transportation	Number (%) of establishments	Duration of meat transportation
Small-scale	39	0 (0.0^{a})	0.0 ± 0.0^{a}	39 (100.0^{a})	23.9 ± 13.1^{a}
Medium-scale	69	7 (10.1^{b})	14.3 ± 13.4^{b}	62 (89.9^{b})	25.7 ± 16.8^{a}
Large-scale	42	14 (33.3^{c})	62.5 ± 46.3^{c}	28 (66.7^{c})	43.8 ± 29.6^{b}
Total	150	21 (14.0)	46.4 ± 44.6	129 (86.0)	29.1 ± 20.7

3.3.1.3. Retail and storage conditions for meat

Sixty-nine percent of the studied establishments reported to be exposing retail meat to ambient temperature and the maximal duration of meat exposition was estimated to 8 h. The average duration of meat exposition (Table 9), was found to be significantly higher in medium establishments compared to small or large retail outlets (small versus medium establishments: MWU test = 531.5, degrees of freedom [df] = 1, $p < 0.001$; small versus large establishments: MWU test = 1217.5, df = 1, $p = 0.381$; medium versus large establishments: MWU test = 536.5, df = 1; $p < 0.001$).

All the studied establishments reported to be storing the non-exposed meat carcasses in the freezer and the average maximal storage duration was estimated to 37.7 h. No significant difference was noted between the

storage durations of meat carcasses in different categories of meat retail establishments (KWH test = 3.6, df = 2, p = 0.146).

Table 9. Meat retail conditions within the establishments of Kigali. Temperature conditions: values are numbers (percentages) of establishments exposing meat under the indicated temperature conditions. Exposition duration: values are means (±standard deviation) of the estimated maximal exposition duration (in hours) for meat within the retail establishment. In the same column, different superscript letters indicate a significant ($p \leq 0.05$) difference.

	n	Meat retail under refrigeration conditions		Meat retail under ambient temperature conditions	
		Number (%) of establishments	Duration of meat exposition	Number (%) of establishments	Duration of meat exposition
Small-scale	39	3 (7.7^{a})	18.0 ± 6.0^{a}	36 (92.3^{a})	7.0 ± 2.9^{a}
Medium-scale	69	16 (23.2^{b})	23.3 ± 3.0^{a}	53 (76.8^{b})	9.0 ± 2.2^{b}
Large-scale	42	27 (64.3^{c})	22.7 ± 3.8^{a}	15 (35.7^{c})	8.3 ± 2.3^{a}
Total	150	46 (30.7)	22.6 ± 3.8	104 (69.3)	8.2 ± 2.6

3.3.1.4. Professional training and hygiene of workers

The overall proportion of retail establishments with trained personnel in meat handling and hygiene was found to be relatively low. No trained staff were reported in all small meat retail establishments, whereas 13.0 and 50.0% of medium and large establishments, respectively, employed trained personnel. In 94% of the 150 studied establishments, personnel in the production area were found to be wearing clean coats, whereas head covers, gloves and masks, were regularly worn by staff in only 66.0, 18.0 and 10.0% of the visited meat retail outlets respectively (Table 10). Nevertheless, regular staff medical check-ups were reported in all small and large meat retail establishments and in 91.3% of the 69 medium retail outlets.

3.3.1.5. Hygiene of the establishment

In all retail establishments, the walls and the floor of the production area were found to be covered with an easy to clean material, in most of the cases porcelain tiles. However, 62.0% of the retail facilities were found to use wooden cutting boards with rough surfaces that are difficult to clean and disinfect. The use of meat cutting boards in polyethylene was observed in only 38.5, 27.5 and 54.8% of the small, medium and large retail outlets respectively.

Though all retail establishments have indicated to regularly clean the retail and production area of the facility, the disinfection was found to be rarely practiced. Only 2.0% of the 150 studied establishments (7.4% of the large

establishments and 0.0% of both the small and medium retail premises) reported disinfecting the production and retail area of the establishment. Nevertheless, pest control measures, by use of insect traps, were applied in all medium and large meat retail facilities and in 61.5% of the small retail establishments.

Table 10. Composition of the working cloth in the studied meat retail facilities. Values are numbers (percentage) of the establishments in which the personnel wear the indicated working cloth. In the same column, different superscript letters indicate a significant difference ($p \leq 0.05$).

	n	Components of the working cloth			
		Coat	Head cover	Mask	Gloves
Small-scale	39	30 (76.9^{a})	15 (38.5^{a})	6 (15.4^{a})	6 (15.4^{a})
Medium-scale	69	69 (100.0^{b})	54 (78.3^{b})	6 (8.7^{a})	15 (21.7^{a})
Large-scale	42	42 (100.0^{b})	30 (71.4^{b})	3 (7.1^{a})	6 (14.3^{a})
Total	150	141 (94.0)	99 (66.0)	15 (10.0)	27 (18.0)

3.3.2. Bacteriological quality of the retailed meat

Table 11 describes the levels of hygiene indicator bacteria, namely the TMC and EEC; as well as the occurrence of *Salmonella* in various types of meat sold in retail outlets of Kigali city. The mean TMC ranged from 6.3 to 9.6 log cfu/g and the mean ECC ranged from 2.9 to 4.4 log cfu/g, whereas *Salmonella* was isolated in 19.6% of all meat samples. The hygiene indicator bacteria loads, were found to be significantly ($p \leq 0.05$) higher in pork and chicken compared to meat from other animal species, whereas the prevalence of *Salmonella* did not vary significantly ($p \leq 0.05$) among the types of meat in which *Salmonella* was detected.

For the same sample, the Spearman's rank correlation between TMC and ECC was found to be positive and significant ($r_s = 0.454$, $n = 270$, $p < 0.01$) as well as the point biserial correlation between ECC and the occurrence of *Salmonella* ($r_{pb} = 0.350$, $n = 270$, $p < 0.01$).

3.3.3. Risk factors for *Salmonella* occurrence

Three variables, namely "meat retail under refrigeration conditions", "use of easy-to-clean meat cutting board in the establishment", as well as "the training of personnel in hygienic meat handling practices", were found to be significantly ($p \leq 0.05$) associated with a decreased risk of *Salmonella* occurrence in retailed meat (Table 12).

Table 11. Bacteriological quality of meat at retail. Hygiene indicator bacteria: values are means (±standard deviation) of bacteriological counts. *Salmonella* occurrence: values are numbers and percentages of *Salmonella*-positive samples. In the same column, different superscript letters indicate a significant difference ($p \leq 0.05$).

	n	Hygiene indicator bacteria		*Salmonella* occurrence	
		TMCs (*)	ECCs (**)	Positive samples (n)	Positive samples (%)
Beef	150	7.0 ± 1.2^{a}	3.4 ± 1.3^{a}	35	23.3^{a}
Goat/Mutton	48	6.3 ± 0.6^{b}	2.9 ± 1.2^{b}	6	12.5^{a}
Chicken	36	9.3 ± 1.3^{d}	4.3 ± 1.2^{cd}	8	22.2^{a}
Pork	24	9.6 ± 1.0^{cd}	4.4 ± 0.5^{d}	4	16.7^{a}
Rabbit	12	6.3 ± 0.6^{abc}	3.7 ± 0.3^{abc}	0	0.0^{b}
Total	270	7.3 ± 1.5	3.5 ± 1.3	53	19.6

(*) TMCs: Total mesophilic counts; (**) ECCs: *E. coli* counts.

Table 12. Risk factors for *Salmonella* occurrence in meat retail establishments of Kigali city.

Variable	Percentage of *Salmonella*-positive establishments (*)		Binary logistic regression		
			Odds ratio	95% CI	p-value
Retail temperature condition for meat					
	Ambient temperature (n = 104)	71.4	1		
	Refrigeration (n = 46)	17.0	0.082	0.032–0.211	0.000
Easy to clean (and disinfect) meat cutting board					
	No (n = 93)	88.9	1		
	Yes (n = 57)	7.0	0.009	0.002–0.037	0.000
Training of personnel in meat handling and hygiene					
	No (n = 120)	57.8	1		
	Yes (n = 30)	18.4	0.165	0.063–0.430	0.000

(*) Meat retailed in a given establishment was considered contaminated by *Salmonella* if at least one meat sample was found to be *Salmonella*-positive.

3.4. Discussion

3.4.1. Meat handling conditions in retail establishments of Kigali city

Beef was found to be the type of meat primarily sold in retail establishments of Kigali. This could be attributed to the cultural considerations of the Rwandan population, as cows are of great socio-cultural importance in Rwandan society (Adekunle, 2007). Another reason would be the lack of public infrastructure available to slaughter other animal species in Kigali city. Consequently, many households in Kigali are unable to afford meat from animals other than bovines, that are generally slaughtered in private small-scale plants (Niyonzima et al., 2016a). Available statistics indicate that in 2013, bovine meat alone counted for 48.7% of the global meat production in Rwanda (FAO, 2015). The reported procurement of raw meat from animals other than bovines directly from farms, by meat retail establishments, appears to be a direct consequence of the lack of public slaughtering facilities for these animal species.

In most of the studied establishments, the transportation of carcasses or meat cuts from the slaughtering place to the retail points was found to be carried out under non-refrigerated conditions. This can be explained by the low financial capacity of a number of meat retail establishments in Kigali, which cannot afford the purchase of meat refrigeration vehicles. The high cost of cold storage equipment, has been identified as one of the key factors hampering the transportation of meat cuts and/or carcasses under refrigeration conditions in developing countries (Kago et al., 2015). Our findings agree with the results from previous studies conducted in various developing countries, such as Ghana (Adzitey et al., 2011) and Kenya (Roesel et al., 2015); where the transportation of meat under non-refrigerated temperature conditions was reported. The average duration of carcass transportation under ambient temperature conditions was found to be about 29 min. As the generation time for *Salmonella* in optimal temperature conditions (35–37°C) is known to be 25 min (Delhalle et al., 2009b), the recorded duration of carcass transportation under ambient temperature, might allow the proliferation of *Salmonella* cells initially present on carcasses or meat cuts. Several studies have reported bacterial proliferation during the carcass transportation stage, when the temperature was not successfully controlled (Lo Fo Wong et al., 2002; Niyonzima et al., 2015).

As during the carcass transportation stage, numerous establishments (69.3%) were found to be exposing retailed meat under non-refrigerated conditions, for an average maximal duration of 8 h. In these establishments, meat cuts were kept frozen and every day, meat to be retailed was hung on hooks at ambient temperature. At the end of the day, the leftovers were frozen and sold the following day. These retail practices might favour the proliferation of microorganisms present in meat cuts, particularly during the period meat is kept at ambient temperature. Several studies have reportedly associated the proliferation of microorganisms to the repeated freezing and thawing of meat products (Oranusi et al., 2014; Wu et al., 2016).

The exposition of meat at ambient temperature observed in some retail establishments can be attributed to the low financial capacity of retailers to afford the purchase of meat refrigeration cabinets, as well as their limited knowledge in hygienic meat handling practices. The meat retail conditions observed in our study, are comparable to the practices reported in other developing countries, such as the Democratic Republic of Congo (Kabwang, 2013), Ethiopia (Haileselassie et al., 2013), and Ghana (Adzitey et al 2011), where they were associated with an increased risk of microbial contamination in the retailed meat.

The knowledge of butcher workers, in meat handling and hygiene was found to be relatively low; as only 20.0% of the studied establishments reported to employ personnel trained in meat handling and hygiene. Our results corroborated the findings from the study conducted in Ethiopia, where trained butcher workers were reported in 38.5% of 26 retail outlets in Mekele city (Haileselassie et al., 2013). Comparable observations were also recorded in western Romania, where only 30.9% of 168 meat handlers, reported to have received professional training (Jianu & Goleț, 2014). Nevertheless, a relatively higher training rate of personnel was recorded in Serbian meat retail establishments, where 52.8% of 116 meat workers, were reported to be trained in meat handling and safety (Smigic et al.,2016).

The wearing of protective clothes other than aprons was found to be rarely practiced, particularly within small meat retail establishments. This could be explained by the limited knowledge of meat handlers in meat hygiene practices. The proportion of hairnet usage (66.0%) recorded in the present study, appears to be low compared to that observed in Romanian establishments, where 89.3% of 168 meat handlers, reported to regularly wear a hairnet, during the meat production and retail activities (Jianu & Goleț, 2014). Nevertheless, our findings are comparable to the results from the studies conducted in Ethiopia (Haileselassie et al., 2013) and Uganda (Muyanja et al., 2011),where hairnets were reported to be regularly worn by 49.3 and 50.2% of meat handlers respectively. The study conducted in the Democratic Republic of Congo, revealed that hairnets were not worn at all, by meat retailers in the markets of Lubumbashi (Kabwang, 2013). Protective clothes, such as proper aprons, hairnets and gloves, are barriers against microorganisms that may be transferred from handlers to meat and should always be worn during the handling of meat, to prevent microbial contaminations from the handler. Previous studies have identified bare hands (Heinz & Hautzinger, 2007), dirty clothes (Cardinale et al., 2005)and worker's hairs (Lues et al., 2006), as probable sources of meat microbial contamination.

Meat handlers may also be carriers of bacterial pathogens, such as *Salmonella* (Gopinath et al., 2012; Niyonzima et al., 2015). Hence, it is important that food handlers carry out a regular medical check-up, to ensure that they are not pathogen carriers. In the present study, the proportion (96.0%) of meat retailers with a medical certificate attesting that they do not carry illnesses susceptible to be transferred to meat, was found to be relatively high. Comparable findings were recorded in Ethiopia, where 84.6% of 26 meat retailers in Mekele city, reported to have

medical certificates (Haileselassie et al., 2013). However, lower compliance rates were reported in other developing countries. The study conducted within street food retailers in Sudan, revealed that only 64% of 54 vendors had a health certificate (Abdalla et al., 2009),whereas meat retailers in Tanzania (Kago et al., 2015) and the Democratic republic of Congo (Kabwang, 2013),were found to be working without any medical certificate.

Meat retail establishments in Kigali city showed important efforts to prevent meat contaminations from the processing environment, as most of the retail outlets were found with infrastructures, such as easy-to-clean walls and floors, within the production area; and reported to be applying pest control measures by using insect traps in both the production area and retail premises. However, these efforts appear to be hampered by the limited knowledge of meat professionals in basic hygienic practices. Indeed, the sanitisation of retail premises and butcher equipment was found to be rarely practiced and a relatively high proportion of retail establishments (62.0%), reported to be using wooden cutting boards (in most instances pieces of tree trunks) that are not easy to sanitise (Carrasco et al., 2012). The lack of knowledge in basic meat hygiene practices within meat retailers, has been reported in other countries, such as the Democratic Republic of Congo (Kabwang, 2013), Ethiopia (Haileselassie et al., 2013), Kenya (Kago et al., 2015), Pakistan (Hassan Ali et al., 2010),and Romania (Jianu & Goleţ, 2014), and was found to be associated with an increased risk of microbial contamination in the retailed meat.

3.4.2. Microbiological quality of the retailed meat

The willingness of the managers to participate in the present study was the principal criteria to select retail outlets, from which meat samples were collected. Consequently, this might have introduced a bias in the observed results. However, as a large percentage (90.7%) of the establishments accepted to participate in the study, and their selection was carried out randomly, the introduced bias is relatively minimal.

The bacteriological quality, particularly the levels of hygiene indicator bacteria in meat sold in retail outlets of Kigali city, was found to vary significantly ($p \leq 0.05$) among the establishments. The observed variability could be explained by the level of hygiene, as well as meat handling practices within the establishment that differ among the retail outlets. Some authors have reportedly associated the microbiological quality of meat at the retail stage, to the hygiene of meat workers and the processing environment (Carrasco et al., 2012; Lo Fo Wong et al., 2002). Furthermore, factors, such as the quality of the used raw material, as well as the transportation conditions for carcasses from the slaughtering place to the retail premises, could have also contributed to the variation of ultimate hygiene indicator bacteria loads, as they are likely to differ among the establishments.

The levels of hygiene indicator bacteria were found to be significantly ($p \leq 0.05$) higher in pork and chicken, compared to meat from other animal species. As meat handling practices within retail establishments were found

to be comparable for all types of meat, the higher bacterial loads observed in pork and chicken samples, could be attributed to the bacterial quality of the pork and chicken carcasses. Studies have indicated that pork and chicken carcasses, generally present higher bacterial loads than carcasses from other animals because their hides are not removed during the slaughtering operations (Bolton et al., 2002a; Borch, Nesbakken, & Christensen, 1996). Furthermore, they undergo a scalding process, which is known to favour the proliferation of microorganisms present on carcasses (Bolton et al., 2002; Borch et al., 1996).

The mean TMC recorded in the present study (7.3 log cfu/g), appears to be relatively high and indicates deficient hygienic practices within the studied meat retail establishments and/or in earlier stages of the meat chain. Our findings corroborate with studies conducted in other developing countries, where the lack of hygienic practices in meat handling was reported. In a study conducted in the markets of Accra (Ghana) for example, the TMC levels recorded in bovine meat samples (2.3–4.4 log cfu/g), were attributed to unhygienic practices and poor handling of meat by butchers (Soyiri et al., 2008). Comparable findings were reported in Pakistan, where a mean TMC of bacteria of 10.2 log cfu/g, was recorded in meat sold in retail shops of Karachi city (Hassan Ali et al., 2010).

The average *E. coli* load in meat sold within retail outlets of Kigali was found to be 3.5 log cfu/g. Similar values have been documented previously. For instance, Soyiri et al. (2008)reported a mean ECC of 3.3 log cfu/g in bovine meat sold in the Ghanaian retail premises and Kabwang (2013)recorded mean *E. coli* loads of4.50, 4.54, 4.43, and 4.44 log cfu/g, respectively, in bovine, goat, pork and chicken meat, retailed in the markets of Lubumbashi (the Democratic Republic of Congo). However, in a study conducted in Belgian meat retail outlets, relatively lower ECC levels (0.21–1.23 log cfu/g) were recorded in pork (Delhalle et al., 2009a).

As for the hygiene indicator bacteria, the prevalence of *Salmonella* recorded in the present study (19.6%), appears to be relatively high when compared with the prevalence observed in developed countries, where hygienic practices in meat handling are reported to be strictly practiced. In the US, for example, a *Salmonella* prevalence as low as 1.02% was recorded in muscle beef cuts collected in retail premises (Vipham et al., 2012),whereas in the European Union countries, a recent report on zoonotic agents and foodborne outbreaks, revealed an average *Salmonella* prevalence of 2.3, 0.5, and 0.1%, respectively, in chicken, pig and bovine meat at retail (EFSA & ECDC, 2015). European regulations, indicate that *Salmonella* must be absent in 25 g of food products destined for human consumption (European Commission, 2005).

The correlation between TMC and ECC, as well as the correlation between ECC and the presence of *Salmonella*, were found to be positive and significant ($p < 0.01$). This indicates that the prevalence of *Salmonella* increases with the levels of hygiene indicator bacteria in meat, and would suggest that efforts to improve hygiene in meat retail units, can significantly reduce the risk of *Salmonella* occurrence in the retailed meat.

3.4.3. Risk factors for *Salmonella* contamination in the retailed meat

Three risk factors were found to be associated with the risk of *Salmonella* occurrence in meat sold within retail outlets of Kigali. The risk of *Salmonella* occurrence in retailed meat was high in the establishments where meat was exposed to ambient temperature compared to the establishments where meat was exposed in refrigerated cabinets. This could be explained by the proliferation of microorganisms including *Salmonella*, initially present in retailed meat pieces, during the time meat is exposed at ambient temperature and the dissemination of these microorganisms through cross-contaminations. Previously, the rupture of the cold chain was reportedly associated with the proliferation of microorganisms including pathogens, such as *Salmonella*, on stored meat (McEntire et al., 2014).

The utilisation of wooden cutting boards, with rough surfaces, which are not easy to sanitise, was also associated with an elevated risk of *Salmonella* occurrence. This could be explained by the fact that materials, such as wood, generally present numerous pores that may trap microorganisms (Carrasco et al., 2012). These pores are not easily accessible to the sanitising agents. Consequently, trapped microorganisms may proliferate and disseminate to the processed products through cross-contaminations. Contaminated equipment, are generally recognised as major sources of cross-contaminations in meat processing units (Small & Buncic, 2009; Warriner et al., 2002). Nevertheless, the risk of *Salmonella* occurrence was found to be significantly reduced within retail establishments, whose personnel were trained in hygienic meat handling practices. These findings highlight the important role of meat handling personnel within retail establishments, in assuring the microbiological quality and safety of the processed products.

3.5. Conclusion

The findings from this study indicate that beef constitutes the main type of meat sold in retail outlets of Kigali. The hygiene indicator bacteria loads in various types of retailed meat, as well as the prevalence of *Salmonella*, were found to be relatively high; indicating the need for hygiene improvements in meat retail establishments and/or in earlier stages of the meat chain. The meat retail conditions, particularly, the exposition of meat to ambient temperature, as well as the lack of professionally trained meat handlers, were identified as the key factors hampering the quality and safety of retailed meat in Kigali, predominantly in small and medium retail establishments. Further studies, addressing the occurrence of *Salmonella* in stages of the chain are needed, to design an accurate risk assessment model for *Salmonella* in the Rwandan meat chain.

