Prevalence, hygienic status and exposure assessment of *Salmonella* spp. in milk and dairy chain in Rwanda

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Omnia labor vincit improbus

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Thesis submitted in fulfillment of the requirements for the degree of Doctor (PhD) in Applied Biological Sciences

Vertaling van de titel :

Prevalentie, hygiëne status en blootstelling van *Salmonella* spp. in de Rwandese melk- en zuivelketen

To refer to this thesis:

Kamana, O. 2015. Prevalence, hygienic status and exposure of *Salmonella* spp. in the milk and dairy chain in Rwanda. Thesis submitted in fulfillment of the requirements for the degree of doctor (Ph.D.) in Applied Biological Sciences. Faculty of Bioscience Engineering, Ghent University.

ISBN number: **978-90-5989-832-5**

ACKNOWLEDGEMENTS

Firstly, I would like to thank my promoter, Prof.dr.ir. Mieke Uyttendaele, for her guidance during my PhD. I still remember when I sent her an e-mail, in 2009 asking for mentorship, and she immediately agreed. From then, working with her was motivating and inspiring. I learnt a lot from her way of managing people and time. I appreciated her way of following my progress with a good understanding of my work conditions in Rwanda. When I needed a prolongation of stay due to practical difficulties she was always willing to support me for all the official authorizations I needed.

Mieke, thanks for leading me and encouraging me and transforming me from a simple ITP trainee to a PhD holder !

Secondly I would like to thank Prof. dr.ir Liesbeth Jacxens who supervised me during this work as co-promoter. She was there day in and day out, following me carefully, and providing skills and knowledge I needed. I learned from her the ability to deliver a properly done work on time. Everytime I had to fly from Kigali, my first commitment was to cope with her weekly deadlines. This helped me to progress and achieve my goals. Especially FOR this last stay from November 2014, I really enjoyed working with her.

Liesbeth ,thank you for helping me to achieve my goals, thanks for your guidance and your daily support !

I would like also to thank my local promoter Prof.dr.rer.nat Anastase Kimonyo who accepted to supervise my local activities and was always available to help me to find my way when I had to be facing practical difficulties on field. I enjoyed working with him and I highly benefited from his valuable experience.

Anastase, thank you for your entire support !

Besides those Professors, I also worked with Dr. ir. Siele, who helped me a lot in my data processing. It was not at all easy for me to re-discover statistics some years ago. She was there all the time , correcting my "poor" English and inspiring me in scientific writing.

Siele, thank you so much ! I will always remember the tips you taught me for scientific writing !

This work would not have been possible without the support from my home institution, the Higher Institute of Agriculture and Animal Husbandry (recently incorporated in the University of Rwanda). I am grateful to late Ir. Makelele Kilyobo, the former director of laboratories , and Ir. Bonaventure Hakizimana, the head of microbiology lab, who were there to give me all the support I needed. I am also thankful for the support I received from the students who worked with me during this work for sample collection and laboratory analyses and for the consumer survey : Eric, Arcade, Evalde, Robert, Sandra,Védaste, Noella, Daniel,Théoneste and Jean-Marie.

I would like to thank the farmers who agreed to allow me to conduct my research in their farms : Agnès, Laurent, Julien, Kalinijabo, Mugabo and Mathias. They were available during my different tours of sampling and made my activities feasible. My gratitude goes also to the managers of the farmers' cooperatives who accepted to let us visit and conduct research in their collection centers.

I would like also to thank the cheese plants and milk shop owners who accepted to receive me and my time for the investigation on microbiological quality and safety : I spent several days with them and I enjoyed their way of understanding what we were doing at that time.

This entire work was financially supported by the Belgian Development Agency (BTC). It was a great pleasure to work in comfortable financial conditions which allowed me to perform all the planned activities. I am especially grateful to Salama, the local BTC scholarship officer who managed to work even in week-ends in order to facilitate my activities.

I would like to express my gratitude to my late parents Protais Kamana and Léoncie Mujawamariya who did not have a chance to see me growing up :

"Chers parents, vous n'avez pas eu la chance de me voir grandir, mais trouvez par ici le fruit de vos efforts. Vous avez guidé mes pas dans ce bas monde est votre plus grand souhait était ma réussite scolaire. Vox vœux ont été exaucés et je vous promets que je vous resterai fidèle".

I am full of joy that I will now be able to tell my family in Rwanda that "it is over". My aunt and uncles were always curious on my frequent travels for PhD. I thank them for all the prayers and I will miss their warm welcoming dinners. I hereby thank also all my cousins and relatives for their prayers. Endless thanks to my brother Thierry and my sister Nicole who are always there to encourage me.

All my "close" friends deserve a particular attention at this moment : Laurent, Clément, Roger-Gérard, Alice, Aline, Aimable, Yves-Laco, Jean-Yves, Karuhije Family, César, Claude, Charles, Segond, Placide,Léonard, Aimé,Igabe, Clarisse ,Jérôme, Colette, Josélyne, Emma, Diane, Fidèle, Eric, Kinyata, Christine, Théo, Didier,Sixte and Gustave. They were always there to support me during this journey and this achievement is not only mine but also theirs.

I am thankful for the medical support I received from my friends, Dr. Savio and Dr. Adelard, when I was facing health issues in 2014. Thank you very much !

I am happy to close this message by expressing my endless thanks to my late grandfather Boniface Kamegeri, from whom I inherited my pride, bravery and determination. I will always remember the day of July 18th 1995 when he accompanied me for my exam for the Junior Seminary....this is the fruit from the seed he sowed on that day !

Olivier Kamana, October 5th 2015

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LIST OF ABBREVIATIONS

ACMSF	: Advisory committee on microbiological safety of food
ADRI	: Association pour le développement rural intégré
ARMV	: Association rwandaise des médecins vétérinaires
BNR	: Banque nationale du Rwanda
°C	: Celsius
CAC	: Codex Alimentarius commission
CCP	: Critical control point
CFU	: Colony forming unit
EAC	: East African community
EC	: European commission
EPR	: Eglise Presbytérienne au Rwanda
EU	: European Union
F	: Farm
FAO	: Food and agriculture organization of the united nations
FDA	: Food and drug administration
FSMS	: Food safety management systems
GMP	: Good manufacturing practices
HACCP	: Hazard analysis and critical control points
HPI	: Heifer project international
IAR	: Ishyirahamwe ry'aborozi mu Rwanda
IRST	: Institut Rwandais de la recherche scientifique et technologique
ISAE	: Institut supérieur d'agriculture et d'élevage
ISAR	: Institut supérieur agronomique du Rwanda

ISO	: International standards organization
LISP	: Livestock infrastructure support program
LWF	: Lutheran world federation
MCC	: Milk collection center
MINAGRI	: Ministry of agriculture and animal resources
MS	: Milk shop
N	: No
NGO	: Non-governmental organization
NISR	: National institute of statistics of Rwanda
PADEBL	: Projet d'appui au développement de l'élevage bovin laitier
PDRCIU	: Projet de développement rural communautaire et des infrastructures de l'Umutara
PRP	: Pre-requisite program
RARDA	: Rwanda animal resource development authority
RBS	: Rwanda bureau of standards
RSB	: Rwanda standards board
RCW	: Rwanda community works
RDCP	: Rwanda dairy competitiveness project
RS	: Rwanda standard
RB S	: Rwanda bureau of standards
RSB	: Rwanda standards board
SCP	: Small cheese plant
SM	: Supermarket
SNV	: Stichting Nederlandse Vrijwilligers
TMC	: Total mesophilic count

UHT : Ultra-High temperature

USA : United States of America

USAID : United States agency for international development

USD : Unites States dollar

WHO : World health organization

WSP : Wholesale point

SUMMARY

Milk and dairy products play a vital role in human diet. In developing countries, milk is among the major sources of income for many (rural) farmers and is an affordable source of animal proteins that can fulfill the rising demand in those countries, as a response to the rapid population growth. For decades, governments have been encouraging milk production increase by setting up a number of development projects and significant achievements were accomplished. In Rwanda, several interventions contributed to increase milk yield and this is expected to continue in the framework of the 2020 Vision, a national development plan aiming to transform the socio-economic conditions of Rwandans by the year 2020, and make Rwanda a middle-income country. Besides the significant increase of milk production, the aspects related to its safety and quality must be considered in parallel, since milk is a highly perishable foodstuff, requiring appropriate handling to prevent food-borne illnesses for consumers. In the African countries including Rwanda, milk increase is not followed by appropriate quality and safety management measures, and there is a situation in which the formal dairy industry coexists with the informal market channeling the major part of milk commercialized raw, boiled or processed towards traditional products mostly fermented. The present PhD study was undertaken in order to contribute to this situation, and its overall objective was the assessment of microbiological quality of milk and dairy products and hygienic status using a food chain approach from farm to fork, leading to an exposure assessment of *Salmonella* spp.

The informal market is predominant in Rwanda, and is estimated to concern more than 90% of the whole milk from farmers grouped in 3 categories (open-grazing, semi-grazing and zero-grazing). The modernization of the chain is ongoing, with around 120 collection centers installed all over the country, and which are the interconnection between the two routes namely the formal and the informal. Processing companies are still few (5 in the time frame of this study) and process milk towards various products such as pasteurized milk, fermented pasteurized milk, cream, butter, etc. Gouda cheese is also processed mostly in the North-West of the country (Gishwati milk basin) in traditional conditions but benefitted from development program supports and have been modernized by receiving trainings on food hygiene and modern and adapted equipment.

Fermented milk, boiled milk and cheese (till 2012) from the informal market are commercialized in milk shops, localized in towns and cities and serve as source of milk for a high number of consumers, whereas processed dairy products are sold in supermarkets via wholesalers directly connected to processing plants (formal market).

According to most international and local standards, the raw milk quality in farms and milk collection centers in Rwanda was almost acceptable. After pasteurization, the total mesophilic count (TMC) and coliforms in milk were only slightly decreased, which indicates ineffective pasteurization and/or post-contamination, resulting in unacceptable microbiological quality of pasteurized milk and derived dairy products according to the international and local standards. Increasing bacterial counts were observed along the retail chain and could be attributed to insufficient temperature control during storage. Milk shops sold boiled and fermented milk of poor and variable microbiological quality in comparison with the pasteurized milk sold in supermarkets. The microbiological load of cheese was the highest of all dairy products, suggesting post-processing contaminations due to unhygienic handling practices. *Listeria monocytogenes* was absent in all samples, possibly due to competition and growth suppression exerted by the indigenous milk and/or fermenting microbiota. *Staphylococcus aureus* was omnipresent, with the highest concentrations found in cheese and fermented milk sold in milk shops, but there was no statistical difference between the different steps of the milk chain. The presence of *S. aureus* is linked with lack of animal health monitoring and/or personnel hygiene. *Salmonella* spp. was isolated from 7.6 % of all samples (n = 330) spread over the whole milk chain, from farm to retail. Retail cheese in Rwandan milk shops contained no *L. monocytogenes* and < 3 log CFU/g *S. aureus*, but a critical 50 % was contaminated with *Salmonella*, presenting a serious food safety issue.

In parallel, the status of hygienic practices was assessed and the level of compliance with hygienic requirements was established, and further linked to microbiological data. It was confirmed that modern farms with appropriate equipment were more complying with hygienic requirements. At collection level, the concerned centers scored similarly with only 26% of compliant answers, due to the lack of maintenance and calibration and trainings of the personnel, among others.

A big difference was observed among processing plants with one outstanding company certified against ISO 22000:2005 and HACCP which complied totally to all internationally demanded requirements of food safety management systems, whereas smaller companies scored with 33% and 29% of compliant answers. In small cheese plants, all plants did not comply with any hygienic requirement, and the personnel were not even aware of basic hygienic principles, making this level of the chain critical. In wholesale points 55% of hygienic requirements were fulfilled and the major issues were regarding the absence of a cleaning and disinfection plan, training of personnel, unfit design, etc. Supermarkets showed more satisfactory results (62%) but the lack of temperature control was the main bottleneck at this stage. In milk shops, which appeared to be another weak point along the chain, only 26% of positive answers were obtained due to the lack of temperature control, personnel hygiene, inappropriate design of the premises, etc.

This study was further directed on the previously identified weak points of the chain, namely small cheese plants and milk shops, in order to get deeper information on the origin of contaminations. In cheese plants, the production was followed from milk reception to final products, and microbiological parameters were analyzed at each step. This study was conducted just after interventions on behalf of the government (2013) and its international development partners and showed noticeable improvements regarding microbiological safety of cheese, and revealed that the personnel hygiene and the processing environment are probably at the origin of occasional *Staphylococcus aureus* and *Salmonella* contamination during processing. In milk shops a similar methodology was used and this study confirmed that the applied heating treatments enable total elimination of *Salmonella*, revealing that the observed bottlenecks result from post-contamination from the environment, an inadequate refrigeration during storage, or insufficient hygiene by the food handlers serving boiled milk in cups in the shops.

All the obtained data on the prevalence of *Salmonella* were completed by additional sampling of cheese and boiled milk in milk shops, and a consumer survey was conducted for both milk and cheese and provided information on consumer behavior and consumption data. It was shown that in milk shops milk is mainly consumed by men (79.7 %) and the daily consumption was the most in practice (33.3 %) with a volume of 400 mL (61.2 %).

Among milk buyers in milk shops, 40.0% is also buying boiled milk for their families resulting in consumption by mainly adults aging between 19-29 years (32.3 %, n=136), and a daily consumption frequency reported by 62.5 % of the survey participants. Among the concerned households (n=36), 66.7 % is re-boiling the milk and 33.3 % is storing it refrigerated before consumption. Gouda cheese is usually sold in supermarkets belonging to the formal market, after modernization of the cheese sub-sector. A cheese portion with a weight of 62.5 g is mainly consumed (80.0 % , n=370), and weekly frequency is most in practice (38.1 %). Finally, a probabilistic exposure model for *Salmonella* was build up by taking into account prior collected prevalence data in milk and Gouda cheese, assumed concentration data, information of the milk chain gathered by observation, and collected consumption data and consumer behavior information. The risk of infection of *Salmonella* by consumption of boiled milk was estimated at 11.08% per serving in milk shops and 4.75% in households. The interventions carried out in the cheese sub-sector by the government after the high prevalence of *Salmonella* in cheese (2012), allowed the reduction of the risk of salmonellosis per year by 30% in case of cheese consumption by adults.

The information provided by this study is of great importance for Rwandan decision makers in food safety and quality. It suggests that the ongoing interventions in milk and dairy products quality and safety should be continued and especially extended to milk shops, since a high number of consumers with a highly consumption frequency is involved. Future research is recommended in what regards the absence of *Listeria monocytogenes* in dairy products and the supposed role played by the indigenous microbiota flora, the effectiveness of food safety management systems (FSMS) being implemented in the Rwandan dairy sector, and the microbiological quality and safety of newly introduced types of cheese.

SAMENVATTING

Melk- en zuivelproducten spelen een vitale rol in het menselijk dieet. In ontwikkelingslanden is melk één van de belangrijkste bronnen van inkomen voor vele (plattelands) boeren en een betaalbare bron van dierlijke proteïnen die kan voldoen aan de stijgende vraag in deze landen als antwoord tegen de snelle populatiegroei. Al tientallen jaren, hebben overheden aangemoedigd om de melkproductie te verhogen door het opzetten van een aantal ontwikkelingsprojecten en belangrijke resultaten werden bereikt. In Rwanda droegen verschillende interventies bij tot een toename van de melkopbrengst en dit zou voortgezet worden in het kader van Vision 2020, een nationaal ontwikkelingsplan met als doel om de socio-economische omstandigheden van de Rwandezen te transformeren tegen het jaar 2020 en van Rwanda een midden-inkomensland te maken. Naast de aanzienlijke toename in melkproductie, moeten de aspecten die verband houden met de veiligheid en kwaliteit in parallel worden beschouwd, omdat melk een zeer bederfelijk levensmiddel is, dat een gepaste behandeling vergt om voedselgebonden ziekten te voorkomen voor de consument. In de Afrikaanse landen, waaronder Rwanda, wordt de toename in melkproductie niet gevolgd door aangepaste kwaliteits- en voedselveiligheidsmaatregelen en heerst er een situatie waarin de formele zuivelindustrie bestaat naast een informele markt waarbij het merendeel van de melk gecommercialiseerd wordt als rauw, gekookt of verwerkt in traditionele (meestal gefermenteerde) producten. De huidige doctoraatsstudie werd ondernomen om bij te dragen aan deze situatie met als algemeen doel om de microbiologische kwaliteit van melk- en zuivelproducten en de hygiënestatus te beoordelen via een voedselketen benadering van boer tot bord, wat geleid heeft tot een blootstellingsinschatting van *Salmonella* spp.

De informele markt overheerst in Rwanda, en wordt geschat op meer dan 90% van de melk van boeren gegroepeerd in 3 categorieën (open-begrazing, semi-begrazing en moderne melkhouderij). De modernisering van de keten is aan de gang, met ongeveer 120 collectiecentra geïnstalleerd over het hele land, en die de koppeling vormen tussen de twee routes, namelijk de formele en de informele. Verwerkende bedrijven zijn er nog steeds weinig (5 in het tijdsbestek van deze studie).

Deze verwerken melk tot diverse producten, zoals gepasteuriseerde melk, gefermenteerde gepasteuriseerde melk, room, boter, enz. Gouda kaas wordt ook verwerkt voornamelijk in het Noordwesten van het land (Gishwati melkbassin) in traditionele omstandigheden, maar profiterend van steun van ontwikkelingsprogramma's en gemoderniseerd door het ontvangen van trainingen rond voedselhygiëne en een moderne en aangepaste uitrusting.

Gefermenteerde melk, gekookte melk en kaas (tot 2012) van de informele markt worden gecommercialiseerd in kleine melkwinkels, gelokaliseerd in steden en dienen als bron van melk voor een groot aantal consumenten, terwijl verwerkte zuivelproducten worden verkocht in supermarkten via groothandelaars die rechtstreeks verbonden zijn aan de verwerkingsbedrijven (formele markt).

Volgens de meeste internationale en lokale standaarden, was de kwaliteit van rauwe melk in boerderijen en collectiecentra in Rwanda bijna aanvaardbaar. Na pasteurisatie, waren het totaal mesofiel kiemgetal en de coliformen in de melk slechts licht gedaald, wat ineffectieve pasteurisatie en / of nabesmetting aangeeft, wat resulteert in onaanvaardbare microbiologische kwaliteit van gepasteuriseerde melk en afgeleide melkproducten volgens de internationale en lokale normen. Toenemende bacteriële tellingen werden waargenomen doorheen de de verdere distributieketen, wat zou kunnen toegeschreven worden aan onvoldoende temperatuurscontrole tijdens de opslag. Melkwinkels verkochten gekookte en gefermenteerde melk van slechte en variabele microbiële kwaliteit in vergelijking met de gepasteuriseerde melk verkocht in supermarkten. De microbiële belasting van kaas was de hoogste van alle zuivelproducten, wat nabesmetting suggereert door onhygiënische praktijken. *Listeria monocytogenes* was afwezig in alle monsters, mogelijk als gevolg van competitie en groei onderdrukking uitgeoefend door de inheemse melkmicrobiota. *Staphylococcus aureus* was alomtegenwoordig, waarbij de hoogste concentraties aangetroffen werden in kaas en gefermenteerde melk verkocht in melkwinkels, maar er was geen statistisch verschil tussen de verschillende stappen van de melkketen. De aanwezigheid van *S. aureus* is gelinkt aan een gebrek aan monitoring van de gezondheid van de dieren en / of hygiëne van het personeel. *Salmonella* spp. werd geïsoleerd uit 7,6% van alle monsters (n = 330), verspreid over de hele melkketen, van boer tot retail. Kaas in Rwandese melkwinkels bevatte geen *L. monocytogenes* en <3 log kve / g *S. aureus*, maar een kritische 50% was besmet met *Salmonella*, wat een ernstige voedselveiligheidskwestie aangeeft.

In parallel werd de status van de hygiënische praktijken beoordeeld doorheen de keten en werd het niveau van naleving van de hygiëne eisen vastgelegd, dit werd verder gelinkt aan microbiologische gegevens. Er werd bevestigd dat de moderne melkbedrijven met de aangepaste uitrusting meer voldeden aan de hygiëne eisen. Op collectieniveau scoorden de collectiecenters gelijkaardig met slechts 26% positieve antwoorden, vanwege onder andere het gebrek aan onderhoud en kalibratie en training van het personeel.

Een groot verschil werd waargenomen tussen de verwerkingsbedrijven, met een uitstekend bedrijf gecertificeerd volgens ISO 22000: 2005 en HACCP dat volledig voldoet aan alle internationale eisen betreffende een voedselveiligheidsmanagementsysteem, terwijl kleinere bedrijven scores hadden van slechts 33% en 29% positieve antwoorden. In kleine kaasbedrijven, voldeden alle bedrijven aan geen enkele hygiëne vereiste en het personeel was zich zelfs niet bewust van basis hygiëneprincipes, wat dit niveau van de keten kritisch maakt. Bij de groothandelaars waren 55% van de hygiëne eisen voldaan en de grootste problemen hadden betrekking tot de afwezigheid van een reiniging en desinfectie plan, opleiding van personeel, onaangepast ontwerp, etc. Supermarkten toonden meer bevredigende resultaten (62%), maar het gebrek aan temperatuurscontrole was hier de belangrijkste bottleneck. In melkwinkels, die kennelijk ook een zwakke schakel in de keten bleken te zijn, werden slechts 26% positieve antwoorden verkregen door het ontbreken van temperatuurregeling, personeel hygiëne, onaangepast ontwerp van het gebouw, etc.

Dit onderzoek werd verder gericht op de eerder geïdentificeerde zwakke punten van de keten, namelijk de kleine kaasbedrijven en melkwinkels, om meer gedetailleerde informatie over de herkomst van besmettingen te krijgen. In kaasbedrijven, werd de productie gevolgd vanaf de melkreceptie tot de eindproducten, en microbiologische parameters werden geanalyseerd bij elke stap. Deze studie werd uitgevoerd net na interventies namens de regering en haar internationale ontwikkelingspartners (in 2013) en toonde merkbare verbeteringen wat betreft de microbiologische veiligheid van kaas, en onthulde dat de hygiëne van het personeel en de omgeving waarschijnlijk aan de basis liggen van occasionele *Staphylococcus aureus* en *Salmonella* besmetting tijdens de verwerking, die eerder in dit werk was geïdentificeerd.

In melkwinkels werd een soortgelijke methodologie gebruikt en deze studie bevestigde dat de toegepaste warmtebehandelingen totale eliminatie van *Salmonella* mogelijk maken, waaruit blijkt dat de waargenomen knelpunten resulteren uit nabesmetting vanuit de omgeving, een onvoldoende koeling tijdens de opslag, of onvoldoende hygiëne van de verkopers die gekookte melk serveren in bekers in de winkels.

Alle verkregen gegevens over de prevalentie van *Salmonella* werden aangevuld met extra bemonstering van kaas en gekookte melk in de melkwinkels, en een consumentenenquête uitgevoerd voor zowel melk en kaas, wat informatie verstrekte over het gedrag van consumenten en consumptie data. Er werd aangetoond dat in melkwinkels melk hoofdzakelijk werd geconsumeerd door mannen (79,7%) op basis van een dagelijkse consumptie (33,3%) met een volume van 400 ml (61,2%). Onder melkkopers in melkwinkels, koopt 40,0% ook gekookte melk voor hun gezinnen resulterend in consumptie van vooral volwassenen tussen 19 en 29 jaar (32,3%, n = 136), op basis van een dagelijkse consumptie (62,5%). Onder de betrokken huishoudens (n = 36), herkookt 66,7% de melk opnieuw en 33,3% slaat de melk gekoeld op voor consumptie. Gouda kaas wordt meestal verkocht in supermarkten die behoren tot de formele markt, na de modernisering van de kaas subsector. Een kaasportie met een gewicht van 62,5 g wordt hoofdzakelijk verbruikt (80,0%, n = 370), met een wekelijkse frequentie (38,1%). Een probabilistisch blootstellingsmodel voor *Salmonella* werd opgebouwd door rekening te houden met vooraf verzamelde prevalentiegegevens in melk en Gouda kaas, veronderstelde concentratie gegevens, informatie over de melkketen verzameld door observatie, en verzamelde consumptie data en consumentengedrag informatie. Het risico op infectie met *Salmonella* werd geschat op 11,08% per portie gekookte melk in melkwinkels en 4,75% in huishoudens. De interventies die in de kaas subsector werden uitgevoerd, door de overheid na de hoge prevalentie die werd teruggevonden in dit onderzoek in 2012, bewerkstelligden een reductie van het risico van salmonellose per jaar van 30% in het geval van kaasconsumptie door volwassenen.

De in deze studie weergegeven informatie is van groot belang voor Rwandese beslissingnemers in voedselveiligheid en -kwaliteit. Het suggereert dat de lopende interventies in melk- en zuivelproducten kwaliteit en veiligheid moeten worden voortgezet en vooral uitgebreid tot melkwinkels, aangezien het hier gaat over een groot aantal consumenten met een hoge consumptie frequentie.

Toekomstig onderzoek wordt aanbevolen rond de afwezigheid van *Listeria monocytogenes* in zuivelproducten en de vermeende rol van de inheemse microbiota, de effectiviteit van de managementsystemen voor de voedselveiligheid in de Rwandese zuivelsector en de microbiologische kwaliteit en veiligheid van nieuwe kaassoorten.

OBJECTIVES AND OUTLINE

Rwanda's economy depends significantly on agriculture, and the livestock sub-sector contributes largely to this. Besides the economic importance, the social importance is also noticeable. Dairy farming is one of the most profitable activities in the rural Rwandan livelihoods. In a context of post-conflict situation, the government of Rwanda in collaboration with different international development partners have put remarkable efforts in the agriculture sector for food security and poverty alleviation purposes. Especially, several programs were implemented to increase milk production. Traditional farming systems are progressively being abandoned, to be replaced by modern ones. Exotic breeds are introduced through artificial insemination and animal health is controlled. As a result, the milk yield is in constant progress, and this falls within the 2020 vision, a national development strategy aiming at transforming the country in a middle-income one by the year 2020.

The ultimate goal of food security purposes is to provide food commodities not only in sufficient quantities but also with high quality for the consumers. In Africa, food safety issues are rarely documented and the focus was made, for long time, on food availability only. The present study was conducted in the Rwandan milk and dairy chain to assess the quality and safety of milk and dairy products along the chain. Being a highly nutritious product, milk is easily contaminated by pathogenic and spoilage microorganisms. It is a necessity to follow milk from the production site i.e. farms to consumer and monitor all the aspects related to hygienic farming practices. Then, post-milking conditions have to be monitored in order to assess conditions of transport from farms to collection and/or processing site. At the collection level the conditions of milk storage are a key point to monitor, given the potentiality of contamination and growth of spoilage and pathogenic microorganisms. The processing conditions also are an important step to follow, to identify possible contamination sources. After processing, the transport conditions to the sale point are also an important element, and finally the retail conditions are the last step to be monitored, as it is the point of contact with consumers.

In the African context generally and particularly in Rwanda, the above mentioned paths are not strictly followed, and parallel circuits are found. They constitute the informal market, which is the most important, as a consequence of lack of adapted infrastructure for collection and processing. This study focuses on both markets, and gives insight in microbiological safety and quality of milk and dairy products with emphasis on *Salmonella* spp., a redoubtable pathogen incriminated in thousands of food-borne illnesses worldwide, regularly. Taking into account the low availability of information in this regard in Africa and Rwanda, this study intends to play a pioneer role for the investigation of microbiological quality and safety of milk and dairy products in Rwanda.

Overall, the present research is divided in six chapters with four objectives shown in the overview below (Figure 0). The first chapter gives a literature review to the study, and the last chapter presents a general discussion and future research perspectives of the research.

The first objective (CHAPTER 2) was the assessment of microbiological quality and safety of the Rwandan milk and dairy chain. A farm-to-fork approach was followed to sample milk and dairy products. Raw milk at farm level and in collection centers, pasteurized milk in processing companies, wholesale points and supermarkets, Gouda cheese from small cheese producers, supermarkets and milk shops, traditionally fermented milk and boiled milk in milk shops were analyzed. Total mesophilic count, coliforms, *Staphylococcus aureus* were investigated as hygiene indicators, whereas *Salmonella* spp. and *Listeria monocytogenes* were checked as pathogenic microorganisms.

The second objective of this study (CHAPTER 3) was to gain insight in hygienic practices along the Rwandan milk and dairy chain to be able to attribute the measured microbiological quality and safety to the level of compliance with hygienic practices. Based on hygienic requirements from literature and guidelines from Codex Alimentarius, hygienic practices were assessed along the chain i.e. farms, collection centers, processing companies, wholesale points, supermarkets, small cheese plants and milk shops. The score on the performance of those different stages of the chain were linked to the previously obtained data on microbiological safety and quality in order to highlight the impact of those hygienic practices on the microbiological quality and safety.

In the third objective (CHAPTER 4) a further investigation of microbiological quality and safety of boiled milk in milk shops and Gouda cheese in processing plants was conducted. The above mentioned microbiological parameters were checked along the production steps of boiled milk with assessment of heating practices and the calculation of process lethality for pathogens, and for the steps of Gouda cheese processing in small cheese plants.

Given the collected data in chapters 2, 3 and 4, an exposure and risk assessment calculation was the fourth objective (CHAPTER 5) in order to gain insight on the probability of infection by *Salmonella*. Therefore, a probabilistic retail-to-consumer exposure model, including prevalence data of *Salmonella* spp., chain information, collected consumption and consumer behavior information was built up and allowed to make an estimate on the risk on illness per serving and per year of the Rwandan population upon consumption of informally marketed milk and formally marketed Gouda cheese.

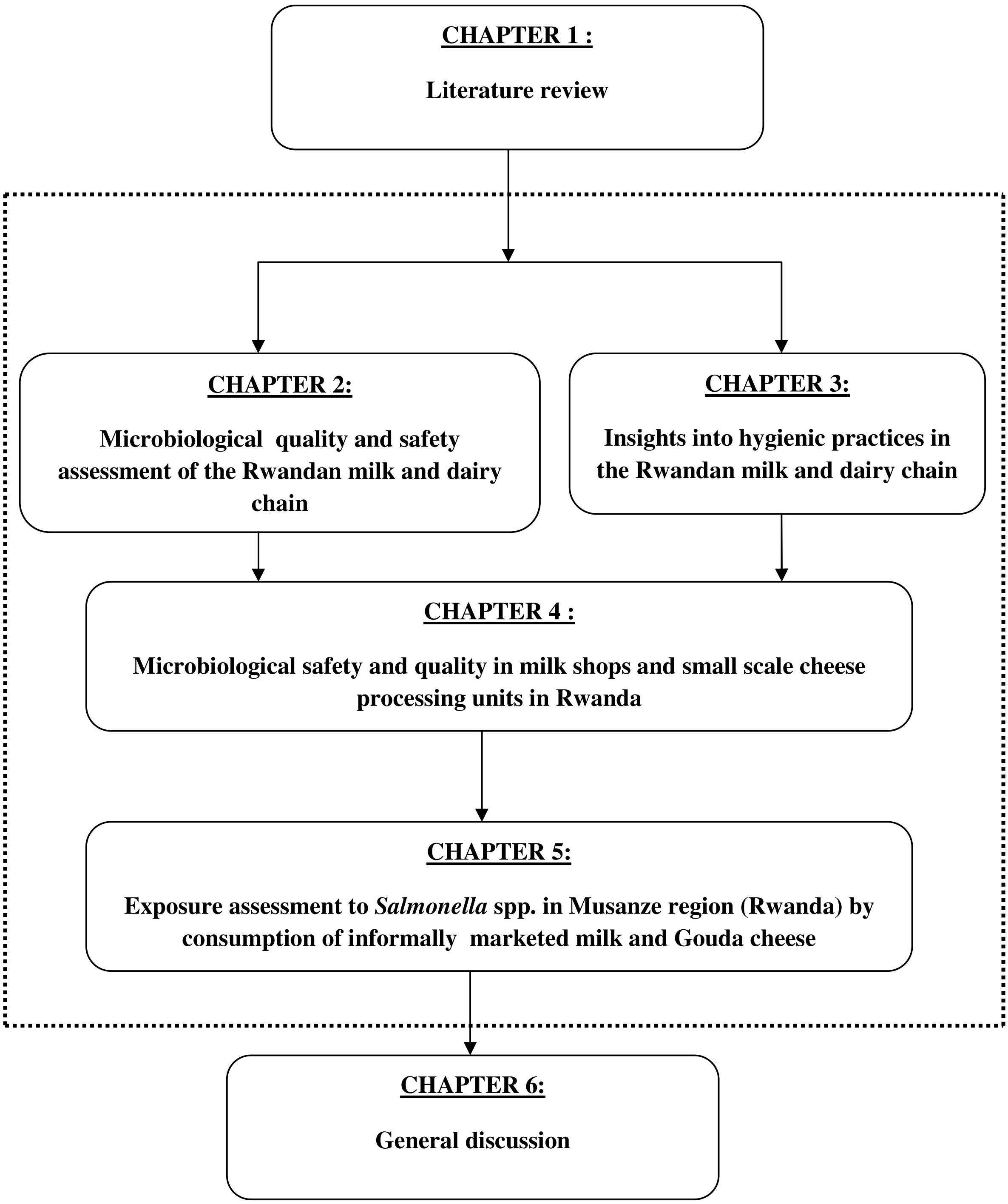


Figure 0 : Overview of the different chapters of the present research

CHAPTER 1: LITERATURE REVIEW

1.1. Milk and dairy products

Milk is the normal mammary secretion of milking animals obtained from one or more milkings without either addition to it or extraction from it, intended for consumption as liquid milk or for further processing (Codex Alimentarius, 1999). It is produced by the mammary glands of many animals like cow, buffalo, goat, sheep, camel, horse, and zebu. Milk is easy to produce, highly nutritious and source of animal fat and proteins worldwide. It contains water, carbohydrates mainly lactose), lipids and proteins. Other numerous elements are present in trace quantities (Walstra, 2013). In this study, the term "milk" is referred to as cow's milk. Raw milk is defined by the European Commission as milk produced by the secretion of the mammary gland of farmed animals and not heated above 40°C or subjected to treatment having equivalent effect (European Commission, 2004b) .