CHAPTER FOUR

Consumption pattern and bacteriological quality of meat consumed within the households of Kigali, Rwanda

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CHAPTER 4. Consumption pattern and bacteriological quality of meat consumed within the households of Kigali, Rwanda.

Abstract

Meat is consumed worldwide as a source of animal proteins, but it is recognized as one of the most important vehicles for food borne infections in humans. This study was conducted to determine the daily intake; the levels of hygiene indicator bacteria, namely the total mesophilic bacteria (TMC) and *Escherichia coli* counts (ECC); and the prevalence of *Salmonella* in meat consumed within the households of Kigali (Rwanda). The survey on meat consumption was carried out in 400 households by using a questionnaire, whereas the bacteriological analyses of meat samples were performed by using conventional culture methods. The results from the survey indicated that beef was the type of meat mostly consumed in Kigali city households, and the daily meat intake significantly varied with the social category of the household. No significant difference was observed between daily meat intakes in different age classes of household members. In the samples where microorganisms were detected, the average levels of TMCs and ECCs in raw meat were found to be 5.4 and 1.6 log cfu/g, respectively, whereas in cooked meat they were significantly reduced to 3.1 and 1.1 log cfu/g, respectively. The prevalence of *Salmonella* was reduced from 21.4% in raw meat to 3.4% in ready-to-eat cooked meat. *Salmonella* was not detected in cooked meat consumed in high-income households. The results from this study highlight the need for hygiene improvements in meat shops as well as in the households of Kigali, particularly those with low and medium incomes.

Key words: meat intake, *Salmonella*, hygiene indicator bacteria, household, Kigali.

4.1. Introduction

Meat is recognized as a nutrient-rich food. It provides valuable amounts of proteins, vitamins such as retinol and vitamin B12, and minerals such as iron, selenium, and zinc, with greater bioavailability than other dietary sources (McAfee et al., 2010). Recent statistics indicate that in developed countries, the average meat consumption is 75.5 kg per year per inhabitant, whereas in developing countries it is estimated at 33.8kg (FAO, 2014). A number of factors, including the number of animals available, religious considerations, and the socioeconomic status of the consumers, were reported to influence the meat consumption pattern (Mann, 2000), but wealth was found to be the main determinant of per capita meat consumption in both developed and developing countries (Speedy, 2003). The consumption of meat is therefore expected to increase as the consumer's financial capacity grows (Heinz & Hautzinger, 2007).

The composition and physical characteristics of meat are favorable to the growth of a wide range of spoilage and pathogenic microorganisms. Thus, meat constitutes an important vehicle for bacterial pathogens responsible for food borne infections in humans (Doulgeraki et al., 2012; Scallan et al., 2011). A study conducted in United States and European countries on the occurrence of food borne outbreaks revealed that 20.7, 11.5 and 7.2% of bacterial food borne outbreaks whose the vehicle was identified were attributable to poultry, beef and pork respectively (Greig & Ravel, 2009). In developing countries, data on food borne infections are scarce. This is principally because of the lack of operational disease surveillance networks as well as an under-reporting of food borne illness cases (Newell et al., 2010; Stevens et al., 2006). However, foodborne infections remain a serious public health concern in these countries. Diarrheal diseases are reported to be one of the main causes of morbidity and mortality in developing countries after malaria and respiratory infections. Among those diseases, salmonellosis is known as the most common food borne disease in both developed and developing countries (CDC, 2013; EFSA & ECDC, 2015).

The contamination of meat by bacterial pathogens such as *Salmonella* can occur at any stage of the meat production chain, including slaughtering, processing, and distribution (Doulgeraki et al., 2012; Nychas et al., 2008). Furthermore, even though the cooking process significantly reduces the load of microorganisms in foods, cooked meat may become re-contaminated by food handlers and the processing environment (Carrasco et al., 2012; Li et al., 2005). The occurrence of pathogenic bacteria such as *Salmonella* in raw and cooked meat has been found to be related to poor hygienic practices at different stages of the meat chain (Cardinale et al., 2005; Delhalle et al., 2009; Wong et al., 2002).

In Rwanda, although it is generally recognized that beef is the most consumed type of meat, as cattle present an important cultural value in the Rwandan society (Adekunle, 2007; Oppong, 2008), no dietary data such as daily intake are available to characterize the consumption of meat. Furthermore, to the best of our knowledge, no published study has assessed the occurrence of pathogenic bacteria such as *Salmonella* in raw or ready-to-eat bovine meat in Rwanda. One study conducted to assess the bacteriological quality of bovine meat from the slaughterhouse to the retail stage in Kigali (Niyonzima et al., 2013) revealed that the majority of samples was non-satisfactory for hygiene indicator bacteria, namely total mesophilic bacteria (TMC) and *E. coli* (ECC) counts, with regard to the European microbiological criteria for food. This would suggest the possible contamination of meat by enteric bacterial pathogens such as *Salmonella* that are generally associated with poor hygienic practices during cattle slaughtering, meat processing, or retail (Jay et al., 2005; Nychas et al., 2008; Rhoades et al., 2009).

The purpose of this study was to determine the daily meat intake in Kigali city households as well as the levels of hygiene indicator bacteria and the occurrence of *Salmonella* in the consumed bovine meat. Data gathered in this

study may be useful for an exposure assessment of Kigali inhabitants to meat borne bacterial pathogens such as *Salmonella*.

4.2. Material and methods

4.2.1. Study area and sampling procedure

The study was conducted in selected households of Kigali (Rwanda). The choice of Kigali is justified by its large population, as it is the most populated city of Rwanda with more than 10% of the national population (National Institute of Statistics of Rwanda, 2012). The total number of households in which the study was conducted was calculated by using the following formula (Yamane, 1967).

$$n = \frac{N}{1 + N\,(e)^2}$$

Where n is the sample size, N is the population size, and e is the level of precision. According to the most recent statistics, the population of Kigali is estimated to be 1,135,428 inhabitants (National Institute of Statistics of Rwanda, 2012). With a precision level e of 0.05, the sample size was estimated to 400 households.

Selected households were grouped into socio-economical categories according to the Rwandan mutual health insurance scheme (Goverment of Rwanda, 2008). Table 13 describes the main characteristics of different categories of households as defined by the Local Development Agency of the Rwandan Ministry of Local Government. Twenty-one percent (21%) of samples were comprised of households with low income, whereas households with medium and high income represented 75% and 4% of the sample size, respectively. These percentages are in accordance with the proportion of different socio-economical categories of households in Kigali. In each category, households were randomly selected from the database of households' socio-economic status (data of the year 2012) provided to us by the Local Development Agency of the Rwandan Ministry of Local Government by using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA).

The survey on meat consumption was conducted in all selected households. However, for microbiological analyses, meat samples were collected from 50 low-income households, 50 middle-income households, and all high-income households (n=17). This approach is in accordance with the European regulation (EC) N° 2073/2005 on microbiological criteria for foodstuffs stating that for *Salmonella* detection a minimum of 50 samples should be analyzed. In the case the total number of samples is less than 50, all samples should be analyzed (European Commission, 2005).In each selected household, one raw meat sample (prior to cooking) and three cooked meat samples from the same meat-based meal were collected for bacteriological analyses.

Table 13. Main characteristics of different categories of households

Category of the households	Main characteristics of the household (*)
Low-income household	Has no farmland or livestock. Does not have or has a poor shelter. The household food needs are not regularly fulfilled. The household cannot afford the payment of school fees for children or basic medical care for any member of the household.
Middle-income household	Has farmland, livestock, and/or practices a revenue-generating activity. Has a shelter and can fulfill regular food needs. Can afford the payment of school fees for children up to the secondary school level and basic medical care for any member of the household.
High-income household	Has farmland, livestock, and/or practices a revenue-generating activity. The household food needs are regularly fulfilled. The household owns one or many houses, land, and/or vehicles. It can afford the payment of school fees for children up to the university level and any medical care for all household members.

(*)Source: Rwanda Ministry of Local Government (2012)

4.2.2. Conduct of the survey

The survey was conducted to assess the consumption of meat at the household level in Kigali. A food frequency questionnaire was administrated to 400 households in three districts of Kigali (Gasabo, Kicukiro, and Nyarugenge). The questionnaire was divided into two sections. The first section provided the socio-demographic information of the household, namely the physical address and the socio-economic category of the household, as well as the number and the age of the household members consuming meat. The second section supplied information on the consumption of meat within the household, including the meat-type preferences, the frequency of consumption, and the quantity of meat consumed.

In each selected household, the consumption of meat was monitored for a period of 30 days between April and August 2014. Information such as the type of meat (lean or offal), the animal species purchased, and the mode of preparation was regularly recorded. The average meat intake for one household member was calculated by dividing the total quantity of meat consumed within the household during the period of the study by the total number of household members consuming meat.

Furthermore, in order to assess the possible variation of the meat consumption rate with the age of the household members, one family member was randomly selected in each household and his/her daily meat intake was regularly recorded during the period of the study. The quantity of meat consumed by the selected household member was recorded as none, half, or the number of meat portions, while taking as reference the average size of the meat portion generally consumed in the household. The weight of the meat portion taken as reference in the household was obtained by dividing 1,000 grams by the estimated number of meat portions gained while cutting 1kilogram of meat at the household level. The estimated daily meat intake *(EDI)*, expressed in grams of meat per day, for the selected household member was therefore calculated as follows:

$$EDI = \frac{N}{n \times d} \times 10^3$$

Where *N* is the total number of meat portions consumed by the selected household member during the period of the study, *n* is the estimated average number of meat portions gained while cutting 1kilogram of meat at the household level, and *d* is the duration of the study in days. The determination of food intake by visual estimation of food portion size has been reported to be an alternative to conventional food weighting methods, which, in some circumstances, are not useful because of cultural considerations (Dhingra et al., 2007).

4.2.3. Microbiological analyses

4.2.3.1. Sample collection

Raw bovine meat samples were collected from registered meat shops of Kigali. After being cut up by the salesman, 1 kilogram of raw meat was placed in a sterile stomacher bag (VWR, Belgium). A portion of approximately 50 grams was aseptically taken from the main sample and placed in a separate sterile stomacher bag for bacteriological analyses, whereas the remaining part was given to the household for preparation. The transport of raw meat and bacteriological samples was carried out in separate cool boxes held at 4°C. The cooked samples were aseptically collected from households (approximatively 30 minutes after cooking) and transported to the laboratory in a cool box. In the laboratory, raw and cooked meat samples were stored in a freezer at -30°C until analysis. The analyses were carried out within the following 24 hours.

4.2.3.2. Analytical methods

Raw and cooked meat samples were analyzed for the TMC, ECC, and *Salmonella* detection. The sample preparation was carried out following the ISO 7218:2007 standard protocol (ISO, 2007). The poor plate method on plate count agar (VWR, Belgium) was used for the determination of TMC according to the ISO 4833:2003 standard protocol (ISO, 2003), whereas the ECC was performed on tryptone bile X-glucuronide agar (Bio-Rad,

France) as described in ISO 16649-2:2001 standard protocol (ISO, 2001). When a sample was found to be below the bacterial enumeration threshold for TMC or ECC, subsequent calculations were performed with the value of the detection limit. According to the ISO 7218:2007 standard, the limit of detection for TMC and ECC in solid samples by using respectively the ISO 4833:2003 and ISO 16649-2:2001 standard protocols is fixed to 1 log cfu/g (ISO, 2007).

*Salmonell*a was detected in 25 grams of meat samples by following the ISO 6579:2002 standard protocol (ISO, 2002) with a non-selective enrichment phase in buffered peptone water (VWR, Belgium), selective enrichment in Rappaport Vassiliadis with soja (Sigma-Aldrich, Belgium), and Muller–Kaufman tetrathionatenovobiocin (Sigma-Aldrich, Belgium) broths, followed by an isolation on xylose-lysine-desoxycholate agar (Sigma-Aldrich, Belgium) and brilliant green agar (Bio-Rad, France). API20E gallery (bioMérieux, France) was used for the biochemical confirmation of characteristic or suspected colonies of *Salmonella*.

4.2.4. Statistical analyses

Statistical analyses were performed using SPSS 16.0 Software (IBM, USA). One-way analysis of variance was used to assess the variability for normally distributed variables, whereas the Kruskal–Wallis (KW) and Mann–Whitney U (MWU) tests were used for non-normally distributed variables. The normality of the distributions was assessed by using the Shapiro–Wilk test (Shapiro & Wilk, 1965). The Pearson Chi-Square (PCS) test was used to compare the variable proportions in different socio-economic categories of households whereas the Spearman rank correlation (r_s) was used to determine the correlation between the meat consumption rate and the bacteriological quality of the consumed meat within the same household. The results from bacterial counts, i.e.TMC and ECC, were transformed into a logcfu scale base 10 before subsequent calculations and statistical treatments.

4.3. Results

4.3.1. Meat consumption in the households of Kigali

Meat was reported to be consumed in 89% of Kigali city households. In the households where meat was not consumed, religious considerations were found to be the predominant reason (88.4%), followed by health issues (9.3%) and ethical considerations (2.3%). The average frequency of meat consumption in all categories of households was found to be once a week. However, this was found to significantly vary in different socio-economic categories of households (low-income households [LI] versus middle-income households [MI]: MWU test=5220.0, degree of freedom [df] =1, p=0.000; MI versus high-income households [HI]: MWU test=236.0, df=1, p=0.000; HI versus LI: MWU test=17.0, df=1, p=0.000). Beef appeared to be the first type of meat consumed in Kigali, as it was reported to be mostly consumed in 98% of 357 households (Table 14).

The consumption of meat from animal species other than bovines appeared to be significantly low (PCS test=329.5, df=1, p=0.000), but no significant difference was observed between the consumption of meat from these animal species at the household level (chicken or small ruminants versus pork: PCS test=1.0, df=1, p=0.317).

Households were found to be composed of five members on average, and no significant difference was observed in the composition of households from different socio-economic categories (KW test=3.5; df=2; p=0.170). The average daily meat intake was estimated at 34.4 grams per person and per day and was found to significantly vary with the socio-economic category of the households (LI versus MI: MWU test=2551.5, df=1, p=0.000; MI versus HI: MWU test=282.0, df=1, p=0.000; HI versus LI: MWU test=2.0, df=1, p=0.000). In all categories of households, the meat intake was composed of both offal and lean meat, but the proportion of offal appeared to be significantly low (MWU test=7358.0, df=1, p=0.000) compared to lean meat (Table 15). The meat intake was not found to vary significantly (KW test=3.9, df=6, p=0.685) between different age classes of household members (Figure 2). The results obtained from the survey on meat consumption reveal that meat is regularly consumed by all social categories of the population in Kigali and beef constitutes the most preferred type of meat.

4.3.2. Levels of hygiene indicator bacteria in raw and cooked meat

Two types of hygiene indicator bacteria — namely the total mesophilic bacteria, known to be an indicator of general hygiene (ISO, 2003), and *E. coli*, reported to be indicator of fecal contamination (ISO, 2001) in food products — were analyzed in both raw meat collected from registered meat shops and cooked meat collected from the households of Kigali. The average numbers of total mesophilic bacteria and *E. coli* in raw and cooked meat samples are given in Table 16.

The average contamination of raw meat by total mesophilic bacteria was found to be 5.4 log cfu/g. The contamination level of meat purchased by households with low and high income was not found to be significantly different (MWU test=315.0, df=1, p=0.113); however, the average contamination level observed in meat bought by middle-income households appeared to be significantly lower than the two other groups (MI versus LI: MWU test=945.5, df=1, p=0.037; MI versus HI: MWU test=226.0, df=1, p=0.004). The average contamination level by *E. coli* was found to be 1.6 log cfu/g in raw meat; however, 63% of 117 analyzed samples appeared to have contamination levels below the detection limit.

Table 14. Frequency of meat consumption and meat type preference in the households. Meat consumption frequency: values are means (± standard deviation) of meat consumption frequencies (per week) within the household. Different superscript letters indicate a significant difference ($p \leq 0.05$). Meat type preferences: values are the percentages (number/total number) of households mostly consuming meat from a given animal species. In the same row, different superscript letters indicate a significant difference ($p \leq 0.05$).

	Meat consumption frequency	Meat type preferences				
		Bovine	Small ruminants	Pork	Chicken	Rabbit
Low-income households	0.54 ± 0.55^{a}	97.1^{a} (68/70)	2.9^{b} (2/70)	0.0^{c} (0/70)	0.0^{c} (0/70)	0.0^{c} (0/70)
Middle-income households	1.06 ± 1.01^{b}	98.1^{a} (265/270)	0.4^{b} (1/270)	0.4^{b} (1/270)	1.1^{a} (3/270)	0.0^{c} (0/270)
High-income households	3.59 ± 1.33^{c}	100.0^{a} (17/17)	0.0^{b} (0/17)	0.0^{b} (0/17)	0.0^{b} (0/17)	0.0^{b} (0/17)
Total	1.08 ± 1.13	98.1^{a} (350/357)	0.8^{b} (3/357)	0.3^{b} (1/357)	0.8^{b} (3/357)	0.0 (0/357)

Table 15. Daily meat intake in the household. Values are the means (± standard deviation) of daily meat intake (g/pers.day) for different categories of households. In the same column, different superscript letters indicate a significant difference ($p \leq 0.05$).

	n	Number of household members	Daily intake		
			Offals	Lean meat	Total
Low-income households	51	4.6 ± 1.9^{a}	1.5 ± 3.7^{a}	12.7 ± 10.2^{a}	14.2 ± 11.1^{a}
Middle-income households	248	4.5 ± 1.8^{a}	3.6 ± 8.6^{a}	30.7 ± 30.3^{b}	34.3 ± 32.2^{b}
High-income households	17	5.4 ± 1.9^{a}	9.7 ± 9.7^{b}	86.4 ± 36.9^{c}	96.0 ± 38.0^{c}
Total	316	4.5±1.9	3.6±8.2	30.8±32.0	34.4±34.3

The average ECCs were significantly higher in meat purchased by households with high income (HI versus LI: MWU test=238.0, df=1, p=0.002; HI versus MI: MWU test=272.0, df=1, p=0.015), whereas ECCs in meat bought by households with low and medium income were not significantly different (MWU test=1133.5, df=1, p=0.332).

In cooked meat, total mesophilic bacteria were detectable in 85.5% of 351 analysed samples with an average contamination level of 3.1 log cfu/g. In different categories of households, the average TMCs were found to be significantly different (LI versus MI: MWU test=963.0, df=1, p=0.048; MI versus HI: MWU test=131.5, df=1, p=0.000; HI versus LI: MWU test=48.5, df=1, p=0.000).*E. coli* was detectable in only 7.7% of 351 samples with an average contamination level of 1.1 log cfu/g. All samples collected from households with high income were found to have ECCs below the detection limit, whereas meat samples collected from households with low and medium income appeared to be contaminated (at a detectable level) by *E. coli* at the proportions of 16% and 2%, respectively. The average ECCs in those two categories of households appeared to be significantly different (MWU test=1079.0, df=1, p=0.018).

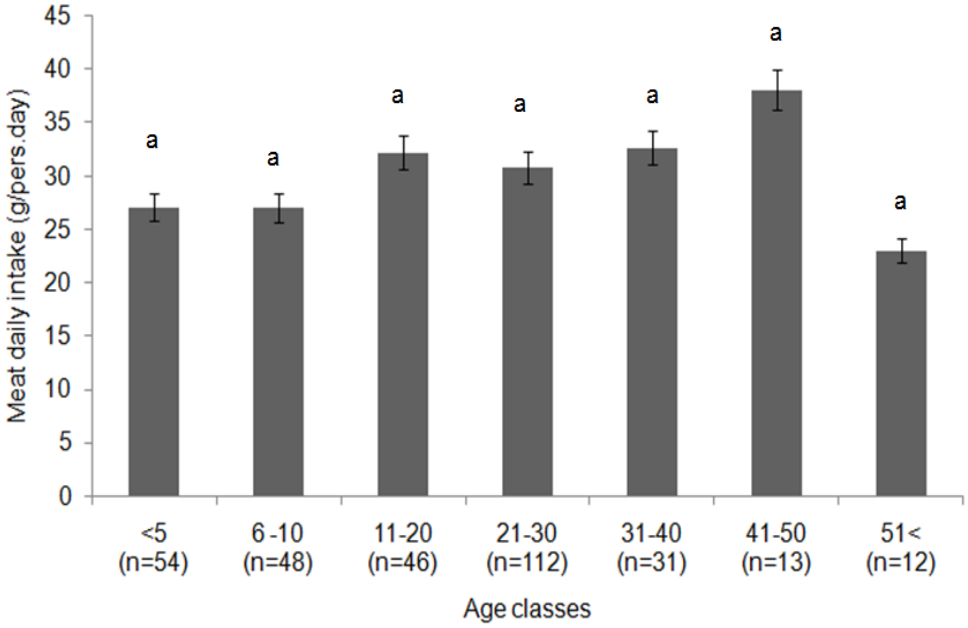

Figure 2. Meat daily intake by age class. Values are mean of meat daily intakes (gram per person and per day) in different classes of age (years). Error bars represent standard error. Age classes with the same superscript letters are not significantly different ($p \leq 0.05$).

By comparing the contamination levels in raw and cooked meat samples, it was noted that, for all categories of households, the load of total mesophilic bacteria was significantly reduced in cooked meat (LI: MWU test=208.0, df=1, p=0.000; MI: MWU test=138.0, df=1, p=0.000; HI: MWU test=0.0, df=1, p=0.000). The average reduction for TMC was 2.3 logarithmic units in all categories of households, with a significantly higher reduction (4.2 log units) in the households with high income (Anova, F=26.0; p[HI versus LI]=0.000; p[HI versus MI]=0.000). TMC reductions observed in other categories of households were not found to be significantly different (p=0.597). The proportion of samples with TMC levels below the detection limit appeared to be increased by 2, 12, and 59% after the cooking process in households with low, medium, and high income, respectively.

The reduction of ECC was found to be significant in meat samples collected from households with medium (MWU test=832.0, df=1, p=0.000) and high (MWU test=6.0, df=1, p=0.035) income. In these two categories, the average reduction of ECCs was found to be 0.4 and 1.4 log units, respectively (reduction by factors 2.5 and 25.1, respectively). However, the ECC reduction (0.2 log units) observed in meat samples collected from the households with low income did not appear to be significant (MWU test=1116.5, df=1, p=0.204). On average, the reduction of ECC in cooked meat samples collected from all categories of households was found to be 0.5 logarithmic units. After the cooking process, the proportion of samples with ECC levels below the detection limit increased from 72 to 84%, 64 to 98%, and 35 to 100% in households with low, medium, and high income, respectively.

Table 16. Numbers of hygiene indicator bacteria in raw and cooked meat. Values are the means (± standard deviation) of bacterial counts (logcfu/g). In the same column, values with the same superscript letters (a, b, c) are not significantly different ($p \leq 0.05$). Different superscript letters (x, y) in the same category of household and for the same bacterial type indicate a significant difference ($p \leq 0.05$).

	n	Raw meat		Cooked meat (*)	
		TMCs	ECCs	TMCs	ECCs
Low-income households	50	$5.5 \pm 0.8^{a(x)}$	$1.4 \pm 0.8^{a(x)}$	$3.6 \pm 1.1^{a(y)}$	$1.2 \pm 0.6^{a(x)}$
Middle-income households	50	$5.2 \pm 0.7^{b(x)}$	$1.5 \pm 0.8^{a(x)}$	$3.1 \pm 1.2^{b(y)}$	$1.1 \pm 0.4^{b(y)}$
High-income households	17	$5.8 \pm 0.7^{a(x)}$	$2.4 \pm 1.5^{b(x)}$	$1.6 \pm 0.8^{c(y)}$	$1.0 \pm 0.0^{c(y)}$
Total	117	$5.4 \pm 0.7^{(x)}$	$1.6 \pm 1.0^{(x)}$	$3.1 \pm 1.3^{(y)}$	$1.1 \pm 0.5^{(y)}$

(*) In a household, bacterial counts are average TMCs and ECCs for three cooked meat samples.

4.3.3. Presence of *Salmonella* in raw and cooked meat

Salmonella was detected in 21.4% of 117 raw meat samples; however in cooked meat, its prevalence was reduced significantly to 3.4% (PCS test=17.358, df=1, p=0.000). In cooked meat samples, the highest prevalence (6%) was observed in meat collected from households with low income. Nevertheless, *Salmonella* was not found in samples collected from households with high income (Table 17). The Spearman correlation revealed that the meat consumption rate within the household was significantly correlated to the bacteriological quality of the consumed meat, in occurrence the total mesophilic bacterial counts (n=117, r_s= – 0.370, p<0.01).

Table 17. Presence of *Salmonella* in raw and cooked meat. Values are percentages of positive samples (number of positive samples/total samples) for *Salmonella*. In the same column, values with the same superscript letters (a, b, c) are not significantly different ($p \leq 0.05$). Different superscript letters (x, y) in the same category of household indicate a significant difference ($p \leq 0.05$).

	Raw meat	Cooked meat(*)
Low-income households	24,0$^{a(x)}$ (12/50)	6,0 $^{a(y)}$ (3/50)
Middle-income households	14,0 $^{a(x)}$ (7/50)	2,0 $^{a(y)}$ (1/50)
High-income households	35,3 $^{a(x)}$ (6/17)	0,0 $^{a(y)}$ (0/17
Total	21,4 $^{(x)}$ (25/117)	3,4 $^{(y)}$ (4/117)

(*) A meat-based meal consumed within the household was considered as contaminated by *Salmonella* when at least one of 3 samples was *Salmonella* positive.

4.4. Discussion

4.4.1. Meat consumption in the households of Kigali city

The findings from this study indicate that meat is consumed by the majority of the population in Kigali city, and beef constitutes the type of meat mainly consumed within the households. The preference for beef could be attributable to cultural considerations of the population, as bovines are of great socio-cultural importance in Rwandan society (Adekunle, 2007; Oppong, 2008). Another explanation would be the unaffordability of meat from other animal species for a number of households in Kigali. The high cost of meat from animal species other than bovines results from the lack of infrastructures to slaughter these animal species. Currently, Kigali city holds two slaughterhouses for bovines and small ruminants; however, no public slaughtering facilities are available for other animal species (Rwandan Ministry of Agriculture and Animal Resources, 2012). Pigs and poultry are commonly slaughtered in private small-scale plants, resulting in a significant increase in the production cost of meat from other animal species compared to bovine meat.

Available statistics (Data for the year 2013) indicate that bovine meat counts for 48.7% of Rwandan meat production, whereas meat from other animal species such as pigs, goats, sheep, chickens, and rabbits represents respectively 14.9, 12.8, 2.9, and 2.9% of the global meat production (FAO, 2015).

The overall daily meat intake of 34.4 grams per person and per day observed in Kigali city households appears to be slightly higher in comparison with the average meat intake of 30.0 grams per person and per day recorded within different age classes of household members (Figure 2). This is mainly attributable to the differences in the methods used to estimate the meat intake. In the former case, the meat intake was determined based on the total quantity of raw meat consumed within the household, whereas in the latter case only the quantity of cooked meat effectively consumed by the focus person in the household was considered. The raw meat purchased for preparation in the household often contains some non-edible parts such as bones or connective tissues that are not effectively consumed, as they are generally removed before the cooking process (Heinz & Hautzinger, 2007). Although the proportion of non-edible parts in the purchased raw meat is generally very small, its consideration in the estimation of the daily meat intake may have led to a slight over-estimation of the consumed meat.

The level of meat intake observed in the present study appears to be low in comparison with the average meat intakes of 209.7 and 93.9 grams per person and per day, respectively, reported in developed and third world countries (FAO, 2014). This could be explained by the fact that in the present study only meat consumed within the households was taken into account, whereas meat-based meals are also consumed outside the household's circle, for example in restaurants or snack bars. Thus, the consideration of meat quantities consumed outside the households may have resulted in higher levels of meat intake. Another explanation for the low meat intake would be the low financial capacity of some households to afford meat. Available statistics (Data for the year 2014) indicate that 39.1% of the Rwandan population lives below the poverty line of 159,375 Rwandan francs (almost 218 US Dollars) per adult equivalent per year (National Institute of Statistics of Rwanda, 2015). Nevertheless, the Rwandan meat consumption rate appears to have considerably increased in recent decades. In the 2000s the national average meat intake (all animal species included) was estimated at 13 grams per person and per day (Speedy, 2003).

4.4.2. Microbiological quality of the consumed meat

The bacteriological analyses revealed that the levels of hygiene indicator bacteria and the prevalence of *Salmonella* in raw meat purchased by different categories of households were variable. The observed variability is mainly attributable to the level of hygiene and meat handling practices that may differ from one butcher's retail shop to another. The ultimate bacteriological quality of meat at the retail stage was reported to be associated with the hygiene of meat handlers and the processing environment (Niyonzima et al., 2015; Soyiri et al., 2008).

Furthermore, other factors such as the quality of raw material used and the storage conditions for both raw material and processed meat products may have contributed to the variation of ultimate levels of TMC and ECC as well as the occurrence of *Salmonella* in raw meat samples, as they are likely to differ from one meat shop to another(Lo Fo Wong et al., 2002; Nychas et al., 2008).

Contrary to other categories of households, the majority of meat consumed in high-income households was composed of packed meat cuts and was purchased in the same meat retail shop. This could explain the high TMC and ECC levels as well as the greater *Salmonella* prevalence observed in meat bought by that category of household. Nevertheless, our results on the microbiological quality and safety of meat consumed within the households with high income should be treated with caution as a very limited number of samples (n=17) was analyzed.

The high levels of total aerobic bacteria and ECC in fresh meat samples indicate deficient hygienic practices in meat retail shops and/or in earlier stages of the bovine meat chain. Alack of hygiene in the meat retail process has been reported in other African countries, such as Botswana, Egypt, Ghana, Nigeria, and Senegal (Gashe & Mpuchane, 2000; Hassanein et al., 2011; Stevens et al., 2006; Tafida et al., 2013).In Ghana, for example, average contamination levels of 4.3 and 3.3log cfu/g for TMC and ECC, respectively, in fresh bovine meat reported in the market of Accra were found to be associated with unhygienic practices and poor handling of beef by butchers(Soyiri et al., 2008). In our study, the observed *Salmonella* prevalence of 21.4% appears to be high in comparison with the prevalences of 2.4 and 9.9% reported in Nigeria (Tafida et al., 2013) and Botswana (Gashe & Mpuchane, 2000), respectively, comparable to the prevalence of 20% reported in Egypt (Hassanein et al., 2011) and low in relation to the prevalence of 87% reported by Stevens et al.(2006) in Senegal. Extremely low prevalences of *Salmonella* in beef are generally observed in developed countries where good hygienic practices prevail along the meat chain. In a baseline study conducted in the United States, Vipham et al. (2012) reported a *Salmonella* prevalence of 1.02% for whole muscle beef cuts sampled at the retail level. Comparable findings have also been reported in European countries. The recent report on zoonotic agents and foodborne outbreaks in European Union countries indicated an average *Salmonella* prevalence of 0.2% in bovine meat at retail (EFSA and ECDC, 2014). According to the European regulations, *Salmonella* must be absent in 25 grams of all foodstuffs intended to be used for human consumption(European Commission, 2005).

The cooking process significantly reduced the levels of hygiene indicator bacteria and the prevalence of *Salmonella*. However, residual average loads of 3.1 and 1.1logcfu/g for TMC and ECC, respectively, as well as a *Salmonella* prevalence of 3.4% were observed in cooked meat samples where these microorganisms were detected. Two reasons could explain the occurrence of bacterial contaminants in cooked meat, namely the inefficiency of the cooking process and the post-cooking contamination of meat. Enteric bacterial pathogens such

as *Salmonella* are generally destroyed at conventional pasteurization temperatures (Jay et al., 2005; Korsak et al., 2004). However, as cooking times and temperatures may vary from one household to another, the possibility of inadequate cooking treatments for meat should not be excluded. Several studies have reported the survival of *Salmonella* in undercooked meat and meat products (Juneja et al., 2001; Roccato et al., 2015; Silva & Gibbs, 2012). Furthermore, cooked meat may get contaminated by microorganisms from handlers or the processing environment. Factors such as kitchen utensils, work surfaces, and personal hygiene were reportedly associated with the presence of *Salmonella* in ready-to-eat meat (Cardinale et al., 2005; Carrasco et al., 2012). In Rwanda, meat is most often prepared though boiling and the cooking duration is relatively long (about 2 to 3 hours). Therefore the occurrence of *Salmonella* in cooked meat could be mainly due to post cooking contamination of meat-based dishes.

A significant negative correlation was noted between the daily meat intake within the household and the levels of total mesophilic bacteria in the consumed meat. This indicates that higher contaminations in cooked meat samples were observed in households where meat was less consumed, in occurrence the households with low income. The high levels of TMC and ECC as well as the greater *Salmonella* prevalence particularly observed in meat consumed in poor households could be explained by the limited financial capacity which does not allow people from these households to afford the required materials for an efficient personal and kitchen hygiene.

4.5. Conclusions

The findings from this study indicate that meat is consumed by the majority of the population in Kigali city, and beef is the favourite type of meat consumed within households. The levels of hygiene indicator bacteria as well as the prevalence of *Salmonella* were found to be relatively high in raw meat sold at different meat retail shops in Kigali. This indicates that general and personal hygiene during meat preparation in retail outlets and/or in earlier stages of the meat chain need to be improved. The significant reduction of hygiene indicator bacteria and the prevalence of *Salmonella* in cooked meat reflect the importance of the cooking step in the control of pathogenic bacteria that could be transmitted to humans through the consumption of contaminated meat. However, the observed residual contamination of cooked meat samples highlights the need for proper cooking and/or hygiene improvements, especially in poor households. Further studies are needed to determine the meat intake and the bacteriological quality of meat consumed outside the household to accurately assess the exposure of the population to *Salmonella* through the consumption of meat.

CHAPTER FIVE

Meat consumption pattern and bacteriological quality of meat consumed outside the home in Kigali, Rwanda

Drafted from:

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CHAPTER 5. Meat consumption pattern and bacteriological quality of meat consumed outside the home in Kigali, Rwanda.

Abstract

Meat-based meals are consumed as a source of animal proteins and constitute one of the leading vehicles for food borne infections in humans. The main objective of this study was to determine the consumption pattern and the bacteriological quality of meat-based meals consumed outside households in Kigali. A survey on meat consumption patterns was carried out in 400 households by using a questionnaire, whereas different meat-based meals were sampled from 150 snack-bars and restaurants. Enumeration of hygiene indicator bacteria (total mesophilic bacteria and *Escherichia coli*) and the qualitative detection of *Salmonella* were carried out by using conventional culture methods. The results indicated that goat was the type of meat mostly consumed outside the household's circle in Kigali and the meat intake varied significantly ($p \leq 0.05$) with the social category of the household. The average levels of total aerobic bacteria and *E. coli* in meat-based meals were found to be 4.7 and 1.4 log cfu/g respectively, whereas *Salmonella* was detected in 11.7% of all meat-based meals. Eight factors mostly linked to the cooking treatments and hygienic handling practices for cooked meals were found to be significantly ($p \leq 0.05$) associated to the risk of *Salmonella* occurrence in meat-based meals consumed outside the household's circle in Kigali. The findings from this study strongly suggest the need for proper cooking and/or hygiene improvements in the establishments selling ready to eat meat-based meals in Kigali, particularly those located in rural localities.

Key words: meat consumption, *Salmonella*, hygiene indicator bacteria, ready to eat food, risk factors, Kigali.

5.1. Introduction

Meat is an important source of valuable proteins for different populations in the world. However, it constitutes an important vehicle for microbial pathogens responsible for food borne infections in humans as its the composition and physical characteristics are favorable to the growth of a wide range of microorganisms including pathogens (Doulgeraki et al., 2012; Scallan et al., 2011). Furthermore, animals often carry germs on the hide or in their digestive tract and cross contaminations during the slaughtering operations are hardly avoidable (Niyonzima et al., 2015).

Salmonellosis is known as one of the leading food borne infections in human (CDC, 2013; EFSA & ECDC, 2015). Though it is recognized that meat is one of the vehicles of *Salmonella* infection in humans, the proportion of human salmonellosis attributable to the consumption of contaminated meat is difficult to estimate accurately. This is mainly due to the fact that only a limited number of illness cases is officially reported. Furthermore, even within the reported cases a very small proportion allows the identification of the food vehicle (Greig & Ravel, 2009; Scallan et al., 2011). The lack of operational disease surveillance systems,

particularly in developing countries, has been identified as the main factor hampering the reporting and determination of causative agents for food borne diseases (Newell et al., 2010; Stevens et al., 2006).

Meat-based meals have been traditionally consumed within the households. However, the consumption of foods including meat-based meals outside the household's circle as ready to eat food; has significantly increased during the last decades in both developed and developing countries. In United States for example, the share of the household budget allocated to food consumption outside the household has increased from 20 to 37% in the period between 1960 and 1990 (Lachat et al., 2012). The rising consumption of foods outside the households is attributable to a number of factors including the affordable prices of ready to eat foods as well as their readily availability to the consumers (Cardinale et al., 2015). Moreover, the urbanization of rural areas and the increase of the financial capacity of city dwellers contribute significantly to the growing consumption of foods outside the household as the wealth was reported to be an important determinant of food consumption away from home (Ma et al., 2006). Ready to eat foods are generally consumed without any other treatment such as cooking intended to eliminate or reduce their microbial load. Therefore, the occurrence of microbial pathogens such as *Salmonella* in those food products constitutes a great public health problem. Several authors have reportedly associated food borne diseases outbreaks to the consumption of contaminated ready to eat foods (Campos et al., 2015; Gurler et al., 2015; Osaili et al., 2014; Yang et al., 2016).

The consumption of meat-based meals within the Rwandan households has been thoughtfully reviewed by Niyonzima et al. (2016) . However, to our knowledge, no published study has yet assessed neither the consumption patterns nor the bacteriological quality and safety of meat-based meals consumed outside the household's circle; though a wide variety of meat-based meals are commonly consumed in the majority of Rwandan snack bars and restaurants.

The objective of the present study was to assess the modes of consumption, and the bacteriological quality and safety of meat-based meals consumed outside the households of Kigali. Data collected through this study can be of significant application in conducting an exposure assessment of Kigali city inhabitants to meat borne bacterial pathogens such as *Salmonella*.

5.2. Material and methods

5.2.1. Meat consumption survey

The present study was conducted in the households of Kigali as it constitutes the most populated city of Rwanda with more that 10% of the national population (National Institute of Statistics of Rwanda, 2012). The number of sampled households was determined by using the formula proposed by Yamane (1967):

$$n = \frac{N}{1 + N\,(e)^2}$$

Where n is the sample size, N is the population size, and e is the level of precision. According to the most recent statistics, the population of Kigali is estimated to be 1,135,428 inhabitants (National Institute of Statistics of Rwanda, 2012). With a precision level e of 0.05, the sample size was estimated to 400 households.

Selected households were grouped into three (3) socio-economical categories namely households with low, medium and high income according to the Rwandan mutual health insurance scheme (Goverment of Rwanda, 2008). The main characteristics of these categories were described in Table 13 (see page 68).

From the database of household's socio-economical status (data for the year 2012) provided to us by the Local Development Agency of the Rwandan Ministry of Local Government, 21% of the sampled households were selected from low income households whereas 75 and 4% were chosen from households with medium and high income respectively. These proportions are in accordance with the percentages of different social categories of households in Kigali. The random selection of households for the survey was carried out by using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA).

In each household, one member aged from 18 years was randomly selected and his/her meat consumption outside the household was monitored through a food frequency questionnaire for a period of 60 days from February to June 2015. The questionnaire was composed of two parts. The first section provided the socio-demographic information including the physical address of the household, the household's social category, as well as the age and gender of the selected household member. The second section supplied meat consumption information namely the type of meat-based dish consumed by the selected household member and the consumption frequency.

The weight of meat components of different meat-based meals consumed by the selected household member was estimated by using two dimensional pictures of cooked meat portions. The use of two dimensional pictures to estimate food intakes has been validated in several studies as an alternative to conventional weighting methods, which, in some circumstances, are not suitable because of cultural considerations (Dhingra et al., 2007; Williamson et al., 2004).

5.2.2. Microbiological analyses

5.2.2.1. Collection of meat samples

Meat samples were collected from the establishments selling meat-based meals in the urban and peri-urban areas of Kigali. From the database of registered establishments selling meat-based meals in Kigali provided to

us by local municipalities, 300 snack bars and restaurants were selected randomly by using Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA). The selected establishments were visited one by one and the objectives of the study were explained to their managers. From those who have agreed to participate in the study (n=203), a second random selection was carried out to only retain 150 snack-bars and restaurants in which the survey and sample collection were performed.

Each snack-bar or restaurant was visited once. Informations regarding the type of meat used, meat handling and storage conditions, management of non-meat ingredients, cooking procedures, management of rest-over, utensil and kitchen hygiene, staff hygiene, cleaning and disinfection procedures and pest control measures were collected through a questionnaire administrated to the manager of the establishment. After the interview, the answers were verified by a direct observation on the site and where necessary the corrections were performed.

In each snack bar or restaurant, two meat-based meals were aseptically collected and placed in separate sterile stomacher bags (VWR, Belgium). The transportation of meat samples to the laboratory was carried out within 2 hours in a cold box with freeze packs. In the laboratory, meat samples were stored in a freezer at -30°C and microbiological analyses were carried out within 24 hours. Table 18 describes the numbers and the types of meat samples collected.

Table 18. Types and numbers of collected meat samples

Type of meat	Cooking process	Number of establishments	Number of samples collected
Bovine meat	Boiling [(a)]	75	150
Goat meat	Grilling	42	84
Pork	Frying	15	30
Chicken	Grilling	10	20
Rabbit	Grilling	8	16

[(a)] In most of the establishments, meat was first fried and then boiled with different ingredients.