Given the aforementioned important nutritive properties, milk is part of the major human diet and is also an income generator commodity. Its world production is in constant progression, and increased of 38% from 1993 to 2013 by reaching more than 600 million tons (FAOSTAT, 2015b). The highest milk producing countries in the World are USA, India, Brazil, China and Russia, and it plays a noticeable role in income generation for many countries. In 2011, the milk industry had an average turnover of 660 billion USD in Asia, 843 billion USD in Europe, 690 billion USD in North America and 1530 billion USD in Oceania , and the global per capita consumption is increasing steadily (Krijgler, 2012). Figure 1.1 represents the global milk consumption and the world population. According to FAO (2015), more than 6 billion people consume milk and their majority are in developing countries.

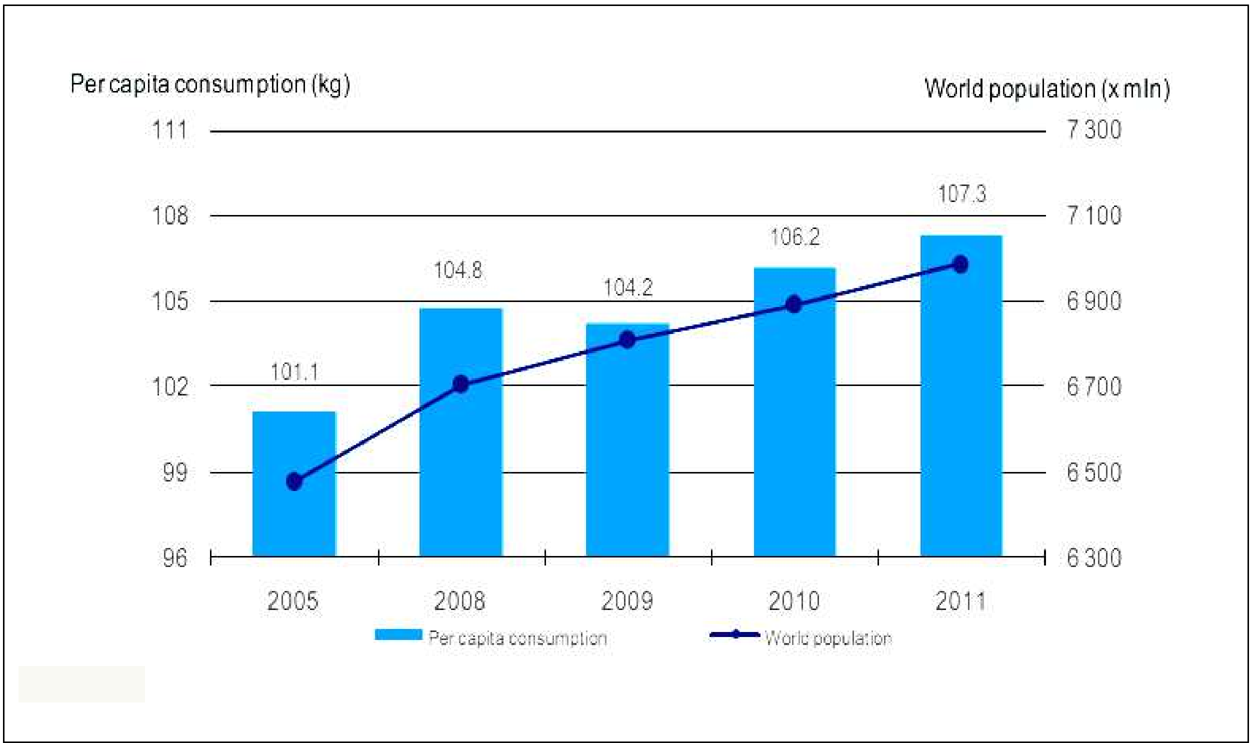


Figure 1.1 : Milk consumption and world population (Source : Krijgler, A., 2012)

Raw milk being highly perishable, food processing allows a longer shelf life and improves microbiological safety. Given its importance in human diet, many products are made of it, offering a wide variety of dairy products such as heat treated milk, cream and butter, milk powder, fermented milk and yoghurt, cheese (soft, semi-hard and hard), etc. Definitions of these products are embedded in multiple Codex Standards such as the "Codex standard for the use of dairy terms-Codex Stan. 206-1999" (Codex Alimentarius, 1999). Given the development of food industries in many countries, focus is made on development of dairy industries by NGOs, and governments to provide local population with animal proteins (Corniaux et al., 2005; Jansen, 1992). In addition, many private businesses also find in the dairy industry an easy and sustainable business (Duteurtre, 2007; Omore, 2004).

1.1.1. Milk production in Africa

As well as for the rest of the world, the African milk production has been in constant progress over the past decades and increased of 123 % from 1993 to 2013 (FAOSTAT, 2015b). In Africa, the farming systems are either pastoral, semi-intensive or intensive (Ndambi et al., 2007). The per capita consumption of milk and milk products is higher in developed countries, but the gap with many developing countries is narrowing. Demand for milk and milk products in developing countries is growing with rising incomes, population growth, urbanization and changes in diets (Delgado, 2003; FAO, 2015). General milk consumption in Africa increased from 35 to about 40 kg per capita in the period of 1990 to 2004 (Ndambi et al., 2007). Although milk production has also been increasing, the demand has increased considerably due to the continuing population growth. Therefore, milk powder is frequently imported to make up the deficit in demand. In 2011, more than 800 000 tons were imported (FAOSTAT, 2015b). The countries with the highest per-capita cow's milk consumption in Sub-Saharan Africa are Sudan (181 kg milk per capita in 2007), Mauritania (135 kg/capita), Kenya (99 kg/capita) and Botswana (89 kg/capita) (FAOSTAT, 2015b). However, in some Western and Central African countries, the milk consumption is still very low (< 10 kg/capita/year), underlining that consumption of milk and milk products is greatly influenced by traditions and cultures (FAOSTAT, 2015b; Ndambi et al., 2007).

1.1.1.1. Pastoral systems

In the pastoral systems, three kinds of farming modes are observed i.e. sedentary, migratory, and transhumant. The main difference between those farming modes, is that sedentary farmers live in the same homes the whole year, whereas migratory and transhumant farmers move. Typical migratory pastoral systems are found in Masaï tribes in Tanzania and Kenya, and Fulani tribes in Ghana and Nigeria. According to Ndambi et al. (2007) and Tonah (2002) the Masaï who live in the sparsely populated semi-arid range-lands of Kenya and Tanzania, practice one of the oldest pastoral systems. The Masai live in extended families of 10-15 people with herds averaging 100-170 cattle and as many sheep and goats also. They consume about 0,85 kg of milk per person daily, and their income come mainly from sales of livestock. The surplus of milk is sold or exchanged for other foodstuffs.

Tonah (2002) describes the dairy production of the Fulani in Ghana as a migratory pastoral system, with habitat changing over time. Fulani settlements consist of several concentric huts arranged to form a single housing unit. In a study on Fulani agro-pastoralists in central Nigeria, Waters-Bayer (1988) found that dairy production units had modal household sizes of 7 - 13 persons, and live in a more sedentary way. The modal herd size is usually between 40 and 60 cattle, with sheep and poultry kept in parallel. During dry periods, animals are grazed away from their homes and grazed near their homes in the rainy season when sufficient pasture is available. The distances covered for the transhumance vary between 5-6 km. The sedentary pastoral system is found in the semi-arid, sub-humid and cool highland zones (Romney et al., 2003) and in most of cases it leads to an improved system where cattle owners also cultivate crops in order to reduce risks, making then an "agro-pastoral" system, on communal grazing land, feeding crop residues and some more supplements to their cattle. This system supports a high number of livestock and poor livestock keepers (De Haan et al., 1997; Thornton, 2002).

1.1.1.2. Semi- intensive systems

In this system, milk production is the major objective , and is located mostly in peri-urban zones with farms owned by business men, civil servants or private individuals who have personnel committed to this activity (Diop and Abellah, 1995). Dairying is done with some degree of intensification by a combination of grazing and concentrate-feeding. In this system, graded or crossbreeding cows are farmed. The crossing is operated between local breeds and exotic ones through artificial insemination, which upgrades the produced volumes and at the same time keeping the adaptability of the animals in changing environmental conditions (Bayemi et al., 2005). Milk production here is much higher than in pastoral systems.

1.1.1.3. Intensive systems

The intensive system is induced by the increasing population growth and urbanization in Africa (Delgado, 2003). The intensification of dairy systems around urban areas goes in parallel of the high demand observed, and farms are small (about 1-2 ha with 1-2 cows generally Holstein Friesian or Ayrshire), and feeding is mainly cut-and-carry with planted Napier grass *(Pennisetum purpureum)* and crop residues, especially from maize and bananas (Ndambi et al., 2007). Contrary to pastoral systems where large proportions and sometimes all the daily milk is consumed at home, only a small portion of milk produced in this system is left for home consumption and the rest sold (De Leeuw, 1998). Larger intensive farms are usually owned by rich individuals, cooperatives or the government.

More investments are also made on buildings and machinery while the use of hired labor is unavoidable. These systems concentrate on the supply of milk in large towns and in most cases have one or more guaranteed delivery sources. There is a higher market orientation in this systems and more emphasis is laid on feeding and breeding management to assure optimal production (Diop and Abellah, 1995).

1.1.2. Milk production in Rwanda

At the Rwandan level, national milk production is far from meeting the demand in animal products. As a result of a very high population growth, the number of consumers has increased rapidly. Also, individual ration of animal proteins, which is already insufficient, decreases as the population increases. Considering its current growth rate of 2.9% which is expected to stabilize at 2% around 2020, the population will be around 14 million by that year. In order to meet the population requirements in animal proteins in the year 2020, the livestock sector will need to produce 483 693 tons of milk, 83 291 tons of meat, 38 546 tons of eggs, 17 362 tons of fish and 11 363 tons of honey (MINAGRI, 2009). The Ministry of Agriculture (MINAGRI), the National Bank of Rwanda (BNR), the Rwanda Animal Resources Development Authority (RARDA), the National Institute of Statistics of Rwanda (NISR) , TECHNOSERVE (an international NGO), and FAO, have data regarding milk production and dairy products but those data are contradicting or lacking for some items (Table 1.1).

Table 1.1 : Milk production in Rwanda (1999-2012)

	Milk production (x 1000 Liters)													
	1999	**2000**	**2001**	**2002**	**2003**	**2004**	**2005**	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
FAOSTAT	110	112	112	112	112	118	121	134	132	145	152	169	187	181
MINAGRI	125	126	125	125	185	178	254	263	296	314	360	362	363	385
TECHNOSERVE		112	112	114	115	134	136	156	191					
BNR	125	126	125	125	220	210	121	263	132	315	360	362	363	396
RARDA										594				
NISR								209		285				
	Milk and dairy products importations (x 1000Tons)													
BNR	1,28	1,35	1,37	1,68	1,65	0,72	0,64	0,51	0,52	0,45	0,42	0,40	0,40	0,40
MINAGRI	1,30	1,50	1,37	1,62	1,65	0,72	0,64	0,58	0,50	0,45	0,40	0,40	0,40	0,40
TECHNOSERVE	2,94	1,54	1,37	1,31	1,67	0,70		0,58	0,51	0,10				

Source : FAOSTAT (2015), MINAGRI (2005 ; 2008; 2012 ; 2013), BNR (2007 ; 2014), TECHNOSERVE (2008), RARDA (2008), NISR (2009).

According to the Rwandan Ministry of Agriculture whose many annual reports were consulted, milk production increased considerably. Data are available from 2000 to 2012 and show a constant progression from more than 120 000 liters to more than 380 000 liters in 2012. The data are the almost the same as published annually by the national bank of Rwanda, although seeing for some years a slight difference, but still the data remain in the same order of magnitude (MINAGRI, 2005, 2008). According to TECHNOSERVE (2008), milk production increase reached more than 190 000 liters in 2007.

This value is lower than the official data from the public bodies, and this is also observed for all the time frame of the project (2000-2007). FAOSTAT (2015a), indicates that milk production increased from 110,000 liters in 1999, to reach more than 180,000 in 2012. Although there is a disparity in available data on milk production, the common fact is the remarkable increase.

In Rwanda, milk is produced through three farming systems namely the open-grazing system, the semi-grazing system and the zero-grazing system. Figure 1.2 represents the images of those systems.

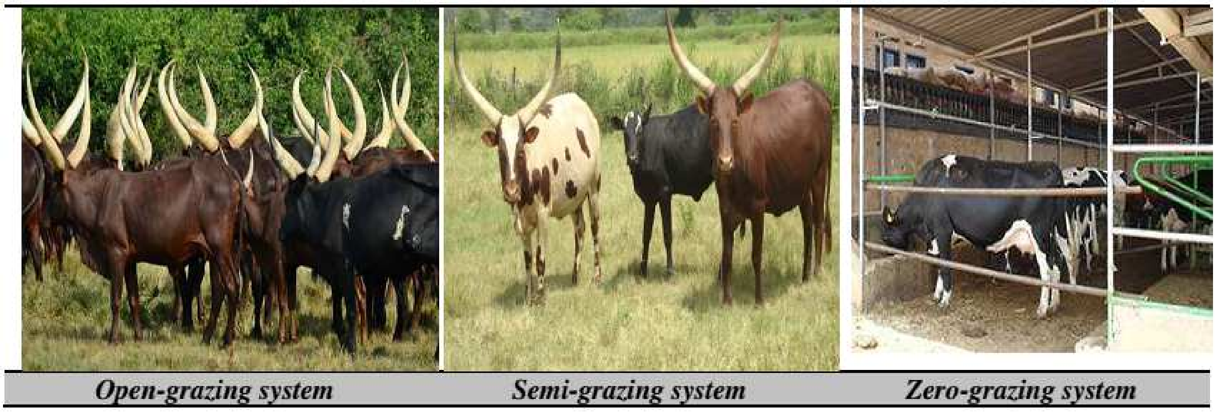


Figure 1.2 : Farming systems in Rwanda

The open-grazing system is the traditional farming system in which cattle are raised in an open ground without intensive care. Many of the farmers in this system are subsistence dairy farmers and have neither the knowledge nor resources to improve yield and market surplus milk. Open range grazing is also utilized in areas where land is more plentiful ; it is possible for exotic cattle to be raised under this system. This system can lead to environmental damage, due to overgrazing land (SNV, 2008; TECHNOSERVE, 2008). Government is encouraging abandonment of this system. The semi-grazing system is a transitional state and encompasses a wide range of farmers. There are the traditional open grazing farmers with insufficient land for grazing but they do not provide adequate forage and are unable to purchase concentrate/feed. Other open grazing farmers are deliberately tending to move towards zero grazing, in particular to increase milk yield and, thus, attempting to use adequate feed. There are also exotic breed dairy farmers who have ample land for grazing and so, spend less money on feed (TECHNOSERVE, 2008).

The zero grazing system is the modern system being encouraged by Rwandan authorities. Cattle are kept in well maintained farms and fed with forage and concentrate, in an intensive way to optimize production. Most of farmers in this category keep cattle in enclosures that are part of the housing unit. Herd sizes range from 1 or 2 cattle to 50 or more, depending on the farmer’s financial resources. In this category farmers raise exotic breeds or improved breeds (TECHNOSERVE, 2008). Some modern farms are equipped with appropriate materials for a good farming.

The Rwandan Ministry of Agriculture subdivides the country in eight different milk basins which are agro-ecological zones according to climatic conditions and dominant farming systems (Figure 1.3). The open grazing system is mostly found in Umutara and Gishwati milk basins, whereas the zero-grazing system is mostly found in Kigali peri-urban farms. The semi-grazing system is found in all milk basins, and is exclusively found in Ngoma, Nyirangarama, Rusizi, Nyanza and Karongi milk basins. The most producing milk basins are Umutara, Gishwati, Kigali and Nyanza (MINAGRI, 2008).

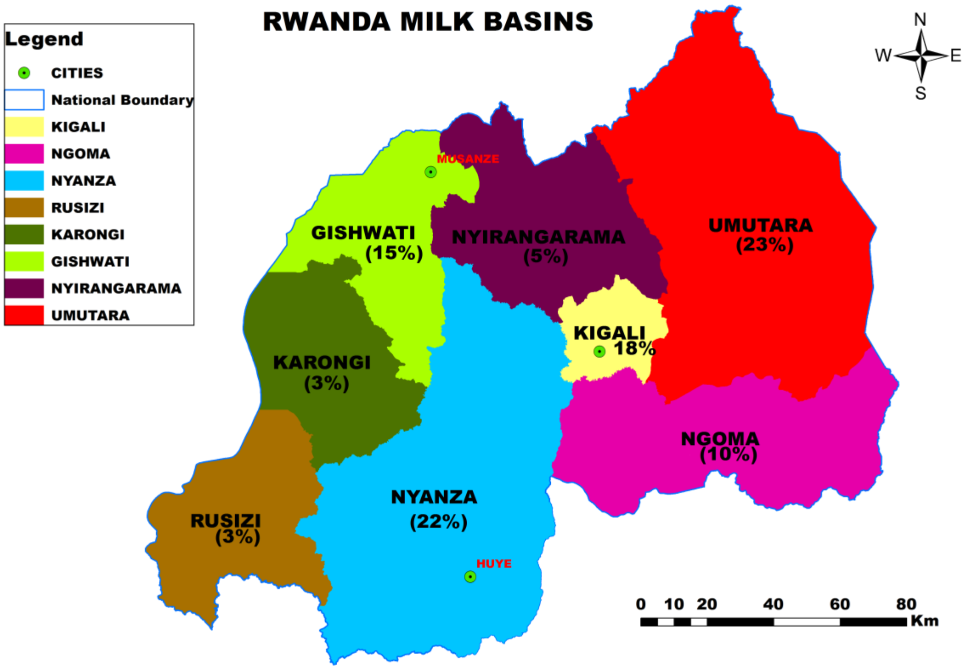


Figure 1. 3 : Milk basins and their production shares in Rwanda

1.1.3. Dairy products in Africa

The African dairy industry is characterized by multinationals processing a wide range of products and employing a high number (more than 200) of personnel. There are also medium-sized companies mostly linked to peri-urban farms and smallholder dairies often located in rural areas, processing a few number of products and employing not more than 15 people (Belli et al., 2013; Breurec et al., 2010; Gran et al., 2002a; Kussaga et al., 2014b) .

The mainly processed products are ultra-high-treated (UHT) milk, pasteurized milk, cream, butter, cheese, ghee, ice cream and yoghurt. This formal processing involves a small share of the whole produced milk not exceeding 20%, and the rest of produced milk passes through an informal market which is highly predominant (Fokou et al., 2010; Omore et al., 2001; Swai and Schoonman, 2011). In the informal market, milk is commercialized either raw or traditionally processed in local products, mostly fermented e.g. "dèguè" in Benin (Tchekessi et al., 2014), "ayib", "ergo" and "ititu" in Ethiopia (Gonfa et al., 2001), "mafi" in South Africa (Beukes et al., 2001), and " fulani" in Burkina Faso (Savadogo et al., 2004).

1.1.4. Dairy products in Rwanda

The number of milk processing industries in Rwanda is low, given that only five plants are operating namely Inyange industries, Blessed dairies, Nyaza dairy plant, Rubilizi dairy and Masaka farms (Karenzi et al., 2013; Modderman, 2010). Inyange industry is the leading dairy industry in Rwanda, located in the suburbs of Kigali city, and having a processing capacity of 50,000 liters per day. Nyanza daily plant was established in 1937 and is the most ancient, processes 5,000 liters daily and is located in the Southern province. Rubilizi dairy is located in the suburbs of Kigali city also, and operates with a capacity of 4,500 liters per day. The Blessed dairies are located in the North-Eastern town of Gicumbi and process 4,500 liters daily, and Masaka farms, the newest one, with a capacity of 3000 liters per day . Pasteurized milk, fermented pasteurized milk, cream and yoghurt are the commonly processed dairy products, excepted Inyange industries which processes UHT milk in addition, Masaka farms which processes yoghurt and luxurious cheeses only, and Blessed dairies which produce yoghurt and fresh cream only.

In Rwanda, the informal market is also reported to be predominant to the detriment of the formal one. The market share of dairy products from milk via the formal market is estimated at 10% by MINAGRI (2008) and 2% by TECHNOSERVE (2008). Besides formal processing plants, Gouda cheese is informally processed in approximately 20 small units, mainly located in the Gishwati region, in the West of the country (Karenzi et al., 2013; Modderman, 2010).

The production capacity of those plants is approximated to be comprised between 150 and 200 litersper day. The informal route involves also the processing of a traditional fermented milk locally called "*ikivuguto*" .

1.2. Organization of the Rwandan milk and dairy chain

The Rwandan dairy chain is characterized by the interconnection between the formal market and the informal one, and the government of Rwanda in collaboration with its development partners is intensifying efforts for modernization and competitiveness increase.

1.2.1. Production, processing and selling

The structure of the entire Rwandan milk and dairy chain is represented in the Figure 1.4. At production level, 35% of the total amount of produced milk is wasted in farms due to lack of refrigeration and hygienic post-milking practices, 16% is consumed and the rest is channeled by the formal market (2%) and in the informal market (47%) (TECHNOSERVE, 2008). Milk collection centers (MCCs) are scattered all over the country, in other to facilitate farmers to access market. In 2009, 110 MCCs were built by the government and its development partners and are managed by the farmers' associations (MINAGRI, 2009). At this level, an interconnection between the informal and the formal markets is observed. On the one hand, milk can be collected from farms and channeled to formal processing plants, and final products are sold in wholesale points and supermarkets. On the other hand, milk can be sold to MCCs and bought from there by mobile traders who resell it to milk shops. The small cheese processing plants who are part of the informal market, deliver products in the supermarkets, and this makes another point of interconnection between the two routes. Formally processed products are sold in wholesale points, before being supplied in supermarkets for retail. Informal products are sold in milk shops, which sell not only milk and dairy products but also other types of foods and beverages, but milk is the main item (SNV, 2008).

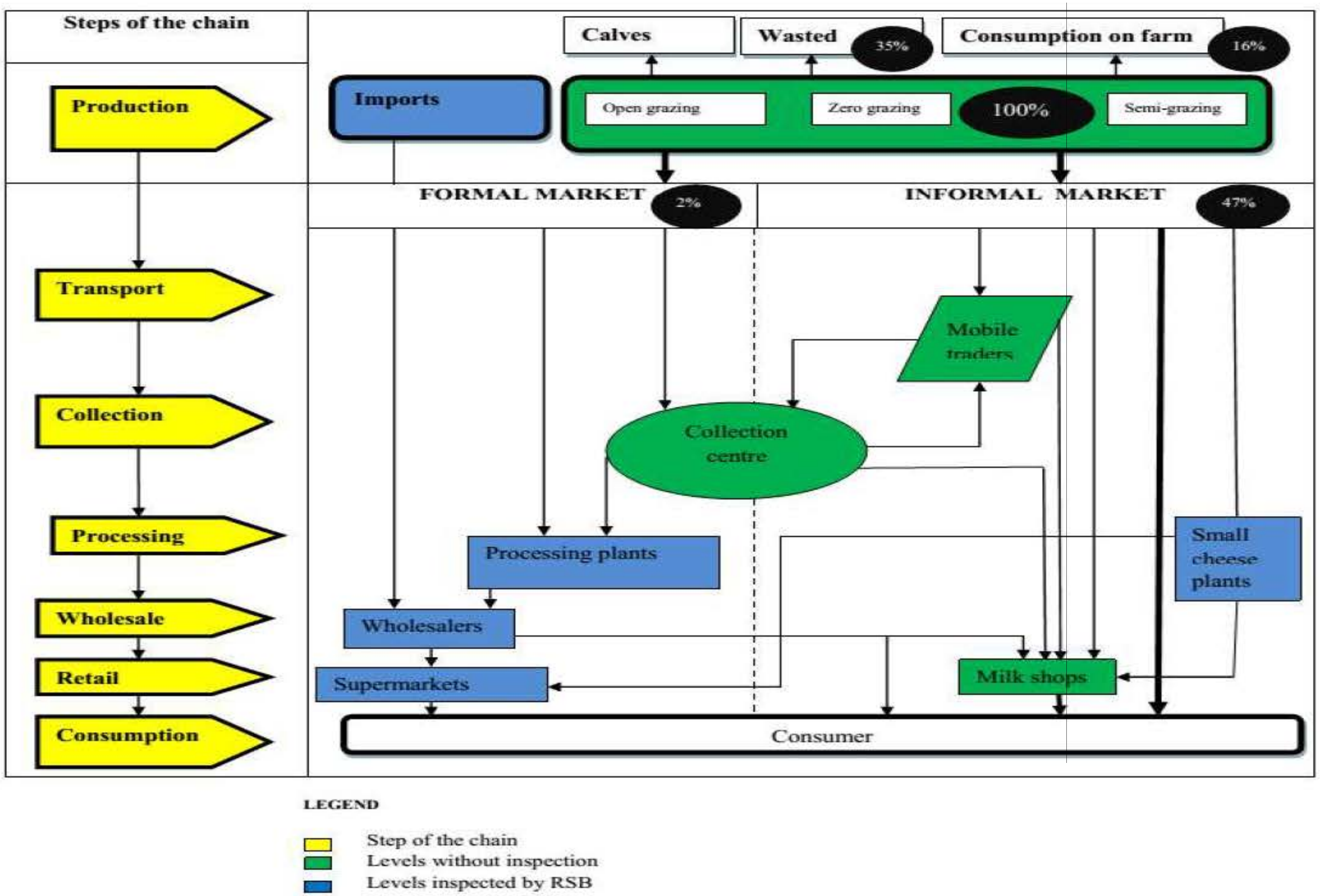


Figure 1.4 : Structure of the Rwandan milk and dairy chain

The increase of milk production without enough capacity in formal dairy processing facilities, enhanced the informal sector to the detriment of the formal one. This structure is similar to the situation found in other Sub Saharan African countries. Studies conducted in Tanzania by Kivaria et al. (2006), Schoder et al. (2013), and Swai and Schoonman (2011); in Kenya by Hooton and Omore (2007), Omore et al. (2001), Sinja et al. (2006) and Thorpe et al. (2000); in Ghana by Donkor et al. (2007); in Sudan by Elmagli et al. (2006); in Niger by Pistocchini et al. (2009); in Ivory Coast by Kouame-Sina et al. (2012); in Mali by Fokou et al. (2010) and Bonfoh et al. (2003); in Uganda by Grillet et al. (2005); in Namibia by Bille et al. (2007); showed the importance of the informal market in volume and turnover, and the marginal proportion of the formal one. Although Rwanda shows some differences with other African countries, especially in terms of rapid socio-economic development and sustainable environmental policies (Republic of Rwanda, 2014), in the agriculture sector and more precisely the dairy sub-sector there is no difference observed between Rwanda and the rest of Sub-Saharan African countries. Except some countries like Nigeria (Ndambi et al., 2007), Chad (Koussou and Grimaud, 2007), Northern Tanzania (Schoder et al., 2013) and Niger (Pistocchini et al., 2009) where traditional transhumant livestock keeping is in practice, milk production in Rwanda is either agro-pastoral or intensive. A technical explanation of the organisation of the Rwandan milk and dairy chain is presented in Figure 1.5.

At farm level, one milking is done per day. Milk is supplied to collection centers by farmers using bikes in general. MCCs are equipped with cooling facilities, which enable them to gather milk for 3 to 4 days before delivery. Dairy farming is not a profession in Rwanda, it is just a subsistence activity, and farms change frequently from one owner to another. Farmers sell milk to mobile traders, to consumers, mobile traders, and deliver also to MCCs. A few number of MCCs are connected to processing plants, and the rest are managed by farmers cooperatives and supply milk to milk shops in Kigali city, the capital of Rwanda, where the demand in milk is high. MCCs sell milk to consumers, milk shops and also mobile traders. Those mobile traders can re-sell milk to consumers and also to milk shops, which sell exclusively to consumers. It appears that consumers can get milk from all the steps of the chain. Another fact to highlight is the role played by mobile traders who are the key actors of the informal market.

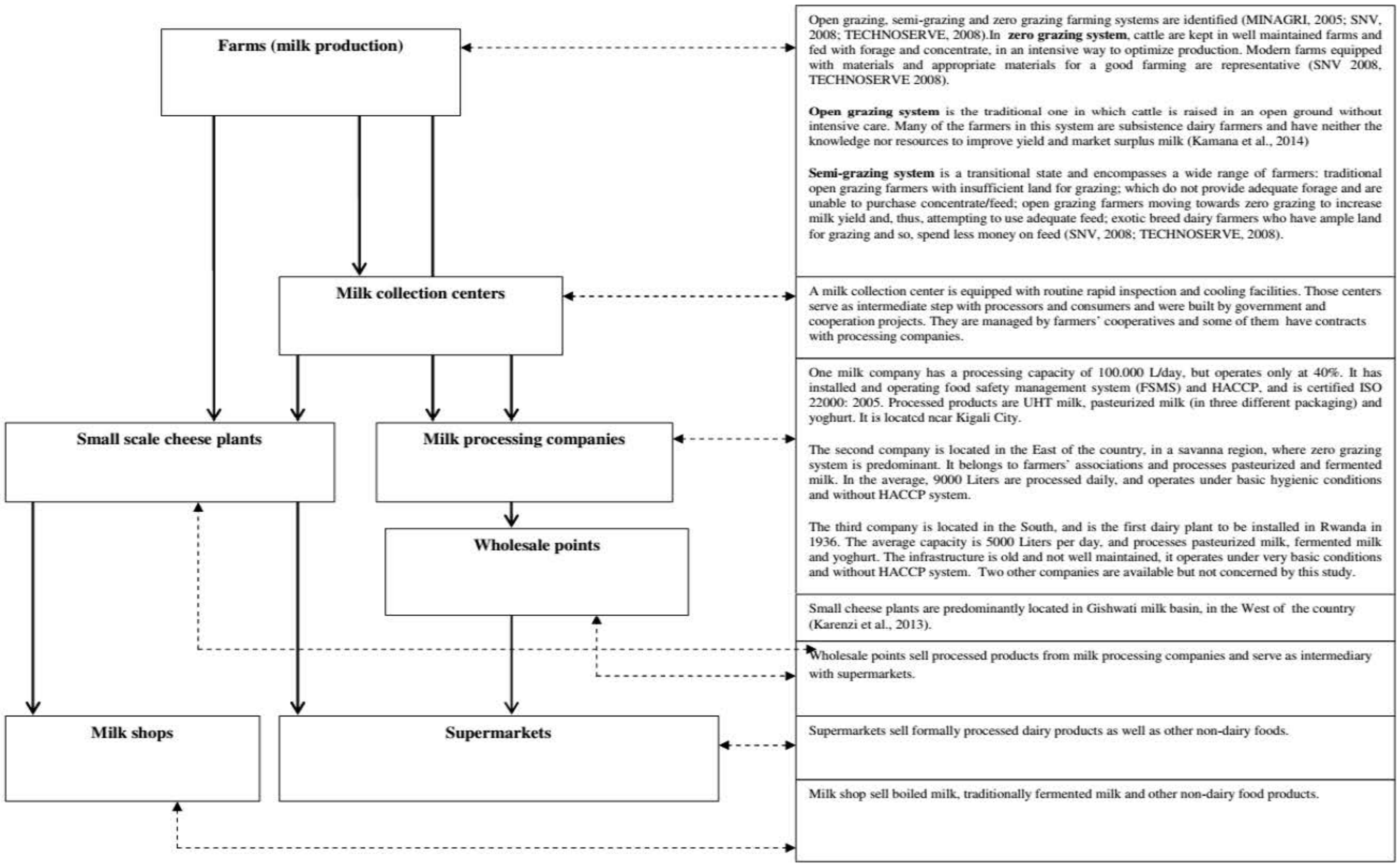


Figure 1.5: Technical explanation of the Rwandan milk and dairy chain

1.2.2. Public and private control and support

In the framework of the 2020 Vision, a national strategy aiming to transform Rwanda into a an emerging country by the year 2020, the agriculture sector in general and the dairy sub-sector in particular is intended to be market oriented (MINAGRI, 2009). For that reason, several development programs have been put in place in order to increase milk production for poverty alleviation and food security. Table 1.2 presents the actors and interventions in the Rwandan dairy chain from 2008 to 2014.

Table 1.2 : Overview of actors and interventions in the Rwandan milk and dairy chain (2008-2014).