5.2.2.2. Analytical methods

Meat samples were analyzed for the total mesophilic bacteria count (TMC); *E. coli* count (ECC) and *Salmonella* detection. The ISO 7218:2007 Standard protocol (ISO, 2007) was followed to prepare meat samples. The determination of total mesophilic bacteria count was performed by using the poor plate method on plate count agar (VWR, Belgium) as described in ISO 4833:2003 Standard protocol (ISO, 2003), whereas the *E. coli* count was carried out on tryptone bile X-glucuronide agar (Bio-Rad, France) by following the ISO 16649-2:2001 standard protocol (ISO, 2001). When a sample was found with bacterial numbers below the enumeration threshold for TMC or ECC subsequent calculations were performed with the value of the

detection limit. The ISO 7218:2007 standard indicates that in solid samples, the limit of detection for TMC and ECC by using respectively the ISO 4833:2003 and ISO 16649-2:2001 standard protocols is 1 log cfu/g (ISO, 2007).

The qualitative detection of *Salmonella* was performed in 25 grams of meat by following the ISO 6579:2002 standard protocol (ISO, 2002) with a non selective enrichment in buffered peptone water (VWR, Belgium), a selective enrichment in Rappaport Vassiliadis with soya (Sigma-Aldrich, Belgium) and Muller-Kaufman tetrathionate novobiocin (Sigma-Aldrich, Belgium) broths and the isolation on xylose-lysine desoxycholate agar (Sigma-Aldrich, Belgium) and brilliant green agar (Bio-Rad, France). The biochemical confirmation of characteristic or suspected colonies of *Salmonella* was performed by using API20E gallery (bioMérieux, France).

5.2.3. Statistical analyses

The results from bacterial counts were transformed into log cfu scale base 10 before subsequent calculations and statistical treatments. One way analysis of variance was used to assess the variability for normally distributed variables, whereas Kruskal-Wallis (KW) and Mann-Whitney U (MWU) tests were used for non-normally distributed variables. The normality of the distributions was assessed by using the Shapiro Wilk's Test, whereas the Pearson Chi-Square (PCS) test was used to compare the variable proportions.

On the same sample, a correlation between the total bacteria and *E. coli* counts (continuous variables) was determined by using the Spearman's rank correlation (r_s) whereas the point-biserial correlation (r_{pb}) was used to determine a correlation between ECC and *Salmonella* occurrence (binary variable).

The determination of risk factors for *Salmonella* occurrence in cooked meat was carried out by using a univariate logistic regression analysis. The outcome variable was the occurrence of *Salmonella* positive meals in the snack bar or restaurant. Meat-based meals in a snack-bar or restaurant were declared contaminated by *Salmonella* if at least one sample was *Salmonella* positive. Potential risk factors were first screened to assess their association to the outcome variable by using the Pearson Chi square test, and only factors significantly ($p \leq 0.05$) associated to the outcome variable were considered eligible for the logistic regression analysis. All statistical analyses were carried out by using SPSS 16.0 Software (IBM, USA).

5.3. Results

5.3.1. Meat consumption outside the household

The results from the survey showed that meat was consumed in 89% of households and goat was found to be the most consumed meat outside the household circle in Kigali. The average meat intake outside the household was estimated to 49.6 grams per person and per day (Table 19) and appeared to significantly

differ with the household social category(low-income households (LI) versus middle-income households (MI): MWU test=369.0, degree of freedom (df) =1, p=0.000; middle-income households (MI) versus high-income households (HI): MWU test=0.0, df=1, p=0.000; high-income households (HI) versus low-income households (LI): MWU test=699.0, df=1, p=0.000). The consumption of meat was significantly high in men comparatively to women (MWU test=9998.0, df=1, p=0.000) and appeared to be increasing with the age (Figure 3). Nevertheless, the localization of the household (urban versus peri-urban zone) was not found to be significantly influencing the meat consumption rate in Kigali (MWU test=8905.0, df=1, p=0.305).

5.3.2. Preparation of meat-based meals

Table 20 describes the level of utilization of meat from different animal species within the establishments selling meat-based meals in Kigali. Bovine and goat meat were found to be the mostly used type of meat as they were respectively reported to be used in 58.7 and 50.0% of the establishments selling meat-based meals in Kigali. Bovine meat appeared to be mostly used in restaurants whereas goat meat was predominantly used in snack-bars. Boiling was found to be the cooking method used in the majority of Kigali city restaurants but in snack-bars, meat-based meals were mostly prepared by grilling (Table 21). The cooking durations were predominantly long in restaurants comparatively to snack-bars (Table 22).

5.3.3. Bacteriological quality and safety of meat-based meals

Meat-based meals including boiled beef, grilled goat brochettes, fried pork, grilled chicken and grilled rabbit were analyzed for the detection of *Salmonella* and the assessment of contamination levels by hygiene indicator bacteria namely total mesophilic bacteria and *E. coli*. Table 23 describes the levels of hygiene indicator bacteria and the occurrence of *Salmonella* in different meat-based meals consumed in Kigali. The mean microbial counts ranged from 3.25 and 6.61 log cfu/g for total mesophilic bacteria and from 1.32 to 1.70 log cfu/g for *E. coli*. *Salmonella* was isolated from 18.7% of snack-bars and restaurants (n=150) and from 11.7% of all meat samples (n=300). A significant Spearman's rank correlation was observed between TMC and ECC (r_S=0.637, n=300, p<0.01) for the same sample and the point-biserial correlation between *E. coli* counts and the occurrence of *Salmonella* appeared to be significant (r*pb*=0.445, n=300, p<0.01). The average TMC level of 6.18 log cfu/g observed in snack-bars appeared to be significantly higher when compared to the average TMC (3.25 log cfu/g) recorded in restaurants (MWU test=1505.0, df=1, p=0.000). However, the levels of ECC in snack-bars and restaurants (1.5 versus 1.3 log cfu/g respectively) were not found to be statistically different (MWU test=2417.5, df=1, p=0.127). The proportion of *Salmonella* positive samples appeared to be significantly (PCS test=11.7, df=1, p=0.001) higher in meat-based meals served in snack-bars (18.0%) than in the restaurants (5.3%).

Table 19. Meat daily intakes in different social categories of households. Values are means (± standard deviation) of meat intakes (g/pers.day). In the same column, different superscript letters indicate a significant difference ($p \leq 0.05$).

	n	Meat daily intake					
		Beef	Goat/ mutton	Pork	Chicken	Rabbit	All species
Low-income households	70	0.4 ± 0.7^{a}	0.2 ± 1.0^{a}	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 1.3^{a}
Middle-income households	270	11.2 ± 13.4^{b}	39.5 ± 54.7^{b}	0.7 ± 2.8^{a}	2.7 ± 9.0^{a}	1.1 ± 5.6^{a}	56.0 ± 57.0^{b}
High-income households	17	53.2 ± 26.2^{c}	62.5 ± 112.5^{c}	10.4 ± 32.7^{b}	42.1 ± 83.5^{b}	2.6 ± 7.3^{a}	150.4 ± 137.7^{c}
Total	357	11.1 ± 16.5	32.9 ± 55.8	1.0 ± 7.6	4.0 ± 21.2	0.9 ± 5.1	49.6 ± 65.5

Table 20. Utilization of meat from different animal species in snack-bars and restaurants of Kigali. Values are numbers (percentages) of establishments using meat from a given animal species for the preparation of meat-based meals.

	n	Type of meat				
		Beef	Goat /mutton	Chicken	Pork	Rabbit
Snack-bars	75	21 (28.0%)	61 (81.3%)	46 (61.3%)	24 (32.0%)	42 (56.0%)
Restaurants	75	67 (89.3%)	14 (13.3%)	0 (0.0)	0 (0.0)	0 (0.0)
Total	150	88 (58.7%)	75 (50.0%)	46 (30.7%)	24 (16.0%)	42 (28.0%)

Table 21. Utilization of different cooking methods in snack-bars and restaurants of Kigali. Values are percentages of snack-bars (of restaurants) using a given cooking method for the preparation of meat-based meals.

	Type of meat				
	Beef	Goat /mutton	Chicken	Pork	Rabbit
Boiling	0.0 (89.6)	0.0 (14.3)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Grilling	85.7 (0.0)	100.0 (0.0)	84.8 (0.0)	25.0 (0.0)	100 (0.0)
Frying	14.3 (10.4)	0.0 (85.7)	15.2 (0.0)	75.0 (0.0)	0.0 (0.0)

Table 22. Estimated cooking duration for meat in snack-bars and restaurants of Kigali. Values are percentages of snack-bars (of restaurants) cooking meat-based meals during a given period.

	Type of meat				
	Beef	Goat /mutton	Chicken	Pork	Rabbit
30-45 min	85.7 (0.0)	95.1 (85.7)	45.7 (0.0)	87.5 (0.0)	92.9 (0.0)
45-60 min	0.0 (6.0)	4.9 (0.0)	43.5 (0.0)	12.5 (0.0)	7.1 (0.0)
60min <	14.3 (94.0)	0.0 (14.3)	10.9 (0.0)	0.0 (0.0)	0.0 (0.0)

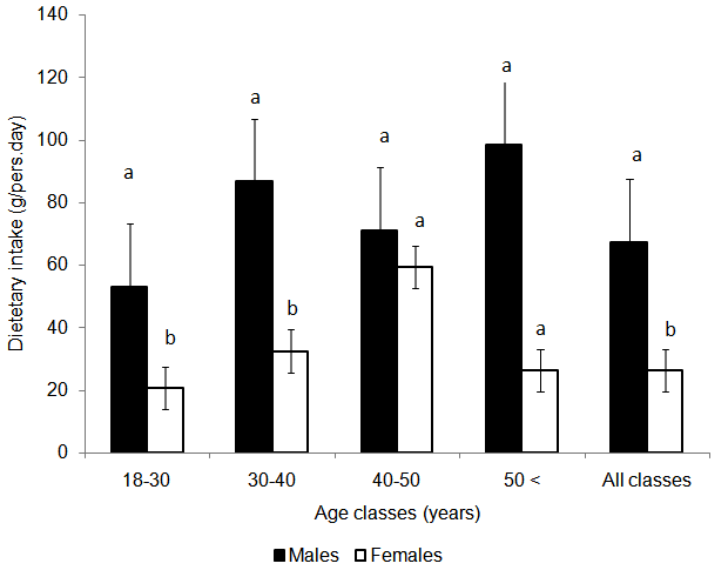

Figure 3. Meat consumption by age and gender category. Values are mean daily meat intakes (g/person). Errors bars represent standard error. For the same age category, different letters indicate a significant difference ($p \leq 0.05$).

Table 23. Bacteriological quality of meat-based meals. Bacteriological counts: values are means (±standard deviation) of bacteriological counts in log cfu/g. *Salmonella* detection: values are numbers and percentages of *Salmonella* positive samples. In the same column, different superscript letters indicate a significant difference ($p \leq 0.05$).

	n	Bacteriological counts		*Salmonella* detection	
		TMC	ECC	Positive samples (n)	Positive samples (%)
Boiled beef	150	3.6 ± 1.9^{a}	1.3 ± 0.4^{a}	8	5.3% ^{a}
Grilled goat meat	84	6.0 ± 3.5^{b}	1.4 ± 0.5^{a}	16	19.0% ^{b}
Fried pork	30	6.6 ± 3.1^{b}	1.3 ± 0.4^{a}	6	20.0% ^{bc}
Grilled chicken	20	6.2 ± 3.4^{b}	1.5 ± 0.5^{a}	3	15.0% ^{abc}
Grilled rabbit	16	6.4 ± 3.5^{b}	1.7 ± 0.7^{a}	2	12.5% ^{abc}
Total	300	4.7 ± 3.1	1.4 ± 0.5	35	11.7%

The microbiological contamination of meat-based meals appeared to be high in rural areas comparatively to urban localities of Kigali. The average TMC level recorded in snack-bars and restaurants of urban Kigali (2.92 log cfu/g) was significantly lower than the one of 6.60 log cfu/g observed in rural areas (MWU test=1686.5, df=1, p=0.000). A comparable trend was observed in ECCs and the occurrence of *Salmonella* (Figure 4).

5.3.4. Risk factors for *Salmonella* contamination in meat-based meals

The binary logistic regression analyses revealed that the risk of *Salmonella* contamination to occur in cooked meat meals was four times higher in snack-bars than in restaurants (OR=4.77; 95% confidence interval = 1.81–12.61; p=0.001). Furthermore, factors such as ‘’not changing the knife while cutting meat from another animal species or non meat ingredients’’, ’’cooking by grilling or frying rather than boiling”, “not wearing specific work clothing while handling meat” and “absence of pest control measures in the kitchen” were found to be associated with an increasing risk of *Salmonella* contamination. However, cooking for a long period, reheating foods prior to serving and a frequent cleaning of the kitchen decreased the risk of *Salmonella* occurrence in cooked meat meals (Table 24).

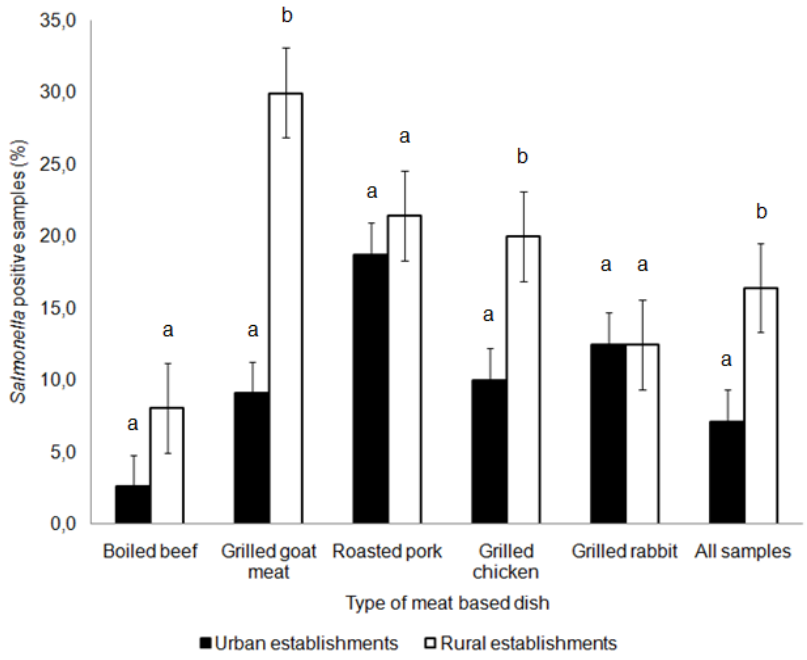

Figure 4. Occurrence of *Salmonella* in meat-based meals sold in urban and rural localities of Kigali. Values are proportions (%) of *Salmonella* positive samples. Errors bars represent standard error. For the same time of meat-based dish, different letters indicate a significant difference ($p \leq 0.05$).

5.4. Discussion

5.4.1. Consumption of meat-based meals

Beef and goat meat were found to be the types of meat mostly consumed outside the household circle in Kigali. The high consumption of these two types of meat could be explained by their affordability to the population of Kigali. Due to the lack of public slaughterhouses for animal species other than bovines and small ruminants in Rwanda in general and particularly in Kigali (Rwandan Ministry of Agriculture and Animal Ressources, 2012); pigs, poultry and rabbits are general slaughtered in private meat abattoirs. This result in an increased production cost for meat from animals other than bovines (and small ruminants) and consequently higher prices on Kigali city markets. Recent statistics indicate that beef and goat meat represent 51.6% of the Rwandan meat production (FAO, 2015).

The consumption of meat in Kigali was found to be high in people from households with high incomes comparatively to those from other social categories. This may be justified by the purchasing power of people from that particular category as the wealth was reported to be the major determinant of meat consumption (McAfee et al., 2010; Sans & Combris, 2015; Speedy, 2003). Our findings are in agreement with the studies conducted in different countries such as United states (Carrie et al., 2011), China (Ma et al., 2006), Brazil (de Carvalho et al., 2014), Equatorial Guinea (East et al., 2005; Fa et al., 2009) and Gabon (Wilkie et al., 2005) where a positive correlation between meat consumption and the wealth of the population was reported.

The meat intake outside the household's circle by females was found to be low comparatively to males probably because, in respect to the Rwandan culture, women do not frequent bars as men do. According to the Rwandan customs, the consumption of alcoholic beverages is generally reserved to men (Adekunle, 2007). Therefore, Rwandan women frequent less the establishments such as snack-bars where alcoholic beverages are more consumed by men. Another reason would be the low financial capacity of Rwandan women to afford the purchase of meat-based meals outside their household. Previous studies highlighted that in developing countries, the purchasing power was significantly low in women comparatively to men (Bastos et al., 2009; Burnet, 2011; Quisumbing et al., 2001). The findings from our study are in agreement with the study by de Carvalho et al. (2014) in Sao Paulo (Brazil) where an average daily meat intake of 200.2g was reported in males against 130.6g in females. A comparable trend was reported by Daniel et al. (2011) in Unites States (158.3 and 103.2g of total meat per day in males and females respectively) and in European countries where the consumption of all categories of pork was reported to be significantly low in women comparatively to men (Verbeke et al., 2010).

Table 24. Risk factors for *Salmonella* occurrence in the establishments selling meat-based meals in Kigali.

Variable	Percentage of *Salmonella* positive establishments (*).		Binary logistic regression		
			Odds ratio	95% CI	p-value
Type of the establishment selling meat-based meals					
	Snack-bar	29.3	4.774	1.81-12.61	0.001
	Restaurant	8.0	1		
Changing knife to cut meat from different animal species and non meat ingredients					
	Yes	3.0	1		
	No	23.1	9.600	1.25-73.56	0.009
Cooking method for meat-based meals preparation					
	Boiling	8.0	1		
	Grilling	28.3	4.547	1.66-12.43	
	Frying	33.3	5.750	1.48-22.39	0.003
Estimated duration of the cooking process					
	30-45 min	30.1	1		
	45-60 min	8.3	1		
	60 min<	7.7	0.193	0.07-0.55	0.002
Previously cooked foods and only reheating at consumption					
	Yes	7.6	0.217	0.08-0.61	0.002
	No	27.4	1		
Frequency of cleaning the kitchen					
	Only once a day	36.8	1		
	Several times	7.5	0.140	0.06-0.36	0.032
Specific work clothing for personnel					
	Yes	4.5	1		
	No	24.5	6.825	1.54-30.16	0.004
Presence of measures against vermin in the establishment					
	Yes	6.8	1.0		
	No	30.7	5.989	2.14-16.80	0.000

(*) Meat-based meals served in a given establishment were considered contaminated by *Salmonella* if at least one meat sample was found to be *Salmonella* positive

The low meat consumption observed in young adults (18-30 years old) may be due to their limited financial capacity to afford the purchase of meat-based meals outside their households comparatively to people from other age categories. Comparable findings were reported in Ireland (Cosgrove et al., 2005) where an average daily meat intake of 128.5g was observed in young adults (18-35 years old) against 134.5g in adults (36-50 years old). However in Brazil (de Carvalho et al., 2014), the consumption of meat (including meat consumed within the household) appeared to be higher among youth than in adults (178.6 g of total meat per day in adolescents versus 167.7 g in adults). The similar trend was reported in United States of America where an average daily meat intake

was estimated to 149.0g in people aged from 20 to 49 years against 130.9 g in people aged from 50 to 69 years (Carrie et al., 2011).

Contrary to other studies conducted in developing countries that have reported low meat intakes in rural communities (Ayele & Peacock, 2003; Delgado, 2003; Heinz & Hautzinger, 2007); the meat intake recorded in peri-urban region of Kigali was found to be equivalent to the one observed in city dwellers. Different studies have reported that people living in rural areas but nearby large cities had the opportunity to get jobs in these cities and their income was found to be comparable to the one of city dwellers (Eliasson et al., 2014; Wang et al., 2011). This could explain the equivalent meat intake recorded in both urban and peri-urban areas of Kigali city as the wealth is recognized to be the main determinant of meat consumption (McAfee et al., 2010; Sans & Combris, 2015; Speedy, 2003).

Previous studies have reported that the average meat intake within the households of Kigali was 34.4 grams per person and per day (Niyonzima et al., 2016a). Therefore, the global meat intake (within and outside the household) is estimated to 84.0 grams per person and per day. Although the meat consumption rate observed in Kigali seems to be low in relation to the average meat intake of 93.9 grams per person and per day recorded in developing countries (FAO, 2014); it appears to have significantly increased with the country's growing economy. In the 2000s, the Rwandan global meat intake was estimated to 13.3 grams per person and per day (Speedy, 2003).

5.4.2. Bacteriological quality of the consumed meat

The selection of the establishments from which meat samples were collected required the willingness of their managers to participate in the study and this could have introduced a bias in the recorded findings. However, as a large proportion (68%) of the establishments has agreed to participate in the study and the selection of these establishments was carried out randomly, the introduced bias is relatively minimal.

The total mesophilic bacteria and *E. coli* counts are respectively recognized as indicator of general hygiene and fecal contamination in foods (ISO, 2001, 2003). In our study, the levels of hygiene indicator bacteria in different meat-based meals were found to be relatively high. This could be attributed to a deficient cooking process and/or a post-cooking contamination of meat-based meals. It is generally recognized that main enteric bacteria are destroyed at conventional pasteurization temperatures (Jay et al., 2005; Korsak et al., 2004). However, as cooking times and temperatures may vary from one meat product or establishment to another, the possibility of inadequate cooking treatments should not be excluded. Several authors have reportedly associated the survival of enteric bacteria in cooked meat to deficient cooking treatments (Breslin et al., 2014; Lahou et al., 2015; Roccato et al., 2015; Silva & Gibbs, 2012). The bacterial contamination of meat products from the handlers or the processing

environment can also occur after the cooking treatment. Factors such as dirty utensils, unclean works surfaces and deficient hygiene for personnel were reported to be associated to the bacterial contamination of cooked meat products (Cardinale et al., 2005; Cardinale et al., 2015).