Organization	Type of organization	Step in the chain	Type of intervention	Provided intervention
ADRI *Association pour le développement rural intégré*	Local NGO	Farm	Genetic improvement	• Training of inseminators • Provision of artificial insemination (AI) kits
ARMV *Association rwandaise des médecins vétérinaires*	Local NGO	Farm	Animal health control	• Provision of vaccines
EPR *Eglise presbytérienne du Rwanda*	Local NGO	Farm	Milk production increase	• Training of farmers on good farming practices
GAHINI Diocese	Local NGO	Farm	Genetic improvement	• Training of inseminators • Provision of AI kits
HPI *Heifer project international*	International NGO	Farm	Genetic improvement	• Training of inseminators • Provision of AI kits
IAR *Ishyirahamwery'aborozi mu Rwanda*	Local NGO	Farm	Farmers' capacity building	• Organisation of farmers in cooperatives
INGABO	Local NGO	Farm	Genetic improvement	• Training of inseminators • Provision of AI kits
IRST *Institut rwandais de la recherche scientifique*	Government institution	Farm	Animal health control	• Use of traditional medicine in disease control
ISAE *Institut supérieur d'agriculture et d'élevage*	Government institution	Farm, collection, processing and selling	Training, research, extension	• Training of agriculture engineers • Research on the entire chain • Training of famers on good farming practices and good manufacturing practices
ISAR *Institut des sciences agronomiques du Rwanda*	Government institution	Farm	Research, extension and training	• Research on genetic improvement, and forage production • Training on pasture improvement and AI

Organization	Type of organization	Step in the chain	Type of intervention	Provided intervention
LAND O'LAKES	International NGO	Farm, Collection, Processing, Wholesale, Retail	Quality improvement	• Training of farmers , milk processors, wholesalers and retailers on GMPs • Reorganization of the cheese processing sub-sector
LISP ***Livestock infrastructure support program***	Government project	Farm, Collection	Milk production increase and quality improvement	• Construction of milk processing plants • Construction of MCCs • Provision of collection equipment
LWF ***Lutheran world federation***	International NGO	Farm	Milk production increase	• Training farmers on good farming practices
ONE COW-ONE FAMILY	Government project	Farm	Milk production increase	• Provision of dairy cows to poor families
PADEBL ***Projet d'appui au développement de l'élevage bovin laitier/Dairy cattle development support project***	Governmentproject	Farm, Collection, Processing	Milk production increase and quality improvement	• Training farmers on good farming practices • Training of inseminators and provision of AI kits • Construction of MCCs • Provision of stainless steel cans • Construction of veterinary clinics and provision of drugs • Training of processors on GMPs
PDRCIU ***Projet de développement des ressources communautaires et des infrastructures de l'Umutara***	Government	Farm, collection	Milk production increase, infrastructure	• Training of inseminators • Construction of MCCs
RCW ***Rwanda community works***	Local NGO	Collection	Marketing	• Support to farmers' cooperatives in milk marketing
SEND A COW	International NGO	Farm	Milk production increase	• Production of semen and AI kits • Provision of dairy cows to poor families
SNV ***Stichting Nederlandse Vrijwilligers***	International NGO	Farm and collection	Value chain organisation	• Organisation of farmers' cooperatives
TECHNOSERVE	International NGO	Farm, Collection, Processing, Wholesale, Retail	Quality improvement, value chain organisation	• Training of farmers , milk processors, wholesalers and retailers on GMPs • Organisation of farmers in cooperatives

All those projects are articulated around a leading program, the "One Cow, One Family" which targets poor households and provide them a heifer for free, and in return the first calf from that cow is given to another household. Beneficiaries are grouped in cooperatives and receive veterinary care on behalf of the government (MINAGRI, 2008, 2009). This program initiated in 2006 and supposed to be terminated by 2015, contributed significantly to the increase of the milk yield. It is expected that 350 000 households will benefit from it (MINAGRI, 2005). Another large scale project, the Dairy Cattle Development Support (PADEBL, a French acronym) was launched under financial support of the African Development Bank, from 2003 to 2012 and provided infrastructures and equipments i.e. MCCs, milk transport bikes, stainless steel cans, etc.(MINAGRI, 2013). In order to maintain the benefits from PADEBL, another project, the Livestock Infrastructure Support Project (LISP) was launched in 2011 under financial support from the African Development Bank, and in parallel, LAND O'LAKES, an international NGO financed by USAID launched the Rwanda Dairy Competitiveness Project (RDCP), by intervening in building the capacity along the dairy value chain, improving food safety and enhancing the quality of dairy products (LANDOLAKES, 2015b). Other projects are run by local and international NGOs and intervene in different areas, among others, training on hygienic practices and good farming practices.

The competent authority in charge of inspections in the Rwandan milk and dairy chain is the Rwanda Standards Board (RSB) (RSB, 2013), formerly the Rwanda Bureau of Standards. The Ministry of Agriculture intervenes also by providing certificates for importations and exportations of animal products (MINAGRI, 2014). Table 1.3 presents the specific standards for milk and dairy products.

Table 1.3 : Overview of standards regarding milk and dairy products in Rwanda

Reference	Standard
	A. EAC standards
RS EAS 68-4: 2007 Ed 2	Milk and milk products-Methods of microbiological examination part 4: Swab tests
RS EAS 81-1: 2007	Milk powders-methods of analysis-part 1: Determination of ash and alkalinity
	B. ISO standards
RS ISO 11866-1: 2005	Milk and milk products— Enumeration of presumptive Escherichia coli—part 1: Most probable number technique using 4—methylumberlliferyr—β—D— glucuronide (MUG)
RS ISO 11866-2: 2005	Milk and milk products-Enumeration of presumptive Escherichia coli-part 2: colonycount technique at 44oC using membranes
RS ISO 1736: 2008	Dried milk and dried milk products—determination of fat content—gravimetric Method (reference method)
RS ISO 1737: 2008	Evaporated milk and sweetened condensed milk—determination of fat content—Gravimetric method(Reference method)
RS ISO 1738: 2004	Butter—Methods of analysis—part 4:Determination of salt content
RS ISO 1739: 2006	Butter—Determination of the refractive index of the fat (Reference method)
RS ISO 1740: 2004	Milk fat products and butter—determination of fat acidity (reference method)
RS ISO 3727-1: 2001	Milk—determination of fat content(routine method)
RS ISO 3727-2: 2001	Butter—Determination of moisture, non—fat solids and fat contents—part 1:determination of moisture content (reference methods)
RS ISO 3727-3: 2003	Butter—determination of moisture, non fat solids and fat contents—part 3:calculation of fat content
RS ISO 3728: 2004	Ice cream and milk ice—determination of total solids content (Reference method)
RS ISO 5537: 2004	Dried milk—Determination of moisture content (Reference method)
RS ISO 5538: 2004	Milk and milk products—Sampling—Inspection by attributes
RS ISO 5541-1-1: 1986 Ed 2	Milks and milks products-Methods of microbiological examination Part 2-1: enumeration of colifoms-Colony count technique at 30 °C
RS ISO 5541-2-2: 1986 Ed 2	Milks and milk products- Methods of microbiological examination part 2-2: Enumeration of coliforms-Most probable number technique at 30°C
RS ISO 5738: 2004	Milk and milk products—determination of copper content—photometric method

Reference	Standard
RS ISO 5764: 2009	Milk –determination of freezing point—thermistor cryoscope method)
RS ISO 6091: 2010	Dried milk—determination of titrable acidity(reference method)
RS ISO 6092: 1980	Dried milk—Determination of titrable acidity(Routine method
RS ISO 6611: 2004	Milk and milk products— Methods of microbiological examination part 3: Enumeration of colony—forming units of yeasts and/or moulds—colony—count technique at 25oC
RS ISO 6731: 2010	Milk, cream and evaporated milk-determination of total solids content (reference method)
RS ISO 6732: 2010	Milk and milk products—determination of iron content—spectrometric Method (reference method)
RS ISO 6734: 2010	Sweetened condensed milk—determination of total solids content (Reference method)
	C. Rwandan standards
RS 193: 2013	Dairy Ice and Ice Cream — Specification
RS 194: 2013	Flavoured Milk — Specification
RS 35: 2012	Unprocessed Whole Milk —Specification
RS 36: 2012	Pasteurized Liquid Milk —Specification
RS 37: 2012	Fermented (Cultured) Milks — Specification
RS 38: 2013	UHT Milk — Specification
RS 39: 2004	Milk powders and Cream powders —Specification
RS 49: 2012	Yoghurt —Specification
RS 50-1: 2005	Cheese —Part1: General Requirements
RS 50-2: 2007	Specification for cheese —Part 2: Gouda Cheese
RS 50-3: 2012	Cheese part 3: Specification for creamy cheese
RS 51: 2005	Butter —Specification
	D. Codex standard
RS CAC/RCP 57: 2004	Code of hygienic practice for milk and milk products

Source : RSB (2015)

Standards concerning laboratory analyses are from the International Standards Organization (ISO) and the East African Community (EAC) and translated in the national legislation, whereas standards regarding hygienic practices are translated from Codex Alimentarius. A process of harmonization of all the Rwandan standards with EAC standards is ongoing and has already been completed for the alcoholic beverages, surface active agents and steel sectors (RSB, 2015a). Given fact that Codex standards highlight general requirements, their translation might lead to misinterpretation, making the operational context risky and more vulnerable to imperfections thus jeopardizing good implementation and thus possibly leading to insufficient control of microbiological contaminations (Kirezieva et al., 2013).

Inspections of milk and dairy products are systematically conducted on importations, by agents present at terrestrial borders and airports. In the domestic market only formally processed products are inspected at production, wholesale and retail levels (supermarkets) (Figure 1.4). For imported products, the importation certificate issued by the Ministry of Agriculture is checked, and samples are taken for microbiological and chemical analyses eventually. The RSB is equipped with five laboratories and two of them namely the microbiology laboratory and the food and agriculture laboratory conducts food analyses. All those laboratories are certified against ISO 17025 standard (RSB, 2015a). The first performs analyses related to spoilage and pathogenic microorganisms, and the second focuses on mycotoxins and other chemical contaminants, especially for tea and coffee for export (RSB, 2013). Although inspections are conducted systematically on all imported food products, there is no systematic monitoring plan for internally produced products, and inspections are performed randomly. The RSB offers third party system certification (HACCP and ISO 22000 : 2005) and product certification (RSB quality mark), and only products under this scheme are inspected on a regular basis i.e. during the certification process, once a semester after certification, and upon certification renewal, which is after three years (RSB, 2015a). A certified product bears a RSB quality mark and conforms to a national, regional or international standard.

1.3. Microbiological hazards associated to milk and dairy products

Milk is sterile when secreted from the in the alveoli of a healthy udder. Microbiological contamination occurs mainly during and after milking. Microorganisms in bulk tank milk originate from the interior of teats, the farm environment and surfaces of milking equipment (Varnam and Sutherland, 1994). Milk can be also contaminated during and after processing (Henson and Traill, 1993).

1.3.1. Sources of contamination

The main source of contamination is the farm environment via dirt e.g. feces and soil attached to the exterior of the udder, which can also inter the teats and cause mastitis (Frank, 2009). Insufficiently cleaned milking equipment can also be at the origin of contaminations, when, during milking, microorganisms adhered to surfaces of those equipments and released into the milk (Varnam and Sutherland, 1994). Aerial contamination is insignificant under normal production conditions (De Leeuw, 1998). The concentration of microorganisms can further increase due to their growth. The microbiological population in bulk tank milk consists of a variety of bacterial species. Most species have a specific origin. For example, the presence of *Staphylococcus aureus* in bulk tank milk will, generally, be traced back to cows suffering from mastitis, and silage is the most likely origin of spores of butyric acid bacteria in bulk tank milk (Hayes et al., 2001).

At processing level, contamination may occur due to post-process contaminants which enter milk after heating, and heat resistant bacteria which survive heating (Rohrbach et al., 1992; Walstra, 2013). Post-pasteurization recontamination occurs most often during the filling process, which is an open process that allows milk to come in contact with the environmental air (Varnam and Sutherland, 1994). At the usual temperature for pasteurization of milk namely 72-75°C, most pathogenic and Gram-negative psychotropic bacteria are eliminated (Walstra, 2013). However, there are a number of survivors from a the natural flora which , given suitable conditions have the ability to cause spoilage (Frank, 2009; Jakobsen et al., 2011). There are two types of heat resistant bacteria : endospore forming genera and highly resistant vegetative genera.

Endospore-forming genera e.g. *Bacillus*, which are readily isolated from small numbers from pasteurized milk, are the most important (Walstra, 2013).

1.3.2. Hygiene indicators

The types of microorganisms present in raw milk are influenced by temperatures and time of storage as well as methods of handling during and after milking (Varnam and Sutherland, 1994). The growth of psychrotropic bacteria is of a major concern when raw milk is kept at low temperature, which is the normal practice in the modern dairy industry (Frank, 2009).

During growth of these bacteria, heat stable enzymes such as proteases and lipases are formed and consequently cause protein and lipid breakdown and related defects (Frank, 2009; Walstra, 2013). Therefore, the metabolic processes of psychrotrophs are considered more important than the total numbers of microorganisms. Species of the *Pseudomonas* genus are most important because of their ability to produce heat-stable enzymes particularly proteases and lipases (Robinson, 2005). The *Enterobacteriaceae* account for 5-33% of psychotropic microflora present in raw milk, and their optimum growth temperature (> 30°C) tends to be higher than that of *Pseudomonas*, but they adapt well to growth at refrigeration temperatures (Varnam and Sutherland, 1994). Other types of psychrotropic bacteria commonly found in milk include *Flavobacterium*, *Achromobacter*, *Aeromonas* , *Alcaligenes* and *Chromobacterium*. Spore-forming bacteria in raw milk are predominantly *Bacillus* spp. (Frank, 2009). All those bacteria are not naturally present in milk, and their presence reflects lack of hygiene during production or processing.

Enterococci are found in high concentrations in human feces, usually between 10^4 and 10^6 bacteria per gram wet weight (Layton et al., 2010), and are also found in animal feces (Harwood et al., 2000; Walters et al., 2009). While enterococci represent in most of cases less than 1% of the flora (Tendolkar et al., 2003), they are normally present in fecal consortium, but are outnumbered by other flora among others *Eshcerichia coli*, clostridia, and the *Bacteroidales* (Zubrzycki and Spaulding, 1962). As a consequence of their ubiquity in human and animal feces and the environment, enterococci have been adopted as indicators of fecal pollution and indeed lack of hygiene.

Staphylococcus aureus is among bacteria present in the human respiratory tract and the microbiological community of the skin, and is also associated with bovine mastitis (Humphreys, 2012; Shyaka et al., 2010). Its presence in dairy products indicates de facto lack of hygiene from milk handlers or dairy farms and/or subclinical mastitis in milking cows. Given its ability to produce toxins which cause staphylococcal poisoning in consumers when it reaches a concentration of 6.5 log CFU/mL in milk (Fujikawa and Morozumi, 2006) , its presence it plays a dual role and can be taken as indicator/or pathogenic microorganism.

1.3.3. Pathogenic microorganisms

Numerous milk-borne pathogens have been isolated from raw milk. The prevalence of these varies considerably, depending on geographical area, season, farm size, number of animals on farm, hygiene and farm management practices (Robinson, 2005; Rohrbach et al., 1992). Although the growth of most of these pathogens in milk is known to be prohibited by cooling and competing on non-pathogenic microorganisms (Frank, 2009), outbreaks of illness caused by *Campylobacter jejuni*, Shiga-toxin producing *E.coli*, *S. aureus*, *L.monocytogenes*, *Salmonella* spp. and *Yersinia enterocolitica* have been reported following consumption of raw milk (Rohrbach et al., 1992). Pathogenic microorganisms in raw milk are of two types : those that are involved in bovine mastitis and those that externally contaminate milk (Walstra, 2013)**.**

Considering the fact that milk is an excellent environment for the growth of different kinds of microorganisms, microbial hazards are the most important concern within the dairy industry (FAO, 2013). The principal pathogens linked with dairy are shown in table 1.4. With regards to milk and dairy products, *Salmonella* and *Listeria monocytogenes* are of main importance and the focus of the present PhD.

1.3.3.1. Salmonella

Salmonella is a genus of rod-shaped bacteria of the *Enterobacteriaceae* family. There are two species of *Salmonella* , *S. bongori* and *S. enterica*, of which there are around six subspecies and enumerable serovars. *Salmonella* are non spore-forming, predominantly motile enterobacteria (Reeves et al., 1989). Many infections are due to ingestion of contaminated food. They can be divided in two groups, namely typhoidal and non-typhoidal *Salmonella* serovars. Non-typhoidal serovars are more common, and usually cause self-limiting gastrointestinal disease.

They can infect a range of animals, and are zoonotic, meaning they can be transferred between humans and other animals (Walstra, 2013). The symptoms of Salmonellosis are diarrhea, nausea, stomach cramps, vomiting and fever (Olsen et al., 2001; Rhoades et al., 2009).

Table 1.4 : Pathogens associated with milk and dairy products

Pathogen	Main source of infection	Main means of on-farm control	Main means of control in processing and food handling
Bacillus cereus	Via milk	No effective control measures presently available	Good manufacturing and hygiene practices. Holding cooked foods at either >60 °C or <4 °C
Brucella abortus	Contact infection handling infected animals/materials). also via raw milk	Herd health management (vaccination, serological screening)	Milk pasteurization .Hygiene for at-risk workers
Cronobacter spp.	Associated with infant formula		Good manufacturing and hygiene controls in the production environment and during rehydration/reconstitution of the product. Control storage temperature and time of reconstituted product
Shiga toxinproducing *Escherichia coli* (STeC) also known as verotoxin-producing *E. coli* (VTeC)	Mainly via raw milk	Hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. Good manufacturing and hygiene practice
Campylobacter jejuni	Mainly via raw milk	Hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization.Good manufacturing and hygiene practices
Listeria monocytogenes	Mainly via raw milk and soft cheeses. also contact infection from handlinginfected animals /materials	Hygienic husbandry, herd health management	Milk pasteurization.Good manufacturing and hygiene practices. Prevention of postprocessing contamination
Mycobacterium bovis	Mainly via raw milk	Hygienic husbandry, herd health management, tuberculin testing and slaughter of positivereactors	Milk pasteurization
Salmonella spp.	Mainly via raw milk	Hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. Good manufacturing and hygiene practices.
Staphylococcus aureus	Mainly via raw milk	Milking hygiene, mastitis control	Milk pasteurization. Good manufacturing and hygiene practices. Process and storage control
Streptococcus zooepidermicus *S. agalactiae*	Mainly via raw milk	Milking hygiene	Milk pasteurization
Yersinia enterocolitica	Mainly via raw milk	Impractical (wide range of animal hosts)	Milk pasteurization
Coxiella burnetii	Via aerosol and milk. also possibly tick bite	Tick control, herd health management	Milk pasteurization.Hygiene precautions for at-risk workers

Source : FAO (2013)

Typhoidal serovars include *S. typhi* and *S. paratyphi*, which are adapted to humans and do not occur in other animals. Typhoid fever is caused by *Salmonella* serotypes which are strictly adapted to humans or higher primates, these include Salmonella typhi, *paratyphi* A, *paratyphi* B and *paratyphi* C (Frank, 2009; Robinson, 2005). In the systemic form of the disease, *Salmonellae* pass through the lymphatic system of the intestine into the blood of the patients (typhoid form) and are carried to various organs (liver, spleen, kidneys) to form secondary foci (septic form). Milk and dairy products are often incriminated in the occurrence of outbreaks of salmonellosis worldwide (De Buyser et al., 2001; Jayarao et al., 2006; Van Duynhoven et al., 2009), and impact losses in human lives and economic performance of the dairy industry. Table 1.5 presents some data on the presence of *Salmonella* in milk and dairy products. Given the importance of this disease, the European and Rwandan legislations suggests its total absence in 25 g, ml or cm^2 of the sampled products or surfaces (European Commission, 2004b; RBS, 2015).

Table 1.5 : Prevalence of *Salmonella* spp. and *Listeria monocytogenes* in milk and dairy products in USA, Belgium and EAC

Country	*Salmonella* (%)		References	*Listeria monocytogenes* (%)		References
	Milk	Dairy products		Milk	Dairy products	
USA	2.60	7.20	Van Kessel et al. (2004) Hedberg et al. (1992)	6.50	7.30	Van Kessel et al. (2004) MacDonald et al. (2005)
Belgium	0.00	0.00	De Reu et al. (2004)	6.30	0.00	De Reu et al. (2002); De Reu et al. (2004)
Kenya	---	0.00	Opiyo et al. (2013)	-	-	-
Uganda	5.00-10.00		Grimaud et al. (2009)	13.00	3.00	Mugampoza et al. (2011)
Tanzania	10.10	66.00	Kussaga et al. (2014b); Schoder et al. (2013)	66.00	32.00	Kussaga et al. (2014b)

1.3.3.2. Listeria monocytogenes

Listeria monocytogenes is the bacterium that causes the infection listeriosis. It is a facultative anaerobic bacterium, capable of surviving in the presence or absence of oxygen (Chambers, 2002; Tamime, 2009).

It can grow and reproduce inside the host's cells and is one of the most virulent food-borne pathogens, with 20 to 30 percent of clinical infections resulting in death. Due to its frequent pathogenicity, causing meningitis in newborns (acquired transvaginally), pregnant mothers are often advised not to eat soft cheeses such as Brie, Camembert, feta, and queso blanco fresco, which may be contaminated with and permit growth of *L. monocytogenes*. Cheeses made from unpasteurized milk (raw and/or thermized) are often incriminated in outbreaks of listeriosis in Belgium, USA and Canada (Verraes et al., 2015) *L. monocytogenes* is a Gram-positive, non-spore-forming, motile, facultatively anaerobic, rod-shaped bacterium. It is catalase-positive and oxidase-negative, and expresses a beta hemolysin, which causes destruction of red blood cells (Robinson, 2005). This bacterium exhibits characteristic tumbling motility when viewed with light microscopy. Although *L. monocytogenes* is actively motile by means of peritrichous flagella at room temperature (20–25 °C), the organism does not synthesize flagella at body temperatures (37 °C) (Frank, 2009; Varnam and Sutherland, 1994). Milk and dairy products are reputed to be regularly involved in multiple listeriosis outbreaks (Jackson et al., 2011; Koch et al., 2010; Ryser and Marth, 2007). *L. monocytogenes* is frequently isolated from milk and dairy products (Table 1.5) and both the European and Rwandan legislation suggest its total absence in 25 gr, ml or cm^2 of the sampled product or surface (European Commission, 2004b; RSB, 2015a).

1.4. Food safety management systems in the dairy chain

The above mentioned occurrences of food-borne diseases in the dairy sector, constitute an important threat for the well-being of consumers. Nowadays, food safety is of great importance in the global food supply chain, and in the world economy one bottleneck in the food chain is a potential source of dangers to the consumers' health (Wilcock et al., 2011). Stakeholders in the food chain such as consumers, inspectors and regulatory agencies have increasingly required that food-manufacturing firms minimize the risk of food safety hazards (Arpanutud et al., 2009). The production of safe food should start from production level to the consumption level, following the food chain approach (European Commission, 2004b).

Codex Alimentarius has established general guidelines embedded in the code of hygienic practices for milk and milk products (CAC, 2004). Nowadays, various internationally acknowledged national systems or private standards are available for guaranteeing food safety. These include HACCP, British Retail Consortium (BRC), Safe Quality Food (SQF), and ISO 22000: 2005 (Jacxsens, 2009; Luning et al., 2008).

HACCP is internationally recognized as a tool enabling food businesses to identify preventively potential failures with regard to safe food production and then to identify prevention strategies (CAC, 2003) . Meanwhile, HACCP are principles are not feasible in the primary sector, due to the absence of adequate and low-cost monitoring tests (Gardner, 1997).
The implementation of FSMS in the African food industry is still low and faces different challenges e.g. lack of risk based legislation frameworks and training (Kussaga et al., 2014a). International standards and HACCP are mainly implemented for exporting companies, whereas companies targeting the domestic market are not often involved (Gran et al., 2002b; Kussaga, 2013). In Rwanda, the implementation of HACCP is still in its inception, and only 22 food companies (among which two dairy companies) are involved in a pilot certification base, undertaken by the RSB (RSB, 2015b).

In order to obtain safe dairy foods it is essential to control and asses all steps from stable to table which could risk the hygiene and safety of the end product. It has been shown that effective implementation of Prerequisite Program (PRPs) in early stages of milk production plus an adequate HACCP system in later process can results on improved quality and hygiene of milk and dairy products (Nada et al., 2012). PRPs can be defined as "every specific and documented activity or facility that is implemented corresponding to the Codex General Requirements of Food Hygiene, the Good Manufacturing Practices, and the legislation, with the purpose to create basic requirements that are necessary for the production and processing of safe foods in all stages of the food chain" (Jacxsens, 2009).

The principal PRPs include: cleaning and disinfection; pest control; water and air quality; temperature control and registration; personnel (facilities, hygiene, health, education); structure and infrastructure; technical maintenance and calibration; waste management; control of raw material; traceability, recall, goods returned, rejections; allergens; physical and chemical contaminates; management of product information; work methodology (Jacxsens, 2009). HACCP is defined as *"a methodology that identifies, evaluates and controls hazards that are significant for food safety"* (Jacxsens, 2009). A HACCP plan can be applied only in a processing plant constructed and functioning in sanitary conditions and environment, meaning that adequate implementations of PRPs are essential for the correct development of a HACCP (Tamime, 2009). According to Jacxsens (2009), the HACCP system consists of the following 7 principles :

- ***Principle 1 : Conduct a hazard analysis*** .The HACCP team should list all of the hazards that may be reasonably expected to occur at each step from primary production, processing, manufacture and distribution until the point of consumption. The HACCP team should next conduct a hazard analysis to identify for the HACCPPLAN, which hazards are of such a nature that their elimination or reduction to acceptable level is essential to the production of a safe food. The HACCP team must then consider what control measures, if any, exist which can be applied to each hazard.

- ***Principle 2 : Determine the critical control points (CCP's)***. Determine the steps/points/procedures at which control can be applied and is essential to prevent or eliminate a food safety hazard or reduce it to an acceptable level. A "step" means any stage in food production and/or manufacture including raw materials, delivery, transport, composition, processing, storage, etc. There may be more than one CCP at which control is applied to address the same hazard. If a hazard has been identified at a step where control is necessary for safety, and no control measure exists at that step, or any other, then the product or process should be modified at that step, or at any earlier stage , to include a control measure.

- ***Principle 3 : Establish critical limit(s)*** : Critical limits must be specified and validated if possible for each CCP. In some cases more than one critical limit will be elaborated at a particular step. Criteria often used, include measurements of temperature, time, moisture level, pH, water activity, available chlorine, and sensory parameters such as visual appearance and texture.

- ***Principle 4 : Establish a system to control of the CCP***: Monitoring is the scheduled measurement or observation of a CCP relative to its critical limits. The monitoring procedures must be able to detect loss of control at the CCP. Further, monitoring should ideally provide this information in time to make adjustments to ensure control of the process to preventing violating the critical limits. Where possible, process adjustments should be made when monitoring results indicate a trend towards loss of control of a CCP. The adjustments should be taken before a deviation occurs. Data derived from monitoring must be evaluated by a designated person with knowledge and authority to carry out corrective actions when indicated. If monitoring is not continuous, then the amount of frequency of monitoring must be sufficient to guarantee the CCP is in control. Most procedures for CCP's will need to be done rapidly because they relate to online processes and there will not be time for lengthy analytical testing. Physical and chemical measurements are often preferred to microbiological testing because they may be done rapidly and can often indicate the microbiological control of the product. All records and documents associated with monitoring CCP's must be signed by the person (s) doing the monitoring and by a responsible reviewing official(s) of the company.

- ***Principle 5 : Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.*** Specific corrective actions must be developed for each CCP in the HACCP system in order to deal with deviations when they occur. The action must ensure that the CCP has been brought under control. Actions taken must also include proper disposition of the affected product. Deviation and product disposition procedures must be documented in HACCP record keeping.

- ***Principle 6 : Establish procedures for verification to confirm that the HACCP system is working effectively.*** Establish the procedures for verification. Verification and auditing methods, procedures and tests, including random sampling and analysis, can be used to determine if the HACCP system is working correctly. The frequency of verification should be sufficient to confirm that the HACCP system is working effectively.

- ***Principle 7 : Establish documentation concerning all procedures and records appropriate to these principles and their application***. Efficient and accurate record keeping is essential to the application of HACCP system. HACCP procedures should be documented. Documentation and record keeping should be appropriate to the nature and size of the operation.

Under a HACCP system, pasteurization should be arranged as a CCP (Critical Control Point). Other typical CCP in milk could be antibiotic detection (Van Schothorst and Kleiss, 1994) and metal detection especially in infant formulas (Simonsen et al., 1987). With regards to cheese making, the fermentation process is typically considered a CCP (Schmidt and Newslow, 2007).

The nutritional properties combined with large economic benefits of milk and dairy products confer them a major role in our everyday life. Numerous hazards threatening consumers should then be specifically addressed, in order to provide safe milk and dairy products. In developing countries especially, a focus is needed on microbiological quality and safety of milk and dairy products.

CHAPTER 2 : Microbiological quality and safety assessment of the Rwandan milk and dairy chain

Redrafted from :

Kamana, O., Ceuppens, S., Jacxsens, L., Kimonyo, A., and Uyttendaele, M. (2014). Microbiological Quality and Safety Assessment of the Rwandan Milk and Dairy Chain. Journal of Food Protection 77, 299-307.

CHAPTER 2 : Microbiological quality and safety assessment of the Rwandan milk and dairy chain

2.1. ABSTRACT

Milk is a valuable and nutritious food product, which can partially fulfill the rising food demand of the growing African population. The microbiological status of milk and derived products was assessed throughout the milk and dairy chain in Rwanda by enumeration of the total mesophilic count (TMC), coliforms, *Staphylococcus aureus* and detection of *Salmonella* ssp. and *Listeria monocytogenes*. The quality of raw milk was satisfactory for the majority of samples, but 5.2 % contained *Salmonella*. At the processing level, the TMC and coliform numbers indicated ineffective heat treatment during pasteurization or post-pasteurization contamination. Increasing bacterial counts were observed along the retail chain and could be attributed to insufficient temperature control during storage. Milk and dairy products sold in milk shops were of poor and variable microbiological quality in comparison with the pasteurized milk sold in supermarkets. Especially the microbiological load and pathogen prevalence in cheese was unacceptably high.

2.2. INTRODUCTION

Milk and derived products constitute an important part of the traditional diet of the African population in many countries, including Rwanda. General milk consumption in Africa increased from 35 to about 40 kg per capita in the period of 1990 to 2004 (FAOSTAT, 2015a). Although milk production has also been increasing, the demand has increased considerably due to the continuing population growth. Therefore, milk powder is frequently imported to make up the deficit in demand. In Africa, milk is consumed in various ways: raw, pasteurized, after (natural) fermentation or after home boiling, e.g. for making tea. The most consumed form varies considerably depending on the culture and nationality. For example, the large majority ($\geq$ 96 %) of consumers in central Kenya consume boiled or pasteurized milk (Arimi et al., 2005) , while people more frequently consume raw milk and/or products derived from unpasteurized milk in rural Kenya, Burkina Faso, Zimbabwe, Tanzania and Ethiopia (Gonfa et al., 2001; Gran et al., 2002b; Mathara et al., 2004; Millogo et al., 2010; Swai and Schoonman, 2011).

The production of fermented milk products has been traditionally and culturally important in Africa, since fermentation of raw milk is a useful way of preserving this highly nutritious but also highly perishable food (Beukes et al., 2001). As a consequence, a wide variety of traditional fermented milk products exists and these are of great dietary and socio-economic importance (Beukes et al., 2001; Gonfa et al., 2001; Gran et al., 2002b; Mathara et al., 2004). Most traditional products are prepared without pasteurization by spontaneous fermentation, commonly initiated by either back slopping i.e fresh milk inoculation with the spoiled one, or by repeated use of the same utensils, in plastic or natural containers such as milk-sacks, calabashes, clay pots, stone jars and baskets (Beukes et al., 2001; Gonfa et al., 2001).

Besides its nutritional importance, milk also serves as a major financial resource in rural areas for trade or to buy other household goods and to pay taxes and (school) fees. For example, milk products contribute approx. 30 % to the family income in Ethiopia (Gonfa et al., 2001). Finally, milk derived products are also used as cosmetics by rural people, e.g. butter as hair and body oil (Gonfa et al., 2001)

Raw milk may be contaminated with pathogens from the farm environment, such as *Salmonella* spp., pathogenic *Escherichia coli* strains (including *E. coli* O157:H7), *Listeria monocytogenes* and *Staphylococcus aureus,* or from infected animals, such as *S. aureus* (mastitis) and *Mycobacterium avium* subsp. *paratuberculosis* (Johne's disease) (Kousta et al., 2010). Pathogens can also enter milk and dairy products through implementation of unhygienic practices, through environmental contamination with utensils, contact surfaces, floor and packaging material and through contaminated ingredients such as contaminated brine or starter cultures.

Normally, pathogenic bacteria in raw milk are eliminated by pasteurization, but insufficient control of the time-temperature treatment may lead to under-pasteurization and insufficient control of the storage temperature may lead to outgrowth of surviving microorganisms and thus reduced safety and shelf-life of the products. As a consequence, milk and dairy products may contain various bacterial pathogens and constitute a vehicle for food-borne disease, since these products are often consumed without further (heat) treatment.

Since the health benefits of milk and dairy consumption should not be compromised by food-borne illness, the microbiological quality and safety of milk and derived products was investigated in the Rwandan milk and dairy chain to reveal its present status.

2.3. MATERIAL AND METHODS

2.3.1 Study area and sampling

The study was conducted along the dairy chain in Rwanda, following a food chain approach, in four milk basins (Kigali, Umutara, Nyanza and Gishwati) and the two largest cities (Kigali and Musanze) (Figure 1-3).

Those milk basins were selected based on their large production and their different geographical areas, representing the diversity of the country. Cities were chosen because of their high populations and the importance of their commercial activities, making them ideal for studying dairy products at wholesale and retail points. The sampling plan of this study is shown in Table 2.1. , indicating the nature and number of samples analyzed. First, at the milk production level, two farms were assessed per farming category, i.e. the zero grazing, the open grazing and the semi grazing system, following a convenience sampling plan, which was also used for the whole chain. Next, eight milk collection centres (two in each milk basin), were sampled once at the start of the raw milk collection in the morning, once at the closure time and in between three times upon milk collection from different suppliers. Collection centers receive milk directly from farms and/or from mobile traders, and are the confluence of the formal and the informal markets (Figure 1.4). At the processing level, three milk processing companies and three small cheese producers were sampled on three different occasions for respectively pasteurized milk and cheese. Also, two wholesale points were sampled twice for pasteurized milk.

Table 2.1 : Overview of the sampling plan conducted along the Rwandan milk and dairy chain

Step of the chain	No.	Sample	No. of samples	Sampling rounds	Total
Farm	6	Raw milk	3	3	54
Milk collection centres	8	Raw milk- Beginning of collection	1	3	24
		Raw milk- Suppliers	3	3	72
		Raw milk- End of collection	1	3	24
Companies	3	Pasteurized milk	3	3	27
Wholesale	2	Pasteurized milk	2	3	12
Small cheese plants	3	Gouda cheese	3	3	27
Supermarkets	6	Pasteurized milk	2	3	36
Milk shops	3	Boiled milk	4	3	36
	2	Fermented milk	1	3	6
		Gouda cheese	1	3	6
	1	Boiled milk	1	3	6
TOTAL					**330**

Finally, at the retail level, three supermarkets and three milk shops were selected in Kigali and three of each retail type in Musanze.