The mean TMC (4.7 log cfu/g) recorded in meat-based meals consumed in Kigali was found to be comparable to the ones recorded in other developing countries. The studies conducted in Cameroon (Yannick et al., 2013) and in Sudan (Abdalla et al., 2009) showed average total bacteria loads of 4.5 and 4.6 log cfu/g in cooked pork and chicken meals respectively. In Taiwan, mean total bacteria loads of 5.7 and 5.6 log cfu/g were respectively reported in street vended chicken and pork meals (Manguiat & Fang, 2013) whereas TMC levels as high as 7.2 log cfu/g were recorded in street meat-based sandwiches in Ethiopia (Muleta & Ashenafi, 2001). Nevertheless, Mosupye and Von Holy (2000) reported lower TMC levels (1.9 -2.6 log cfu/g) in beef and chicken-based street foods sold in Johannesburg (South Africa).

Concerning *E. coli*, a mean count of 1.38 log cfu/g was recorded in the present study. Our findings are comparable to *E. coli* loads of 1.3 and 1.4 log cfu/g respectively reported in grilled pork and chicken meals in Philippines (Manguiat & Fang, 2013). However, higher counts were reported in Lubumbashi (Democratic Republic of Congo) where *E. coli* loads as high as 4.2 log cfu/g were recorded in street goat brochettes (Kabwang, 2013) and in Sudan, where a mean *E. coli* load of 3.2 log cfu/g was observed in street cooked beef and chicken meals (Abdalla et al., 2009).

As for hygiene indicator bacteria, the occurrence of *Salmonella* was found to significantly ($p \leq 0.05$) vary in different types of meat-based meals. The prevalence of *Salmonella* was found to be significantly lower in boiled beef comparatively to grilled or fried meat. This could be attributed to higher time and temperature treatments applied to beef during the cooking process. In our study, all beef samples were collected in restaurants where beef is generally prepared in sauces and the cooking duration is relatively long. Ninety four per cent of 75 restaurants have reported to boil beef for more than one hour whereas in snack-bars, meat was grilled or fried for shorter periods. The temperature and the cooking duration were found to be important factors in the destruction of microorganisms in foods and several studies have reported the survival of *Salmonella* in undercooked meat products (Breslin et al., 2014; Lahou et al., 2015; Roccato et al., 2015). Another reason for higher prevalence of *Salmonella* in grilled or fried meat would be their contamination from the non meat components of the dish. In most snack-bars of Kigali, grilled or fried meat was generally served with uncooked vegetable salads that could be a source of *Salmonella* through cross-contaminations. Several studies have reported the occurrence of *Salmonella* in ready to eat raw vegetable salads (Gómez-Aldapa et al., 2013; Gurler et al., 2015; Wijnands et al., 2014).

In our study, the overall *Salmonella* prevalence in ready to eat meat-based meals was found to be 11.7%. Ours findings are comparable to the prevalence of 15.1 % in grilled goat brochettes (n=139) and 10.1% in street poultry meals (n=444) respectively reported in Democratic Republic of Congo (Kabwang, 2013) and Senegal (Cardinale et al., 2005). Nevertheless, *Salmonella* prevalence as low as 2.6% in ready to eat pork based meals (n=117) and 0.2% in beef based meals (n=478) were reported in China (Yang et al., 2016) and Jordan (Osaili et al., 2014) respectively.

The levels of hygiene indicator bacteria and the occurrence of *Salmonella* were found to be significantly higher in meat-based meals sold in peri-urban areas of Kigali comparatively to urban localities. . This could be associated to the lack of hygiene and sanitary facilities such as proper running water in rural areas, but most importantly to less frequent sanitary controls by competent authorities in rural meat selling establishments. Our findings are in agreement with the study conducted in Lubumbashi (Democratic Republic of Congo) that revealed that hygiene conditions in the restaurants were less satisfactory in rural areas than in cities (Kabwang, 2013).

A significant and positive correlation was observed between total mesophilic bacteria and *E. coli* counts for the same sample and between ECC and the occurrence of *Salmonella*, indicating that the prevalence of *Salmonella* increases with the levels of hygiene indicator bacteria in meat samples. This would suggest that hygiene improvements in the establishments selling meat-based meals can significantly contribute in reducing the risk of *Salmonella* contamination in meat-based meals.

5.4.3. Risk factors for *Salmonella* contamination in meat-based meals

Eight risk factors were found to be significantly associated with the occurrence of *Salmonella* in meat-based meals served in snack-bars and restaurants of Kigali. The risk of *Salmonella* contamination was high in meat-based meals sold in snack-bars than in restaurants. This can be explained by the fact that, meat-based meals served in the restaurant (in most cases sauces) undergo a long heat treatment susceptible to destroy all bacterial pathogens present into meat. In snack-bars, the cooking durations were relatively shorter and may suggest a poor cooking and a possible survival of *Salmonella* in cooked meat. Furthermore, uncooked vegetable salads generally served together with meat-based meals in snack-bars could have significantly contributed to the increased risk of *Salmonella* occurrence.

Not changing the knife while cutting meat from another animal species or non meat ingredients such as vegetable, was found to be associated to a high risk of *Salmonella* contamination in meat-based meals. In kitchens where diverse meat-based meals are prepared, raw materials of various kinds are generally handled closely together to create numerous opportunities for cross contaminations. Therefore, the utilization of the same knife for cutting different raw material may contribute significantly to the dissemination of bacterial pathogen such as

Salmonella into the processed meat products. Previous studies have reportedly associated *Salmonella* cross contaminations during the processing of meat products to the use of contaminated cutting equipments particularly the knives (Hald et al., 2003; Mackey & Derrick, 1979).

The absence of pest control measures in the kitchen compartment of restaurants and snack-bars increased the risk of *Salmonella* contamination in the served meat-based-meals. This could be explained by the possible dissemination of *Salmonella* on raw material and/or cooked meat meals through cross contamination by pests. Vermin such as flies were found to be implicated in the dissemination of bacterial pathogens on food products from the soiled environment (Jay et al., 2005; Niyonzima et al., 2015). Nevertheless, the risk of *Salmonella* contamination was reduced by a frequent cleaning of the kitchen and utensils used for meat preparation. Our findings corroborated the studies by Christison et al.(2007,2008) where the kitchen utensils, food handlers and work surfaces were identified as potential sources of bacterial contaminations in ready to eat foods in Johannesburg (South Africa). It was also found that reheating meat-based meals prior to serving resulted in reducing the risk of *Salmonella* contamination. This could be attributed to the additional thermal destruction of residual *Salmonella* cells and/or new bacterial contaminants introduced into meat after the cooking process. The post cooking contaminations in ready to eat foods including meat have been reported in different countries such as Madagascar (Cardinale et al., 2015), Senegal (Cardinale et al., 2005), South Africa (Mosupye & Von Holy, 2000) and Trinidad (Badrie et al., 2003).

5.5. Conclusions

The findings from this study indicate that meat is regularly consumed outside the household's circle in Kigali and goat brochettes constitute the mostly consumed meat-based dish. The levels of hygiene indicator bacteria in different meat-based meals as well as the prevalence of *Salmonella* appeared to be relatively high. Therefore, there is a need to improve hygienic meat handling and/or cooking practices in the establishments selling meat-based meals in Kigali to limit the transmission of bacterial pathogens to humans through the consumption of contaminated meat.

CHAPTER SIX

Quantitative risk assessment of human salmonellosis attributable to the consumption of meat-based meals

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CHAPTER 6. Quantitative risk assessment of human salmonellosis attributable to the consumption of meat-based meals.

Abstract

Meat constitutes one of the major vehicles for human *Salmonella* infections. The objective of this study was to assess the risk of developing *Salmonella* illness associated to the consumption of meat-based meals in Kigali city inhabitants through a quantitative microbial risk assessment (QMRA) model and to determine through the built model the efficacy of different interventions aimed at reducing the risk of *Salmonella* along the Rwandan meat chain. In the present study, the risk of *Salmonella* illness attributable to the consumption of meat-based meals was assessed through a QMRA model by using the Codex alimentarius approach and three main risk exposure path ways (namely beef consumption within the household as well as beef and goat meat consumption outside the household) were considered. The estimated risk of human salmonellosis associated to the consumption of meat-based meals in Kigali city inhabitants was found to vary between 1.73 and 3.36% in different risk exposure path ways and females as well as young adult consumers appeared to be less exposed to the risk. The analysis of intervention scenarios aimed at reducing the risk of human salmonellosis showed a relative risk reduction ranging from 22.7 to 83.1% and the reduction yield was significantly higher when different interventions were simultaneously applied at various stages of the meat chain. Data gathered through this study would be helpful in monitoring the risk of *Salmonella* illness attributable to the consumption of meat-based meals in Rwanda and in other countries where the meat production chains are comparable.

Key words: meat, quantitative risk assessment, *Salmonella*, risk factors, model, Rwanda

6.1. Introduction

Salmonellosis is recognized to be one of the major bacterial food borne diseases worldwide and meat constitutes one of the vehicles of human *Salmonella* infections (Greig & Ravel, 2009; Majowicz et al., 2010). The contamination of meat by *Salmonella* can occur at any stage of the meat production chain from the slaughterhouse to the consumption and it is generally associated to deficient hygiene practices in meat handling.

The consumption of meat and meat products has increased in both developed and developing countries during the last decades, mainly as a result to the growing purchasing power of populations as well as the changes in consumers habits generally associated to the urbanization of rural communities (Heinz & Hautzinger, 2007; Ma et al., 2006). The recent statistics indicate that the world meat consumption rate estimated to 43.4 Kg per person and per year (data of the year 2016), was significantly low in previous years and is expected to increase with the countries growing economies (FAO, 2016; Sans & Combris, 2015). In Rwanda, the consumption of meat has also

evolved in a comparable trend as the current meat consumption rate is estimated to be six times higher comparatively to that recorded during the 2000s (Niyonzima et al., 2017a; Speedy, 2003).

Previous studies have reported the isolation of *Salmonella* species in meat-based meals consumed within the households as well as in various ready to eat meat-based dishes generally commercialized in snack-bars and restaurants of Kigali city (Niyonzima et al., 2016a, 2017a). Furthermore, *Salmonella* has been detected in stool samples from patients with diarrheal gastroenteritis in Rwanda (Kabayiza et al., 2014; Lévy et al., 1986; Nzabahimana et al., 2014) indicating the occurrence of *Salmonell*a illness in the Rwandan population.

However, despite the evidence of *Salmonella* occurrence in both patients and meat-based dishes, no study, to the best of our knowledge, has yet assessed the risk of *Salmonella* illness that might be associated to the consumption of meat-based meals in Rwanda though their consumption is continuously growing particularly in highly populated cities such as Kigali. The present study was therefore carried out to assess quantitatively the risk of salmonellosis attributable to the consumption of meat-based meals in Kigali city inhabitants and to determine the efficacy of different intervention scenarios intended to mitigate that risk. Data gathered in this work shades light on the contribution of meat-based meals in the transmission of food borne bacterial pathogen such as *Salmonella* in Rwanda and in countries with growing economies in general, and would be helpful in monitoring the risk of food borne diseases associated to these pathogens.

6.2. Material and methods

6.2.1. Study area and consumer population

The study was conducted in Kigali city, Rwanda. The choice of Kigali was justified by its large population as it represents more than 10% of the Rwandan population. The recent statistics indicate that the population of Kigali is estimated to 1,135,428 inhabitants (National Institute of Statistics of Rwanda, 2012). As the meat consumption rate is reported to be positively correlated to the consumer's financial capacity (de Carvalho et al., 2014; Manyori et al., 2017; McAfee et al., 2010), the population of Kigali city was subdivided into three socio-economical categories (namely low, medium and high income households) according to the existing Rwandan mutual health insurance scheme (Goverment of Rwanda, 2008). The main characteristics of different socio-economical categories of the population in Kigali city are described in Table 13 (see page 68). Considering the meat consumption rate of 89% and the proportion of different socio-economical categories in the population of Kigali city (21% for low income, 75% for middle income and 4% for high income households), the population consuming meat was estimated to 212,211; 757,898 and 40,421 inhabitants respectively in low, medium and high income households of Kigali city (Niyonzima et al., 2016a).

6.2.2. Risk assessment model

The quantitative risk assessment model proposed by Evers et al. (2010) was adapted to the Rwandan context and used in the present study. The adapted model starts from the end of the animal slaughtering process at the abattoir level and ends with the number of human salmonellosis cases attributable to meat consumption within and outside the household. The model is deterministic and covers the meat distribution aspects, kitchen meat preparation and cross contamination as well as the dose-response relationship. In performing the risk assessment, the Codex Alimentarius approach including the hazard identification, hazard characterization, exposure assessment and the risk characterization stages was followed (Codex Alimentarius Comission, 2009).

6.2.3. Hazard identification

Salmonella is a Gram negative bacillus belonging to the family of *Enterobacteriaceae*. It is a non sporulating bacteria and measures 2 to 5µm of length and 0.7 to 1.5µm of diameter, with peritrichous flagella when motile (Gopinath et al., 2012; Korsak et al., 2004). The optimal growth temperature for *Salmonella* is reported to vary from 35 to 37°C whereas its optimal pH ranges from 6.5 to 7.5. Under optimal growth conditions, its generation time is estimated to be 25 minutes (Delhalle et al., 2009b). *Salmonella* may be harbored by the digestive tract of domestic and wild animals and is generally isolated from a wide range of food of animal origin including meat (Niyonzima et al., 2015). In food products, *Salmonellae* are reported to be heat sensitive as they are killed at normal pasteurization temperatures (Bogard et al., 2013; Lahou et al., 2015; Roccato et al., 2015). Furthermore, the growth of *Salmonella* is inhibited in foods with NaCl concentration greater than 4%, in foods with a water activity less than 0.94 or in food products at a storage temperature below 5°C (Jay et al., 2005; Korsak et al., 2004). Nevertheless, although significant reductions in *Salmonella* numbers in frozen foods were reported in a number of studies (Chaves et al., 2011; Niemira et al., 2003), it should be noted that freezing does not constitute a sanitizing strategy for contaminated meat and meat product (Escartín et al., 2000).

6.2.4. Hazard characterization

Human infection by *Salmonella* occurs through fecal-oral route, mainly as a result to the consumption of contaminated food products ; though human contamination by contact with infected animals is also possible (Korsak et al., 2004). After ingestion of contaminated foods, follow three phases of subclinical infection including the colonization of the intestine and adhesion to the intestinal wall, invasion of the intestinal wall, and finally the dissemination of *Salmonella* in the mesenteric lymph nodes and other organs (Berends et al., 1996). The illness symptoms appear generally between 12 and 36 hours following the ingestion of the contaminated foods. These

include fever, abdominal cramps, diarrhea as well as vomiting and usually last from 4 to 7 days (Delhalle et al., 2009a; Gopinath et al., 2012).

The infective dose for *Salmonella* is reported to be variable and this is attributable to a number of factors including the variable pathogenicity of different *Salmonella* strains (Marcus et al., 2000), the sensitivity of hosts (high sensitivity in young, old, pregnant or immune-compromised people) as well as the nature of the food vehicle. The high fat or protein content of certain foods sources was reported to protect *Salmonella* against stomach acidity (Álvarez-Ordóñez et al., 2011). Volunteers feeding trials indicated that *Salmonella* doses as high as 5 to 7 log colony forming units were generally ingested to initiate illness in humans (Blaser & Newman, 1982; Korsak et al., 2004). However, investigations from outbreaks revealed that much lower doses such as 7 colony forming units might trigger *Salmonella* illness in human (Teunis et al., 2010b). The average minimal dose capable to cause illness in the half of the exposed population (ID50) was estimated to be 9661 colony forming units (Evers & Chardon, 2010; Manyori et al., 2017).

6.2.5. Exposure assessment

6.2.5.1. Meat consumption data

Data on meat consumption was derived from two studies conducted in Kigali city inhabitants (Niyonzima et al., 2016a, 2017a). The findings from these studies indicated that bovine and goat meat were the mostly consumed types of meat in Kigali city. However, as the consumption of goat meat within the households was found to be marginal, three risk exposure pathways namely bovine meat consumption within the household, bovine meat consumption outside the household as well as the consumption of goat brochettes outside the household were considered in the present study. The average meat daily intakes in different categories of the Kigali city inhabitants are described in Table 25.

6.2.5.2. Meat Contamination data

6.2.5.2.1. Prevalence and concentration of *Salmonella* in meat

The study conducted to assess the bacteriological quality of meat consumed within the households of Kigali city revealed an average *Salmonella* prevalence of 21.4% in bovine meat at retail level whereas in goat meat it was estimated to be 12.5% (Niyonzima et al., 2016a, 2016b).

As to the best of our knowledge no data on the concentration of *Salmonella* in meat sold in Kigali city retail premises were available, data from a study conducted in Lusaka province (Zambia) were used, assuming that the meat retail hygienic practices in both countries are comparable (Haileselassie et al., 2013; Mrema et al., 2006).

The Zambian study (Manyori et al., 2017) reported the concentration of *Salmonella* in meat at retail to vary from 0.5 to 1.1 log cfu/g and an average *Salmonella* concentration of 0.9 log cfu/g was used in the present study.

At the slaughterhouse level, Niyonzima et al. (2017b) reported a *Salmonella* prevalence of 21.3% and 8.0 respectively in bovine and goat carcasses at the end of the slaughtering process in Kigali city. Furthermore, the concentrations of total *E. coli* in bovine and goat meat at the slaughterhouse level were found to be respectively 1.5 and 1.2 log units lower, when compared to their concentrations in meat at the retail stage (Niyonzima et al., 2016b, 2017b). Assuming that the meat concentration in *Salmonella* cells between the slaughtering and the retail stages increases of the same magnitude as *E. coli* (Delhalle et al., 2009b), the concentration of *Salmonella* in bovine and goat carcasses at the end of the slaughtering process was estimated to –0.6 and –0.3 log cfu/g respectively.

Table 25. Meat dietary intake in Kigali city inhabitants. Values are the means daily meat intake (g/pers.day) for different categories of households in Kigali city.

Risk exposure pathway	Household category	Daily intake	Reference
Bovine meat consumption within the household			
	Low income households	14.2	Niyonzima et al. (2016)
	Middle income households	34.3	Niyonzima et al. (2016)
	High income households	96.0	Niyonzima et al. (2016)
Bovine meat consumption outside the household			
	Low income households	0.4	Niyonzima et al. (2017)
	Middle income households	11.2	Niyonzima et al. (2017)
	High income households	53.2	Niyonzima et al. (2017)
Goat meat consumption outside the household			
	Low income households	0.2	Niyonzima et al. (2017)
	Middle income households	39.5	Niyonzima et al. (2017)
	High income households	62.5	Niyonzima et al. (2017)

6.2.5.2.2. Kitchen preparation and cross contamination

To the best of our knowledge, no published data on kitchen cross contaminations in Rwanda were available. Therefore, data from the study conducted in Lusaka province (Zambia) were used in our study, assuming that the cooking practices in these countries are comparable. This study estimated to 40 and 45% the proportion of meat

portions susceptible to contaminate the environment through cross contaminations in the home and restaurant's kitchen respectively, whereas the proportion of *Salmonella* colony forming units from a meat portion, susceptible to contaminate the cooking environment, was assumed to be 30% (Manyori et al., 2017).

Meat-based meals consumed within and outside the households of Kigali city were reported to be cooked well-done (Niyonzima et al., 2016a, 2017a). Thus, in the present study, *Salmonella* was assumed to do not survive in cooked meat. Several studies have reported the total destruction of *Salmonella* in well-done cooked meat (Bogard et al., 2013; Lahou et al., 2015; Roccato et al., 2015).

In a study conducted to assess the cross contamination and transfer rates of *Salmonella* to nonmeat foods and kitchen utensils during the cooking process, Ravishankar et al. (2010) estimated to 10 and 30% respectively, the proportions of *Salmonella* colony forming units transferred to cooking utensils and nonmeat foods (lettuce) prepared with contaminated chicken meat. These transfer rates were used in the present study as the proportion of *Salmonella* colony forming units that end up ingested by the consumer, as they are susceptible to contaminate meat-based dishes after the cooking process. In snack-bars and restaurants of Kigali city, meat-based meals were reported to be generally served with raw vegetable salads where as within the households meat was predominantly consumed in sauces (Niyonzima et al., 2016a, 2017a). Therefore, in the present study, the *Salmonella* transfer rate of 10 % attributable to the contamination from cooking utensils was used in the home risk exposure pathway whereas that of 30% attributable to the contamination from nonmeat foods was utilized in the snack-bar/restaurant risk exposure pathway.

6.2.5.2.3. *Salmonella* infection and illness

The *Salmonella* ID50 (infective dose 50) of 9661 colony forming units estimated by Evers et al. (2010) was used in the present study, and it was assumed that the totality of infected population would get ill after ingestion of a such *Salmonella* dose (Manyori et al., 2017).