Supermarkets were sampled twice for pasteurized milk and four milk shops were sampled twice for boiled milk and two of them were sampled once for cheese and the two others were sampled once for fermented milk, while three boiled milk samples were taken from two other milk shops. The formal route of milk is comprised of milk processing plants, wholesale points and supermarkets, while small cheese producers and milk shops are part of the informal one (Table 2.1). This sampling schedule was repeated three times in the time period January to May 2012, resulting in 330 samples analyzed for the microbiological parameters described below.

2.3.2. Microbiological analyses

Samples were collected under aseptic conditions and transported in an ice box to be analyzed for the total mesophilic count (TMC), coliforms, *S. aureus*, *Salmonella* and *L. monocytogenes.* The TMC was determined using the pour plate method on plate count agar (Oxoid, Cambridge, UK) according to the ISO 4833:2003 standard protocol (ISO, 2003) . The total coliform enumerations were performed using the pour plate method on violet red bile lactose agar (Oxoid) following ISO 4831:2006 (ISO, 2006) . The presence of *S. aureus* was determined using the spread plate technique on Baird-Parker agar plates (Oxoid) and presumptive colonies confirmed by the coagulase test, as described in ISO 6888-1:1999 (ISO, 1999).

The presence of *Salmonella* was detected in 25 mL or 25 g samples according to ISO 6579:2002+A1:2007 (ISO, 2002) i.e. by nonselective enrichment in buffered peptone water (Oxoid), cultivation on modified semisolid Rappaport Vassiliadis (Oxoid), selective isolation on xylose lysine desoxycholate agar (Oxoid), and purification of presumptive isolates on nutrient agar (Oxoid), and finally, confirmation of typical colonies by the triple sugar iron test. *L. monocytogenes* was detected in 25 ml or 25 g by following the ISO 11290-1 protocol (ISO, 1996) with a pre-enrichment in half-Fraser broth (Oxoid), selective enrichment in Fraser broth (Oxoid), cultivation on Brilliance Listeria agar (Oxoid), and confirmation of typical colonies by a catalase test, hemolysis, carbohydrate use, and Camp tests.

2.3.3.Temperature and pH

The temperature and pH of the samples were determined using a thermometer (HANNA HI 955501) and a pH meter (HANNA HI 207) respectively.

2.3.4. Statistical analyses

Data were analyzed with SPSS Statistics 20. ANOVA and post-hoc Bonferroni test were performed on normally distributed variables with equal variances, while Kruskal-Wallis and Mann-Whitney U tests were used for non-normally distributed variables. Calculation of the 95% confidence intervals for pathogen prevalence was calculated according to the Wilson score method without continuity correction (Wilson, 1927). Microbiological counts were log transformed prior to statistical analyses and calculations. For coliforms and *S. aureus* counts, statistical analyses and graphical presentations were performed considering enumerable samples only, i.e. the above the lower quantification limit of 1 log CFU/mL and 2 log CFU/mL respectively.

2.4. RESULTS AND DISCUSSION

The structure of the Rwandan dairy chain from production to consumption is presented in Figure 1.4. Milk from farms follows two routes, namely through the formal and the informal markets. However, there are interconnections between those two routes, making the overall milk supply chain in Rwanda complex. The informal market is the predominant one (MINAGRI, 2008), while the importance of industrially processed milk varies according to the different sources. According to the Rwanda Animal Resources Development Authority (RARDA) (RARDA, 2009), 10 % is processed industrially; whereas TECHNOSERVE (2008) mentions that the formal market share is only 2 %.

Similarly, the dairy industry in Tanzania was reported as predominantly informal (80 to 90 % of all milk) , because informal milk markets provide social and economic benefits such as higher farm gate prices, creation of employment and competitive consumer prices (Swai and Schoonman, 2011).

2.4.1. Microbiological quality of raw milk

The average TMC of raw milk was 5.2 (± 0.2) log CFU /mL when collected at farm level (n = 54) and increased slightly to 5.5(± 0.2) log CFU /mL when collected at milk collection centres (n = 120). No significant difference (p=1.000) between raw milk from different suppliers or at different times of collection, i.e. the start vs. the end of collection, was noted at the collection centres (Figure 2.1). Time of collection could vary from 2 h to 3 h. The average pH of raw milk decreased significantly to 6.0 at the collection level in comparison with that at the farm level (6.4) and showed a high between-sample variation (Figure 2.2b). The milk temperature at farms was high, namely 36.0 °C, since it was taken just after milking (Figure 2.2a).

Raw milk temperature decreased at the collection level due to the presence of cooling facilities, but a high variability was noted among suppliers. This high variability can be explained by the fact that raw milk is supplied at milk collection centres by farmers and mobile traders in stainless steel cans using bicycles or pick up vehicles without refrigeration. The milk temperature thus depends on the time needed to transport the milk from the farm to the milk collection centre and on the ambient temperature.

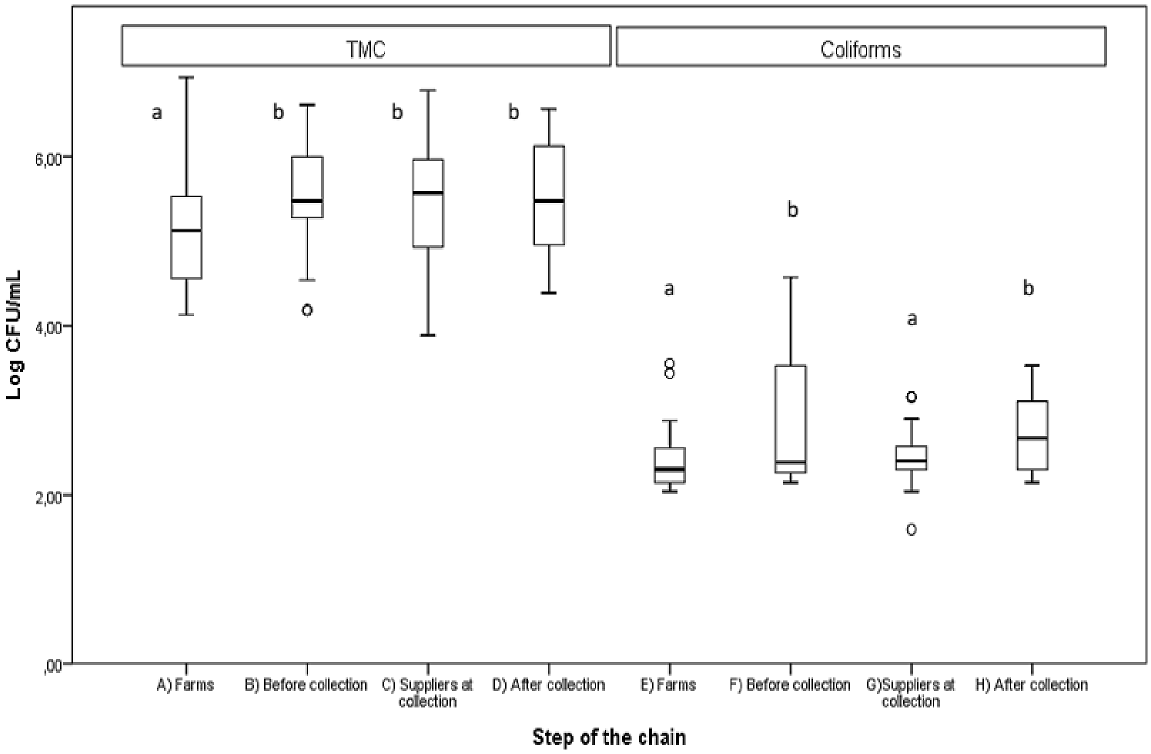
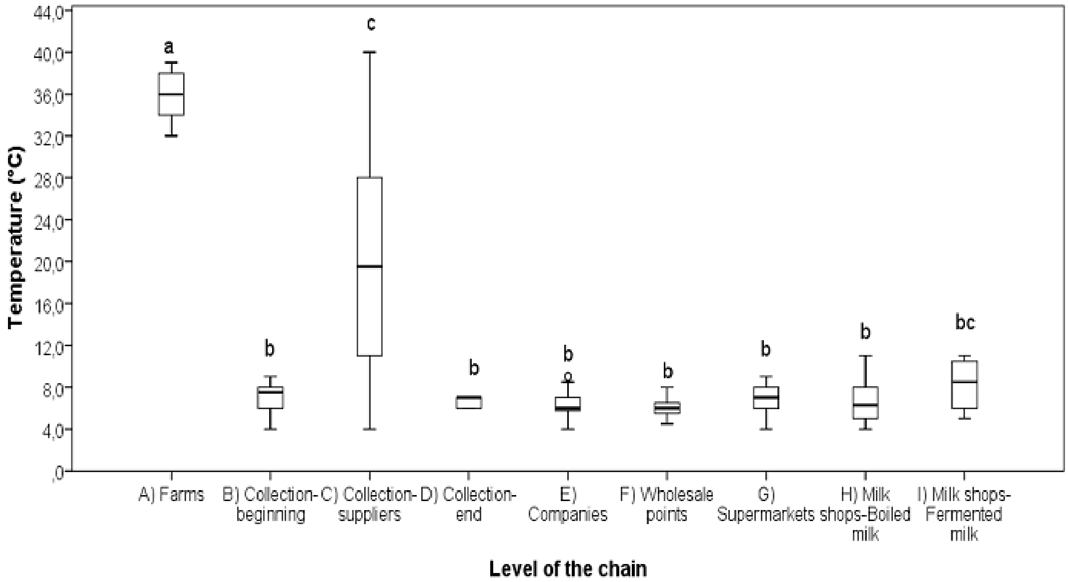


Figure 2.1 : Microbiological quality of raw milk at the farm and collection center level. Different letters indicate statistically significant differences (p<0.05).

a



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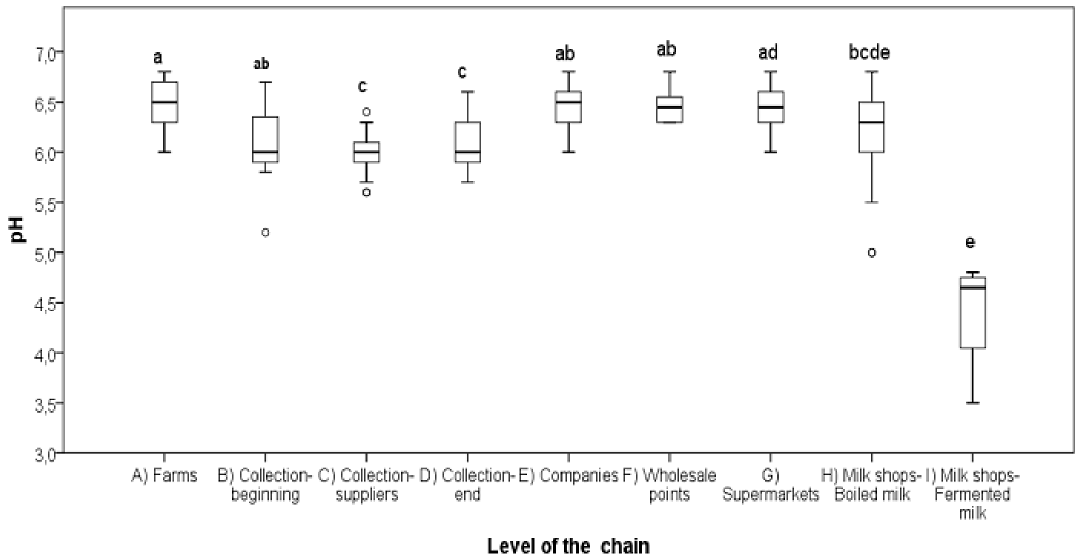

Figure 2.2 : The pH (a) and the temperature (b) of milk sampled along the dairy chain.

The microbiological load of raw milk differed significantly (p = 0.001) among the different farm types (Figure 2.3). The average total mesophilic count (TMC) of raw milk from the zero grazing system (Farm 1 and 2) was significantly lower than that from the semi-grazing system (Farm 3 and 4), but not significantly different from that of the open grazing system (Farm 5 and 6). No significant differences were found in the coliforms (p =0.725) and *S. aureus* counts among the different farm types. Coliforms were enumerated in 92.5% of samples , and *S. aureus* in 42.6% of samples (n=54). In the zero grazing system, exotic (to Rwanda) or improved cattle breeds are kept in well maintained enclosures, fed with forage and raised in an intensive live-stock production system to optimize their milk production. These modern farms are equipped with milking machines and cooling facilities. In addition, animal health and personnel hygiene are monitored. In contrast, the open grazing system is the traditional one in Rwanda, in which cattle of local breeds are raised on an open pasture without intensive care, whereas the semi-grazing system is a transitional farming system.

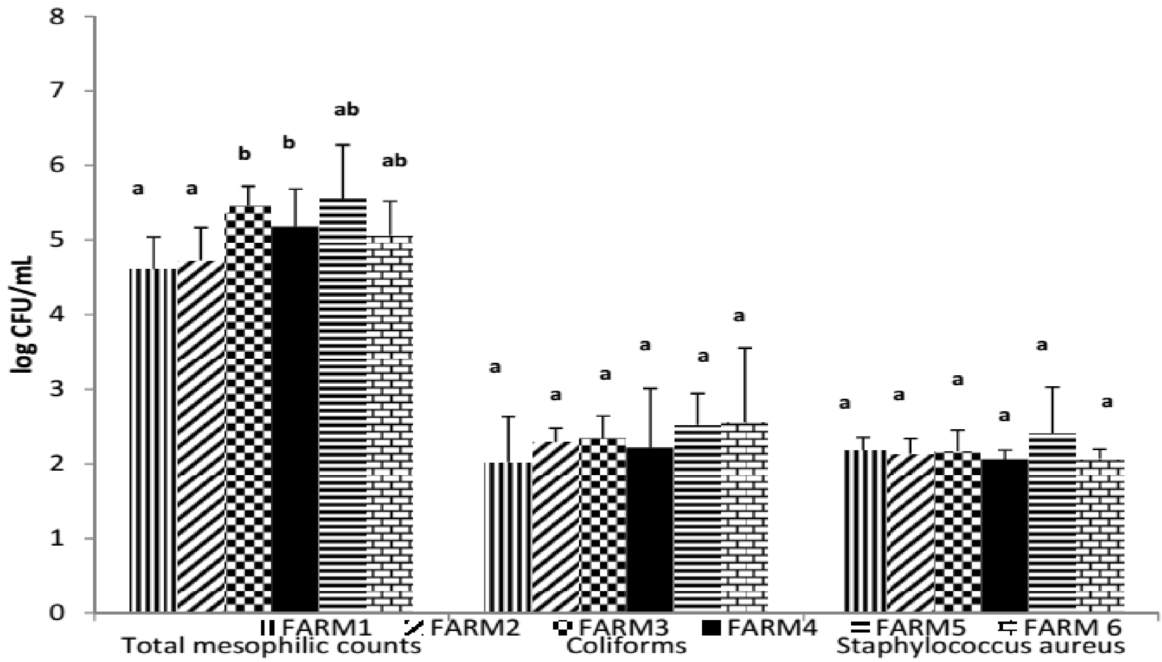

Figure 2.3 : Microbiological quality of raw milk at farms with different farming systems. Different letters indicate statistically significant differences (p<0.05).

European Union (EU) requirements for raw milk intended to be heat-treated or processed into dairy products include the upper limit of 5 log /CFU mL for the total plate count (European Commission, 1989, 1992, 2004b). Similarly, the US national standards for milk sanitation have established a total plate count limit of 5.0 log CFU/mL for raw milk prior to pasteurization for the individual producers and 5.5 log CFU/mL after commingling of raw milk (Food and Drug Administration, 2009). Finally, the Zimbabwe Dairy Regulations also stipulate < 5.0 log CFU /mL for TMC for Grade A milk, in addition to < 3.0 log CFU /mL for coliforms and < 1.0 log CFU /mL *E. coli* (Republic of Zimbabwe, 1977).

Less stringent conditions are dictated by the East African Community Standard (EACS) for satisfactory quality of raw milk, namely < 4.7 log CFU /mL coliforms (EAC, 2006).The TMC of raw milk quality in farms and milk collection centers ranged from 4.1 log CFU /mL to 6.94 log CFU /mL and 15% of all coliform counts were < 3.0 log CFU/ mL .Therefore, raw milk quality in farms and milk collection centers was almost satisfactory, since the microbiological parameters were only slightly above most international standards. In comparison, the TMC of raw milk ranged from 5.3 to 7.8 log CFU /mL in Botswana, from 5.8 to 8.6 log CFU /mL in Trinidad, from 6 to 8 log CFU /mL in Burkina Faso and was on average 8.1 log CFU /mL in urban Mali and 6.4 log /CFU mL in Zimbabwe (Aaku et al., 2004; Adesiyun et al., 1995; Bonfoh et al., 2002; Mhone et al., 2011; Millogo et al., 2010). Moreover, raw milk contained on average 5.5 log CFU/ mL coliforms in milk storage tanks at farms in the Côte d'Ivoire and 6.0 log CFU /mL during sale (Kouame-Sina et al., 2010), while retail raw milk in Tanzania contained on between 6.2 and 6.6 log CFU/mL coliforms (Swai and Schoonman, 2011). In general, the microbiological quality of raw milk in Africa does not comply with the international standards mentioned above, probably due to the lack of sufficient temperature control and hygiene.

2.4.2. Microbiological quality of pasteurized milk

Formal raw milk processing consists of pasteurization in companies. After processing, the TMC of pasteurized milk was decreased to 4.6 (± 0.6) log CFU/mL and the number of coliforms was lowered from 2.7 log CFU /mL to 2.5 log CFU /mL (Figure 2.4).

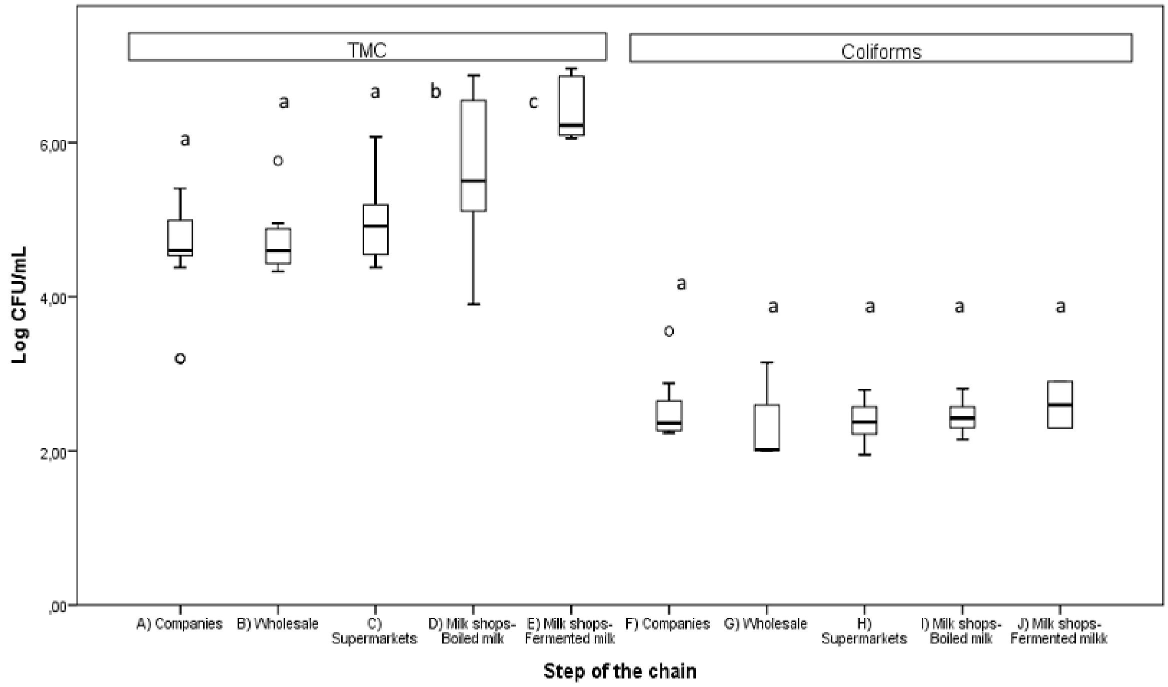

Figure 2.4 : Microbiological quality of processed milk i.e. pasteurized milk in companies, wholesale points and supermarkets; boiled milk and fermented milk in milk shops. Different letters indicate statistical significant differences (p<0.05).

In companies, 29.6% of samples (n=27) were above the quantification limit for coliforms in pasteurized milk at company level. These high total counts in pasteurized milk indicate ineffective pasteurization and/or post-pasteurization contamination. Therefore, hygienic conditions during the production processes should be improved and the production processes should be regularly monitored at their critical control points. Milk sampled at the processing companies had an average pH of 6.4 and an average temperature of 6.3 °C (Figure 2.2), which indicates effective temperature control in the companies. The milk quality also differed significantly according to the processor ($p < 0.001$) (Figure 2. 5).

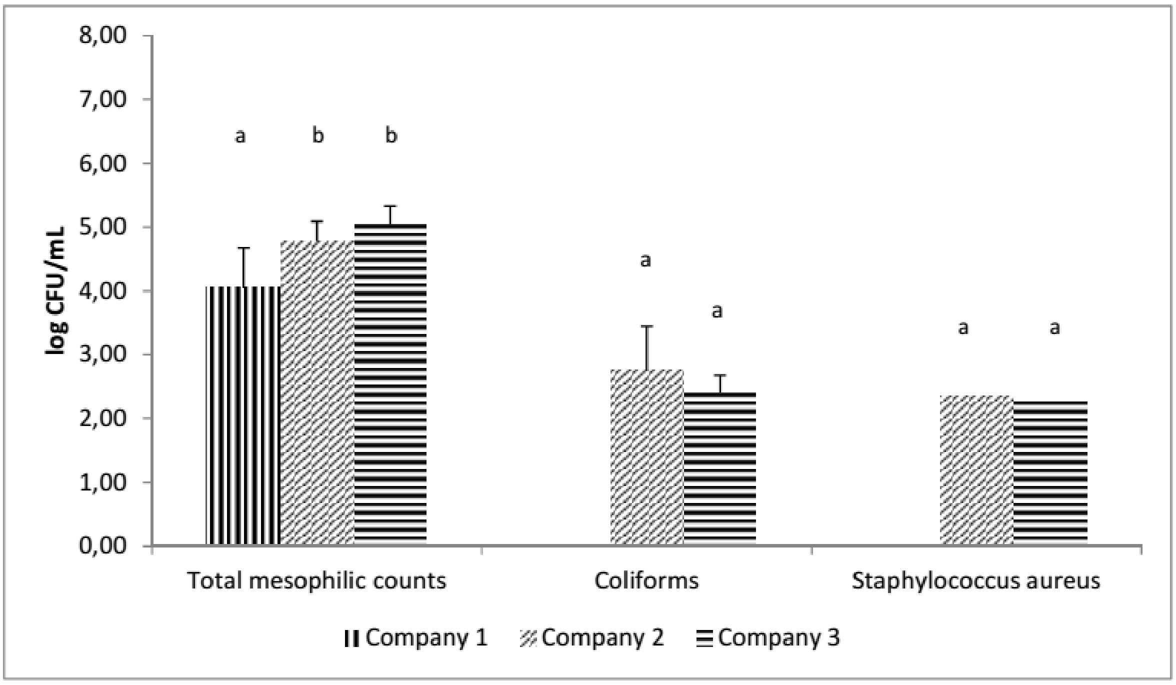

Figure 2.5 : Microbiological quality of pasteurized milk in three different milk processing companies. The quality was higher in company 1 where no coliforms and *S. aureus* were enumerated. Different letters indicate statistically significant differences (p<0.05).

Company 1 had the lowest average of TMC, (4.04 log CFU/mL) Company 2 and Company 3, had 4.78 log CFU/mL and 5.05 log CFU/mL respectively. Company 1 is the leading milk processing company in Rwanda, in terms of processing capacity and the variety of products. In contrast to companies 2 and 3, company 1 follows up the suppliers' raw milk quality, has implemented HACCP and obtained certification, which are probably the reasons for the observed differences in microbiological milk quality (Nada et al., 2012).

The pasteurized milk is supplied by companies to wholesale points, where milk samples showed similar values for all measured milk parameters as that in companies. The observed similarities in companies and wholesale points can be explained by two reasons. First, they collaborate for marketing purposes and try to attract clientele by providing safe products sold in a clean environment. Second, the competent authority, namely the Rwandan Bureau of Standards (RBS), controls the microbiological quality and the implementation of Good Manufacturing Practices (GMP) in companies and whole sale points and to a much lesser extent in retail points.

The pasteurized milk from wholesale points is sold in supermarkets, where an increase of average TMC (+ 0.3 log CFU/mL to 4.9 log CFU/mL), coliforms (+ 0.02 log CFU/mL to 2.17 log CFU/mL) and temperature (+ 0.7 °C to 7.1 °C) was observed, while the average pH remained constant (6.4) (Figure 2.4. and 2.2). The increase of bacterial counts and temperature in supermarkets is linked to breaking of the cold chain due to the lack of energy for continuously keeping the refrigerators at the required temperatures, meaning that refrigerators are switched off at night time to save energy.

According to the US national milk standards, pasteurized milk and milk products should contain < 4.3 log CFU/mL or g total bacteria and < 1 log CFU/mL or g coliforms after pasteurization. Therefore, the microbiological quality of pasteurized milk and derived dairy products was not acceptable in Rwanda, especially since these values continued to increase along the milk chain, resulting in 4.9 log CFU/mL for TMC and 2.1 log CFU/mL for coliforms. In comparison, the TMC of pasteurized milk ranged from 2.0 to 4.3 log CFU/mL in Botswana and was on average 6.6 log CFU/mL in Zimbabwe (Aaku et al., 2004; Mhone et al., 2011).

2.4.3. Microbiological quality of boiled and fermented milk in milk shops

Besides supermarkets, milk is also offered to consumers in milk shops. However, instead of pasteurized milk, they offer boiled and fermented milk, which are produced onsite in these shops and showed considerably increased average values for TMC and coliforms, respectively to 5.6 logCFU/mL and 2.5 log CFU/mL for boiled milk, in compared with 5.0 and 2.1 log CFU/mL for pasteurized milk sold in supermarkets (Figure 2.4). In milk shops, milk is supplied by different actors (Figure 1.4) and checked for density and pH. After boiling, one part is refrigerated in containers and another is cooled to approximately 40 °C for a short time, followed by inoculation with spoiled milk and kept at room temperature for 24 h and thus becomes fermented milk. After fermentation, it is kept refrigerated and served cold. From milk reception to serving, many activities are carried out informally and unstandardized.

Firstly, milk with low density can be accepted at reception for a cheaper price. Secondly, the boiling is an uncontrolled process and the used recipients and containers are cleaned in variable ways. Thirdly, milk is sold until it is finished instead of considering its shelf life and fourthly, personnel hygiene is not under control. The high temperature variation in milk shops is due to the frequent opening and closing of refrigerators to serve clients and due to the addition of freshly boiled milk to the refrigerators, causing temperature fluctuations in the stored milk products. Due to all these reasons, a high variability in boiled and fermented milk quality was noticed, i.e. the average TMC varied > 1.0 log CFU/mL over the three rounds of sampling in multiple milk shops (Figure 2.6).

The high number of milk shops makes it difficult for the competent authorities to perform the appropriate number of controls.

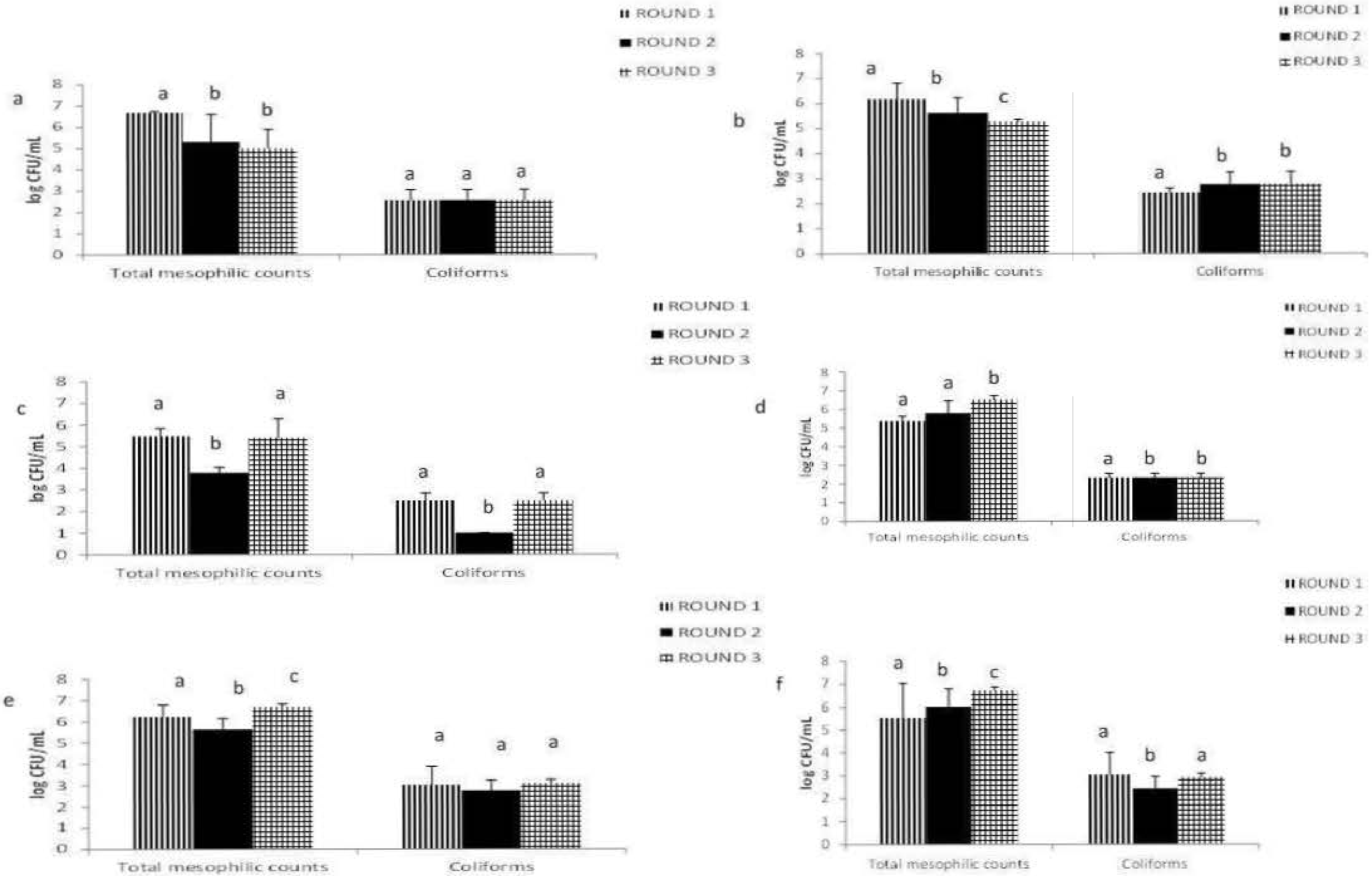

Figure 2.6 : Variability in the microbiological quality of boiled milk offered for sale in six milk shops (a-f) during the three different sampling rounds conducted during the study. Different letters indicate statistically significant differences (p<0.05)

2.4.5 Microbiological quality of cheese at the processing and retail level

The microbiological load of cheese was the highest of all dairy products (TMC > 6.0 log CFU/g, Figure 2.7) due to fermentation. No significant differences were found (p =1.000) in the microbiological load of Gouda cheese from small traditional cheese making factories mainly located in the West, in the Gishwati milk basin (Figure 2.8).

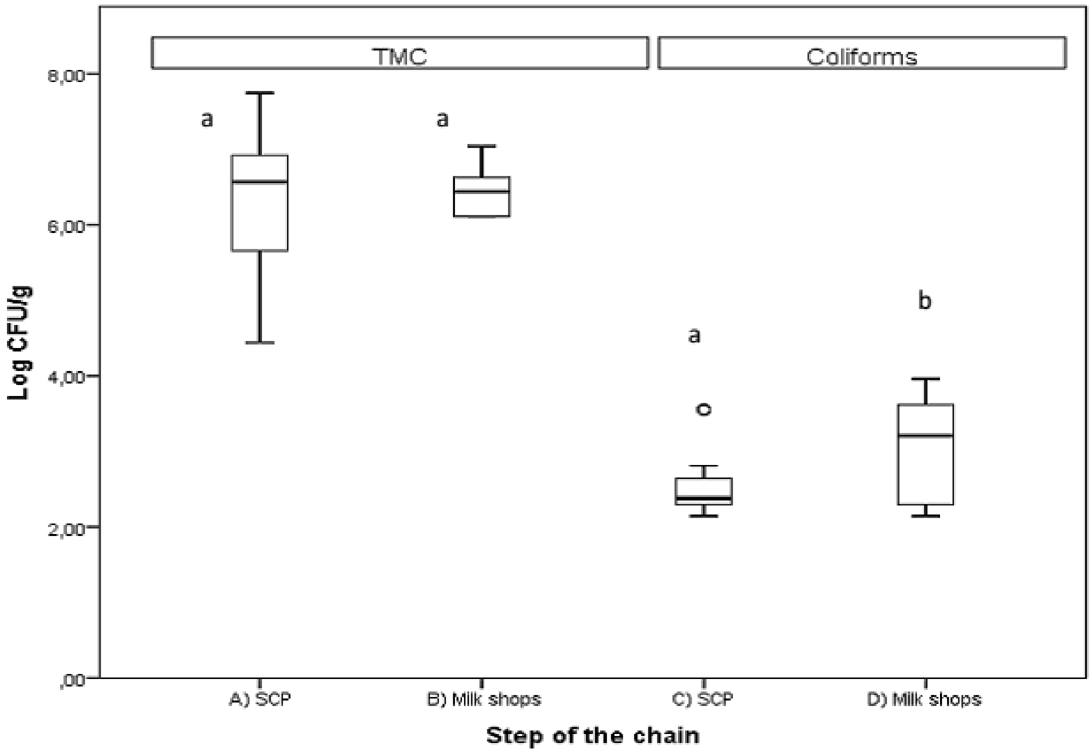

Figure 2.7 : Microbiological quality of cheese at production and retail levels. Different letters indicate statistically significant differences (p<0.05)

Coliforms were present in high amounts (2.5 log CFU/g, 100% of samples ,n=27, were above the quantification limit) in cheese sampled from the small cheese producers due to suboptimal production environment conditions, i.e. inadequate personnel hygiene, the absence of effective cleaning and disinfection, use of wooden materials which hamper efficient cleaning and disinfection, the design of the premises without separation between clean and dirty areas and the use of untreated water. Cheese sold in milk shops showed an increase in coliforms from 2.5 log CFU/mL to 3.0 log CFU/mL and *S. aureus* from 2.3 log CFU/mL to 2.9 log CFU/mL, in comparison to cheese sampled at the small cheese producers (Figure 2.7, Table 2.2), probably due to poor hygienic conditions during transport and sale, resulting in additional microbiological multiplication or post-processing contaminations.