6.2.6. Risk characterization

Input data for different risk exposure pathways were introduced in the built model and the output were recorded as the number of human salmonellosis cases attributable to the consumption of a given meat product per year (360 days). The probability of developing *Salmonella* illness attributable to meat consumption through a given risk exposure pathway was then calculated by using the following formula:

$$P_i = \frac{N_i}{N_e} \times 100$$

Where, P_i is the probability of *Salmonella* illness in percentage, N_i is the number of *Salmonella* cases in the exposed population and N_e the number of inhabitants in the exposed population.

6.2.7. Scenario analysis

The impact of different scenarios aimed to reduce the risk of human salmonellosis attributable to the consumption of different meat product in Kigali, was assessed through the built risk assessment model. The model was first run with the actual input data for a given exposure pathway to provide the baseline results. Then after, selected input variables were modified and the effect of these modifications on the ultimate model output was measured.

The relative reduction of the probability of *Salmonella* illness attributable to a given intervention scenario was calculated by using the formula:

$$RR = \frac{(N_{base} - N_{inter})}{N_{base}} \times 100$$

Whereas *RR* is the relative reduction of the risk of *Salmonella* illness (in percentage) attributable to the applied intervention scenario, N_{base} is the number of *Salmonella* illness cases recorded before the application of the intervention scenario (baseline results) and N_{inter} is the number of *Salmonella* illness cases recorded after the application of the intervention scenario.

The yield of the applied intervention scenario was then calculated as follow:

$$IY = \frac{RR}{RL}$$

Where *IY* is the yield of the intervention scenario, *RR* is the relative reduction of the risk of *Salmonella* illness attributable to the applied intervention scenario, *RL* is the reduction level of the modified input parameter in percentage. The yield of intervention scenario was reported to be a useful indicator to assess the effectiveness of interventions applied to reduce the risk of microbial pathogens in food chains (Delhalle et al., 2009b).

6.3. Results

6.3.1. Exposure assessment

Table 26 describes the exposure level of different categories of Kigali city inhabitants to the risk of getting *Salmonella* illness after the consumption of meat-based meals within and outside the home. The total number of

salmonellosis cases attributable to the consumption of beef-based meals within and outside the home in Kigali city was estimated to 17,446 and 19,198 cases per year respectively; and the probability of *Salmonella* illness associated to these two exposure pathways was not found to be significantly different ($p<0.05$). Nevertheless, the risk of *Salmonella* illness attributable to the consumption of goat brochettes outside the home (estimated to 3.34%) appeared to be significantly ($p<0.05$) higher comparatively to the one associated to the consumption of beef based meals within and outside the home in Kigali city.

In all risk exposure pathways, the probability of *Salmonella* illness to occur was found to be increasing with the socio-economical category of meat consumers. Furthermore, the magnitude of risk exposure was significantly higher ($p<0.05$) in men comparatively to women, and was found to be increasing as the age of the consumer grows (Figure 5).

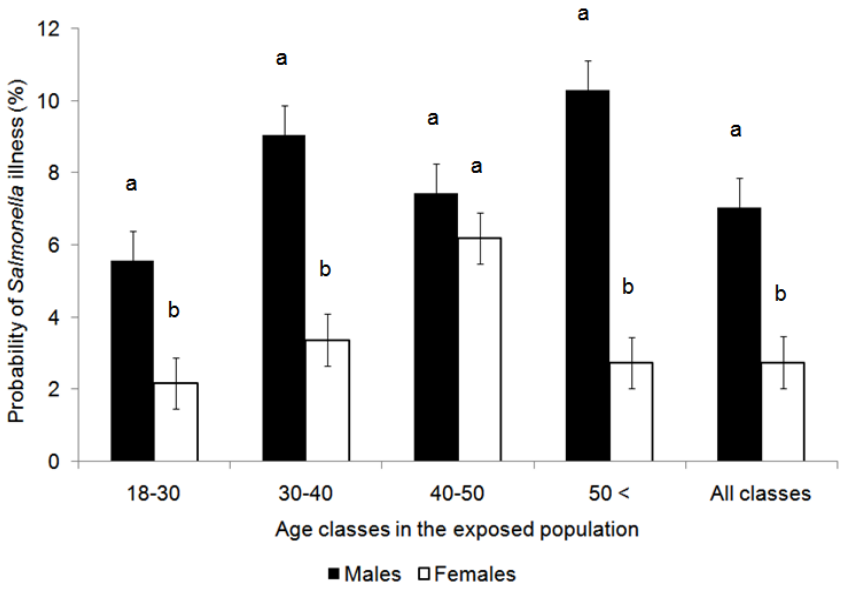

Figure 5. Exposure of different age classes of Kigali city inhabitants to the risk of salmonellosis attributable to the consumption of meat-based meals. Values are the probabilities of *Salmonella* illness in different age classes of Kigali city inhabitants. Error bars represent standard error. For the same age class, different superscript letters indicate a significant difference ($p<0.05$).

6.3.2. Scenario analysis

The effect of intervention scenarios to reduce the risk of human salmonellosis associated with the consumption of meat-based meals in Kigali city was assessed. The assessed intervention scenarios include the reduction of the prevalence and the concentration of *Salmonella* in meat carcasses at the end of the slaughtering process as well as in meat cuts at retail, the reduction of the probability of post cooking contamination in the kitchen as well as different combinations of these interventions. The application of intervention scenarios, under different exposure pathways, yielded a relative reduction of human salmonellosis cases ranging from 22.7 to 83.1%.

Table 26. Baseline results of exposure assessment. In the same column and for the same risk exposure pathway, different superscript letters indicate a significant difference ($p<0.05$).

Risk exposure pathway	Category of the household	Model outputs	
		Number of illness cases per year	Probability of illness (%)
Bovine meat consumption within the household			
	Low income households	1599	0.75[a]
	Middle income households	3790	1.82[b]
	High income households	2057	5.09[c]
	All categories	17446	1.73
Bovine meat consumption outside the home (snack-bar/restaurant)			
	Low Income households	152	0.07[a]
	Middle income households	15197	2.01[b]
	High income households	3846	9.51[c]
	All categories	19195	1.90
Goat meat consumption outside the home (snack-bar/restaurant)			
	Low Income households	44	0.02[a]
	Middle income households	31284	4.13[b]
	High income households	2655	6.57[b]
	All categories	33983	3.36

The reduction of salmonellosis cases observed when interventions were applied simultaneously at different stages of the production chain, was found to be significantly higher comparatively the one recorded when the interventions were applied individually (Table 27, Table 28 and Table 29).

6.4. Discussion

The present study was aimed at assessing the risk of *Salmonella* illness associated to the consumption of meat-based meals in Kigali city inhabitants through a quantitative microbiological risk assessment (QMRA) model. The QMRA model construction requires a number of data from various sources, and when data for some variables are not available, assumptions need to be made to build the entire model (Delhalle et al., 2009b). In our study, data from scientific peer-reviewed literature was used as an alternative for model variables whose data on Rwanda was not available. Therefore, the outputs of the built model should be interpreted with caution, as they might not represent accurately the Rwandan meat chain due to the lack of data. Nevertheless, the present study attempted to simulate the Rwandan meat production chain and allowed to identify concrete intervention scenarios for reducing the risk of human salmonellosis attributable to the consumption of meat-based meals in Kigali city (Rwanda).

6.4.1. Exposure assessment

The findings from this study indicate that the risk of human salmonellosis associated to the consumption of meat-based meals in Kigali city is relatively low (1.7–3.4%). This is mainly attributable to the low meat intake and the meat cooking practices observed in the population of Kigali city. A recent study reported that the average meat daily intake in Kigali city inhabitants is estimated to be 84.0 grams per person (Niyonzima et al., 2017a) and this consumption rate appears to be relatively low in comparison with the average world consumption rate estimated to 122.6 grams of meat per person and per day (FAO, 2016). The low meat consumption rate observed in Kigali city inhabitants could be explained by the low financial capacity of the population, as the consumer's wealth was reported to be the major determinant of meat consumption in both developed and developing countries (Carrie et al., 2011; Heinz & Hautzinger, 2007; McAfee et al., 2010; Speedy, 2003). Furthermore, as in other African countries, meat-based meals in Kigali city were reported to be mainly cooked through boiling for several hours (2-3 hours) and this might have contributed to the reduction of the risk of *Salmonella* occurrence in cooked meat-based-meals (Manyori et al., 2017; Niyonzima et al., 2016a). Several authors have reported the total destruction of *Salmonella* in foods cooked at normal pasteurization temperatures (i.e. 70°C for 2 minutes) (Delhalle, et al., 2009b; Korsak et al., 2004).

Table 27. Results of the intervention scenarios to reduce the risk of human salmonellosis attributable to the consumption of bovine meat within the household. Illness cases: values are numbers of human salmonellosis cases associated to the consumption of meat-based meals in Kigali city inhabitant per year. Relative reduction: values are the relative reduction of salmonellosis cases in percentage comparatively to the baseline results.

Intervention scenario	Reduction level	Scenario outputs		
		Illness cases	Relative reduction	Intervention yield
Base line results		17446		
1. Reduction of *Salmonella* concentration on carcasses at the end of the slaughtering process.	Reduction of 25%	13085	25.0	1.0
	Reduction of 50%	8697	50.1	1.0
	Reduction of 75%	3301	81.1	1.1
2. Reduction of *Salmonella* prevalence in meat at retail.	Reduction of 25%	13482	22.7	0.9
	Reduction of 50%	8209	52.9	1.1
	Reduction of 75%	4362	72.0	1.0
3. Reduction of post cooking contamination probability in the kitchen.	Reduction of 25%	13085	25.0	1.0
	Reduction of 50%	8697	50.1	1.0
	Reduction of 75%	3301	81.1	1.1
4. Increased efforts along the meat production chain	Reduction of 25% in both scenarios 2 and 3.	9019	48.3	NA
5. Increased efforts along the meat production chain	Reduction of 25% in scenarios 1, 2 and 3.	7361	57.8	NA

NA: Not Applicable

Table 28. Results of the intervention scenarios to reduce the risk of human salmonellosis attributable to the consumption of bovine meat outside the household. Illness cases: values are numbers of human salmonellosis cases associated to the consumption of meat-based meals in Kigali city inhabitant per year. Relative reduction: values are the relative reduction of salmonellosis cases in percentage comparatively to the baseline results.

Intervention scenario	Reduction level	Scenario outputs		
		Illness cases	Relative reduction	Intervention yield
Base line results		19195		
1. Reduction of *Salmonella* concentration on carcasses at the end of the slaughtering process.	Reduction of 25%	14398	25	1.0
	Reduction of 50%	9600	50	1.0
	Reduction of 75%	4800	75	1.0
2. Reduction of *Salmonella* prevalence in meat at retail.	Reduction of 25%	14800	22.9	0.9
	Reduction of 50%	9033	52.9	1.1
	Reduction of 75%	4798	75.0	1.0
3. Reduction of post cooking contamination probability in the kitchen.	Reduction of 25%	14398	25	1.0
	Reduction of 50%	9600	50	1.0
	Reduction of 75%	4800	75	1.0
4. Increased efforts along the meat production chain	Reduction of 25% in both scenarios 2 and 3.	11.102	42.2	NA
5. Increased efforts along the meat production chain	Reduction of 25% in scenarios 1, 2 and 3.	2329	87.9	NA

NA: Not Applicable.

Table 29. Results of the intervention scenarios to reduce the risk of human salmonellosis attributable to the consumption of goat meat outside the household. Illness cases: values are numbers of human salmonellosis cases associated to the consumption of meat-based meals in Kigali city inhabitant per year. Relative reduction: values are the relative reduction of salmonellosis cases in percentage comparatively to the baseline results.

Intervention scenario	Reduction level	Scenario outputs		
		Illness cases	Relative reduction	Intervention yield
Base line results		33983		
1. Reduction of *Salmonella* concentration on carcasses at the end of the slaughtering process.	Reduction of 25%	25494	25.0	1.0
	Reduction of 50%	17001	50.0	1.0
	Reduction of 75%	8503	75.0	1.0
2. Reduction of *Salmonella* prevalence in meat at retail.	Reduction of 25%	25502	25.0	1.0
	Reduction of 50%	16992	50.0	1.0
	Reduction of 75%	8510	75.0	1.0
3. Reduction of post cooking contamination probability in the kitchen.	Reduction of 25%	25494	25.0	1.0
	Reduction of 50%	17001	50.0	1.0
	Reduction of 75%	8503	75.0	1.0
4. Increased efforts along the meat production chain	Reduction of 25% in both scenarios 2 and 3.	19131	43.7	NA
5. Increased efforts along the meat production chain	Reduction of 25% in scenarios 1, 2 and 3.	14344	57.8	NA

NA: Not Applicable.

The risk of *Salmonella* illness associated with the consumption of goat brochettes outside the household was found to be two times higher comparatively to the one attributable to the consumption of beef within and outside the home. This could be explained by the fact that goat brochettes are generally composed of 5 to 7 meat portions and their consumption results in a significantly higher meat intake comparatively to the consumption of bovine meat, which is generally consumed in sauces. The recent meat consumption survey revealed that the daily consumption of goat meat was three times higher in comparison to bovine meat, and represented 66.3% of the global meat consumption outside the home in Kigali city inhabitants (Niyonzima et al., 2017a). Additionally, grilled goat brochettes were in most of times served with raw vegetables salads and this might be a source of *Salmonella* through cross contaminations. Several studies have reportedly associated human salmonellosis to the consumption of uncooked vegetable salads (Gadaga et al., 2008; Gómez-Aldapa el al., 2013; Gurler et al., 2015; Wijnands et al., 2014).

In most of developing countries including Rwanda, the purchasing power of female consumers was reported to be relatively low comparatively to men (Bastos et al., 2009; Burnet, 2011; Quisumbing et al., 2001). This could explain the low exposure of female inhabitants of Kigali city to the risk of *Salmonella* illness through meat-based meals consumed outside the home, as most of them cannot afford the purchase of these meals. Another reason would be the cultural considerations that limit women to frequent establishments such as snack-bars where meat-based-meals are generally consumed outside the home. According to the Rwandan customs, the consumption of alcoholic beverages is generally reserved to men (Adekunle, 2007), and this makes that women frequent less often than men the establishments such as snack bars where meat-based meals are sold together with alcoholic beverages. The relatively low exposure observed in young adults (18-30 years old) and in people from low and medium income households could be attributed to their limited financial capacity to afford the purchase meat-based meals. Various studies have reportedly associated the increase of meat consumption rate to the growth of the consumer's financial capacity (Heinz & Hautzinger, 2007; McAfee et al., 2010; Sans & Combris, 2015).

The findings from this study indicate that the risk of human salmonellosis associated to the consumption of meat-based meals in Kigali is relatively low and the estimated risk is mainly dependent of the current meat consumption rate in Kigali city inhabitants. Moreover, recent meat consumption surveys indicate that the current meat daily intake in Rwandan population is six times higher comparatively to that recorded in 2000s and is expected to increase with the countries growing economy (Niyonzima et al., 2017a; Speedy, 2003). Therefore, intervention strategies should be developed in order to face the risk of human *Salmonella* illness expected to grow with the increasing meat consumption rate in Kigali city inhabitants.

6.4.2. Scenario analysis

In the present study, different intervention scenarios aimed at reducing the risk of human salmonellosis attributable to the consumption of meat-based meals in Kigali city inhabitants were assessed. The reduction of *Salmonella* loads on carcasses at the end of the slaughtering process was found to decrease significantly the risk of human salmonellosis in Kigali city inhabitants. This could be attributed to the importance of the slaughtering process in regards to the ultimate microbiological quality and safety of meat, as the abattoir represents the first stage of the chain where the contamination of meat by *Salmonella* might occur (Niyonzima et al., 2015; Wong et al., 2002). Our results are in agreement with the findings of Grijspeerdt et al. (2007) who reported a significant reduction of human salmonellosis risk (68.5 to 99.9%) in Belgian pork meat consumers, through a decrease of *Salmonella* Typhimurium loads on pig carcasses by 0.5 to 2 logarithmic units.

The relative reduction of human salmonellosis risk associated with the decrease of *Salmonella* prevalence in retailed meat, highlights the important contribution of meat handling and storage practices within the retail establishments of Kigali city, on the final microbiological quality and safety of the commercialized meat pieces. Previous studies have identified factors such as the exposition of retailed meat at ambient temperature, the limited utilization of meat cutting boards in easy to clean material and the lack of training in hygienic meat handling practices for personnel; to be associated with an increased risk of *Salmonella* occurrence in meat commercialized within retail establishments of Kigali city (Niyonzima et al., 2016b). Therefore, improvements of these particular practices would contribute a lot in reducing the risk of *Salmonella* illnesses through the consumption of meat-based meals in Kigali city inhabitants.

Although various authors reported the growth of *Salmonella* during the transportation and the storage of meat before the preparation at home (Abdunaser et al., 2009; Delhalle et al., 2009b; Wong et al., 2002), these stages appeared to have a minimal effect on the risk of human salmonellosis associated with the consumption of meat-based-meals in Kigali city. In fact, due to the lack of cold storage equipments, the storage of raw meat within the households was generally not practiced. Most of inhabitants were generally buying meat in a limited quantity that they can prepare the same day. Furthermore, the cooking modes practiced in Rwanda were found to be destroying *Salmonella* cells that might have contaminated meat during the pre-cooking stages (Niyonzima et al., 2016a, 2017a). Therefore, the post cooking contamination appeared to be the most probable source of *Salmonella* in cooked meat-based meals consumed in Kigali city.

In our study, reducing the magnitude of post cooking cross contaminations of meat-based meals in the kitchen reduced significantly the risk of human *Salmonella* illness. Our findings are in agreement with the results from the study conducted in Belgium, where a relative reduction of human salmonellosis risk by 17 to 73 % was recorded

through the reduction of cross contaminations in the kitchen (Delhalle et al., 2009b). This would emphasize the importance of including consumers in the programs aimed at controlling microbial food borne diseases such as *Salmonella* illnesses. Furthermore, the higher yield in reducing human salmonellosis risk observed when the intervention strategies were applied concurrently at different stages of the chain; would suggest the need for a holistic approach, with combined efforts along the meat chain, to efficiently control the risk of *Salmonella* illness associated to the consumption of meat-based-meals in Kigali city.

6.5. Conclusion

The findings from this study indicate that the current risk of human salmonellosis associated to the consumption of meat-based meals in Kigali city is relatively low. However, as the level of meat consumption in Kigali city inhabitants is continuously increasing with the growing country's economy, there is a need to develop intervention strategies to face the risk of *Salmonella* illness expected to increase with the meat consumption rate. The applied interventions should involve all actors of the meat chain including the meat processors, retailers as well as consumers.

CHAPTER SEVEN: GENERAL DISCUSSION

CHAPTER 7. General discussion

The aim of the present study was to shade light on factors associated to the contamination of meat by *Salmonella* at different stages of the meat chain in Rwanda as well as assessing the risk of developing *Salmonella* illness through the consumption of meat-based meals in Kigali city inhabitants. In this section, the risk and protection factors of *Salmonella* occurrence in meat from the slaughtering stage to the consumption of meat-based meals within and outside the households of Kigali city are discussed. This discussion addresses also the effect of various intervention scenarios aimed at mitigating the risk of meat-borne *Salmonella* illness in Kigali city inhabitants.

7.1. Microbiological contamination of carcasses within slaughtering establishments

The study addressing the slaughtering practices and the microbiological quality of goat and bovine carcasses (Study N°1) was carried out in three establishments corresponding to the totality of officially registered slaughtering establishments for cattle and goats in Kigali. The choice of cattle and goat slaughtering establishments to study the microbiological contamination of meat in Kigali was justified by the fact that bovine and goat meat were the types of meat consumed the most in Kigali (Niyonzima et al., 2016a, 2017a).

The levels of hygiene indicator bacteria as well as the prevalence of *Salmonella* in slaughtered carcasses were found to vary from one establishment to another. The mean total bacterial count generally recognized as an indicator of general hygiene in the slaughtering establishment (Delhalle et al., 2008) varied from 2.9 to 5.7 and from 3.9 to 5.2 log cfu/cm^2 respectively in bovine and goat carcasses; whereas the mean *E. coli* counts known to be indicator of fecal contamination (ISO, 2001) ranged from 1.1 to 2.4 log cfu/cm^2 in bovine carcasses and from 1.6 to 1.8 log cfu/cm^2 in goat carcasses. *Salmonella* was detected in 21.3 and 8.0% of the analyzed bovine and goat carcasses respectively.

The level of bacterial contamination recorded in bovine and goat carcasses prepared within the slaughtering establishments of Kigali appeared to be relatively high and suggests the need of hygienic improvement in animal slaughtering and meat handling practices in some cattle and goat abattoirs of Kigali. According to the European regulation on microbiological criteria for foodstuffs (European Commission, 2005), bovine and goat carcasses are considered satisfactory when the mean total bacterial count is lesser than 3.5 log cfu/cm^2, acceptable when it ranges between 3.5 and 5.0 log cfu/cm^2 and unsatisfactory when it is greater than 5.0 log cfu/cm^2. Furthermore, *Salmonella* must be absent in the tested area of each carcass. Thus, only one of the evaluated slaughtering establishments was found with satisfactory carcasses for the total mesophilic bacteria whereas all of them were unsatisfactory for the occurrence of *Salmonella* with regard to the European regulations.

The prevalence of *Salmonella* on bovine carcasses prepared within the abattoirs of Kigali city (21.3%, n=150) appeared to be relatively low in comparison with that recorded in other developing countries such as Nigeria (Jajere et al., 2015) and Senegal (Stevens et al., 2006) where prevalences as high as 61.1% (n=54) and 42.8% (n=236) were respectively reported and associated to unhygienic slaughtering practices. However, the studies conducted in cattle slaughtering premises in Algeria (Nouichi & Hamdi, 2009) and Tunisia (Oueslati et al., 2016) showed a lower *Salmonella* prevalence in bovine carcasses (7.0 and 5.7% respectively). In goat carcasses, the prevalence recorded in our study (8.0%, n=150) was relatively low comparatively to that reported by Jajere et al. (2015) in Nigerian slaughterhouses (24.2%, n=66) and that of 32.2% (n=121) observed by Duffy et al. (2009) at two Australian goat abattoirs. However, it appears to be higher than that of 1.1% (n=90) observed in an ovine slaughterhouse in Algeria (Nouichi & Hamdi, 2009).

The limited number of the studied slaughtering establishments in Kigali city (n=3) did not allow to establish wheatear there is or not a statistical association between the animal slaughtering techniques practiced within these establishments and the occurrence of *Salmonella* in the prepared bovine or goat carcasses. However the slaughtering practices observed in some particular establishments could explain the higher levels of hygiene indicator bacteria as well as the prevalence of *Salmonella* recorded in goat and bovine carcasses. These include among others the exsanguination of animal lying on the ground, the manual skinning and the sanitation of slaughtering equipments with cold running water.

7.1.1. Sticking and exsanguination operations

The sticking step constitutes one of the slaughtering stages where microbiological contamination of processed carcasses occurs. Carcasses are most likely contaminated from unsanitized equipments such as knives and the soiled slaughtering environment. In the establishments where the exsanguination is performed on animals laying on the ground, the microbial contamination of the sticking wound is most likely to occur, as slaughtered animals struggle in the blood lying on the floor, throughout the entire duration of the bleeding period. Additionally, this practice appears to favor the soiling of animal hides by blood, which in turn increases the risk of carcass contamination during the skinning operations (McEvoy et al., 2000; Serraino et al., 2012). Several animal slaughtering protocols recommend the suspension of animals during exsanguination operations to minimize the risk of microbial contamination of the sticking wound as well as facilitating the bleeding process (FAO, 2006; Niyonzima et al., 2015).

7.1.2. Skinning and evisceration processes

The hide removal and evisceration steps are other slaughtering stages where carcass microbiological contaminations are most likely to occur, as hides and visceral contents constitute the major sources of carcass microbial contaminants (Sheridan, 1998; Wheatley, Giotis, & McKevitt, 2014). The slaughtering establishments found with highly contaminated carcasses in Kigali city (establishments A and C) appeared to be practicing the manual skinning during hide removal operations. This technique was reportedly associated to a higher risk of hide-to-carcass microbial contamination in comparison with the mechanical skinning by using a hide puller found to be practiced in the establishment B (Niyonzima et al., 2015). Our findings corroborated the FAO Good Practices in meat industry (FAO, 2006) that recommend the mechanical skinning by using a hide puller to minimize the hide-to-meat microbial contamination during skinning operations. Furthermore, the ligature of esophagus and rectum previously reported to reduce the risk of microbial contamination of carcasses from the visceral content during the evisceration process (Nesbakken et al., 1994; Sheridan, 1998), were found to be practiced only in the establishment B. This could explain the high levels of hygiene indicator bacteria as well as the prevalence of *Salmonella* in goat and bovine carcasses prepared within the establishments A and C, where that particular technique was not regularly practiced.

7.1.3. Sanitation of slaughtering equipments

The efficient sanitation of slaughtering equipments such as knives constitutes an important measure to prevent microbial cross-contaminations on processed meat carcasses. In our study, two slaughtering establishments (A and C) were found to be sanitizing slaughtering knives by simple cold water rinses. This knife sanitation technique appears to be inefficient and might have contributed to the occurrence of highly contaminated carcasses in these establishments. In a study conducted to determine the efficacy of sanitation procedures for slaughtering equipments, de Jong et al. (2008) indicated that cold water rinses did not reduce significantly the load of *Campylobacter jejuni* on meat cutting boards, however dipping knives in hot water at 82.2°C for 15 seconds reduced the loads of mesophilic bacteria and *Salmonella* DT104 by 3.1 and 2.3 logarithmic units respectively (Taormina & Dorsa, 2007). Several authors recommended the sanitation of knives and/or other slaughtering equipments with hot water at 82.0°C and above or another sanitizing system with an equivalent effect (European Commission, 2004; Goulter et al., 2008; Leps et al., 2013).

7.1.4. Hygiene of staff and processing environment

The hygiene of personnel and the working environment are one of the key determinants of the ultimate microbial quality and safety of the processed food products. Although number of hygienic practices regarding personnel and

the processing environment were comparable in all slaughtering establishments evaluated, some such as the disinfection of the slaughtering premises and the wearing of mask and gloves by personnel with direct contact with meat, were only reported in one establishment (establishment B); and might have contributed to the lower levels of hygiene indicator bacteria recorded on meat carcasses prepared in that particular establishment. The disinfection of processing premises was reportedly associated to a decreased risk of microbial contamination of the processed food products (Holah, 2014; Møretrø et al., 2012). Additionally, the regular wearing of gloves and masks by meat handlers appears to be an interesting measure to prevent meat microbial contamination by handlers as bare hands as well as uncovered oral and nasal cavities constitute an important source of microbial contaminants in food processing units (Bassis et al., 2014; Nel et al., 2004).

The findings from the study N°1 showed that the levels of hygiene indicator bacteria as well as the prevalence of *Salmonella* on bovine and goat carcasses processed within the slaughtering establishments of Kigali city were relatively high. This indicates a strong need for improvements in hygiene as well as in slaughtering practices within the abattoirs of Kigali city. The satisfactory levels, in regards with hygiene indicator bacteria, recorded on carcasses processed in some particular establishments; indicate that obtaining carcasses with high microbiological quality is possible, although important efforts are still needed, particularly in small scale establishments. Furthermore, the positive and significant correlation observed between the loads of hygiene indicator bacteria and the occurrence of *Salmonella* in the processed carcasses, would suggest that the safety of goat and bovine carcasses in Kigali, would be increased through hygiene improvements within the slaughtering establishments.

7.2. Microbiological contamination of meat cuts within retail establishments

The study N°2 addressed the meat handling conditions from the abattoir to the retail establishments as well as the microbiological quality and safety of the retailed meat. The findings from this study indicated that, in most of retail establishments of Kigali city, the transportation and exposition of retailed meat were carried out under ambient temperature conditions. This practice mostly attributed to the low financial capacity of retail establishments to afford the purchase of refrigerated cabinets (Kago et al., 2014; Niyonzima et al., 2015); was reportedly associated to the proliferation of microorganisms on retailed meat. Comparable meat handling practices were also reported in other developing countries such as Ethiopia (Haileselassie et al., 2013), Ghana (Adzitey et al., 2011), Kenya (Roesel et al., 2014), and the Democratic Republic of Congo (Kabwang, 2013) where they were associated to the proliferation spoilage microorganisms as well as the occurrence of microbial pathogens into the retailed meat.

The knowledge of workers in hygienic meat handling practices was also found to be relatively low particularly in small and medium retail establishments. This was highlighted by the low proportion of establishments whose

employees in the production area were regularly wearing protective clothes such as hairnets or gloves, that are recognized to be protecting handled food products against microbial contaminants from the workers (Heinz & Hautzinger, 2007; Lues et al., 2006). Additionally, the disinfection of the production and marketing areas was found to be rarely practiced in the evaluated meat retail establishments, and might have negatively affected the microbiological quality of the retailed meat cuts.

Nevertheless, it should be noted that meat retail establishments in Kigali city showed important efforts in preventing microbial contaminations from the processing environment through infrastructures such as easy to clean walls and floors, the application of pest control protocols, as well as the regular medical checkups for meat handling personnel; but these efforts seemed to be not sufficient to significantly reduce the levels of hygiene indicator bacteria as well as the prevalence of *Salmonella* in retailed meat.

The levels of hygiene indicator bacteria in meat cuts retailed within the establishments of Kigali (7.3±1.5 and 3.5±1.3 log cfu/g for total mesophilic bacteria and *E. coli* counts respectively) were found to be relatively high. This could be mainly associated to the unhygienic meat handling practices within the retail establishments and/or in earlier stages of the meat production chain. Our results corroborated the findings from the studies conducted in other developing countries such as the Democratic Republic of Congo (Kabwang, 2013), Ghana (Soyiri et al., 2008) and Pakistan (Hassan Ali et al., 2010) where higher loads of hygiene indicator bacteria in retailed meat cuts were mainly attributed to unhygienic meat handling practices. The prevalence of *Salmonella* in retailed meat cuts was also relatively high (19.6%) and appeared to be positively correlated to the loads of hygiene indicator bacteria.

The logistic regression analysis allowed to identify the factors associated to the risk of *Salmonella* occurrence in meat cuts retailed within the establishments of Kigali. These include the temperature conditions of meat during retailing, the cleanability of meat cutting boards as well as the training of meat handling personnel in hygienic practices.

7.2.1. Meat retail conditions

The risk of *Salmonella* occurrence was high in meat cuts retailed within the establishment where meat was exposed at ambient temperature. This could be explained by the proliferation of microorganisms initial present in retailed meat cuts during the time it is exposed at ambient temperature and their dissemination through cross contaminations. The assessment of meat retail conditions in Kigali city revealed that in 69.3% of the evaluated retail establishments, meat cuts were exposed under ambient temperature conditions for a period of 7 to 8 hours. These retail conditions appeared to be favorable to the proliferation of *Salmonella,* at its generation time in optimal temperature conditions (35-37°C) was reported to be only 25 minutes (Delhalle, et al., 2009b). Several

authors recommend the exposition meat cuts under refrigeration conditions at temperatures not more than 3 and 7°C respectively for offal and other types of meat; in order to prevent microbiological proliferations onto retailed meat cuts (European Commission, 2004; Lo Fo Wong et al., 2002).

7.2.2. Cleanability of meat cutting equipments

The utilization of wooden meat cutting boards (in most instances pieces of tree trunks) was found to be also associated to an increased risk of *Salmonella* occurrence in retailed meat cuts. This could be explained by the fact that material such as wood with rough surfaces, present numerous pores that may trap microorganisms and make that they are not easily accessible by sanitizing agents. Thus, trapped microorganisms may proliferate and get disseminated through cross contaminations (Carrasco et al., 2012). The utilization of contaminated equipments was reportedly associated to an increased risk of microbiological cross-contaminations in meat processing establishments (Heinz & Hautzinger, 2007; Small & Buncic, 2009; Warriner et al., 2002b).

7.2.3. Training of meat handling personnel

The risk of *Salmonella* occurrence in meat cuts was significantly reduced in retail establishments whose the personnel was trained in hygienic meat handling practices. This highlights the important contribution of trained meat handling personnel in assuring the microbiological quality and safety of the processed products. Our findings corroborate the results from previous studies that have reportedly associated the limited food safety knowledge and practices of personnel in food processing units, to a decreased quality of the processed products (Haileselassie et al., 2013; Muyanja et al., 2011; Samapundo et al., 2015).

The findings from the study N°2 revealed that, despite the efforts made by meat retail establishments in Kigali city to prevent meat contamination from the processing environment such as processing facilities with easy to clean walls and floor, application of pest control protocols, regular medical checkups for meat handling personnel, etc.; the levels of hygiene indicators bacteria as well as the prevalence of *Salmonella* in retailed meat cuts were still relatively high. This highlights the need for further improvements in hygienic meat handling practices. Important efforts should be particularly convoyed in addressing the issue of exposing meat cuts under retail at ambient temperature and training meat handlers in basic hygienic practices; as these factors were found to be associated to an increased risk of *Salmonella* occurrence in retailed meat.

7.3. Meat consumption patterns and microbiological quality of cooked meat-based meals

The studies N°3 and 4 addressed the consumption patterns of meat-based-meals in Kigali city inhabitants as well as the microbiological quality and safety of the consumed products either at the household level or within snack-bars and restaurants.

7.3.1. Meat-based meals consumed within the households

At the household's level, beef was found to be the type of meat consumed the most. This could be mainly attributed to the fact that cattle are of great socio-cultural importance in Rwandan society (Adekunle, 2007). The estimated meat daily intake in evaluated households was varying with the socio-economical category of the household. Our findings corroborated the results from previous studies that reported a significant and positive correlation between the meat consumption rate and the financial capacity of consumers (McAfee et al., 2010; Sans & Combris, 2015; Speedy, 2003). Meat was in most instances consumed in sauces, which is the most appropriate culinary form to accompany rice or maize pastas dishes generally consumed at household level in central African countries (Kabwang, 2013).

The cooking process of meat-based meals practiced within the households of Kigali city appeared to significantly reduce the load of hygiene indicator bacteria (total mesophilic bacteria and *E. coli* counts) as well as the prevalence of *Salmonella* in cooked meat. This might be mainly attributable to the long cooking durations (about 2 to 3 hours) generally used for the preparation of meat-based dishes. Comparable cooking practices for meat-based dishes were reported in other African countries such as the Democratic republic of Congo (Kabwang, 2013) and Zambia (Manyori et al., 2017), where they were associated to a total destruction of vegetative bacterial cells in cooked meat. Furthermore, previous studies have reported that enteric bacteria such as *Salmonella* were generally destroyed at conventional pasteurization temperatures (Jay et al., 2005; Korsak et al., 2004). Therefore, the recorded microbial contamination of some meat-based meals prepared in low and medium income households would suggest a post-cooking contamination.

The microbiological contamination of cooked meat-based meals particularly observed in low income-households, could be explained by the limited financial capacity of people from these households to afford the required material for an efficient kitchen and personal hygiene. Our findings are in agreement with previous studies conducted in various developing countries where diarrheal diseases associated to unhygienic practices were found to be most prevalent in poor households (Guerrant et al., 1999; Zwane & Kremer, 2007).

The study N°3 revealed that beef was the type of meat consumed the most within the households of Kigali city. Additionally, the cooking method practiced at the household level was found to reduce significantly the loads of

hygiene indicator bacteria as well as the prevalence of *Salmonella* in cooked meat-based meals. Nevertheless, the occurrence of *Salmonella* recorded in some cooked meat-based meals suggests a post cooking contamination; and calls for improvements in personal and kitchen hygiene particularly in poor households.

7.3.2 Meat-based meals consumed outside the home

In Kigali city, the consumption of meat-based meals outside the home was found to be particularly practiced by people from middle and high income households. This could be explained by the fact that the cost of these meals in snack-bars and restaurants was relatively high and not affordable for the majority of Kigali city inhabitants from poor households. Our observations corroborated the findings from previous studies, that identified the wealth as one of the major determinants of food consumption away from home (Ma et al., 2006; Maupeu & Wa-Mûngai, 2006). Grilled goat brochettes were found to be the meat-based-meal principally consumed outside the home in Kigali, and contrary to the consumption of meat-based meals within the households, the daily meat intake outside the home appeared to be low in young adults and female consumers. This could be attributed to the low financial capacity of young adults and female consumers to afford meat-based meals outside the home. Furthermore, as according to the Rwandan customs, the consumption of alcohol is generally reserved for men (Adekunle, 2007), female consumers do not frequent as men do, establishments such as snack-bars and restaurants, where meat-based meals are generally sold together with alcoholic beverages.

The prevalence of *Salmonella* in the consumed meat-based products was found to be positively correlated to the loads of hygiene indicator bacteria, and appeared to vary from one product to another. The prevalence of *Salmonella* was lower in boiled meat-based meals comparatively to the grilled or fried ones. This might be attributed to the higher time and temperature treatments applied to boiled meals during the cooking process. In the majority of establishments of Kigali city (94%), meat was boiled for more than one hour; whereas the grilling and frying process for meat was performed for shorter periods, suggesting the possible survival of *Salmonella* in grilled or fried meat. Our findings are in agreement with previous studies that reported the survival of *Salmonella* in undercooked meat, particularly in large meat pieces such as whole chicken or steaks (Breslin et al., 2014; Lahou et al., 2015; Roccato et al., 2015). Another source of *Salmonella* in fried and grilled meat might be the non-meat components of the meal. In fact, most of the evaluated establishments were found to serve grilled or fried meat with raw vegetable salads that could be a source of *Salmonella* through cross-contamination. Several authors have reported the isolation of *Salmonella* in ready-to-eat vegetable salads (Gómez-Aldapa et al., 2013; Wijnands et al., 2014).

The logistic regression analysis allowed the identification of a number of factors significantly associated to the risk of *Salmonella* occurrence in meat-based meals consumed outside the home in Kigali city, and most of these

factors were linked to the applied cooking practices and kitchen cross-contaminations. Thus, the findings from the study N°4 highlighted an important need for hygiene improvements during meat preparation and service as well as the cooking processes within the establishments selling meat-based meals of Kigali; to mitigate the risk of salmonellosis attributable to the consumption of contaminated meat in Kigali city inhabitants.

7.4. Risk assessment of meat borne *Salmonella* illness in Kigali city inhabitants

The study N°5 assessed the level of exposure of Kigali city inhabitants to the risk of *Salmonella* illness associated to the consumption of meat-based meals. In this study, three risk exposure pathways were considered namely the consumption of beef-based meals within the household, the consumption beef-based meals outside the home as well as the consumption of goat meat outside the home as bovine and goat meat were reportedly the types of meat consumed the most in Kigali (Niyonzima et al., 2016a, 2017a).

In all evaluated risk exposure pathways, the probability of *Salmonella* illness to occur was found to be increasing with the meat consumption rate in different socio-economical categories of consumers. This could be explained by the fact that the meat consumption rate is positively correlated to the financial capacity of consumers (Sans & Combris, 2015), and the level of risk exposure is a directly dependent of the food intake (Evers & Chardon, 2010; Manyori et al., 2017). Furthermore, the level of risk exposure in young adults and female consumers; appeared to be relatively low, as a direct consequence of low meat intakes in these particular categories of Kigali city inhabitants. Similarly, the consumption of grilled goat brochettes outside the home was associated to an increased risk of *Salmonella* illness in Kigali city inhabitants comparatively to other risk exposure pathways, as the meat intake and the prevalence *Salmonella* associated to that particular meal were higher (Niyonzima et al., 2017a).

The important limitation of the present study was the lack of data on Rwanda for some variables of the quantitative risk assessment model. As an alternative solution, data from the published literature were used in place; and consequently, the outputs of the model might not represent accurately the Rwandan meat chain. However, the missing data were identified and further research in that direction would contribute to improve the quality and accuracy of the model outputs.

Another limitation would be the assumption in the QMRA model, that the used cooking practices in Kigali city allow the total destruction of *Salmonella* and that the observed contamination is attributable to a post-cooking contamination of meat-based meals. The used information on culinary practices in Rwanda derived from two studies conducted within the households as well as in collective catering establishments in Kigali city, whose data was collected through interviews (Niyonzima *et al.*, 2016a, 2017a). The methodology used for data collection might have introduced a bias in the recorded findings, as the interviewees could have provided erroneous information on their culinary practices in order to protect the image of their household or catering establishment.

However, as the culinary habits recorded in Rwanda were found to be similar to that practiced in other African countries with comparable cultures (Kabwang, 2013; Manyori *et al.*, 2017), the introduced bias seems to be minimal.

Although the current risk of *Salmonella* illness associated to the consumption of meat-based meals in Kigali city inhabitants appears to be relatively low (1.7-3.4%); it is expected to increase with the rising meat consumption rates in the Rwandan population, associated to the country's growing economy. Therefore, there is an important need for developing appropriate intervention strategies along the Rwandan meat chain to mitigate the raised risk of *Salmonella* illness that might occur in the near future.

7.5. Scenario analysis for risk mitigation along the meat chain

The study N°5 described the results of five scenarios aimed at mitigating the risk of *Salmonella* illness attributable to the consumption of meat-based meals in Kigali city inhabitants. The evaluated scenarios concerned different stages of the meat chain namely abattoirs, retail establishments as well as the consumer's kitchen.

The first scenario dialed with the reduction of *Salmonella* concentration on goat and bovine carcasses at the end of slaughter. The results from the model showed that the reduction of *Salmonella* concentration on goat and bovine carcasses at the end of slaughter by 25 to 75% would reduce the risk of salmonellosis up to 81.1%. These results corroborate the findings from previous studies where abattoirs were identified as one of the key stages in the control of microbiological contaminations along the meat chain (Bollaerts et al., 2010; Delhalle, et al., 2009a). The reduction of *Salmonella* concentration on carcasses slaughtered in abattoirs of Kigali would require important efforts through the slaughtering processes, and the study N° 1 has offered important orientations on the slaughtering steps requiring a particular interest. Furthermore, the low level of microbial contamination observed on carcasses prepared in some establishments, indicate that obtaining goat and bovine carcasses with low bacterial loads is possible in slaughtering establishments of Kigali city.