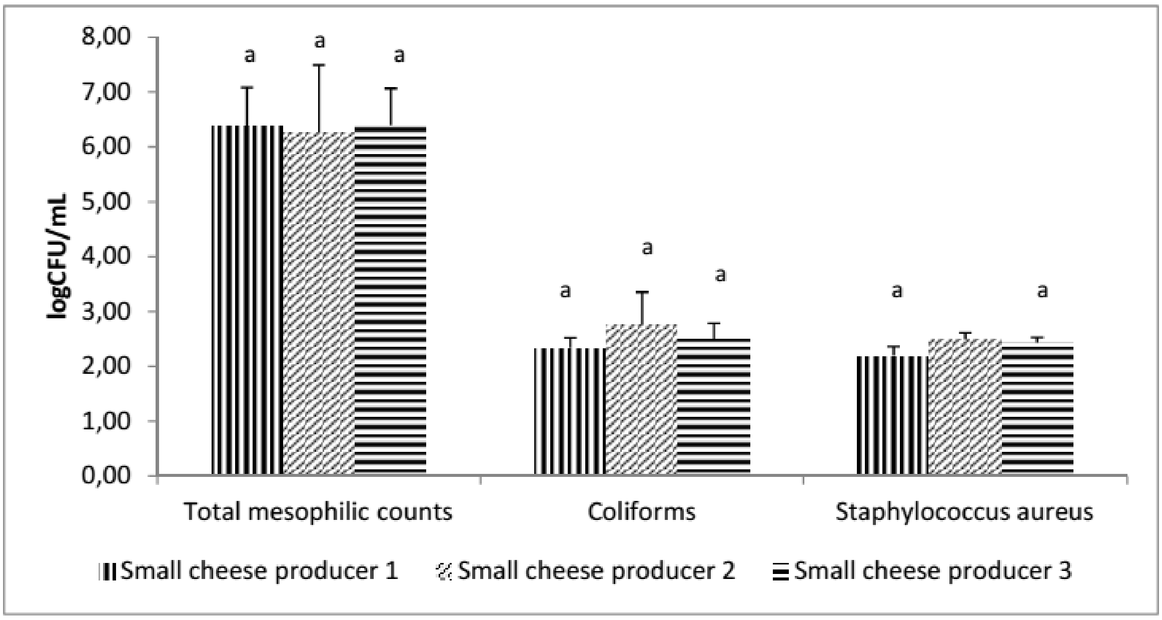

Figure 2.8 : Microbiological quality of cheese in different small cheese producers. No statistical significant differences were observed ($p<0.05$)

2.4.6 Microbiological safety of milk and dairy products.

Listeria monocytogenes was absent in all samples (Table 2.2). In comparison, *L. monocytogenes* was also not found in raw, cultured pasteurized milk or naturally soured milk in Zimbabwe (Gran et al., 2003). In contrast, the prevalence of *L. monocytogenes* in raw milk was 1.9% in Malaysia, 6.3% in Belgium and 0.6% in China, while for cheese it ranged from 3.3% to 17.4% in various European countries (Chye et al., 2004; De Reu et al., 2004; Ning et al., 2013; Rudolf and Scherer, 2001). The complete absence of *L. monocytogenes* in this study and that of Gran et al. (2003) might be the result of competition and growth suppression exerted by the indigenous milk microbiota. It has been reported that the growth of *L. monocytogenes* in pasteurized semiskimmed milk was reduced with 2 log CFU/mL in comparison with that in UHT-treated milk due to the inhibitory effects of the bacteria naturally present in milk (Bovill et al., 2000).

Table 2.2. Levels of *Staphylococcus aureus* and presence of *Salmonella* spp. and *Listeria monocytogenes* along the milk and dairy chain in Rwanda

	Farms	Collection centers	Companies	Small cheese producers	Wholesale	Supermarkets	Milk shops Fermented milk	Milk shops Boiled milk	Milk shops Cheese	Total
Staphylococcus aureus (log CFU/mL)	2.40	2.52	2.35	2.37	2.39	2.44	2.57	2.30	2.90	
	42.6% (23/54)	55.8% (67/120)	18.5% (5/27)	77.7% (21/27)	41.6% (5/12)	58.3% (21/36)	50.0% (3/6)	57.1% (24/42)	100.0% (6/6)	53.0% (175/330)
Salmonella spp.	5.5% (3/54)	5.0% (6/120)	0.0% (0/27)	14.8% (4/27)	0.0% (0/12)	0.0% (0/36)	0.0% (0/6)	21.4% (9/42)	50.0% (3/6)	7.5% (25/330)
Listeria monocytogenes	0% (0/54)	0% (0/120)	0% (0/27)	0% (0/27)	0% (0/12)	0% (0/36)	0% (0/6)	0% (0/42)	0% (0/6)	0% (0/330)

Thus, the presence of low numbers of *Listeria* may lead to false negative detection results due to the lack of growth during enrichment. Further research on this topic is warranted.

S. aureus was present along the whole chain, resulting in 53.0 % prevalence with the highest counts in cheese and fermented milk sold in milk shops (Table 2.2). There was no statistical difference between the different steps of the chain (p=0.07). In comparison, the prevalence of *S. aureus* in raw milk was 93.0% in Belgium (De Reu et al., 2004) and from 5.4 to 25.0 % in cheeses from various European countries (Kousta et al., 2010). A study of retail cheese in the UK showed that 2.0 % of the cheese from pasteurized milk was of unsatisfactory quality due to high levels (> 3 log CFU/g) of *S. aureus* and/or *E. coli* (Little et al., 2008). The prevalence of *S. aureus* raw milk was 94.3 % in Trinidad, 82 % in Zimbabwe, 72.0 % at milk collection centres in Ethiopia, 43.5 % in dairy farms in Ethiopia and 20.0 % in the Côte d'Ivoire (Adesiyun et al., 1995; Gran et al., 2003; Kouame-Sina et al., 2010; Makita et al., 2012). Moreover, high concentrations of *S. aureus* were found in these milk samples, namely between 5.2 and 6.0 log CFU/mL in Trinidad, on average 5.2 log CFU/mL in Zimbabwe, 4.9 log CFU/mL in Mali and 3.9 log CFU/mL in the Côte d'Ivoire (Adesiyun et al., 1995; Bonfoh et al., 2002; Gran et al., 2003; Kouame-Sina et al., 2010; Mhone et al., 2011).

The problem associated with high populations of *S. aureus* is the potential production of heat and acid resistant staphylococcal enterotoxins, which are the cause of staphylococcal food poisoning. Therefore, the major concern should be to keep *S. aureus* levels in food low at all times. Moreover, *S. aureus* could also serve as a hygiene indicator, since high levels indicate improper (unhygienic) handling or lack of refrigeration during storage, enabling outgrowth. According on EU regulations, a cheese batch which contains *S. aureus* in concentrations > 5.0 log CFU/g also needs to be analyzed for the presence of *S. aureus* entertotoxins and be withdrawn or recalled from the market if positive (European Commission, 1989, 1992, 2004b).

Toxins were produced by ≥ 6.5 log CFU/mL *S. aureus* in sterilized milk at temperatures > 14 °C (Fujikawa and Morozumi, 2006). *S. aureus* toxin production in raw milk before consumption or processing can be prevented by cooling, emphasizing the need for cooling facilities to ensure not only the quality but also the safety of milk and derived products in Africa.

Subclinical mastitis and milk adulteration were identified as the main reasons for the observed contamination of milk products with the pathogen *S. aureus* (Adesiyun et al., 1995; Bonfoh et al., 2002; Omore et al., 1996).

S. aureus is one of the most common causes of bovine mastitis and is commonly part of the microbiological community of the human skin and respiratory tract (Humphreys, 2012). Therefore, *S. aureus* in raw milk is linked to lack of animal health monitoring and/or personnel hygiene.

Salmonella spp. was isolated from 7.6 % of all samples (n = 330, Table 2.2). At the farm level, the overall prevalence of *Salmonella* was 5.6 % (95 % confidence interval: 1.9 % - 15.1 %). However, this pathogen was only isolated from raw milk originating from open grazing farms (3 positives among the 18 samples taken). This could be explained by the river in the vicinity of these farms. The river is a known source of typhoid fever in humans (personal communication) and was also used as a drinking water source for the cows of those farms. At the next step in the milk chain, the milk collection centres, *Salmonella* was found in 5.0 % (95 % confidence interval: 2.3 % - 10.5 %) of the raw milk samples.

None of the pasteurized or fermented milk samples was contained *Salmonella* (95 % confidence interval: 0.0 % - 7.4 % for pasteurized milk and 0.0 % - 39.0 % for fermented milk), while 21.4 % (95 % confidence interval: 11.7 % - 35.9 %) of the boiled milk sold in milk shops were positive, clearly indicating post-pasteurisation contamination. The high *Salmonella* spp. prevalence in milk shops is probably due to the variety of milk origins, the poor hygienic conditions and manual contamination by the personnel.

Finally, 14.8 % (95 % confidence interval: 5.9 % - 32.5 %) of the cheese at the production level contained *Salmonella* and 50.0 % (95 % confidence interval: 18.8 % - 81.2 %) at the retail level in milk shops. In comparison, *Salmonella* was not isolated from raw, cultured pasteurized milk or naturally soured milk in Zimbabwe (Gran et al., 2003) . The prevalence of *Salmonella* ranged from 0.2 to 6.1 % in European raw milk and no *Salmonella* was found in European cheeses (Kousta et al., 2010; Little et al., 2008).

According to the microbiological criteria in European Recommendations 2004/24/EC (European Commission, 2004a) and 2005/175/EC (European Commission, 2005), retail cheese made from pasteurized milk should contain < 3 log CFU/g *S. aureus*, < 2 log CFU/g *L. monocytogenes* at time of consumption and no *Salmonella* per 25 g. Retail cheese in Rwandan milk shops contained < 3 log CFU/g *S. aureus* and no *L. monocytogenes*, thus complying with these criteria. However, half of the sampled retail cheese was contaminated with *Salmonella*, which thus presents a serious safety issue in the Rwandan cheeses.

2.4.7 Conclusion

Taking into account that refrigeration was generally absent or insufficiently applied, the microbiological quality of milk and dairy products in Rwanda was acceptable. Taking into account that refrigeration was generally absent or insufficiently applied, the microbiological quality of milk and dairy products in Rwanda was acceptable except for the contamination with *Salmonella* found in approximately 5% of the raw milk samples.

Although only one milk processing company implemented HACCP, these companies produced milk products of high quality without *Salmonella*. However, the formal market share is estimated at maximally 10 %. More quality and safety issues were found on the informal market, especially with *Salmonella* in cheese and boiled milk sold in small milk shops. To improve the status of these informal dairy products, much more resources and investments are required, which poses a tremendous challenge.

CHAPTER 3 : Insights into hygienic practices in the Rwandan milk and dairy chain

Redrafted from:

Kamana, O., Jacxsens, L., Kimonyo, A., and Uyttendaele, M. (2015). Insights into hygienic practices in the Rwandan milk and dairy chain (Submitted).

CHAPTER 3 : Insights into hygienic practices in the Rwandan milk and dairy chain

3.1 ABSTRACT

The present study aimed to assess the status of hygienic practices in the Rwandan milk and dairy chain. A checklist was developed to assess the compliance with good hygienic practices. Data collection was done based on interviews and site visits in farms, collection centers, milk processing companies, small scale cheese plants, wholesale points, supermarkets and milk shops. These results of the status of good practices were further linked to actual microbiological data. It was concluded that there is a major gap in compliance with hygienic practices leading in particular to higher contaminated products in cheese processing.

3.2 INTRODUCTION

Milk and dairy products are highly nutritive, and this makes them ideal for growth of spoilage and pathogenic microorganisms (Frank, 2009) . Pathogenic bacteria include among others *Listeria monocytogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp. and *Yersinia enterocolitica*, and are associated with outbreaks of illnesses (intVeld, 1996; Kousta et al., 2010; Varnam and Sutherland, 1994). In African countries, limited information is available on reported foodborne outbreaks related to milk and dairy products (FAO, 2005; Mensah et al., 2012; Oguntoyinbo, 2014; WHO, 2012). Raw milk may be contaminated by the environment and/or by infected animals. Contamination may also occur in milk and dairy products as a consequence of improper hygienic practices during storage on farm, transport and at processing level (LeJeune and Rajala-Schultz, 2009; Millogo et al., 2010). Several studies are conducted in African countries to assess the status of good practices in the dairy chain i.e. at farm and processing levels by Belli et al. (2013) in Cameroun, Breurec et al. (2010) in Senegal, Mhone et al. (2011) and Gran et al. (2002a) in Zimbabwe, Kouame-Sina et al. (2010) in Ivory Coast, Elmagli et al. (2006) in Sudan, and by Millogo et al. (2010) in Burkina Faso.

These studies reveal that most often even basic good practices such as proper sanitation, temperature control, infrastructure and equipment, personal hygiene, are often not in place or insufficiently applied at farm, processing or during sales of milk and dairy products.

In addition, a full food safety management system based upon the Hazard Analysis Critical Control Point (HACCP) principles is often not established by the processing companies in the African dairy industry (Kivaria et al., 2006; Kussaga et al., 2014b; Mwangi et al., 2000).

Most frequent mentioned bottlenecks are lack of capacity and awareness of food business operators, lack of qualified personnel, lack of infrastructure (e.g. roads, collection centers, etc.), lack of governmental structures such as appropriate legislation, governmental food inspection organizations which put a pressure on companies, lack of laboratory capacity to perform monitoring and verification of the actual microbiological status of food products put on the market and lack of resources along the chain with food business operators or governments (Amoa-Awua et al., 2007; Bagumire et al., 2010; Jirathana, 1998; Macheka et al., 2013; Muchangos et al., 2012; Ropkins and Beck, 2000; Sarter et al., 2010; Schillhorn van Veen, 2005).

Compliance with good practices is prone to have a direct impact on the quality, hygiene and safety of produced food products as demonstrated by Sampers et al. (2010) in poultry processing in Europe, by Petruzzelli et al. (2014) in school catering facility and by De Giusti et al. (2010) in fresh ready-to-eat vegetables processing in Italy, by Islam et al. (2013) in home-processed weaning foods in Bangladesh, by Kokkinakis et al. (2011) in ice cream production in Greece, by Khatri and Collins (2007) in the Australian meat industry, by Amoa-Awua et al. (2007) in traditional maize processing in Ghana, and by Kussaga et al. (2014b) in milk processing companies in Tanzania.

Monitoring studies of hygiene and safety of milk and dairy products at various retail selling points have been reported by Bonfoh et al. (2006) in Mali, by Koussou and Grimaud (2007) in Chad, by Swai and Schoonman (2011) in Tanzania, and at consumer level by Lues et al. (2003) in South Africa, and by Kamana et al. (2014) in Rwanda. These papers report on lack of good hygienic practices implementation causing post-processing contamination problems with African dairy and cheese products.

The parallel circuit of an informal market of dairy products, being often quite large in volumes and value in these countries, is an additional hurdle for control on implementation of good practices by governmental organizations or is not accessible for support by NGOs (Grace et al., 2007; Kilango et al., 2012). Up to now, no information is available related to the status of good practices in the dairy chain in Rwanda, although several interventions by the government or NGOs are undertaken during the last few years e.g. introduction of stainless steel recipients to transport milk from farm to milk collection centers by the Dairy Cattle Development Support Project (MINAGRI, 2009).

The aim of the present study was to assess the status of good practices along the dairy chain in Rwanda in multiple stages of the chain and in different food businesses, to link these outputs to actual microbiological data on hygiene and safety of the same food businesses as published by Kamana et al. (2014) (Chapter 2). The results of the present survey are also discussed by taking into consideration past or current running projects by the government or NGOs to improve the hygienic situation.

3.3. MATERIALS AND METHODS

3.3.1 Characterization of the Rwandan dairy chain and selection of companies for the study

The study was conducted along the milk and dairy chain in Rwanda from primary production to distribution to consumers (Figure 1.4 and Figure 1.5). Farmers can be divided in three categories : open grazing, semi-grazing and zero grazing farming systems (MINAGRI, 2005; SNV, 2008; TECHNOSERVE, 2008). For each category two farms were selected in the study (F1, F2 being zero grazing farms, F3, F4 semi-grazing and F5, F6 open grazing farms).

After transportation, milk is collected in one of the 110 milk collection centers (MCC) that were built in 2009 all over the country to support farmers and help their cooperatives to access market. Collection activities are carried out by farmers themselves, transporting milk in stainless steel cans by bike or vehicle (MINAGRI, 2009). Eight MCCs across the country are included into this study (MCC 1 up to 8, by taking two in each milk basin, namely Kigali, Nyanza, Umutara and Gishwati).

Processing companies get milk mainly from collection centers, but also from farmers directly. Processing companies are still few (only five over the whole country). In this study, three processing companies are included (C1 in Kigali City, C2 in Umutara and C3 in Nyanza), processing raw milk towards pasteurized milk and packed in Tetra Pack boxes (all companies) and plastic gallons (only C3).

Also three small scale cheese processing plants, mainly located in Gishwati area, in the West of the country, are uptaken in the study (SCP1, SCP2 and SCP3). Gouda cheese is processed in traditional way (RARDA, 2009). Wholesale points are big markets, from where retailers purchase dairy products. The assessment was carried out in two wholesale points, located in two largest cities of the country, namely Kigali and Musanze (WSP1 and WSP2). At retail level, the study was conducted in six supermarkets (SM1 up to 6) and six milk shops (MS1 up to 6), in Kigali and Musanze, by taking in each category, four in Kigali and two in Musanze. Supermarkets are found in cities and sell processed milk products and traditional cheese. Milk shops sell processed milk and also unprocessed milk (fermented or not) preserved by boiling. (USAID, 2008).

3.3.2 Assessment of good (hygienic) practices

3.3.2.1 Design and validation of checklists

A checklist was developed, and tailored to all the steps of the chain, i.e. primary production, milk collection centers, milk processing plants, cheese plants, wholesale shops, supermarkets and milk shops. The consulted reference documents were the code of hygienic practice for milk and milk products of Codex (CAC/RCP 57-2004), translated in national legislation in Rwanda (RS: 63/2005) (RBS, 2005), and information on good practices and HACCP from literature (e.g. Amoa-Awua et al., 2007; Jacxsens et al., 2009; Sampers et al., 2010). A trial survey was conducted prior to the final setting up of the checklist, and this was done in one actor at each step level, from farms to milk shops.

At farm level, twenty five questions were asked and related to four categories: milking practices and facilities, animal health management and feeding, milk post-harvest handling and farm management and sanitation. At collection level, nineteen questions were asked, regarding milk hauling, work methodology, cleaning and disinfection, and temperature control. In processing companies and small cheese plants, twenty seven questions were asked about good practices and HACCP. At wholesale level, nine questions were asked regarding temperature control, maintenance and calibration and work methodology. In supermarkets, eight questions were asked about work methodology, maintenance and calibration, and temperature control.

Finally, thirteen questions were asked in milk shops, about cleaning and disinfection, work methodology, personnel hygiene and training, and work methodology (Annex 1).

3.3.2.2. Assessment of good practices

The checklist was designed for interviews with the responsible food business operators of the companies or farms, and comprised questions asked to get the answer "Yes" (Y) in case of compliance with good practices, and "No" (N) in the contrary. Three visits were conducted per premise between January and May 2012. The answers from interviewees were confirmed by on-site observations. After the third visit, a final conclusion on presence or absence of the particular practice was made.

3.3.3. Data interpretation

Frequency tables were used to interpret data from the assessment. Tables were designed to show the percentage of positive answers per category in the chain and individually per company.

3.3.4 Overview on intervention projects in the Rwandan milk and dairy chain

An overview was made to identify the ongoing projects during the study period (February-May 2012) for the Rwandan dairy chain development (Table 1.2). A total of twenty projects were identified. The majority of those projects are NGOs (n=13/20) intervening mostly in the primary sector, for food security purposes to enhance the volume of milk production (n=12/13). However, some of those projects intervene for food safety reasons, even though it is not their main objective (e.g. TECHNOSERVE, LAND O' LAKES). This collection is made by interviewing diverse stakeholders e.g. university professors, Rwandan Bureau of Standards, and search on internet. The outcomes from those interventions were used to interpret the obtained results on compliance to good practices.

3.4. RESULTS AND DISCUSSION

3.4.1 Compliance with good practices in the dairy supply chain

At farm level in total over the six farms and twenty-five questions raised with regard to compliance with good practices, 36% positive answers (n= 54/150) were obtained. Modern farms with zero grazing (F1 and F2) seem to function both best with 92% positive answers (n= 23/25), whereas other farms with semi and open grazing systems (F3, F4, F5, and F6) showed lower (but similar) scores on compliance with good practices (Table 3.1). F1 and F2 have appropriate infrastructure and equipment for hygienic milking (Q1, 2, 3), and good hygienic practices such as personnel hygiene (Q23) and sanitation (Q20) are implemented and monitored (Figure 3.1). The absence of an appropriate hygienically designed and maintained infrastructure/equipment (Q1,2,3), animal health monitoring (Q4, 5), and cooling facilities (Q13, 17, 21) are the main weak points in other farms (F3 up to F6). None of the farms had trainings in place and personal hygiene and health follow-up for the workers (Q22, 24). Farms rely predominantly on their empirical knowledge. Two good practices namely 'treatment with veterinary drugs' (Q9) and 'milk storage cans/tanks construction' (Q12) have compliance over all farms. This can be attributed to the fact that interventions were previously conducted to improve the status of these good practices at farm level (Table 1.2). A governmental intervention on the provision of veterinary drugs in each administrative sector has led to the better and stricter follow up of veterinary drugs, and the supply of stainless steel cans, instead of unhygienic difficult to clean plastic jars, on a large scale by the Dairy Cattle Development Support Project to be used for milk collection (MINAGRI, 2009). After this intervention, the government of Rwanda is still providing these stainless steel cans for a low price and home delivery (personal observation).

Table 3.1 : Compliance with good practices at farm level

	Compliance with good practices	**Frequencies (n=6)**		**Farms**					
		Y	**N**	**F1 Y**	**F2 Y**	**F3 Y**	**F4 Y**	**F5 Y**	**F6 Y**
	a. Milking practices and facilities								
1	Are the areas including premises used for the production of milk designed in a manner that minimizes the introduction of hazards into milk?	2	4	x	X				
2	Are the premises used for production of milk well maintained, in order to minimize introduction of hazards into milk?	2	4	x	X				
3	Are the areas including premises used for the production of milk situated and, to the extent practicable, used in a manner that minimizes the introduction of hazards into milk?	2	4	x	X				
	b. Health management and feeding								
4	Is animal health status of milking cows well managed in order that addresses of concerned for human health?	2	4	x	X				
5	Does milk come from animals in good health conditions?	2	4	x	X				
6	Considering the end use of milk, are forage managed accordingly, so that they can't cause any hazard introduction?	2	4	x	X				
7	Are animals watered with a good quality water?	2	4	x	X				
8	Is there an effective pest control programme?	2	4	x	X				
9	Are the animals treated with veterinary drugs, tested and authorized?	6	0	x	X	X	x	x	x
10	Is milking carried out in such a manner that minimizes contamination of the milk being produced?	2	4	x	X				
11	Is the timeout respected after treatment?	2	4	x	X				
	c. Post-harvest handling								
12	Are milk storage tanks and cans designed, constructed, maintained and used in a manner that avoids the introduction of contaminants into milk and minimize the growth of micro-organisms ?	6	0	x	X	X	x		x
13	Is milk stored in a manner that will avoid and minimize any increase of microbiological load of milk?	2	4	x	X				
14	Is milk transported in a manner that will avoid and minimize any increase of microbiological load of milk?	2	4	x	X				

	Compliance with good practices	Frequencies		Farms											
15	Is milking equipment designed, constructed, installed, maintained and used in a manner that will avoid the introduction of contaminants into milk?	2	4	x		x									
16	Is Milking equipment operating in a manner that avoids damage to udder and teats and that avoids the transfer of disease between animals through the milking equipment?	2	4	x		X									
17	Are premises for the storage of milk and milking-related equipment situated, designed, constructed, maintained and used in a manner that avoids the introduction of contaminants ?	2	4	x		X									
18	Is milk stored in a manner that minimizes the growth of micro-organisms ?	2	4	x		X									
	d. Farm management and sanitation														
19	Is milk collected, transported and delivered without undue delay?	2	4	x		X									
20	Are all premisses and materials, cleaned and disinfected in a proper way?	2	4	x		X									
21	Is milk delivered in a manner that avoids the introduction of contaminants into milk and minimizes the growth of micro-organisms ?	2	4	x		X									
22	Is the personnel hygiene checked?	0	6												
23	Are there means to assure personnel hygiene?	2	4	x		X									
24	Are there trainings for the personnel?	0	6												
25	Is there any system of documentation and record keeping?	2	4	x		X									
	TOTAL (%)	**36**	**64**	**92**	**8**	**92**	**8**	**8**	**92**	**8**	**92**	**8**	**92**	**8**	**9 2**

However, the obtained results from the surveys (Table 3.1) demonstrate clearly that it is not feasible for Rwandan semi traditional and traditional farmers to work out and implement all these good practices on their own efforts. Not only in Rwanda but also other African countries are suffering in this situation due to lack of resources (Belli et al., 2013), lack of knowledge (Grace et al., 2007) and lack of awareness (Bonfoh et al., 2002; Gran et al., 2002a). Figure 3.2. presents the milking conditions in Rwandan farms.

	Zero grazing system (Farms 1 and 2)	Semi grazing system and open grazing system (Farms 3,4,5,6)
Milking facilities		
Milk recipients and storage at farm		
Milk transport to MCC		

Figure 3.1 : Illustration on milking practices in Rwandan farms

Here comes an important role for farmers associations, NGOs and government to play to improve the hygienic status at farm level. Studies conducted in Botswana, Cameroun, Tunisia, Malaysia and Belgium demonstrated that hygienic practices at farm level are necessary to start with a good quality/safety in the dairy chain (Aaku et al., 2004; Belli et al., 2013; Chye et al., 2004; De Reu et al., 2004).

In Rwanda, most of the farmers do belong to a farmer association or a cooperative to gain market access and to stimulate the formal milk market (SNV, 2008). So, there is a potential platform ready to further enhance good practices at farm level by multiple interventions both technological such as cooling facilities and sanitation or more managerial such as training and awareness (Luning and Marcelis, 2009). Farm 1 and 2 were modern farms supplying milk in the formal market, while the rest of farms belong to the informal one. At farm level, the inspections from the competent authorities are not in place. In Rwanda, inspections are carried out by the Rwanda Standards Board (Formerly Rwanda Bureau of Standards), which is the competent authority in charge of standardization, research and inspection (RSB, 2013).

The inspections are conducted at processing, wholesale and retail levels in the dairy chain, and in parallel the Ministry of Agriculture delivers animal products certification services including enforcement of sanitary laws, monitoring and surveillance of animal diseases (MINAGRI, 2014). Besides those two bodies, environmental health officers under the Ministry of Health operate hygiene inspections at retail level (personal observations).

Enforcement of good hygienic practices and agricultural practices by governments by means of inspections, has been demonstrated to be effective in following and implementing good practices and improve hygiene status as demonstrated in several countries such as England and Wales by Bailey and Garforth (2014), The Netherlands by Noordhuizen and Metz (2005), Brazil by Costa et al. (2013) , and Peru by Fuentes et al. (2014). In the eight visited milk collection centers remarkable similarities were observed, with regard to milk reception and storage. At this level only 26 % (n = 40/152) positive answers were obtained over all 19 questions (Annex 1). All collection centers are equipped with appropriate cooling tanks, cooling facilities and the premises are designed in a manner that minimizes the contamination and/or growth of micro-organisms in milk (Q4, Q5 Q17).

All milk collection centers are similarly scoring on this questionnaire due to the fact that the Dairy Cattle Development Support Project in partnership with NGOs namely Send a Cow, Heifer International and TECHNOSERVE constructed 110 milk collection centers over the country quite recently from 2007 to 2009 (MINAGRI, 2009) (Table 1.2). The layout, management and implementation of good practices and start-up training to personnel of the milk collection centers were part of that project.

Milk is brought to collection centers by farmers without refrigeration (see Figure 3.2). A slight microbial growth was observed during the transport due to short distances and transport in hygienic and protecting stainless steel cans (Chapter 2). Milk is received, weighted and cooled in stainless steel tanks. The collection takes between three and five hours, in the beginning of the process milk is at approx.18 (±0.4) °C and drops to 6-7°C (Chapter 2) . The main negative answers on the questionnaire on the level of the milk collection centers, were regarding training of the personnel (Q7) since the new recruited staff did not receive trainings after the project was finished in 2009, absence of maintenance and calibration (Q18), and pest control plans (Q16) (Annex 1).

In Rwanda, the capacity of the milk processing plants is still low and so, milk is leaving the formal market to the informal market at this level, because mobile milk hawkers buy the milk and bring it to the milk shops (personal observations). The milk collection centers are part of the formal market but are also the interconnection with the informal market as presented in Chapter 2.

The competent authorities do not carry out inspections at this level of the chain, and this is not only for Rwanda, but also the situation for other West and Eastern African countries (Grace et al., 2007; Oloo, 2010; Sarter et al., 2010).

Big differences were observed in good practices between the three milk processing companies. In total, 56% (n= 45/81) positive answers were obtained (Table 3.2) over the three companies to which the questions on compliance to good practices were raised. Company 1 performed with 100% positive answers, whereas Company 2 and Company 3 performed only with 33% and 29% respectively. The implementation of HACCP and certification against ISO 22000:2005 in Company 1 can explain the 100% compliance whereas Company 2 and Company 3 are on the basic hygiene level without elaborating on the HACCP principles (Q13, 14) are the reasons for the observed differences (Table 3.2). It has been demonstrated before that certification of a food safety management system is enhancing compliance to good practices and HACCP (Ali and Fischer, 2005; Costa Dias et al., 2012; Nada et al., 2012; Opiyo et al., 2013; Vilar et al., 2012).

In Company 2 and 3 even the pasteurization process (time/temperature conditions) and cooling capacity of the milk (open tank) are not monitored carefully (personal observations). These two activities are inherent necessary to obtain good quality/safety of pasteurized milk (Stabel, 2001).

Table 3.2 : Compliance with good practices in milk processing companies

No.	Compliance with good practices	Frequencies (n=3)		Companies		
		Yes	No	C1	C2	C3
	I. **Good manufacturing practices**					
1	Is there an effective cleaning and disinfection plan?	3	0	Y	Y	Y
2	Is there an effective pest control plan?	3	0	Y	Y	Y
3	Is water quality managed in a proper way?	1	2	Y	N	N
4	Is the temperature controlled in order to keep the cold chain along the entire production process?	1	2	Y	N	N
5	Are all workers trained on FSMS?	3	0	Y	Y	Y
6	Is personnel hygiene checked and monitored?	1	2	Y	N	N
7	Is there an effective maintenance and calibration plan?	1	2	Y	N	N
8	Is there a waste management plan?	1	2	Y	N	N
9	Is any milk control carried out prior to processing?	3	0	Y	Y	Y
10	Is there a traceability /recall plan?	1	2	Y	N	N
11	Are measures taken to avoid physical and chemical contaminations?	3	0	Y	Y	Y
12	Is there a precised work methodology?	3	0	Y	Y	Y
	II. HACCP					
13	Is HACCP implemented	1	2	Y	N	N
14	Does the plant have any certification?	1	2	Y	N	N
15	Is there a person (or group of persons) in charge of quality management?	3	0	Y	Y	Y
16	Are the products enough known and specified?	1	2	Y	N	N
17	Is the intended use of products taken in account?	1	2	Y	N	N
18	Are flow diagrams available for all products?	1	2	Y	N	N
19	Are flow diagrams followed as they are set up?	1	2	Y	N	N
20	Are all dangers identified and documented?	1	2	Y	N	N
21	Are CCPs known and documented?	1	2	Y	N	N
22	Are critical limits for each CCP available?	1	2	Y	N	N
23	Is there a monitoring system for each CCP?	1	2	Y	N	N
24	Is there a corrective action plan?	1	2	Y	N	N
25	Are procedures of verification and validation available?	1	2	Y	N	N
26	Are documentation and record keeping carried out?	3	0	Y	Y	Y
27	Are products stored in a manner that avoids contamination and adulteration?	3	0	Y	Y	Y
	TOTAL (%)	**56**	**44**	**100**	**33**	**29**

Governmental inspection by the Rwanda Standards Board on good practices is randomly conducted at this level in all three companies; however, inspections are less stringent in smaller companies (personal observations). Processing companies belong to the formal market.

In the three small scale cheese processing plants, all the visited processors did not comply with any of the basic hygiene requirements nor were they aware about HACCP principles (n = 3, 0/36 questions) (total number of questions to be answered was 36, Annex 1).

Gouda cheese is traditionally processed, in an unfit environment, with little or no implementation of adherence to good hygienic practices. Due to a high *Salmonella* prevalence in their final products (presence in 25g cheese in 50% of the samples; based on 6 samples) (Kamana et al., 2014) followed by a ban from the market in 2013, the Rwandan government has foreseen training in hygiene, provision of rubber boots and gloves in 2012-2013 in collaboration with Land O'Lakes, an international NGO (Table 1.2). However, further interventions are necessary to improve the hygiene status in the traditional small scale cheese plants. In other African countries, limited information is available for the cheese sector, especially Gouda cheese. Studies conducted by Belli et al. (2013) in Cameroun, Ashenafi (1990) in Ethiopia, Mankai et al. (2012) in Tunisia and Wahba et al. (2010) in Egypt showed that processing environment and conditions affect the quality and safety of cheese. In Rwanda, governmental inspection is performed in the small scale cheese companies, but given their high number approximated to 30 (Karenzi et al., 2013) and their scattered localization the frequency of visits is low, randomly conducted approximately once a year. This situation is leading to less pressure and necessity to implement the good practices and to invest in food quality/safety (Henson and Traill, 1993; Starbird, 2000; Tompkin, 2001).

In the two wholesale points in total a 55% compliance (n = 10/18) of the good practices was obtained (Annex 1) and a similar situation was observed for these two wholesale points in the survey. Though the temperature is under control (Q3), it was noted there was no effective cleaning and disinfection plan in place (Q1), insufficient hygienic design of the premises (Q9), lack of hygiene training of the personnel (Q4) and lack of a calibration and maintenance plan was noticed (Q6). The existence of an effective pest control plan (Q2), the presence of facilities for workers to achieve hygiene requirements (Q5), a traceability and recall plan (Q7) and a precise work methodology (Q8) were other positive points noted.

Wholesale points are regularly inspected on good practices by the government which overall should lead to better compliance to good practices (Starbird, 2000), although in the present survey there is still room for considerable improvement.

At retail level, a similar situation was observed in all six supermarkets visited ending up with 62% (n=30/48) positive answers. The main difference between these supermarkets and the prior step in the dairy supply chain, the two wholesale points, was the absence of temperature control in the supermarket (Q3). Also at this retail level, governmental inspection is performed but there is still a need for improvement in hygienic control measures to be implemented. Wholesale shops and supermarkets are the last step of the formal market.

In milk shops, only 23% positive answers (n = 18/78) were obtained (Annex 1) and similar answers were detected over all six milk shops visited. During storage of milk (raw or boiled milk) in the milk shops, the cold temperature (6.4°C±1.4) is apparently reasonably under control (Chapter 2), and milk physical quality is checked by measuring the density and the pH prior to boiling and/or fermentation processes.

The lack of trainings for the personnel (Q4), cleaning and disinfection plans (Q1), and a waste management plan (Q7) to be in place, the non respect of temperature limits e.g. refrigerators frequently open to serve clients and milk refrigerated just after boiling, which causes temperature fluctuations are identified as being among the weak points in compliance to good practices at the retail level here being the milk shops. Also the time/temperature conditions during boiling are not monitored and different sources of heat and heat transfer are applied (such as gas, fire or electric). Milk shops are small scale shops, which are shifting frequently from owner, and are buying milk in the informal market via mobile hawkers. So, this level of the dairy chain is more vulnerable due to less sustainable structures (Bonfoh et al., 2003). However, it is an important source of milk in urban Rwandan areas so safety of the boiled/fermented milk is necessary (Karenzi et al., 2013; Modderman, 2010; USAID, 2008).

3.4.2 The impact of observed compliance to good practices on microbial results of the dairy products

The observed results on implementation and compliance to good practices along the dairy chain in Rwanda are assumed to reflect also on the microbial quality and safety of the milk and dairy products in the dairy chain in Rwanda.

During the visits and observations at the various actors in the Rwandan dairy chain in 2012, also samples were taken and analyzed for total mesophilic counts (TMC), coliforms, *Staphylococcus aureus*, *Salmonella* spp. and *Listeria monocytogenes* as well as pH and temperature upon sampling. The results of these analysis are extensively described in Chapter 2. Results of this study are also briefly summarized in Table 3.3 and discussed against our findings on compliance with good practices.

Table 3.3: Relation between compliance with good practices and the implication on microbial parameters, temperature and pH (after Kamana et al., 2014

Step in the chain	% of compliance to good practices		Products	Microbial level (log CFU/mL)				PH	Temperature (°C)
	Yes	No			Minimum	Maximum	Average (St.dev.)		
Farms	36	64	Raw milk (n=54)	TMC	4.13	6.94	5.20(±0.60)	6.48 (±0.23)	35.85 (±1.89)
				Coliforms	<1.00	3.44	2. 70(±0.63)		
				S. aureus	<2.00	3.53	2.40(±0.14)		
				Salmonella spp*.	-	-	5.55%		
				*L. monocytogenes**	-	-	0.00%		
Milk collection centers	15	85	Raw milk (n=120)	TMC	3.88	6.79	5.50 (±0.20)	6.07 (±0.27)	15.58 (±10.01)
				Coliforms	<1.00	3.55	2.67 (±0.57)		
				S. aureus	<2.00	3.89	2.52 (±0.40)		
				Salmonella spp.			5.00%		
				L. monocytogenes			0.00%		
Milk processing companies	56	44	Pasteurized milk (n=27)	TMC	3.20	5.40	4.62 (±0.61)	6.40 (±0.28)	6.31 (±1.34)
				Coliforms	<1.00	3.55	1.46 (±0.76)		
				S. aureus	<2.00	2.36	2.35 (±0.12)		
				Salmonella spp.	-	-	0.00%		
				L. monocytogenes	-	-	0.00%		
Small cheese plants	0	100	Cheese (n=27)*	TMC	4.44	7.75	6.35 (±0.86)	-	-
				Coliforms	2.15	3.55	2.53 (±0.42)		
				S. aureus	<2.00	2.61	2.37 (±0.22)		
				Salmonella spp.	-	-	14.81%		
				L. monocytogenes	-	-	0.00%		
Wholesale	80	20	Pasteurized milk (n=12)	TMC	4.33	5.76	4.71 (±0.40)	6.46 (±0.16)	6.04 (±0.90)
				Coliforms	<1.00	2.15	1.58 (±0.74)		
				S. aureus	<2.00	2.72	2.39 (±0.24)		
				Salmonella spp.	-	-	0.00%		
				L. monocytogenes	-	-	0.00%		
Supermarkets	78	32	Pasteurized milk (n=36)	TMC	4.31	6.08	4.95 (±0.47)	6.45 (±0.24)	7.14 (±1.28)
				Coliforms	<1.00	2.79	2.17 (±0.14)		
				S. aureus	<2.00	2.68	2.44 (±0.19)		
				Salmonella spp.	-	-	0.00%		
				L. monocytogenes	-	-	0.00%		

Step in the chain	**% of compliance to good practices**		**Products**	**Microbial level (log CFU/mL)**				**PH**	**Temperature (°C)**
Milk shops	25	75	Boiled milk (n=42)	TMC	3.48	6.83	5.69 (±0.89)	6.10 (±0.72)	6.57 (±1.66)
				Coliforms	<1.00	3.80	2.42 (±0.65)		
				S. aureus	<2.00	3.50	2.30 (±0.33)		
				Salmonella spp.	-	-	21.40%		
				L. monocytogenes	-	-	0.00%		
			Fermented milk (n=6)	TMC	6.06	6.96	6.38 (±0.37)	4.40 (±0.48)	7.33 (±2.58)
				Coliforms	2.30	2.90	2.60 (±0.33)		
				S. aureus	<2.00	2.81	2.57 (±0.45)		
				Salmonella spp.	-	-	0.00%		
				L.monocytogenes	-	-	0.00%		
			Gouda cheese** (n=6)	TMC	6.11	7.04	6.46 (±0.35)		
				Coliforms	2.15	3.96	3.07 (±0.72)		
				S.aureus	2.45	3.45	2.92 (±0.02)		
				Salmonella spp.	-	-	50.00%		
				L. monocytogenes	-	-	0.00%		

(*) : in 25 g

(**) :log CFU/g

At farm, the TMC for raw milk were on average 5.20 log CFU/mL, coliform numbers were 2.39 log CFU/mL, and *Staphylococcus aureus* was 2.17 log CFU/mL. *Salmonella* spp. was isolated in 5.55% samples, while *Listeria monocytogenes* was absent (Table 3.3). The average pH was 6.48 (±0.23), and the temperature was 35.85 (±1.89)°C. According to different standards, raw milk quality at farm level was almost satisfactory, since the acceptance limits for these parameters are only slightly exceeded (Kamana et al., 2014) (Chapter 2). However, modern farms (F1 and F2) have lower microbial loads (4.61 and 4.73 log CFU/mL respectively), which can be linked also with their higher performance scores on good practices (92%) (Table 3.1 and Kamana et al., 2014). For Farms 3, 4, 5, and 6, the lower compliance to good practices (8%) might be at the origin of their higher TMC (Table 3.1 and 3.3), since their TMC were 5.47, 5.55, 5.07, and 5.18 log CFU/mL respectively (Kamana et al., 2014). Although microbial quality of Rwandan raw milk can still be improved in comparison to local and international standards, it showed lower microbial loads than other African countries. The TMC of raw milk at farm level ranged from 5.3 log CFU/mL to 7.8 log CFU/mL in Botswana, from 6 to 8 log CFU/mL, in Burkina Faso, and was on average 8.1 log CFU/mL in Mali and 6.4 log CFU/mL in Zimbabwe (Aaku et al., 2004; Bonfoh et al., 2002; Mhone et al., 2011; Millogo et al., 2010). Those differences might be attributed, among others, to the interventions from the Rwandan government and its partners in supplying to farmers storage stainless steel cans, easy to clean and suitable to minimize the contaminations from the environment.

The high number of coliforms might be resulting from the lack of personnel hygiene, since in all semi grazing and open grazing farms, there are no facilities for hand washing prior to manual milking (Q23), and the lack of animal health monitoring (Q5) might be at the origin of the presence of *Staphylococcus aureus*, since this bacteria is associated with mastitis and/or personnel hygiene (Motarjemi et al., 2014). The presence of *Salmonella* spp. might be due to the vicinity with a river known to be causing typhoid fever in humans (personal communication) or to a fecal contamination from the environment. The pH was in the normal range (Walstra, 1999) but the temperature was high 35.85(±1.89) °C, as it was measured immediately after milking.

In milk collection centers, the average TMC was 5.22 log CFU/mL, the coliform numbers were 2.67 log CFU/mL, *Staphylococcus aureus* was 2.28 log CFU/mL, 5% of the 120 samples were *Salmonella* spp. positive, and *Listeria monocytogenes* was absent from all samples (Kamana et al., 2014). The average pH was 6.07(±0.27) and the temperature dropped till 15.58 (±10.01) °C. The load of TMC was below the treshold limit of 6.3 log CFU/mL according to the East African Community Standard (EAC, 2006), and the coliform numbers and *S. aureus* were lowered. However, between farm level and level of milk collection centers, there was no microbiological growth observed. In Moroccan collected milk the TMC were > 6.0 log CFU/mL in wet and cold months (climatic conditions similar to Rwanda), and coliform numbers >4.5 log CFU/mL (Sraïri et al., 2009). The lower microbial loads in Rwandan collected milk, might be resulting from the implementation of the two-stage dairy collection system and the installation and operation of 110 milk collection centers all over the country, so that farmers spend less time to reach the cooling facilities (Karenzi et al., 2013; SNV, 2008) (Table 1.2). Only 26 % of positive answers were given on the questionnaire on good practices at the level of the milk collections centres (Annex 1). Despite this result, overall an acceptable result in milk safety and quality is reached.

In milk processing companies, the TMC of pasteurized milk was 4.62 log CFU/mL, the coliform numbers were 1.46 log CFU/mL, *Staphylococcus aureus* was 2.05 log CFU/mL, and *Listeria monocytogenes* and *Salmonella* spp. were absent in all 27 samples (Table 3.3, after Kamana et al., 2014). The pH average was 6.40 (± 0.28) and the temperature was 6.41 (±1.34) °C.

The US indicate that the TMC for pasteurized milk and dairy products should be <4.3 log CFU/ mL or /mL, and coliforms <1 log CFU/mL (FDA, 2014). In general, the pasteurized milk in Rwanda does not comply with the standards and is unsatisfactory and indicating that pasteurization/cooling and hygienic practices are not under control. However, Company 1 scored with 100% positive answers, and the specific TMC average for this company was 4.0 log CFU/mL. This outstanding level resulted from the implementation of HACCP and ISO 22000:2005 certification, given that the implementation of an effective food safety management system leads to dairy products with improved and satisfactory quality (Nada et al., 2012).

The size of the company also might be influencing the quality of pasteurized milk, since it has been demonstrated that small scale companies are more likely to fail in meeting quality requirements (Opiyo et al., 2013) even in a highly industrialized country as Japan (Sampers et al., 2012). The high microbial loads in the two other companies might be due to ineffective pasteurization and/or post-pasteurization contamination, as was detected by the interviews in the companies (Table 3.2.). In comparison, the TMC of pasteurized milk ranged from 2.0 to 4.3 log CFU/mL in Botswana, and was on average 6.6 log CFU/mL in Zimbabwe (Aaku et al., 2004; Mhone et al., 2011). At processing level, more enforcement of HACCP and monitoring of CCP pasteurization and cooling is necessary to gain better results and more stable results on the microbial quality of the milk after processing.

At wholesale level, microbiological counts were similar to those in processing plants, also the temperature (6.4±0.3 °C at processing level and 6.0±0.9 °C at wholesale level).

Those similarities are explained by the close links between milk processors and wholesale vendors, for marketing purposes and also the packaging concept (closed packages) which are protecting the milk from contaminations. The high frequency of routine inspections from the competent authorities, namely the Rwanda Standards Board, makes the wholesale points rigorous in hygiene keeping. A large effort is made at wholesale level to have a cold and stable temperature of the milk. At retail level, pasteurized milk in supermarkets showed also similar microbial loads (Table 3.3). The temperature average is 7.14(±1.2) °C. In supermarkets, refrigerators are often switched off at night time to save energy. Both at wholesale and retail level a moderate compliance to good practices were obtained (respectively 62% and 55 %).

Besides supermarkets, milk is also offered to consumers in milk shops. However, instead of pasteurized milk, they offer boiled and fermented milk, which are produced onsite in these shops and showed consierably increased average values for TMC and coliforms, respectively to 5.69 log CFU/mL and 2.42 log CFU/mL for boiled milk, in comparison with pasteurized milk sold in supermarkets containing 4.95 log CFU/mL (Table 3.3, after Kamana et al., 2014). The high number and high turnover of milk shops makes it difficult for the competent authorities to perform the appropriate number of controls.

In small cheese plants, the TMC for cheese was on average 6.35 log CFU/mL, the coliform counts were 2.53 log CFU/mL, and *S. aureus* was 2.32 log CFU/mL. *Salmonella* spp. was isolated in 14.81% samples, and *Listeria monocytogenes* was absent in all 27 samples (Table 4, after Kamana et al., 2014). The microbiological load of cheese was the highest of all dairy products due to fermentation.

Coliforms were present in high amounts (2.5 log CFU/g) in cheese sampled from the small cheese producers due to suboptimal production environment conditions, i.e. inadequate personnel hygiene (Q6), the absence of effective cleaning and disinfection (Q1), use of wooden materials which hamper efficient cleaning and disinfection (Q27), the design of the premises without separation between clean and dirty areas (Q27) and the use of untreated water (Q3). Overall, the compliance to good practices was for the cheese factories (0%). Cheese sold in milk shops showed an increase in coliforms from 2.53 log CFU /g to 3.07 log CFU/g and *S. aureus* from 2.32 log CFU/g to 2.92 log CFU/g, in comparison to cheese sampled at the small cheese producers (Table 3.3), probably due to poor hygienic conditions during transport and sale, resulting in additional microbiological multiplication or post-processing contaminations (Annex 1). According to the microbiological criteria in European Regulation 2005/2073/EC (European Commission, 2005), cheese made from pasteurized milk should contain < 3 log CFU/g *S. aureus*, < 2 log CFU/g *L. monocytogenes* at time of consumption and no *Salmonella* per 25 g. Cheese in Rwandan milk shops contained < 3 log CFU /g *S. aureus* and no *L. monocytogenes* in 25 g, thus complying with these criteria. However, half of the sampled retail cheese was contaminated with *Salmonella*, which thus presents a serious safety issue in the Rwandan cheeses. This caused a cheese ban on the market in 2013 by the government.

3.5 CONCLUSION

In developing countries, interventions in the dairy chain have been focusing mostly on production aspects to improve the food security situation (Grace et al., 2007; Hooton and Omore, 2007; Lore et al., 2006; Ndambi et al., 2007; Unnevehr, 2015). Meanwhile, the increase of milk production could go in parallel with the implementation of good practices along the chain, in order to maximize the double excepted benefits from milk production: poverty alleviation and nutrition. In Rwanda, the gaps in hygiene practices affect the quality of milk and dairy products. At farm level, the interventions could be focused on the training of personnel, and animal health monitoring. Though veterinary drugs are used under strict control from the competent authorities, a regular follow-up is necessary, especially for mastitis prevention.

The implementation of collection centers all over the country contributed significantly in facilitating farmers to access market, and milk quality was improved. However, efforts need to be concentrated in the maintenance of those infrastructures, and continual training of workers could be organised, since there is no sustainable management plan for milk collection centers. At production level, the certification and HACCP implementation showed tangible results in one company. This could be mandatory for all the companies in order to improve quality of pasteurised milk. At retail and wholesale level the situation is also due to some improvement although not actually reflected in microbial quality and safety. The frequency of inspections from the competent authorities could be increased, and workers should receive training on milk quality for milk shops. Cheese plants are the hugest food safety issue identified in the Rwandan dairy chain. This as well as the milk shops sector is in need to be reorganised and professionalized. The traditional cheese making processors could be guided into more professional processors with attention to GMP and HACCP principles and thus improved quality and safety of cheese marketed.

The recently organised trainings and other interventions (provision of gloves and adapted shoes for workers) to improve working conditions could be more formalized and organized on a regular basis. The government could increase controls and inspection to improve quality.

This requires an effective commitment on behalf of the Government and its partners, especially development NGOs and training institutions. The workers in the dairy sector could be trained on GMPs, and HACCP could be gradually introduced in small scale processing units.

CHAPTER 4 : Microbiological quality and safety in milk shops and small scale cheese processing units in Rwanda

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Redrafted from :

Kamana, O., Ceuppens, S., Jacxsens, L., Kimonyo, A., and Uyttendaele, M. (2015). Microbiological quality and safety in milk shops and small scale cheese processing units in Rwanda (Submitted).

CHAPTER 4 : Microbiological quality and safety in milk shops and small scale cheese processing units in Rwanda

4.1 Abstract

The present study aimed to investigate the microbiological quality and safety of boiled milk and Gouda cheese along the processing steps, in order to identify the appropriate further interventions to be taken to provide safer dairy products in Rwanda. It was noted that despite the high microbial loads in raw milk, the applied heat treatment during processing is effective by reducing those loads considerably and in calculating the process lethality for it was indeed shown that the boiling process is sufficient to ensure a 6 log reduction of *Salmonella* and *Listeria monocytogenes*. Therefore, the observed increase again in microbial loads afterwards, and the occasional presence of in particular coliforms and *Staphylococcus aureus* in boiled milk is linked with post-contamination from the environment, an inadequate refrigeration during storage, or insufficient hygiene by the food handlers serving boiled milk in cups in the shops. In small cheese processing plants also it was noted that the personnel hygiene and the processing environment are probably at the origin of occasional *S. aureus* and *Salmonella* contamination during processing. *L. monocytogenes* was not detected in any of the product samples or environmental or hand swab samples. To further increase quality and safety of boiled milk and Gouda cheese in Rwanda a focus on aspects of basic hygiene requirements and appropriate refrigeration is recommended.

4.2. INTRODUCTION

Being part of the African traditional diet, milk is consumed in different ways, according to traditions and cultures (Ndambi et al., 2007). Processed dairy products, mainly pasteurized milk are omnipresent in Africa, and are mostly consumed in urban areas (Sinja et al., 2006; Staal et al., 1997) but are often of poor and inconsistent quality (Gran et al., 2003; Kamana et al., 2014; Lore et al., 2006).

Besides formally pasteurized milk, raw milk and/or traditional products mostly fermented derived from unpasteurized milk are often consumed in African countries (Abdelgadir et al., 1998; Savadogo et al., 2004; Tchekessi et al., 2014). In Sub-Saharan Africa, the informal market is predominant to the detriment of the formal one (Grace et al., 2007; Leksmono et al., 2006; Metzger et al., 1995). Channeled dairy products through this market do not undergo controls on behalf of the competent authorities, making them vulnerable and potentially of poor quality.

Milk and dairy products being highly nutritious, are ideal for growth of spoilage and pathogenic microorganisms such as *Salmonella*, pathogenic *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*, which may contaminate them at farm from cow and the environment during milking or at further steps during transport, distribution or further processing (Hayes et al., 2001; Kousta et al., 2010). In Rwanda, informally marketed dairy products are boiled milk, fermented milk (locally known as "*inshyushyu*" and "*ikivuguto*" respectively) and Gouda cheese (Kamana et al., 2014; Karenzi et al., 2013; Modderman, 2010). Boiled milk and fermented milk are processed on site, i.e. milk shops, whereas Gouda cheese comes from traditional small cheese plants. A previous study conducted by Kamana et al. (2014), showed that boiled milk and Gouda cheese sold in milk shops were of poor quality and unsafe with 9 out 42 samples of boiled milk and 3 out of 6 samples of Gouda cheese being contaminated by *Salmonella* (Chapter 2). The present study aimed to investigate the microbiological quality and safety of boiled milk and Gouda cheese along the processing steps, in order to identify the appropriate further interventions to be taken to provide safer dairy products in Rwanda.

4.3. MATERIAL AND METHODS

4.3.1. Characteristics of milk shops and small cheese plants involved in this study

This study was conducted in three milk shops (MS) and three small cheese plants (SCP). All small cheese plants and milk shops were chosen for having been part of the previously conducted study by Kamana et al. (2014) (chapter 2). The selected milk shops are located in Musanze, the second largest city of Rwanda, and sell various food products i.e. dairy and non-dairy, but milk is the common and most popular item.

MS1 is the most popular shop in the city, and is located near the taxi station in a highly busy area, mostly frequented by passengers and bus drivers. It employs four persons and the volume of milk sold daily varies from 100 to 120 liters. MS2 is located near the Musanze central market, in vicinity of other many milk shops. Its clientele comprises mainly market vendors and buyers and other different categories of street walkers, employs three people, and the daily volume sold is comprised between 50 and 60 liters. The third milk shop (MS3) is located in a more residential area and its clientele comprises mainly households, and sells between 20 and 25 liters daily. Only two people are employed in MS3.

For all milk shops, milk is bought in the morning by mobile traders, gathered in smaller containers, boiled, allowed to cool, and sold refrigerated. The entire process is described in Figure 4. 1.

The selected small cheese plants are located in Gishwati milk basin, in the West of Rwanda. The volume of daily processed milk varies between 150 and 200 liters. Gouda cheese is traditionally processed with rudimentary technology, by unqualified personnel (12-15 persons per cheese plant) using raw milk from neighboring farms. After reception, the raw milk is boiled, left at ambient temperature and inoculated with fermented milk and calcium chloride, sodium nitrate and rennet. After approximately one hour the curd and the whey are separated and the whey is drained. Thereafter the curd is stirred, put in moulds, and soaked in brine for 5 days and the cheese is ripened for a minimum of 21 days, and is daily turned during this period.

In milk shops, milk samples were collected at reception, after boiling, and at the time of selling.

At reception and after boiling, 3 samples were taken at each step, and 6 samples of boiled milk in a glass or cup were taken at the time of selling, making a total of 12 milk samples. Swab samples were also taken from the hands of workers in the milk shop (6 samples) and from the cups used in milk selling (6 samples). This type of sampling was repeated on three different days in all three milk shops, thus obtaining in total 108 milk samples and 108 swab samples of hands of food handlers or of cups used for serving the boiled milk.

In the small cheese plants, three milk samples were taken both at reception, and after boiling.

In the subsequent steps the curd was sampled before and after pressing, also three samples each, and finally three samples of the final ready-to-sell Gouda cheese were taken.

During the visits to the small cheese plants also swab samples were taken from the hands of workers (6 samples), the surface of the moulds used to give shape to the cheese (3 samples) and the surface of the shelves used for ripening (3 samples).

No	Step	Activity	Some illustrations of the steps in the milk boiling practices in milk shops
1	Receipt of raw milk	Milk is received in the morning generally from 7:00 AM to 9:00 AM, brought to milk shops by mobile traders and/or farmers. Mobile traders are intermediate between farmers and collection centers. Milk is supplied in stainless steel cans carried on bicycles. The quantities supplied by mobile traders vary from 20 to 30 liters. The number of suppliers varies depending on the size of the shop and needed quantities.	
2	Bulking	After reception the raw milk is gathered in smaller containers (3.5 to 5 liters). Those aluminum containers are put on the bare ground, prior to boiling. This takes from 10 to 90 minutes.	
3	Boiling	Milk is boiled in the containers used for bulking, using different sources of energy i.e. fire wood, electricity and charcoal. The boiling time varies between 490 and 780 seconds. The process is stopped when milk foams.	
4	Cooling	After boiling, the milk still in the same container used for boiling is put back on the ground, or the milk is immediately distributed in smaller containers which will be refrigerated thereafter. The time for cool-down varies from 30 to 90 minutes.	

No	Step	Activity	Some illustrations of the steps in the milk boiling practices in milk shops
5	Refrigeration	Milk is refrigerated. The temperature in the fridges is from 4 to 9°C. Milk is served according to the demand of consumers.	
6	Selling	Milk is sold by pouring a full glass or cup, and the price varies according to the quantity (from 0.4 to 0.5 liters).	

Figure 4.1: Milk boiling practices in milk shops from receipt of raw milk to sales of boiled milk

Again, the sampling was repeated on three different days, making in total 54 milk samples, 54 curd samples, 27 cheese samples and 108 environmental swab samples (hands or food contact surfaces). Sampling of both the small cheese plants and the milk shops was conducted in the period from October 2012 to April 2013.

4.3.2. Microbiological analyses

Samples were collected under aseptic conditions and transported in an ice box to be analyzed for the total mesophilic count (TMC), coliforms, *S. aureus*, *Salmonella* and *L. monocytogenes.* The TMC was determined using the pour plate method on plate count agar (Oxoid, Cambridge, UK) according to the ISO 4833:2003 standard protocol (ISO, 2003) . The total coliform enumerations were performed using the pour plate method on violet red bile lactose agar (Oxoid) following ISO 4831:2006 (ISO, 2006) . The presence of *S. aureus* was determined using the spread plate technique on Baird-Parker agar plates (Oxoid) and presumptive colonies confirmed by the coagulase test, as described in ISO 6888-1:1999 (ISO, 1999). The presence of *Salmonella* was detected in 25 ml, 25 g or 25 cm^2 samples according to ISO 6579:2002+A1:2007 (ISO, 2002) i.e. by nonselective enrichment in buffered peptone water (Oxoid), cultivation on modified semisolid Rappaport Vassiliadis (Oxoid), selective isolation on xylose lysine desoxycholate agar (Oxoid), and purification of presumptive isolates on nutrient agar (Oxoid), and finally, confirmation of typical colonies by the triple sugar iron test. *L. monocytogenes* was detected in 25 ml, 25 g or 25 cm^2 by following the ISO 11290-1 protocol (ISO, 1996) with a pre-enrichment in half-Fraser broth (Oxoid), selective enrichment in Fraser broth (Oxoid), cultivation on Brilliance Listeria agar (Oxoid), and confirmation of typical colonies by a catalase test, hemolysis, carbohydrate use, and Camp tests. Horizontal methods for sampling techniques using cotton swabs on surfaces in food industry (ISO 18593:2004) were used to sample food contact surfaces and hands of operators (ISO, 2004).

The temperature and pH of the samples were determined using a thermometer (HANNA HI 955501) and a pH meter (HANNA HI 207), respectively.

Data were analyzed with SPSS Statistics 21. Analysis of Variance and the post hoc Bonferroni tests were performed on normally distributed variables with equal variances. Kruskal-Wallis and Mann-Whitney U tests were used for non-normally distributed variables.

4.3.3. Calculation of the process lethality

The process lethality (F_{70}) for *Salmonella* and *L. monocytogenes* was determined for the boiling process in milk shops. To measure the temperature evolution, a data logger (Testo175-T3, Testo NV/SA, Ternat, Belgium) was introduced in the milk containing recipient, and temperature measurements were recorded every 10 seconds. The F_{70} value for the boiling time of the milk was calculated as :

$$F_{70} = \int_0^t 10^{\frac{(Tt - Tref)}{z}} dt$$

Where T_t is the temperature at the core of the milk at time t, and T_{ref} is the reference temperature. In this study T_{ref} was 70°C and the maximum z-values for *Salmonella* (8.0°C) and *L. monocytogenes* (9.3°C) were selected from the data available at the website of ILT-NRW (2015). Because the lethal effect below 55.0°C is negligible, only T_t ≥ 55.0°C were used to calculate the process lethality (Claeys, 1998).

4.4. RESULTS

4.4.1. Evolution of pH, temperature and microbiological quality and safety of milk in milk shops

The pH of milk remained more or less constant and comparable in all milk shops throughout the whole process of receipt, storage, boiling and serving of milk with the pH ranging from pH 6.0 to 6.8 (mean pH = 6.4 ± 0.2). Figure 4.2 represents the evolution of temperature in the milk shops. The average temperature of raw milk at reception was the lowest in MS1 (9.1 °C) and significantly lower than the average temperature at MS2 and MS3 (10.5 for both). Also after boiling and cool-down again the lowest average temperature was observed in MS1.

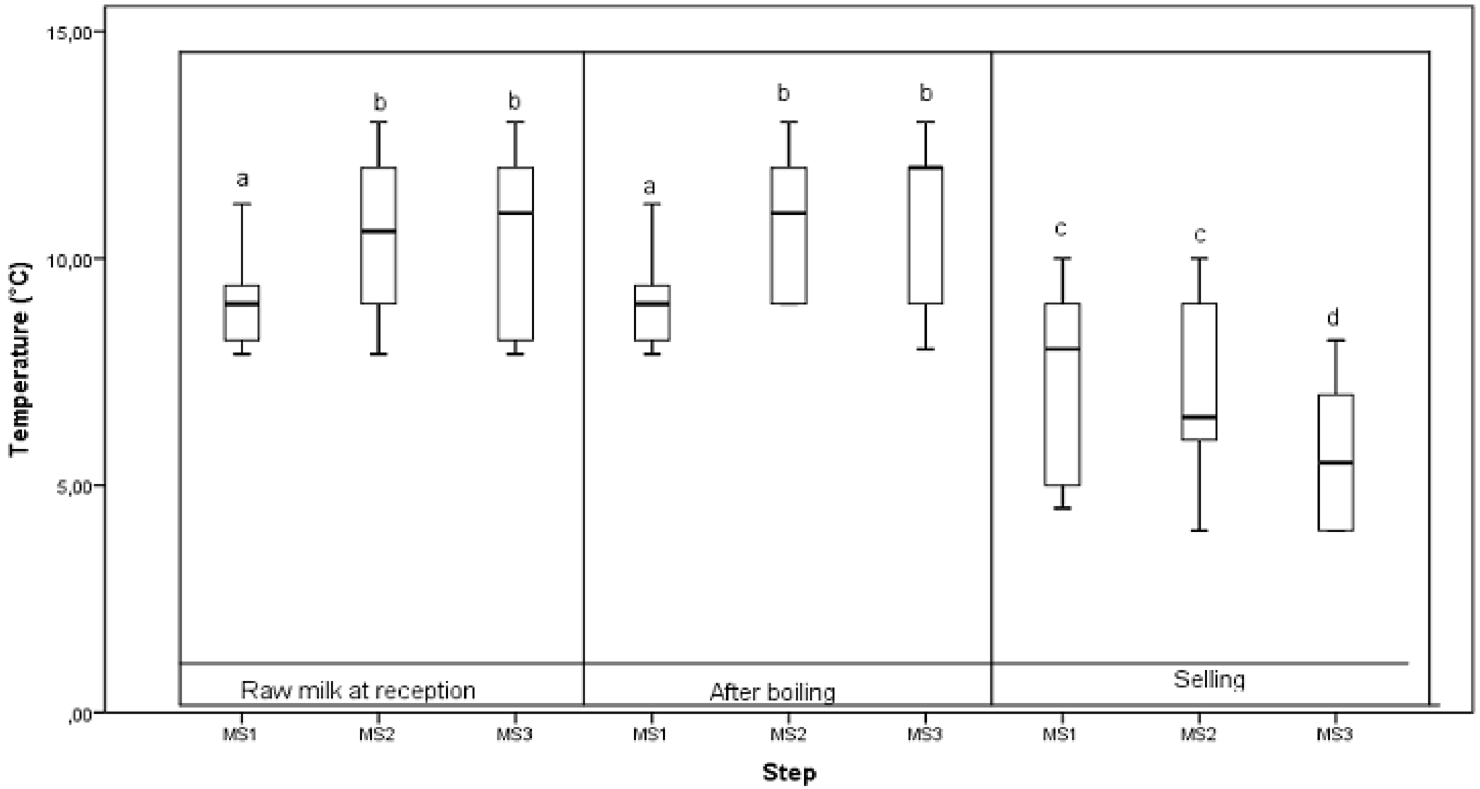

Figure 4.2 :Temperature of the milk from reception to after boiling and at time of selling in milk shops. Different letters indicate statistical significant differences($p<0.05$).