The second scenario addressed the reduction of *Salmonella* prevalence in meat cuts under retail within the establishments of Kigali and appeared to significantly contribute to the reduction of salmonellosis cases attributable to the consumption of meat-based meals. The microbiological quality of meat cuts under retail is reportedly dependant of the quality of the used raw material in occurrence meat carcasses; but also relies on meat handling practices used within the retail establishment (Lo Fo Wong et al., 2002; Niyonzima et al., 2015). The study N°2 identified factors such as the exposition of retailed meat cuts at ambient temperature, the cleanability of meat cutting equipments as well as the limited knowledge of workers in hygienic meat handling practices to be associated to an increased risk of *Salmonella* occurrence in retailed meat cuts. Therefore, efforts addressing these

particular risk factors should be deployed to assure a significant reduction of the prevalence of *Salmonella* in meat cuts retailed within the establishments of Kigali.

The third scenario concerned the microbiological contamination of meat-based meals within the consumer's kitchen. The findings from studies N°3 and 4 revealed that the cooking methods practiced in Kigali city reduced significantly the probability of *Salmonella* occurrence in the consumed cooked meat-based meals, and the cross contamination appeared to be the main determinant of *Salmonella* occurrence in cooked meals. According to the model, the cross contaminations during the cooking and serving processes should be reduced to significantly mitigate the risk of *Salmonella* illness associated to the consumption of meat-based meals. Therefore, the consumer must be informed through education campaigns of his important role in controlling microbial food borne diseases such as salmonellosis.

The fourth and fifth scenarios assessed the effect of a simultaneous application of control measures at various stages of the meat production chain. According to the model, these scenarios resulted in higher risk reduction yields in comparison to other measures when they were applied alone. This important finding indicates that an efficient control of food borne diseases such as salmonellosis cannot be achieved through an individual application of control measures at different segments of the chain; but through a coordinated and holistic approach involving all actors of the production chain; including meat processors, retailers, consumers and surveillance authorities.

CONCLUSION & PERSPECTIVES

CONCLUSION AND PERSPECTIVES

The present study addressed the factors associated to the risk of *Salmonella* occurrence in meat at different stages of the chain from the slaughterhouse to the consumption, and assessed the risk of *Salmonella* illness in Kigali city inhabitants attributable to the consumption of meat-based meals. Although the prevalence of *Salmonella* was relatively high in the consumed meals, the risk of *Salmonella* illness attributable to the consumption of these meals appeared to be relatively low in Kigali city inhabitants; principally because of the low meat intake particularly in poor households. However, as the rate of meat consumption is increasing with the country's growing economy; the findings from this study revealed an important need for intervention measures along the meat chain to mitigate the risk of meat-borne human salmonellosis, expected to increase with the financial capacity of Kigali city inhabitants. For a better reduction of the risk, the used interventions must be applied simultaneously and involve all actors of the meat chain from the slaughterhouse to the consumer.

At the slaughtering level, interventions should concern the strengthening of existing slaughtering establishments through capacity building in good slaughtering and hygienic practices, as well as the construction of abattoirs for animal species other than ruminants, particularly poultry and pigs. The existing carcass inspection protocols at the slaughterhouse level should also be improved by introducing the regular microbiological analysis of processed meat carcasses. These analyses could help a lot the concerned establishments as well as competent authorities to check if they comply with national standards regarding the quality and the safety of meat.

In meat retail establishments, interventions should be particularly oriented toward the improvement of hygienic meat handling practices as well as temperature conditions for the commercialized meat cuts. A special emphasis should be put a on the use of low temperatures during the storage as well as the exposition of retailed meat pieces. The control of meat retail establishments, by competent authorities, for compliance to national standards in meat hygiene should also be enhanced particularly in peri-urban regions.

The establishments of collective catering such as snack-bars and restaurants must also be involved in the control of meat borne salmonellosis. In these establishments, interventions measures should be specially oriented towards the training of employee in good cooking and hygienic practices respectively, to assure the total destruction of *Salmonella* as well as reducing the risk of post-cooking contamination in cooked meat-based meals. The competent authorities must particularly assure that the authorized catering establishments comply with the national standards in term of hygienic infrastructures.

Although all actors of the meat production chain are of great importance in the control of meat-borne salmonellosis, consumers appear to be of a particular importance as they are the last beneficiaries of meat-based

meals. Therefore, significant efforts should be devoted to raising consumer's awareness about their role in the control of food borne diseases and particularly salmonellosis. Consumers should be especially informed of appropriate cooking practices to assure the destruction of *Salmonella* spp. during the preparation of meat-based meals at the household's level, as well as basic hygienic practices to prevent post-cooking contaminations in prepared meals. The sensitization of consumers should be designed in a manner to reach all socio-economical categories of the population particularly poor households. Thus, the monthly community work generally organized in each village in Rwanda, would be a great occasion to demonstrate good cooking and hygienic practices for meat. The public health officials would help in the preparation of teaching modules and organizing the training of village officials in charge of social affairs, who in turn would organize the sensitization activities at the village level.

The present study was the first research contribution to shade light on the risk of *Salmonella* illness attributable to the consumption of meat based meals in Kigali (Rwanda). However, it cannot pretend having fully explored all risk factors along the meat production chain. In fact, the used risk assessment model started only at the slaughterhouse level and appeared to be hampered by the lack of data for some model variables. Therefore, further studies addressing the pre-slaughter segment of the chain as well as the model variables where data were identified as missing, could contribute a lot in enhancing the accuracy of the model outputs. Furthermore, epidemiological studies comparing *Salmonella* strains isolated from meat at different levels of the chain to these isolated from human salmonellosis patients should be conducted to confirm the contribution of meat-based-meals to the human salmonellosis in Kigali city inhabitants.

Additionally, the present risk assessment study dialed only with *Salmonella* species though there are other bacterial pathogens of food safety interest in the meat chain such as pathogenic *E.coli*. Therefore, this study should be a starting point for further microbiological risk assessment studies on meat or other food commodities in Rwanda.

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APPENDICES

APPENDICES

QUESTIONNAIRE N°1. Facteurs de risque et/ou de protection de la contamination de la viande dans les abattoirs.

Section A. Identification

Nom de l'Etablissement	**Adresse de l'établissement**	
	District	
Nom de la personne de contact	**Secteur**	
	Cellule	

Section A. Chaine d'abattage

1. Quelle est le volume d'abattage par semaine de votre établissement ?
2. Quelle est la provenance des animaux abattus dans votre établissement ?
3. Comment les animaux sont –ils acheminés à l'abattoir ?
4. Pour combien de temps les animaux sont-ils gardés en attente avant l'abattage ?
5. Au parc de stabulation, le jeun de 24 heures est-il observé ?
6. Un nettoyage et une désinfection du parc de stabulation sont – ils réalisés régulièrement ? Si oui à quelle fréquence ?
7. Quels sont les produits de nettoyage et de désinfection utilisés ?
8. L'inspection *ante mortem* des animaux est-elle systématiquement réalisée ?
9. Les animaux venant du parc de stabulation, sont-ils lavés avant l'entrée au bloc d'abattage ? Si oui par quelle méthode ?
10. Les animaux sont-ils étourdis avant l'abattage ? Si oui par quelle méthode ?
11. L'égorgement et la saignée, sont-ils réalisés sur les animaux suspendus ou sur les animaux étendus au sol ?
12. Au cours de la saignée, le sang est-il collecté ? si oui par quelle méthode ?
13. Les couteaux d'égorgement, sont-ils stérilisés ? Si oui à quelle température[1] et à quelle fréquence ?
14. Combien de temps[2] passe t-il entre l'étape de la saignée et celle de l'écorchage ?
15. L'écorchage des animaux, est il réalisé manuellement ou à la machine ?
16. Les couteaux d'écorchage, sont-ils stérilisés ? Si oui à quelle température[3] et à quelle fréquence ?
17. Existe-t-il une séparation dans l'espace entre la zone réservée au de traitement des peaux et la chaine de préparation des carcasses ?
18. La ligature de l'œsophage et de l'anus sont-elles réalisées avant l'éviscération ?
19. Les couteaux d'éviscération, sont-ils stérilisés ? Si oui à quelle température [4] et à quelle fréquence ?
20. Existe-il dans l'abattoir une ligne d'abattage sur laquelle sont préparées les carcasses accidentellement contaminées ?
21. Que faites-vous de la carcasse accidentellement contaminée au cours de l'éviscération?
22. Existe-t-il une séparation dans l'espace entre la zone réservée au traitement des viscères et la chaine de préparation des carcasses ?
23. La fente des carcasses bovines est elle réalisée systématiquement ?

[1] Mesure de la température de stérilisation des couteaux.
[2] Mesure du temps entre la saignée et écorchage à chaque visite
[3] Mesure de la température de stérilisation des couteaux
[4] Mesure de la température du stérilisateur des couteaux

24. Procédez-vous par un nettoyage et/ou une désinfection de la scie utilisée pour la fente des carcasses ? Si oui comment et à quelle fréquence ?
25. Les carcasses bovines sont – elles lavées après la fente ? Si oui par quelle méthode ?
26. Effectuez-vous les analyses de l'eau en contact avec la viande ? Si oui à quelle fréquence et quels sont les paramètres envisagés ?
27. Procédez-vous par une réfrigération systématique des carcasses ?
28. Contrôlez-vous régulièrement la température des carcasses au cours de la réfrigération ? Si oui à quelle fréquence ?
29. Combien de temps faut-il aux carcasses réfrigérées pour attendre 7°C à cœur?
30. Au cours du processus d'abattage, procédez-vous au changement de crochets de suspension des carcasses ? Si oui à quelles étapes d'abattage ?

Section B. Personnel de l'abattoir

31. Le personnel de l'abattoir a-t-il reçu une formation ou information sur les bonnes pratiques d'hygiène ? Si oui laquelle ?
32. Le personnel de l'abattoir dispose t-il d'une tenue de travail réservée au travail des viandes ?
33. A quelle fréquence le personnel change –t – il de tenue de travail ?
34. Effectuez- vous un contrôle de l'hygiène personnelle et vestimentaire des employés ? Si oui par quelle méthode et à quelle fréquence ?
35. Existe-il des mesures en application dans votre établissement, garantissant que le personnel ne contamine pas la viande qu'il manipule, par des germes dont il serait porteur ? Si oui lesquelles ?

Section C. Nettoyage et désinfection

36. A quelle fréquence effectuez-vous un nettoyage et /ou une désinfection de la ligne d'abattage et de tous les équipements en contact direct avec la viande ? Quels sont les produits utilisés ?
37. A quelle fréquence effectuez-vous un nettoyage et / ou une désinfection des locaux et des équipements qui ne sont pas en contact direct avec la viande ? Quels sont les produits utilisés ?
38. L'abattoir réalise-t-il des contrôles de l'efficacité du nettoyage et de la désinfection? Si oui par quelles méthodes et à quelle fréquence ?

Section D. Gestion de déchets et lutte contre les animaux nuisibles

39. Existent-ils des mesures pour rassembler, stocker et évacuer les déchets de façon à ce que ceux-ci ne constituent pas une source de contamination de la viande (zone de rassemblement et fréquence d'évacuation) ? Si oui décrivez-les.
40. Existent-ils des mesures application dans votre établissement, pour maîtriser ou prévenir le risque d'animaux nuisibles sur le site de production ou dans l'établissement ? Si oui lesquelles ?

QUESTIONNAIRE N°2. Facteurs de risque et/ou de protection de la contamination de la viande dans les ateliers de découpe.

Section A. Identification

Nom de l'Etablissement	Adresse de l'établissement	
	District	
Nom de la personne de contact	Secteur	
	Cellule	

Section B. Matières premières

1. Par quel moyen les carcasses bovines sont – elles transportées de l'abattoir à votre établissement ?
2. Quelle est la durée du transport des carcasses bovines de l'abattoir à votre établissement ?
3. Contrôlez-vous la température des carcasses au cours du transport et/ou celles des enceintes utilisées pour le transport des carcasses ? Si oui à quelle fréquence ?
4. A quelle température conservez-vous les carcasses[5] bovines dans votre établissement ?
5. Contrôlez-vous régulièrement la température des carcasses dans votre chambre froide? Si oui à quelle fréquence ?

Section C. Chaine de découpe

6. Quelle est la durée de l'opération de découpe pour un lot de produit ?
7. Les couteaux et autres instruments de découpe sont ils régulièrement stérilisés ? Si oui à quelle température[6] et à quelle fréquence ?
8. Effectuez-vous les analyses de l'eau en contact avec la viande ? Si oui à quelle fréquence et quels sont les paramètres envisagés ?
9. A quelle fréquence effectuez-vous un nettoyage et /ou une désinfection de la chaine de découpe et de tous les équipements en contact direct avec la viande ? Quels sont les produits utilisés ?
10. A quelle fréquence effectuez-vous un nettoyage et / ou une désinfection des locaux et des équipements qui ne sont pas en contact direct avec la viande ? Quels sont les produits utilisés ?
11. L'atelier réalise-t-elle des contrôles de l'efficacité du nettoyage et de la désinfection? Si oui par quelles méthodes et à quelle fréquence ?

Section D. Personnel

12. Le personnel de l'atelier a-t-il reçu une formation ou information sur les bonnes pratiques d'hygiène ? Si oui laquelle ?
13. Le personnel de l'atelier dispose t-il d'une tenue de travail réservée au travail des viandes ?
14. A quelle fréquence le personnel change –t – il de tenue de travail ?
15. Effectuez- vous un contrôle de l'hygiène personnelle et vestimentaire des employés ? Si oui par quelle méthode et à quelle fréquence ?
16. Existe-il des mesures en application dans votre établissement, garantissant que le personnel ne contamine pas la viande qu'il manipule, par des germes dont il serait porteur ? Si oui lesquelles ?

[5] Mesure de la température des carcasses

[6] Mesure de la température de stérilisation des couteaux

Section E. Produits finis

17. Les produits fini sont – ils conservés séparément des matières premières ?
18. A quelle température[7] conservez-vous les produits finis ?
19. Contrôlez-vous régulièrement la température des chambres froides ou autres enceintes frigorifiques dont vous disposez ? Si oui à quelle fréquence ?
20. Existe-t-il des mesures en application dans votre établissement, permettant de garantir que les produits les plus anciens sont expédiés les premiers ? Si oui lesquelles ?

Section F. Gestion de déchets et lutte contre les animaux nuisibles

21. Existent-ils des mesures pour rassembler, stocker et évacuer les déchets de façon à ce que ceux-ci ne constituent pas une source de contamination de la viande (zone de rassemblement et fréquence d'évacuation) ? Si oui décrivez-les.

22. Existent-ils des mesures application dans votre établissement, pour maîtriser ou prévenir le risque d'animaux nuisibles sur le site de production ou dans l'établissement ? Si oui lesquelles ?

[7] Mesure de la température des produits finis

QUESTIONNAIRE N°3. Facteurs de risque et/ou de protection de la contamination de la viande dans les établissements de la vente au detail

Section A. Identification

Nom de l'Etablissement	Adresse de l'établissement	
	District	
Nom de la personne de contact	Secteur	
	Cellule	

Section B. Conditions de transport, stockage et commercialisation de la viande

1. Quelle est la quantité de viande bovine commercialisée dans votre établissement par semaine ?
2. Par quel moyen les carcasses bovines sont – elles transportées de l'abattoir à votre établissement ?
3. Quelle est la durée du transport des carcasses bovines de l'abattoir à votre établissement ?
4. Contrôlez-vous la température des carcasses au cours du transport et/ou celles des enceintes utilisées pour le transport des carcasses ? Si oui à quelle fréquence ?
5. A quelle température[8] conservez-vous les carcasses bovines dans votre établissement ?
6. Contrôlez-vous régulièrement la température des carcasses dans votre chambre froide? Si oui à quelle fréquence ?
7. Dans quelles conditions de température la viande est elle vendue (à température ambiante, vitrine réfrigérée) ? Combien de temps la viande peut elle rester dans ces conditions ?
8. Contrôlez-vous régulièrement la température[9] des vitrines réfrigérées ? Si oui à quelle fréquence ?

Section C. Hygiène des locaux et des équipements de préparation de viandes

9. Les couteaux et autres instruments[10] de découpe sont ils régulièrement stérilisés ? Si oui à quelle température et à quelle fréquence ?
10. A quelle fréquence effectuez-vous un nettoyage et /ou une désinfection des équipements en contact direct avec la viande ? Quels sont les produits utilisés ?
11. A quelle fréquence effectuez-vous un nettoyage et / ou une désinfection des locaux et des équipements qui ne sont pas en contact direct avec la viande ? Quels sont les produits utilisés ?
12. La boucherie réalise-t-elle des contrôles de l'efficacité du nettoyage et de la désinfection? Si oui par quelles méthodes et à quelle fréquence ?

Section D. Personnel

13. Le personnel a-t-il reçu une formation ou information sur les bonnes pratiques d'hygiène ? Si oui laquelle ?
14. Le personnel dispose t-il d'une tenue de travail réservée au travail des viandes ?
15. A quelle fréquence le personnel change –t – il de tenue de travail ?
16. Effectuez- vous un contrôle de l'hygiène personnelle et vestimentaire des employés ? Si oui par quelle méthode et à quelle fréquence ?

[8] Mesure de la température des carcasses
[9] Mesure de la température des produits en vente
[10] Mesure de la température de stérilisation des couteaux

17. Existe-il des mesures en application dans votre établissement, garantissant que le personnel ne contamine pas la viande qu'il manipule, par des germes dont il serait porteur ? Si oui lesquelles ?

Section E. Gestion de déchets et lutte contre les animaux nuisibles

18. Existent-ils des mesures pour rassembler, stocker et évacuer les déchets de façon à ce que ceux-ci ne constituent pas une source de contamination de la viande (zone de rassemblement et fréquence d'évacuation) ? Si oui décrivez-les.

19. Existent-ils des mesures en application dans votre établissement, pour maîtriser ou prévenir le risque d'animaux nuisibles sur le site de production ou dans l'établissement ? Si oui lesquelles ?

QUESTIONNAIRE N°4. Modes de consommation et de préparation de viandes au sein du ménage et dans les établissements de la restauration collective.

Section A. Identification et caractéristiques du ménage (ou établissement)

Adresse du ménage	
District	
Secteur	
Cellule	

Catégorie d'âge des membres du ménage			
6 -15 ans		36 -45 ans	
16 -25 ans		46-55	
26 -35 ans		56 et plus	

Section B. Habitudes du consommateur (Achat, conservation et préparation de la viande)

1. Ou achetez-vous fréquemment la viande ?
    a. Au marché central
    b. A la boucherie de votre localité
    c. Au supermarché
    d. Autre à préciser

2. Quel type de viande achetez-vous fréquemment ?
    a. La viande maigre non conditionnée
    b. La viande maigre conditionnée
    c. La viande hachée non conditionnée
    d. La viande hachée conditionnée
    e. Les reins
    f. Le foie
    g. Les intestins
    h. L'estomac

3. Quelle est la quantité de viande bovine vous achetez par mois ?

4. Pour combien de temps conservez la viande dans votre domicile avant sa préparation ?
    a. 1 heure
    b. 2 heures
    c. 2 jours
    d. Plus de 2 jours

5. Par quel moyen conservez-vous la viande à domicile ?
    a. A la température ambiante
    b. Au réfrigérateur
    c. Au congélateur
    d. Autres à préciser

6. Quelle est la méthode de préparation de viande utilisez-vous fréquemment dans votre domicile ?
    a. Bouillir dans l'eau
    b. Frire dans l'huile
    c. Bouillir puis frire
    d. Autre à préciser

Section C. Consommation de la viande par ménage (réservée aux ménages)

6. A quelle fréquence consommez-vous de la viande bovine dans votre ménage ?
    a. Chaque jour
    b. 3 fois la semaine
    c. 1 fois la semaine
    d. 2 fois le mois
    e. 1 fois le mois
    f. Autre à préciser
7. Quelle est la quantité de viande bovine consommée par une personne de votre ménage âgée de 6 à 15 ans par jour (une photo d'une portion de viande maigre de 50g)?
    a. La moitié de la portion
    b. Une portion
    c. Deux portions
    d. Autre à préciser
8. Quelle est la quantité de viande bovine consommée par une personne de votre ménage âgée de 16 à 25 ans par repas (une photo d'une portion de viande maigre de 50g) ?
    a. La moitié de la portion
    b. Une portion
    c. Deux portions
    d. Autre à préciser
9. Quelle est la quantité de viande bovine consommée par une personne de votre ménage âgée de 26 à 35 ans par repas (une photo d'une portion de viande maigre de 50g) ?
    a. La moitié de la portion
    b. Une portion
    c. Deux portions
    d. Autre à préciser

10. Quelle est la quantité de viande bovine consommée par une personne de votre ménage âgée de 36 à 45 ans par repas (une photo d'une portion de viande maigre de 50g) ?
    a. La moitié de la portion
    b. Une portion
    c. Deux portions
    d. Autre à préciser

11. Quelle est la quantité de viande bovine consommée par une personne de votre ménage âgée de 46 à 55 ans par repas (une photo d'une portion de viande maigre de 50g) ?
    a. La moitié de la portion
    b. Une portion
    c. Deux portions
    d. Autre à préciser
12. Quelle est la quantité de viande bovine consommée par une personne de votre ménage âgée de plus de 56 ans par repas (une photo d'une portion de viande maigre de 50g) ?
    a. La moitié de la portion
    b. Une portion
    c. Deux portions
    d. Autre à préciser