Overall there was more variation noted of the temperatures achieved for milk in MS2 and MS3 at these two steps than was the case in MS1. However, at the time of selling, when boiled milk had been put in the refrigerator for an usually longer period of time, MS1 showed the highest average temperature (7.5°C) and MS 3 achieved the lowest (significantly different ($p=0,004$)) average temperature of 5.6 °C but in all cases quite high variations in temperatures measured for the boiled milk upon serving was observed.

The microbiological quality of milk along the boiling process and the cleanliness of the environment are presented in the Figure 4.3. Statistical significant differences were observed for the raw milk at reception among milk shops for TMC ($p=0.001$) as well as for coliforms ($p=0.003$). The highest average counts of TMC and coliforms in raw milk were found in MS1 with 6.4 log CFU/mL and 2.8 log CFU/mL respectively. After boiling, coliforms were not detected anymore and the average TMC decreased by more than 1 log CFU/mL in all milk shops and now MS1 showed the minimal value obtained (4.1 log CFU/mL) which was statistically different from TMC from the other two milk shops ($p=0.008$).

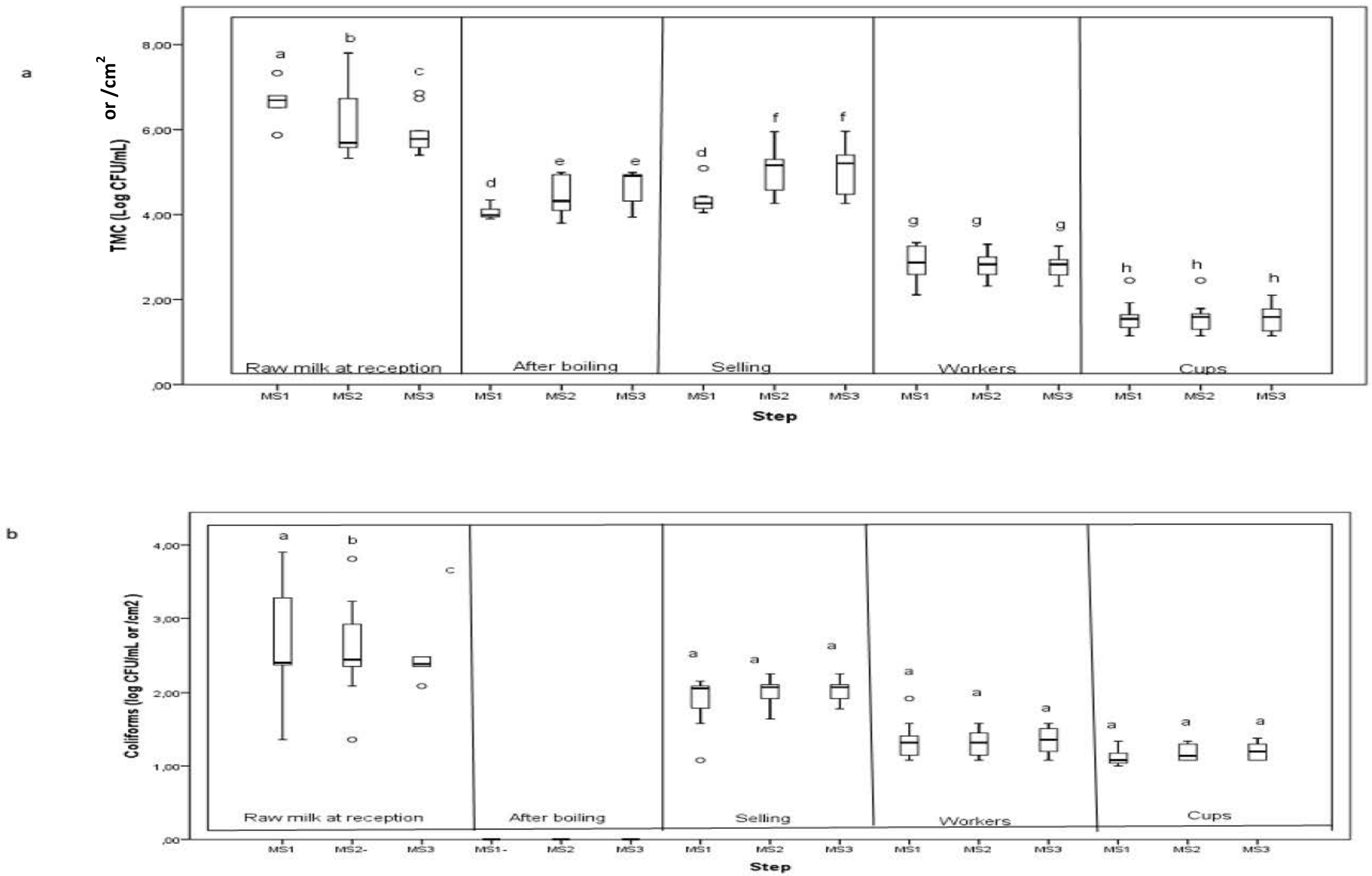

Figure 4.3 : Microbiological quality of milk along the stages in milk shops and assessment of hygiene of workers' hands and cups for serving boiled milk . Different letters indicate statistically significant differences (p<0.05).

The evolution of temperature during the boiling process of the milk is presented in Figure 4.4 The boiling process took 7.8 to 13.0 minutes (median time of the 9 batches being 8.8 minutes) to achieve foaming of the milk and a maximum observed temperature ranging from 89 to 106.2 °C (median temperature of the 9 batches being 102°C).

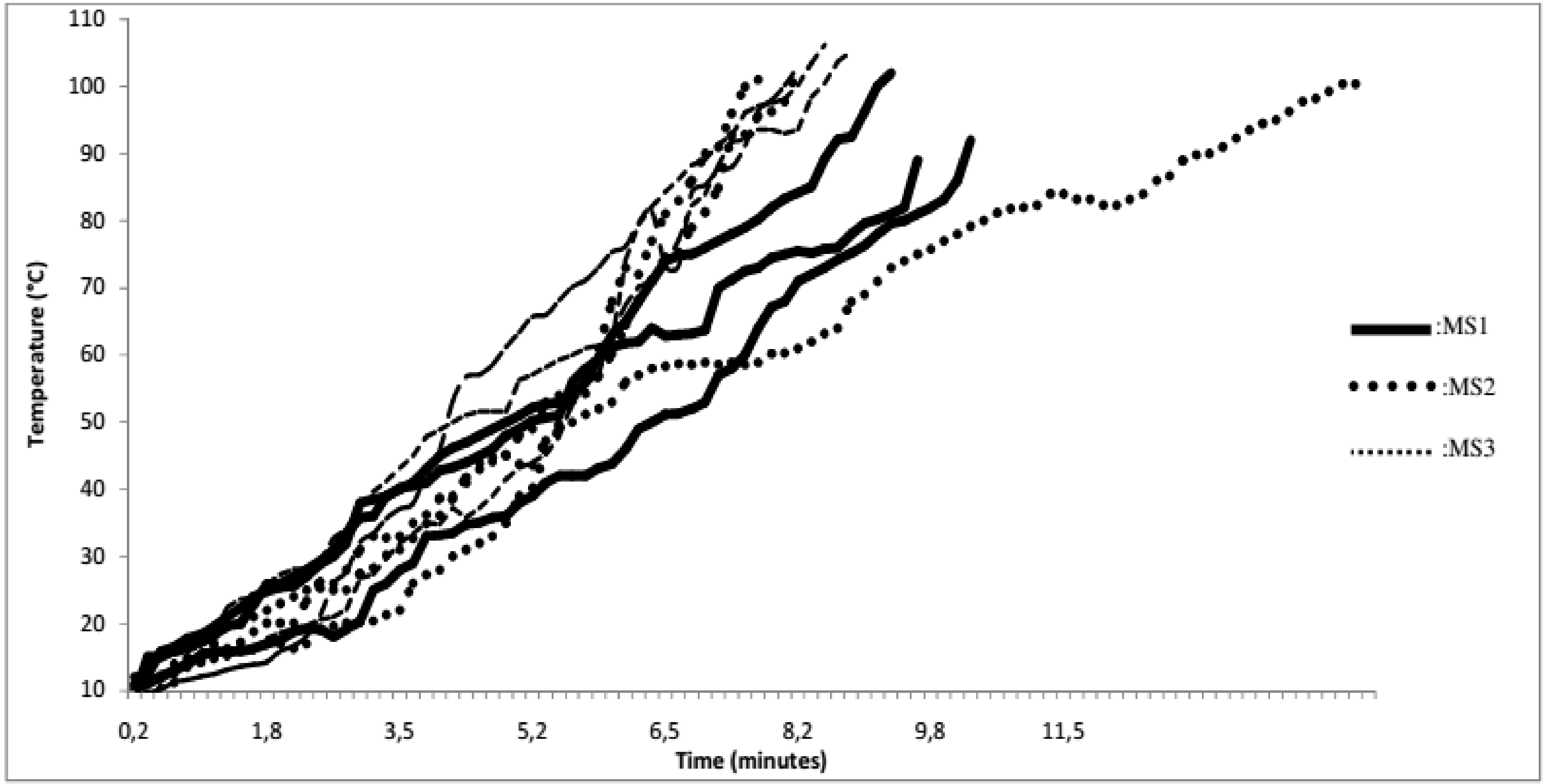

Figure 4.4 : The temperature profile of the boiling process of milk in milk

The process lethality expressed as F_{70} or the equivalent number of minutes at 70°C obtained for the boiling process are presented in Table 4.1. For a sufficient inactivation of *L. monocytogenes* (6 log reduction) a heat treatment equivalent to 2 minutes at 70°C, thus a F_{70}-value of 2, is usually recommended (ACMSF, 2007). For *L. monocytogenes*, the minimum obtained F_{70} value was 62.2 minutes in MS1, and the maximum was 1.1 10^4 minutes in MS3. With regard to *Salmonella*, the minimum F_{70} value was 34.3 minutes obtained in MS1, whereas the maximum was 2.9 10^3 minutes obtained in MS3. From the results it can be concluded that very high F_{70}-values are obtained and thus residual presence of none of both pathogens, nor any coliforms is expected in the boiled milk. The only residual TMC contamination in boiled milk would relate to spore-forming micro-organisms. However, the boiled milk is kept in open containers during cool down or in some cases is transferred after boiling to some close containers, but still there is opportunity for post-contamination with micro-organisms from the milk shop environment of workers' hands.

Table 4.1 : The process lethality for *Salmonella* and *Listeria monocytogenes* during boiling of milk in milk shops

				***Salmonella* Z max.= 9.3°C**		***L. monocytogenes* Z max = 8.°C**	
Milk Shop	**Repe-tition**	**MaximumT (°C)**	**Time of heating (minutes)**	**F70 (minutes)**	**Time for F70 ≥6D (minutes)**	**F70 (minutes)**	**Time for F70 ≥6D (minutes)**
MS1	**A**	89.0	10.0	3.4 10^1	9.5	6.2 10^1	9.3
	B	102.0	9.7	9.8 10^2	8.2	3.2 10^3	7.8
	C	92.0	10.0	6.5 10^1	9.3	13.7 10^1	9.8
MS2	**A**	100.4	13.0	1.3 10^3	9.9	4.1 10^3	9.3
	B	102.6	7.8	1.0 10^3	6.8	3.3 10^3	6.7
	C	102.0	8.2	1.2 10^3	7.1	4.2 10^3	7.0
MS3	**A**	106.2	8.5	2.9 10^3	6.5	1.1 10^4	6.3
	B	103.0	8.2	1.2 10^3	6.8	4.2 10^3	6.7
	C	105.2	8.8	2.3 10^3	7.3	9. 0 10^3	7.3

When selling the boiled milk, a slight increase in TMC was observed in all milk shops, but still with the average TMC being significant (p=0,007) lower in MS1 (4.4 log CFU/mL) than the other two milk shops. The coliform counts in boiled milk being served were again enumerable in 80% of samples indicating indeed some level of post-contamination with the maximum average value of 2.1 log CFU/mL in MS3. Some coliforms were also observed on the workers' hands (38.5% of samples) and occasionally on the cups for serving boiled milk as part of the average TMC which was close to 2.8 log CFU/mL in all milk shops. In raw milk at reception in milk shops, *S. aureus* was found in all milk shops but in low numbers, with the maximum number obtained being 2.5 log CFU/mL in MS1. No more *S. aureus* were enumerated after boiling in all milk shops, again indicating the sufficient heat treatment to inactivate all vegetative potential pathogenic micro-organisms. But at the time of selling 3 out of 54 samples showed presence of *S. aureus* in the boiled milk served in MS1 with an average of 2.2 log CFU/mL (for the 3 samples) indicating post-contamination. In MS2, 1 out of 54 samples from the workers' hand swabs showed presence of *S. aureus* with 2.1 log CFU/cm^2 being found. *Salmonella* spp. and *L. monocytogenes* as important food borne pathogens were however absent from all milk samples taken in every milk shops and none of these pathogens could be detected on workers' hands or cups used for serving the boiled milk.

4.4.2. Microbiological quality and safety throughout the Gouda cheese production

The microbiological quality from raw milk to Gouda cheese and cleanliness of the workers' hands food contact materials involved in the cheese production is represented in Figure 4.5. The average TMC of raw milk at reception in all samples was ca. 6.0 log CFU/mL but coliform counts in raw milk were significant different among the three small cheese processing plants with the highest average coliform numbers (3.3 log CFU/mL) as well as variation in numbers being observed in SCP2. After boiling, the average TMC of the milk decreased in all cheese production sites, reaching the minimum average value of 4.0 log CFU/mL in SCP3, and a statistical significant difference observed among all cheese production plants. After boiling of the milk, as expected, no coliforms were enumerated anymore.

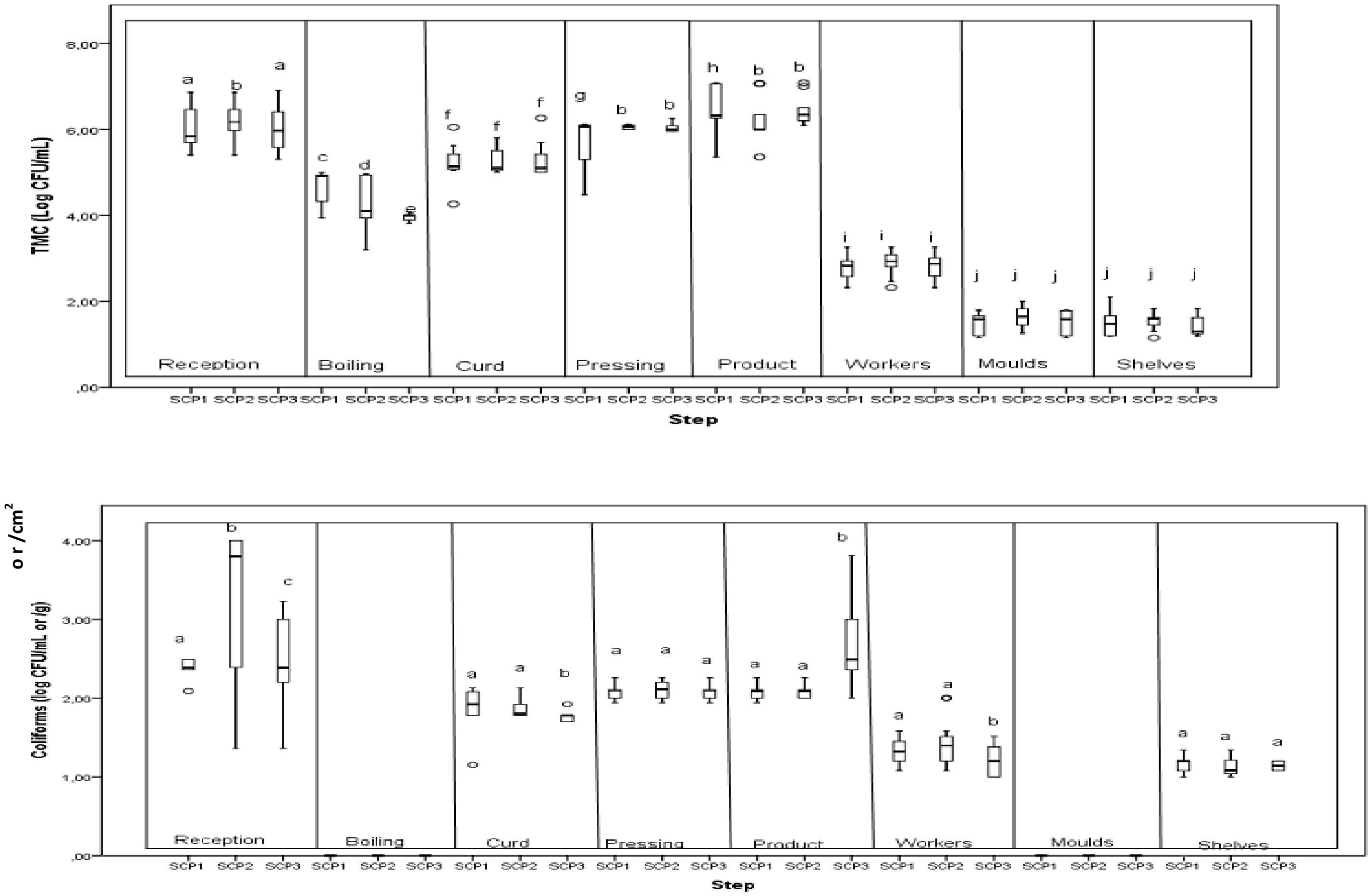

Figure 4.5 : Microbiological quality of milk and cheese, workers' hands and food contact materials along the stages of Gouda cheese processing in small cheese plants. Different letters indicate statistical significant differences (p<0.05)

In the subsequent step, the average TMC of the curd was situated at ca. 5.0 log CFU/mL, and the group of coliforms could be enumerated again, with numbers close to 1.8 log CFU/mL. The average TMC values continued to increase after pressing the curd, reaching an number of ca. 5.9 log CFU/mL (with high variation in numbers noted in particular in SCP1, which might explain the statistical significant difference noted in TMC of the pressed cheese for SCP1 and the two others cheese producing plants (p=0,003). The coliform numbers increased also slightly during pressing of the cheese, probably due to a concentration effect, and exceeded 2.0 log CFU/mL in all cheese producing plants. In the final Gouda cheese, both TMC and coliforms average values kept these prior observed numbers with a maximum value of 6.4 log CFU/g for TMC and 2.7 log CFU/g for coliforms in SCP3.

The results of cleanliness of the workers' hands showed similar average values of TMC (2.8 log CFU/cm^2) in all cheese processing sites, and also similar numbers of coliforms were enumerated from all samples, with the maximum average value of 1.4 log CFU/cm^2 in SCP2. On the cheese contact materials namely moulds and shelves, the average TMC were close to 1.5 log CFU/cm^2 in all cheese processing sites, but coliforms were not enumerated when the moulds were swabbed, although in all sites coliforms were found on shelves with average values slightly above the quantification limit. At this level, coliforms were enumerated in 76% of samples.

S. aureus was also present in raw milk destined to cheese processing with the maximum average of 2.3 log CFU/mL found in SCP2, but was absent from all samples after boiling and the curd, although again numbers slightly above the quantification limit were found after pressing the curd (in 12 % of samples) as well as on workers' hands in all cheese processing sites (10% of samples). In the final Gouda cheese, *S. aureus* was found in all cheese processing sites up to the maximum of 2.4 log CFU/gr in SCP1. A potential issue with *Salmonella* spp. was noted in SCP1, with one *Salmonella* positive sample (n= 54) for raw milk at reception, another positive sample from the curd (after pressing, n= 27) and another positive sample in sampling the final product (n=27). In SCP3, one *Salmonella* positive sample was found on swabs from a worker's hand (n=54). *L. monocytogenes* was absent from all product or swab samples taken during the processing of Gouda cheese at all three cheese production sites.

4.5 DISCUSSION

Our study investigated the microbiological quality and safety of boiled milk and Gouda cheese along the processing steps in respectively milk shops and small cheese processing plants taking raw milk for the informal milk market in Rwanda. Although the TMC in raw milk destined for boiling in milk shops exceeded the limits according to the U.S. national standards which recommend the maximum upper limit of 5.5 log CFU/mL for raw milk after collection (Food and Drug Administration, 2009) and exceeded the European Union requirements which recommend not more than 5.0 log CFU /mL for raw milk intended to be heated (European Commission, 2004b), local standards were still met as the East African Community (EAC) requires an upper limit of 6.3 log CFU/mL for Grade 3 raw milk (EAC, 2006). A similar situation prevails for coliforms which were below the maximum limit of 4.7 log CFU/mL established by EAC. In milk shops, raw milk is supplied by mobile traders who buy it from farms and/or milk collection centers (Kamana et al., 2014). Thus, its quality is highly variable due to its different origins with various post-milking hygienic conditions. In comparison, higher TMC values were found in raw milk from informally marketed milk in Mali (8.1 log CFU/mL), Tanzania (6.9 log CFU/mL) and Chad (from 7.4 to 7.6 log CFU/mL) (Bonfoh et al., 2003; Kivaria et al., 2006; Koussou and Grimaud, 2007). The lower counts found in Rwanda might be linked with the interconnection between the formal and the informal markets, given that milk collection centers are equipped with cooling facilities (Kamana et al., 2014; Karenzi et al., 2013; Modderman, 2010).

The boiling practice reduced significantly the microbial loads, as highlighted by several studies (Agarwal et al., 2012; Metwally et al., 2011; Tremonte et al., 2014). The obtained temperature profiles during boiling and calculated process lethality (F_{70} values) indicated in all cases highly sufficient heat treatment to enable at least 6 log reduction of both *Salmonella* and *L. monocytogenes*. According to Farber et al. (1988), *L. monocytogenes* does not survive classical milk pasteurization for 15 seconds at 75°C. For *Salmonella*, no survival was observed after 16.2 seconds at 74°C by d'Aoust et al. (1987). Thus, the applied heat treatment in milk shops was largely effective, since the lethality of pathogens was achieved and highly exceeded and thus residual presence of none of these pathogens, nor any other vegetative pathogenic bacteria or coliforms are expected to be present in the boiled milk after this treatment.

However, after boiling there is opportunity for post-contamination with micro-organisms from the milk shop environment of workers' hands.

The observed increase in TMC and coliforms in boiled milk at serving could be attributed to post-contamination, as well as slight increase in numbers might also be expected due to some extent ineffective refrigeration as the temperature of refrigerated boiled milk at selling was above 5°C in all milk shops. Ineffective temperature control and insufficient clean environmental conditions are among the commonly observed risk factors for unsafe milk and dairy products in the informal market in Africa (Bonfoh et al., 2003; Donkor et al., 2007; Millogo et al., 2010).

In small cheese production plants, the microbiological quality of raw milk at receipt was also exceeding US and European standards but complied to local EAC standards. The observed increase in microbiological loads along the process might be linked with fermentation and to some extent also contaminations from the production environment, given the importance of manual operations and the cleanliness of contact materials which is not monitored. The deficient hygienic manufacturing practices are materialized by the absence of a cleaning and disinfection plan, the use of untreated water and wooden food contact materials not easily cleanable. This resulted in final Gouda cheese products exceeding local standards. The Rwanda Standards Board recommends an upper threshold value for cheese of 5 log CFU/g for TMC and less than 100 cfu/g (the detection limit) for coliforms (RBS, 2015). In other African countries, limited information is available for the cheese sector, especially Gouda cheese. Studies conducted by Belli et al. (2013) in Cameroun, Ashenafi (1990) in Ethiopia, Mankai et al. (2012) in Tunisia and Wahba et al. (2010) in Egypt showed that the sanitary conditions of the processing environment affects the quality and safety of cheese.

The omnipresence of *S. aureus* in raw milk for both small cheese processing plants and milk shops might be linked with the prevalence of mastitis which is endemically present in African countries including Rwanda (Byavu et al., 2000; Kateete et al., 2013; Shyaka et al., 2010), and with manual milking happening in the many small farms.

In final products namely boiled milk and Gouda cheese, its occurrence could be to some extent associated with the poor personnel hygiene and different operations carried out manually, without prior strict hygienic practices. Meanwhile, the observed levels of *S. aureus* in boiled milk at selling and Gouda cheese are far below the 6.5 log CFU/mL at which *S. aureus* was found to produce toxins (Fujikawa and Morozumi, 2006). In milk shops, *Salmonella* was absent along the process, in contrast to the previous study by Kamana et al. (2014) presented in Chapter 2. This difference might be attributed to the reinforcement of randomly conducted inspections by environmental health officers from the Ministry of Health which put a pressure on milk shops workers for basic hygiene requirements in the framework of food-borne diseases prevention (RBC, 2015). The repeated isolation of *Salmonella* in SCP1 might be attributed to poor hygienic conditions, since this particular cheese processing plant was not effectively using the means provided by the interventions for the professionalization of the cheese sub-sector (personal observations). In fact, an intervention by a private NGO, namely the second phase of the project on the Dairy Competitiveness Program focused on the improvement of cheese making by providing means for safer environment and trainings on good practices (LANDOLAKES, 2015b). The isolation of *Salmonella* from one worker's hand in SCP3 shows also that there is still a gap in basic personnel hygiene requirements.

4.6. CONCLUSION

This study aimed to understanding the origins of in a prior study (Kamana et al., 2014) (chapter 2) insufficient quality and safety of informally marketed boiled milk and Gouda cheese in Rwanda. After following the steps of milk boiling in milk shops from raw milk reception to selling and Gouda cheese processing from raw milk to final products, it appears that the despite the high microbial loads in raw milk, the applied heat treatment is effective by reducing those loads considerably and exceeding the lethality rate for enteric bacterial pathogens.

Therefore, the observed increase again in microbial load, and the occasional presence of in particular coliforms and *S. aureus* in boiled milk is linked with post-contamination from the environment, personnel hygiene or inadequate refrigeration. In small cheese processing plants also it was noted that the personnel hygiene and the processing environment are probably at the origin of occasional *S. aureus* and *Salmonella* contamination during processing. The interventions from the Rwandan government and its partners for the basic hygiene requirements in milk shops and the professionalization of the cheese sub-sector contributed in reducing contaminations in comparison to the previous study by Kamana et al. (2014).

But there is still a gap in the aspects regarding appropriate refrigeration in milk shops and personnel hygiene and environmental hygiene for both small cheese processing plants and milk shops and these aspects are prone to further improvements and is recommended to be the focus in the future for safer boiled milk and Gouda cheese in Rwanda.

CHAPTER 5 : Exposure assessment to *Salmonella* in Musanze region (Rwanda) by consumption of informally marketed milk and Gouda cheese

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Redrafted from :

Kamana, O., Jacxsens, L., Kimonyo, A., and Uyttendaele, M. (2015). Exposure assessment to Salmonella in Musanze region (Rwanda) by consumption of informally marketed milk and Gouda cheese (Submitted).

CHAPTER 5 : Exposure assessment to *Salmonella* in Musanze region (Rwanda) by consumption of informally marketed milk and Gouda cheese

5.1 ABSTRACT

Exposure to *Salmonella* by boiled milk and Gouda cheese of consumers in Musanze district in Rwanda is assessed. A consumer survey (n = 90) revealed that boiled milk in milk shops, belonging to the informal market, is mainly consumed by men (79.7 %), on a daily basis (33.3 %) with a volume of 400 mL (61.2 %). Among milk buyers in milk shops, 40.0% is also buying boiled milk for their families resulting in consumption by mainly adults aging between 19-29 years (32.3 %, n=136), and a daily consumption frequency is reported by 62.5 % of the survey participants. Among the interviewed households (n=36), 66.7 % is re-boiling the milk and 33.3 % is storing it refrigerated before consumption. Gouda cheese is usually sold in supermarkets (formal market). A cheese portion with a weight of 62.5 g is mainly consumed (80.0 % , n=370), and weekly frequency is most in practice (38.1 %). A probabilistic exposure model was build up taking into account prior collected prevalence data of *Salmonella* contamination in boiled milk and Gouda cheese in Rwanda, assumed concentration of *Salmonella*, information of the milk chain gathered by observation, and collected consumption data and consumer behavior information. Finally, the risk of infection upon serving and per year were calculated for different categories of consumers. It appears that even though *Salmonella* is eliminated by milk boiling, the risk of infection still persists due to post-contamination in the milk shops (11.08% per serving), but is decreased by re-boiling in households if consumed by adults or children to 4.75% per serving. For cheese consumption, this study revealed that the risk of infection was lowered by multiple food safety interventions for cheese producers such as trainings on food hygiene and provision of modern equipments improving thus the situation from before to after 2012 by 30% per year in case of adults.

5.2. INTRODUCTION

In sub-Saharan Africa and other developing countries in general, milk is of great importance for both rural and urban populations as a source of (animal) proteins (Delgado, 2003; Duteurtre, 2007). Its popularity lies in traditional and cultural habits especially for pastoral communities whose milk production and related farming activities constitute the backbone of their livelihoods (Muriuki, 2001; Ndambi et al., 2007; Thorpe et al., 2000). For decades, efforts have been concentrated on milk production increase in Africa for food security purposes, without consideration of food safety aspects (Grace et al., 2007). The rapid urbanisation in those countries led to significant changes in the dairy farming systems, with the implementation of dairy industries processing a wide range of milk and dairy products (Corniaux et al., 2005; Dieye et al., 2005; Kussaga et al., 2014b). This modernization being still in progress, a parallel circuit of milk and dairy products emerged and its share in the whole dairy value chain is predominant (Omore et al., 2001; Sinja et al., 2006; Staal, 2006). The co-existence of those two different markets leads to the presence of formally processed milk and dairy products and informal products mostly traditional and/or fermented which don't undergo official controls and this makes them vulnerable and often of poor quality as highlighted by several studies e.g. Akabanda et al. (2010) in Ghana, Savadogo et al. (2004) in Burkina Faso, Tchekessi et al. (2014) in Benin, Fokou et al. (2010) in Mali and Schoder et al. (2013) in Tanzania.

Milk-borne salmonellosis accounts for approximately 48.0 % of all milk-borne outbreaks reported in different industrialized countries (De Buyser et al., 2001). Although in developing countries limited information is available on food-borne diseases (Mensah et al., 2012; Mwamakamba et al., 2012; WHO, 2012), the prevalence of *Salmonella* in milk and dairy products indicates a high probability of salmonellosis occurrence.

In Rwanda, where the co-existence of the formal and informal markets as well as in other developing countries was noticed, a study conducted by Kamana et al. (2014) revealed that 21.2 % (n=33) of Gouda cheese and 6.1 % (n=297) of milk were contaminated by *Salmonella* along the milk and dairy chain, from primary production to final consumption.In the African context, limited studies are conducted to assess the exposure on milk-borne pathogens and potential disease.

In Ivory Coast, it was demonstrated that 51.6 % of interviewees consumed raw milk with a probability of 29.9 % for ingestion of milk contaminated by *Escherichia coli*, *Staphylococcus aureus*, and *Enterococcus* spp., and about 652 consumers were estimated to ingest contaminated milk daily (Kouame-Sina et al., 2012). In Ethiopia, Makita et al. (2012) found that the annual incidence rate of staphylococcal poisoning was 20.0 % per 1000 people consuming informally marketed home-made yoghurt, and the probability of purchasing milk contaminated with *S. aureus* was 22.7 % with 217 people likely to purchase contaminated boiled milk informally marketed in Tanzania (Kilango et al., 2012).

The present study aims to develop a probabilistic shop-to-consumer exposure model, including prevalence data of *Salmonella*, milk chain information, collected consumption and consumer behavior information in order to gain insight in the risk on illness per serving and per year of the Rwandan population in Musanze region, consuming informally marketed milk and formally marketed Gouda cheese. The outcomes of the model provide useful information and serves as a case study for future mitigation strategies to decrease the burden of salmonellosis in the African dairy chain.

5.3. MATERIAL AND METHODS

5.3.1. Microbiological analyses

In addition to previously obtained prevalence data (Kamana et al., 2014, Kamana et al., 2015) (Chapter 2, chapter 4), microbiological analyses were performed to isolate *Salmonella* from boiled milk, purchased in the milk shops (n=36) and Gouda cheese (n=36) purchased in supermarkets in Musanze town. These latter samples were collected while conducting the interviews in these shops to obtain information on consumption patterns and consumer behavior. For this purpose, 4 samples were collected per milk shop (n=3) and supermarket (n=3) and the sampling was conducted in 3 rounds.

Milk samples were collected in previously sterilised test tubes, and cheese samples were collected with sterile aluminium foil and were transported to the lab at ca. 4.0° C for 30 minutes in an ice box for microbiological analyses immediately upon arrival at the lab.

The presence of *Salmonella* was detected in 25 ml (or g) samples according to ISO 6579:2002+A1:2007 (ISO, 2002) i.e. by nonselective enrichment in buffered peptone water (Oxoid), cultivation on modified semisolid Rappaport Vassiliadis (Oxoid), selective isolation on xylose lysine desoxycholate agar (Oxoid), and purification of presumptive isolates on nutrient agar (Oxoid), and finally, confirmation of typical colonies by the triple sugar iron test.

5.3 2. Consumption survey and consumer handling practices

5.3.2.1 Set-up of the survey

In order to get insight into the consumer habits and handling practices, a survey was conducted in Musanze town, the second largest town of Rwanda (Republic of Rwanda, 2014). Two questionnaires were developed for this purpose, i.e. for milk consumption and for cheese consumption. The questionnaire used to collect information on milk consumption consisted of two sections (Annex 1): (i) milk consumption in milk shops, with information related to the identification of the consumers namely age and gender, volume consumed and frequency of consumption; (ii) milk consumption in households, including identification of consumers (age and gender of the family members of the person who purchased the boiled milk in the milk shops,), volume and frequency of milk consumption at home, mode of transport and distance covered from milk shop to home, and handling practices at home including storage conditions, whether or not re-boiling of the milk before consumption, and whether o not other foods were mixed with the milk before consumption. For cheese consumption data, the questionnaire (Annex 2) comprised also two sections but cheese is sold in supermarkets and only consumed at home : (i) consumer identification with age, gender, the frequency of consumption and the quantity consumed; (ii) information on handling practices in households and details on the consumers identification in case of consumption by many consumers at home. A trial survey was conducted prior to the effective start of the data collection, in order to make the questionnaires more applicable and consistent. Therefore, a total of 30 persons were interviewed for milk consumption in milk shops, and 10 for cheese consumption. Those interviews enabled us to have a general view on the quantities of boiled milk consumed in milk shops and in households; different volumes of used recipients were identified ranging between 100 mL and 750 mL

(Figure 5.1). Examples of cheese portions were identified during this preliminary survey and weighed between 62.5 g and 250 g.

In addition to quantities, different ranges of distances covered by milk buyers were identified, mode of re-boiling in households were checked, consumers were put in different categories of age, and a final version of the questionnaires fit for the survey was set up. The outcomes of these trial surveys are not included further in this paper but leaded to the two questionnaires (Annex 1 and 2).

Data for consumption and consumer behavior were collected in three different milk shops, and three supermarkets chosen for having been part of the previous sampling on microbiological quality and safety and hygiene assessment (Kamana et al., 2014, Kamana et al., 2015). Figure 2 represents the used approach for data collection in both milk shops and supermarkets. In milk shops, 90 respondents were interviewed, and 54 of them were only consuming boiled milk in milk shops, whereas 36 were buying boiled milk for home consumption. In households, data for 136 consumers were collected, making in total 190 data of boiled milk consumers. In the case of cheese, all buyers (n=90) were purchasing cheese for home consumption in families, and data for in total 370 consumers were collected. The survey was conducted from January to March 2014.

Figure 5.1 : Identification of different recipients used for milk consumption in households during survey of milk consumer handling practices in Musanze region of Rwanda

5.3.2.2. Data cleaning and processing

Data from questionnaires were compiled using Microsoft Excel 2009 sheet and grouped in three categories namely data for boiled milk consumption in milk shops, data for boiled milk consumption in households and data for cheese consumption in households. Statistical analyses were performed using SPSS Statistics 21. Analysis of Variance and the post hoc Bonferroni tests were performed on normally distributed variables with equal variances. Kruskal-Wallis and Mann-Whitney U tests were used for non-normally distributed variables. Statistical tests were conducted to evaluate significant difference between age, gender in frequency and volume of consumption.

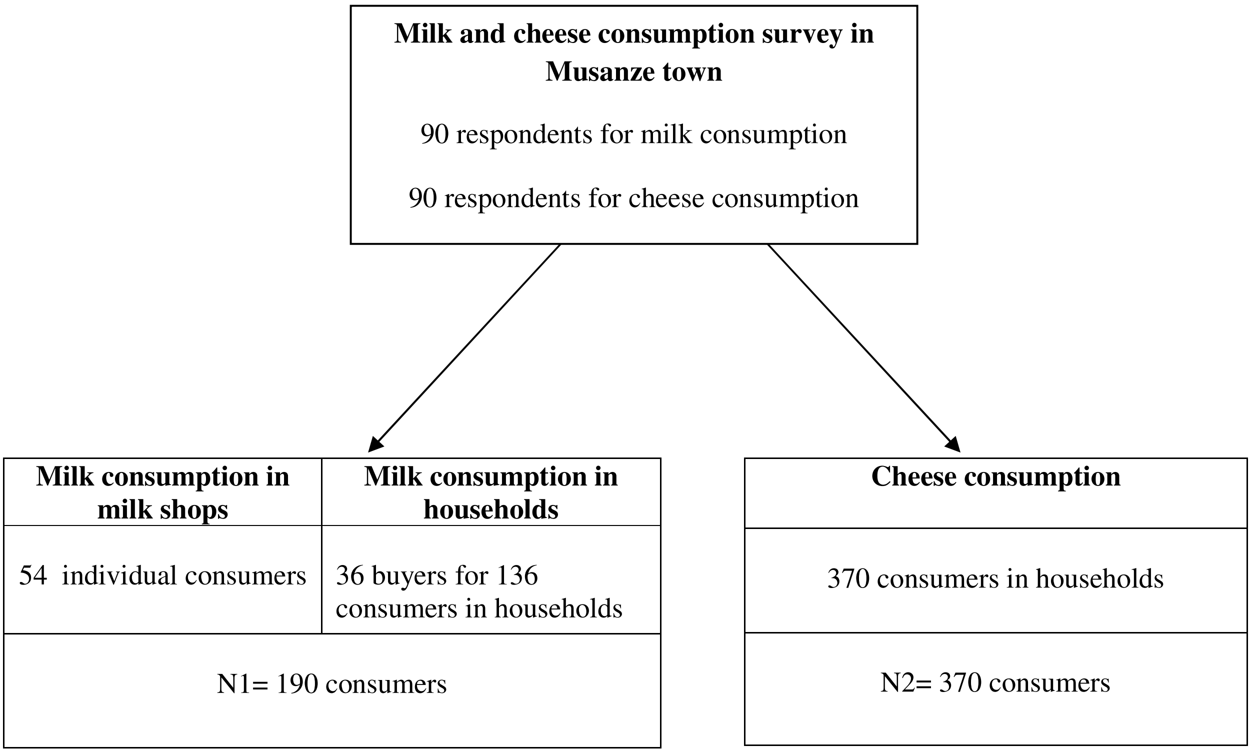

Figure 5.2 : Data collection for milk and cheese consumption in Musanze town

5.3.3. Exposure and risk model

5.3.3.1. Set-up of exposure assessment model from shop to consumption

A modular process model was designed to simulate the steps undergone by milk and cheese from purchase to consumption (Figure 5.3). Milk bought in milk shops undergoes two ways : on-site consumption or home consumption. In the first case, milk is directly consumed by buyers, whereas in the second case it is transported home, where it is consumed by different family members with re-boiling or not. In case of direct consumption, the risk of illness faced by consumers depends immediately on the presence of *Salmonella* in served boiled milk in the shop. Once milk bought and brought home, it was assumed that no growth and/or cross-contamination by *Salmonella* is happening, given the short distances covered for transport home.

For home consumption, milk is kept refrigerated or not. In this case too, an assumption was made on the absence of contamination and/or cross-contamination, taking into account the short time between purchase and consumption.

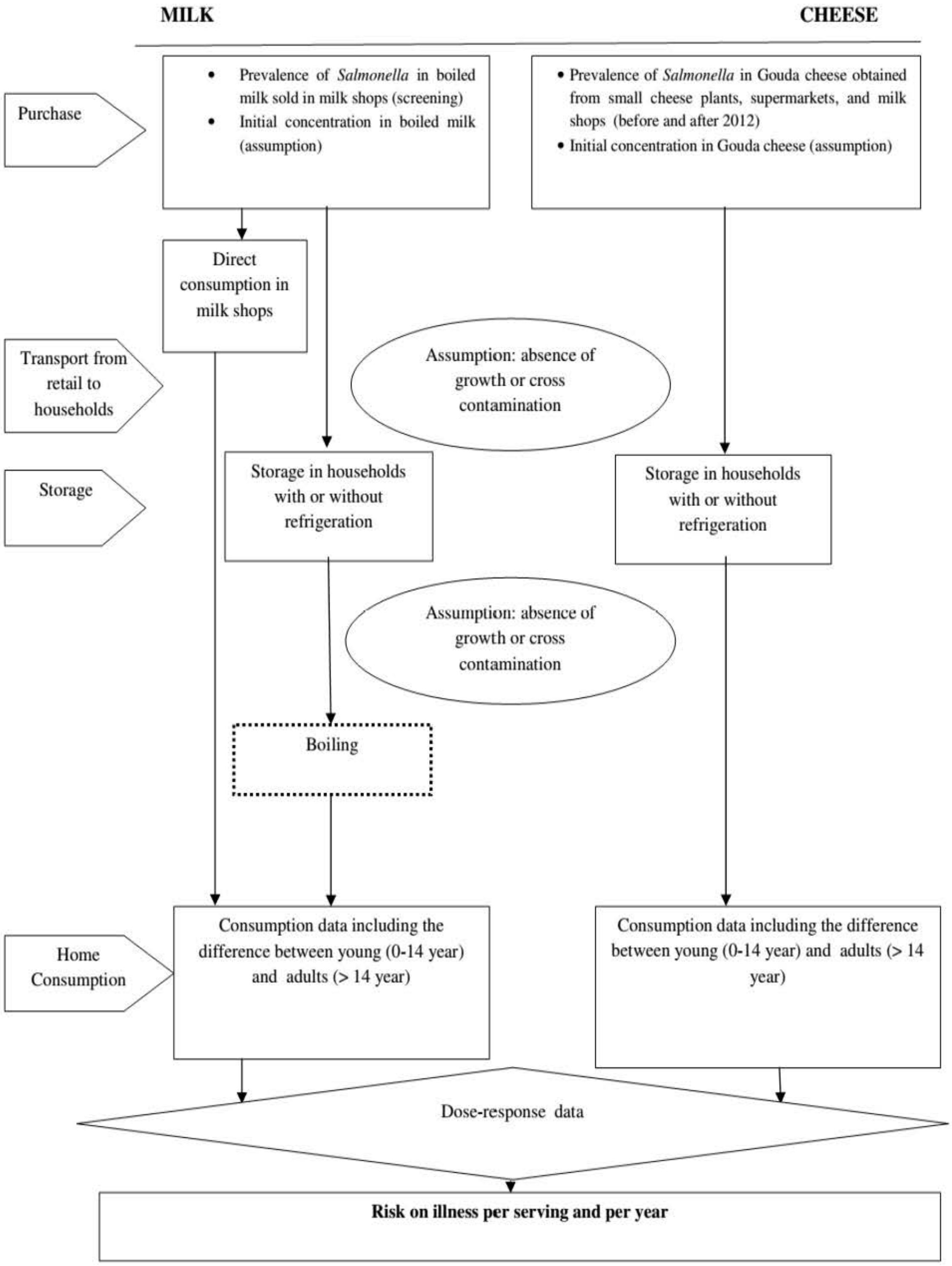

Figure 5. 3 : Schematic overview of retail-to-consumer process model in *Salmonella* exposure assessment of boiled milk and Gouda cheese in Rwanda

In fact, the milk shops are located in the town, and milk buyers are town residents living in vicinity of those milk shops, buying milk just before consumption. The initial concentration of *Salmonella* was assumed to be 0.1 in minimum, 5 most likely and maximum 100 CFU/mL (or gr). The boiling effect was considered to be effective in eliminating *Salmonella* totally based on previous findings indicating that *Salmonella* does not survive a heat treatment of 16.2 seconds at 74.0°C (d'Aoust et al., 1987). This was confirmed by prior study conducted by Kamana et al. (2015) which stipulated that the observed current boiling practices in the Rwandan context (using charcoal and fire wood) enabled at least a 6 log reduction of *Salmonella.* It has been reported that the heat treatments in use in the African informal dairy sector were effective in eliminating milk-borne parhogens (Arimi et al., 2005; Hetzel et al., 2005). For cheese bought in supermarkets and brought home for consumption, it was also assumed that no growth and/or cross-contamination was happening, and is consumed directly. Consumption data and consumer handling practices (e.g. re-boiling the milk) were applied as collected from the survey to introduce in the exposure assessment model. Data from the current consumption survey for both milk and cheese consumption in Musanze region in Rwanda were analyzed to look for statistical significant differences in groups of consumers regarding volumes and frequencies of consumption (age, gender). Different models on dose-response available for *Salmonella* (Blaser et al., 1982; Blaser and Newman, 1982; Oscar, 2004) were taken into account to lead to a final estimation of the risk on illness per serving and per year.

5.3.4. Prevalence of Salmonella,mathematical model and risk simulations

Table 5.1 : Prevalence of *Salmonella* in boiled milk (25 mL) and Gouda cheese (25 g). Boiled milk samples were collected in milk shops in years 2012, 2013 and 2014 whereas cheese samples were collected in small cheese plants and milk shops in 2012, in small cheese plants in 2013 and supermarkets in 2014.

	Year	Boiled milk	Gouda cheese	Reference
Prevalence	2012	21.4% (9/42)	21.2% (7/33)	Kamana et al.,2014
	2013	0.0% (0/18)	3.7% (1/27)	Kamana et al.,2015
	2014	5.5% (2/36)	0.0% (0/36)	Current study
Total		11.4% (11/96)	8.3% (8/96)	

A mathematical model was designed in a Microsoft Excel spreadsheet for further simulations using @Risk (Version 6, Palisade, USA) software. Monte Carlo simulations were run three times using 50,000 iterations. For milk consumption in milk shops, input data were initial prevalence and concentration of *Salmonella* in served milk, consumption frequency, volumes consumed and data on dose-response on salmonellosis. The exposure per serving (output, S6), expressed as CFU/ml or g, was obtained by integrating the distribution of concentration of *Salmonella* (S2), the distribution on volume consumed (S4), and the distribution of prevalence (S1) (Table 5.2). The risk on illness per serving (output, S11, S12, S13) was obtained by taking into account the exposure per serving (S6) and the available data on dose-response for salmonellosis (three scenarios) (S7, S8, and S9).

Table 5.2 : Inputs, applied functions, units, sources of information and defined outputs applied for the exposure model for *Salmonella* in boiled milk consumed in milk shops

	Parameter	Function	Detail	Unit	Source
S1	**Prevalence**	RiskDiscrete ({0;1};{0,886;0,114})	Fractions of positive samples	Presence in 25 mL	Table 5.1 based on 11/96 positive samples
S2	**Concentration of *Salmonella***	RiskPert(0,1; 5; 100)	min = 0,1 most likely = 5 max = 100	CFU/mL	Assumption
			Consumption in milk shops		
S3	**Consumption frequency**	RiskDiscrete({requencies per year};{fractions of consumers})	a1=12,a2=24,a3=52,a4=104,a5=260,a6=365,a7=730,b1=0,0556,b2=0,0926,b3=0,0926,b4=0,0926,b5=0,2407,b6=0,3333,b7=0,0926	Times/year	Consumer survey, this paper
S4	**Consumption volume**	RiskDiscrete({volumes};{fractions of consumers})	a1=500,a2=400,b1=0,3889,b2=0,6111	mL	Measured, this paper and Figure 5.1
S5	**Exposure per serving (without prevalence)**	RiskOutput ("exposure per serving without prevalence")+S2*S4			
S6	**Exposure per serving with prevalence**	IF(S1=0;0;S5)			
S7	**Dose-response *Salmonella***	RiskUniform (10^5-10^{10})		CFU/serving	Blaser and Newman 1989
S8	**Dose-response *Salmonella***	RiskUniform(10-10^6)		CFU/serving	Oscar, 2004
S9	**Dose-response *Salmonella***	RiskUniform(0-10^3)		CFU/serving	Blaser et al.,1982
S10	**Risk on illness per serving - Dose-response 1**	RiskOutput("risk on illness per consumption ")+S6/S7			
S11	**Risk on illness per serving - Dose- response 2**	RiskOutput("risk on illness per consumption ")+S6/S8			
S12	**Risk on illness per serving - Dose- response 3**	RiskOutput("risk on illness per consumption ")+S6/S9			

	Parameter	Function	Detail	Unit	Source
S13	**Risk on illness per year -Dose response 1**	=RiskOutput("risk on illness per consumption per year")+S10*S3			
S14	**Risk on illness per year -Dose-response 2**	=RiskOutput("risk on illness per consumption per year")+S12*S3			
S15	**Risk on illness per year -Dose-response 3**	=RiskOutput("risk on illness per consumption per year")+S13*S3			

Finally the risk on illness per year (S14, 15, and S16) was obtained by integrating those data with the distribution of consumption frequency (S3). In case of milk consumption in households (Table 5.3), a similar methodology was followed, and conducted for the young consumers (age group 0-14 year) on the one hand and the adults (> 14 year) on the other hand, because a significant difference from the consumer survey was detected between those two age groups. The effect of *Salmonella* elimination while boiling (expressed as a discrete distribution) was taken into account for the calculation of the risk on illness per serving during home consumption of milk. For cheese consumption, two scenarios were considered (Table 5.4). The first scenario was based on available microbiological prevalence data in the context of informally marketed cheese in 2012, as described by Kamana et al. (2014). The second scenario was considered by using microbiological data obtained after the professionalization of the cheese sector in 2013-2014. In both scenarios, the situation for the young (age group 0-14 year) and adults (> 14 year) consumers was assessed because a significant difference was detected between those two age groups, and the risk on illness per serving and per year was calculated.

Table 5.3 : Inputs, applied functions, units, sources of information and defined outputs applied for the exposure model for *Salmonella* in boiled milk consumed in households

	Parameter	Function	Detail	Unit	Source
S1	Prevalence	RiskDiscrete({0;1};{0,886;0,114})	Fractions of positive samples	Presence in 25 mL	Table 1 based on 11/96 positive samples
S2	Concentration of *Salmonella*	RiskPert(0,1; 5; 100)	min = 0,1 most likely = 5 max = 100	CFU/mL	Assumption
			Consumption in households		
S3	Consumption frequency-Young	Risk Discrete({Frequencies per year};{fractions of young consumers})	a1=12,a2=24,a3=52,a4=104,a5=260,a6=365, b1=0,b2=0,b3=0,b4=0,1176,b5=0,1372,b6=0,7254,b7=0,0196	Times/year	Consumer survey, this paper
S4	Consumption frequency-Adults	Risk Discrete({Frequencies per year};{fractions of adult consumers})	a1=12,a2=24,a3=52,a4=104,a5=260,a6=365, b1=0,b2=0,0588,b3=0,1294,b4=0,941,b5=0,1058,b6=0,5647,b7=0,0470	Times/Year	Consumer survey, this paper
S5	Consumption volume-Young	Risk Discrete({volumes};{Fractions of young consumers})	a1=750,a2=500,a3=475,a4=450,a5=400,a6=380,a7=350,a8=320,a9=300,a10=250,a11=200,a12=125,a13=100,b1=0,b2=0,b3=4,b4=7,b5=0,b6=0,b7=0,b8=7,b9=6,b10=7,b11=1,b12=19,b13=0	mL	Consumer survey, this paper
S6	Consumption volume-Adults	Risk Discrete({volumes};{fractions of adult consumers})	a1=750,a2=500,a3=475,a4=450,a5=400,a6=380,a7=350,a8=320,a9=300,a10=250,a11=200,a12=125,a13=100,b1=7,1,b2=9,4,b3=4,7,b4=0,b5=7,1,b6=14,1,b7=2,4,b8=0,b9=10,6,b10=16,5,b11=16,5,b12=2,4,b13=9,4	mL	Consumer survey, this paper
S7	Exposure per serving-Young (without prevalence)	Risk Output("exposure per serving young")+S2*S5			
S8	Exposure per serving-Adults (without prevalence)	Risk Output("exposure per serving young")+S2*S6			
S9	Exposure per serving with prevalence-Young	IF(S9=0;0;S7)			

	Parameter	Function	Detail	Unit	Source
S10	Exposure per serving with prevalence-Adults	IF(S9=0;0;S8)			
S11	Re-Boiling	RiskDiscrete({0;1};{0,412;0,588})	Fraction re-boiling		Consumer survey, this paper
S12	Re-Boiling effect-Young	IF(S11=0;S9;0)	If re-boiling is occurring, complete elimination of *Salmonella* in milk		Kamana et al. (2015)
S13	Re-Boiling effect-Adults	IF(S11=0;S10;0)	If re-boiling is occurring, complete elimination of *Salmonella* in milk		Kamana et al. (2015)
S14	Dose-response *Salmonella*	RiskUniform (10^5-10^{10})		CFU/serving	Blaser and Newman 1989
S15	Dose-response *Salmonella*	RiskUniform(10-10^6)		CFU/serving	Oscar, 2004
S16	Dose-response *Salmonella*	RiskUniform(0-10^3)		CFU/serving	Blaser et al.,1982
S17	Risk on illness per serving Young-Dose-response 1	RiskOutput("risk on illness per serving ")+S9/S14*S12			
S18	Risk on illness per serving - Young-Dose- response 2	RiskOutput("risk on illness per serving ")+S9/S15*S12			
S19	Risk on illness per serving - Young-Dose- response 3	RiskOutput("risk on illness per serving ")+S9/S16*S12			
S20	Risk on illness per serving Adults -Dose response 1	RiskOutput("risk on illness per serving")+S10/14*S13			

	Parameter	Function	Detail	Unit	Source
S21	Risk on illness per serving -Adults-Dose-response 2	RiskOutput("risk on illness per serving")+S11/15*S13			
S22	Risk on illness per serving Adults-Dose-response 3	RiskOutput("risk on illness per serving")+S12/16*S13			
S23	Risk on illness per year Young- Dose-response 1	RiskOutput("risk on consumption per year young ")+S17*S3			
S24	Risk on illness per year Young-Dose-response 2	RiskOutput("risk on consumption per year young ")+S18*S3			
S25	Risk on illness per year Young-Dose-response 3	RiskOutput("risk on consumption per year young")+S19*S3			
S26	Risk on illness per year Adults -Dose response 1	RiskOutput("risk illness per year adults")+S20*S4			
S27	Risk on illness per year Adults-Dose-response 2	RiskOutput("risk illness per year adults")+S21*S4			
S28	Risk on illness per year Young-Dose-response 3	RiskOutput("risk illness per year adults")+S22*S4			

Table 5.4 : Inputs, applied functions, units, sources of information and defined outputs applied for the exposure model for *Salmonella* in Gouda cheese taking into account the situation before and after the interventions by the government to improve hygiene and knowledge in small scale cheese making companies.

	Parameter	Function	Detail	Unit	Source
S1	Prevalence in final product (scenario1 : small cheese plants, milk shops before interventions)	RiskDiscrete({0;1};{0,788;0,212})	Fractions of positive samples	Presence in 25 g	Kamana et al, 2014 (Table 1)
S2	Prevalence in final product (scenario 2: small cheese plants, supermarkets after interventions)	RiskDiscrete({0;1};{0,984;0,16})	Fractions of positive samples	Presence in 25 g	Data collection 2014 (Table 5.1)
S3	Concentration of *Salmonella*	RiskPert (0,1; 5; 100)	Min = 0,1 Most likely = 5 Max = 100	CFU/g	Assumption
		Consumption Data			
S4	Consumption frequency-Young	RiskDiscrete({Frequencies per year};{fractions of young consumers})	a1=12,a2=24,a3=52,a4=104,a5=365,a6=730, b1=0,10,b2=0,16,b3=0,26, b4=0,17,b5=0,10, b6=0,05,b7=0	Times/year	Consumer survey, this paper
S5	Consumption frequency-Adults	RiskDiscrete({Frequencies per year};{fractions of adult consumers})	a1=12,a2=24,a3=52,a4=104,a5=365,a6=730, b1=0,55,b2=0,48,b3=0,138,b4=0,27,b5=0,13, b6=0,05,b7=0	Times/Year	Consumer survey, this paper
S6	Consumption quantity-Young	RiskDiscrete({quantities};{Fractions of young consumers})	a1=250,a2=125,a3=0,625, b1=0,01,b2=0,03,b3=0,080	G	Consumer survey, this paper
S7	Consumption quantity-Adults	RiskDiscrete({quantiries};{fractions of adult consumers})	a1=0,250,a2=0,125,a3=0,625 b1=0,15,b2=0,22,b3=0,249	G	Consumer survey, this paper
S8	Exposure per serving-Young (without prevalence)	RiskOutput("exposure per serving young")+S3*S6			

	Parameter	Function	Detail	Unit	Source
S9	Exposure per serving-Adults (without prevalence)	RiskOutput("exposure per serving adult")+S3*S7			
S10	Exposure per serving with prevalence-Young-Scenario1	IF(S1=0;0;S8)			
S11	Exposure per serving with prevalence-Young-Scenario 2	IF(S2=0;0;S8)			
S12	Exposure per serving with prevalence-Adults-Scenario 1	IF(S1=0;0;S9)			
S13	Exposure per serving with prevalence-Adults-Scenario-2	IF(S2=0;0;S9)			
S14	Dose-response *Salmonella*	RiskUniform (10^5-10^{10})		CFU/serving	Blaser and Newman 1989
S15	Dose-response *Salmonella*	RiskUniform(10-10^6)		CFU/serving	Oscar, 2004
S16	Dose-response *Salmonella*	RiskUniform(0-10^3)		CFU/serving	Blaser et al.,1982
S17	Risk on illness per serving Young-Scenario 1-Dose-response 1	RiskOutput("risk on illness per consumption sc1 young-dose response Bleser and Newman")+S10/S14			
S18	Risk on illness per serving -Scenario 1-Young-Dose- response 2	RiskOutput("risk on illness per consumption sc1 young-dose response Oscar")+S10/S15			
S19	Risk on illness per serving -Scenario 1-Young-Dose- response 3	RiskOutput("risk on illness per consumption sc1 young dose response Blaser")+S10/S16			
S20	Risk on illness per serving Young-Scenario 2-Dose-response 1	RiskOutput("risk on illness per consumption sc2 young dose response Blaser and Newman")+S11/S14			
S21	Risk on illness per serving -Scenario 2-Young-Dose- response 2	RiskOutput("risk on illness per consumption sc2 young dose response Oscar")+S11/S15			

	Parameter	Function	Detail	Unit	Source
S22	Risk on illness per serving - Scenario 2-Young-Dose- response 3	RiskOutput("risk on illness per consumption sc2 young dose response Blaser")+S11/S16			
S23	Risk on illness per serving Scenario1-Adults -Dose response 1	RiskOutput("risk on illness per consumption sc1 adults dose response Blaser and Newman")+S12/S14			
S24	Risk on illness per serving Scenario 1-Adults-Dose-response 2	RiskOutput("risk on illness per consumption sc1 adults dose response Oscar")+S12/S15			
S25	Risk on illness per serving Scenario 1-Adults-Dose-response 3	RiskOutput("risk on illness per consumption sc1 adults dose response Blaser")+S12/S16			
S26	Risk on illness per serving Scenario2-Adults -Dose response 1	RiskOutput("risk on illness per consumption sc2 adults dose response Blaser and Newman")+S13/S14			
S27	Risk on illness per serving Scenario 2-Adults-Dose-response 2	RiskOutput("risk on illness per consumption sc2 adults dose response Oscar")+S13/S15			
S28	Risk on illness per serving Scenario 2-Adults-Dose-response 3	RiskOutput("risk on illness per consumption sc2 adults dose response Blaser")+S13/S16			
S29	Risk on illness per year Scenario 1-Young- Dose-response 1	RiskOutput("risk on illness per consumption per year sc1 young dose response Blaser and Newman")+S17*S10			
S30	Risk on illness per year Scenario 1 -Young-Dose- response 2	RiskOutput("risk on illness per consumption per year sc1 young-dose response Oscar")+S18*S4			
S31	Risk on illness per year Scenario 1-Young-Dose- response 3	RiskOutput("risk on illness per consumption per year sc1 young dose response Blaser")+S19*S4			
S32	Risk on illness per year Scenario 2-Young- Dose-response 1	RiskOutput("risk on illness per consumption per year sc2 young dose response Blaser and Newman")+S20*S4			

	Parameter	Function	Detail	Unit	Source
S33	Risk on illness per year Scenario 2 -Young-Dose- response 2	RiskOutput("risk on illness per consumption per year sc2 young dose response Oscar")+S21*S4			
S34	Risk on illness per year Scenario 2-Young-Dose- response 3	RiskOutput("risk on illness per consumption per year sc2 young dose response Blaser")+S22*S4			
S35	Risk on illness per year Scenario-1Adults -Dose response 1	RiskOutput("risk on illness per consumption per year sc1 adults dose response Blaser and Newman")+S23*S5			
S36	Risk on illness per year Scenario 1-Adults-Dose-response 2	RiskOutput("risk on illness per consumption per year sc1 adults dose response Oscar")+S24*S5			
S37	Risk on illness per year Scenario-1Young-Dose- response 3	RiskOutput("risk on illness per consumption per year sc1 adults dose response Blaser")+S25*S5			
S38	Risk on illness per year Scenario-2Adults -Dose response 1	RiskOutput("risk on illness per consumption per year sc2 adults dose response Blaser and Newman")+S26*S5			
S39	Risk on illness per year Scenario 2-Adults-Dose-response 2	RiskOutput("risk on illness per consumption per year sc2 adults dose response Oscar")+S27*S5			
S40	Risk on illness per year Scenario-2Adults-Dose- response 3	RiskOutput("risk on illness per consumption per year sc2 adults dose response Blaser")+S28*S5			

5.4. RESULTS

5.4.1. Consumption and consumer behavior of respondents

5.4.1.1. Boiled milk consumption in milk shops and households

In milk shops, majority of consumers of boiled milk were men, and predominant age observed was comprised between 19 and 29 (Table 5. 5). Two volumes of milk namely 400 and 500 mL were sold, and the glass of 400 mL was the most consumed. The most indicated frequency of consumption was daily. In case milk is purchased for home consumption, it is consumed mostly by female with the age ranging from 19 to 29 (Table 5.6). Statically significant differences were noted among the young and adults both in frequency of consumption and consumption volume (p= 0.001). The volume of 250 mL was the most consumed and a daily frequency of consumption was the most indicated. Milk is transported from milk shops to home by mostly stainless steel cans, for mainly short distances comprised between 100 and 500 meters (Table 5. 6). The majority of household don't refrigerate milk after purchase but, 66.7% of milk buyers boil it before consumption, using fire wood in most of cases.

Table 5.5 : Milk consumption in milk shops based on data collection in Musanze region in Rwanda (mode indicated in bold)

	Number of respondents (individual respondents) : 54						
Gender	*Female* 20.3% (11/54)				***Male*** **79.7%** **(43/54)**		
Age (Years)	*0-14* 9.2 % (5/54)		*15-18* 14.8% (8/54)	***19-29*** **51.8%** **(28/54)**	*30-65* 22.2% (12/54)	*>65* 1.8% (1/54)	
Volume (mL)		***400*** **61.2%** **(33/54)**		*500* 38.8% (21/54)			
Frequency	*Monthly* 3.7% (3/54)	*Bi-monthly* 9.3% (5/54)	*Weekly* 9.3% (5/54)	*2-3 times a week* 9.3% (5/54)	*4-6 times a week* 24.0% (13/54)	***Daily*** **33.3%** **(18/54)**	*2-times daily* 9.3% (5/54)

Table 5 .6: Consumer handling practices and milk consumption in households based on data collection in Musanze region in Rwanda (Modes indicated in bold)

	Number of Respondents (families) : 36 **Individual respondents : 136**			
Transport mode	*Plastic container*	*Stainless steel can*	*Other*	
	47.2 % (17/36)	**52.8 %** **(19/36)**	0% (0/36)	
Distance covered (meters)	*<100*	*100-500*	*600-1000*	*>1000*
	11.1% (4/36)	**80.5%** **(29/36)**	8.4% (3/36)	0% (0/36)
Refrigeration at home	*Refrigeration*	*Absence of refrigeration*		
	33.3% (12/36)	**66.7%** **(24/36)**		
Boiling at home	*Boiling*	*Absence of boiling*		
	66.7% **(24/36)**	33.3% (12/36)		
Way of boiling	*Electricity*	*Fire wood*	*Charcoal*	
	12.5% (3/24)	**58.3%** **(14/24)**	29.2% (7/24)	
Milk mixed with other foods	*Mixed*	*Not mixed*		
	33.3% (12/36)	**66.7%** **(24/36)**		
Foods mixed with milk	*Tea*	*Corn flakes*	*Other foods*	
	75% **(9/12)**	25% (3/12)	0% (0/12)	

				Number of respondets (Families) :36 **Individual respondents : 136**									
Gender		*Male*		*Female*									
		46.3 % (63/136)		**53.7%** **(73/136)**									
Age (Years)	*0-14*		*15-18*	*19-29*	*30-65*	*>65*							
	37.5 % (51/136)		13.2% (18/136)	**32.3%** **(44/136)**	16.2 (22/136)	0.8 (1/136)							
Volumes (mL)	*750*	*500*	*475*	*450*	*400*	*380*	*350*	*320*	*300*	*250*	*200*	*125*	*100*
	1.5% (2/136)	4.4% (6/136)	11.0% (15/136)	8.8% (12/136)	5.9 (8/136)	5.9% (8/136)	4.4% (6/136)	11.0% (15/136	5.9 (8/136)	**15.4%** **(21/136)**	11% (15/136)	5.1% (7/136)	5.1% (7/136)
Frequency	*Monthly*	*Bi-monthly*		*Weekly*	*2-3 times a week*		*4-6 times a week*		*Daily*	*2-times daily*			
	0% (0/136)	3.7% (5/136)		8.0% (11/136)	10.3% (14/136)		11.8% (16/136)		**62.5%** **(85/136)**	3.7% (7/136)			

Given that the majority of consumers purchase milk daily, it was assumed that milk is bought just before consumption and the storage time was negligible. In most of cases milk is consumed without being mixed with other foods like tea and weaning foods.

5.4.2. Cheese consumption

Cheese is consumed in households in three different portions, and the portion of 62.5 g, is the most consumed (Table 5.7). Cheese consumers who are mostly women, and who are in the category of 19-29 years, consume it on a weekly basis. Refrigeration is not in place in the half of cases, and cheese is consumed mostly without being mixed with other foods e.g. pasta. Statistical significant difference was observed between the young (0-14 years) and the rest of consumers (> 14 years) (p= 0.034), and no statistical significant difference was observed between men and woman (p= 0.725).

5.5. Exposure assessment and risk on illness by consumption of milk and cheese in Musanze region in Rwanda

The results on risk on illness per serving and per year by boiled milk consumption directly in milk shops are presented in Table 5.8. Depending on the dose-response used, the risk on illness per serving is comprised between 0.000 % and 11.080%; the risk on illness per year is between 0.004% and 11.407% . In households, the risk on illness per serving in the young category of consumers is ranging between 0.03% and 4.75%, and once calculated for a year, it is comprised between 2.32% and 4.75% (Table 5.9). For the adult consumers, the risk on illness per serving is ranging between 0.04% and 4.75%, and is calculated between 2.47% and 4.75% for a year (Table 5.8). For cheese consumption, the risk on illness per serving and per year for both adults and young was reduced after 2012 (Table 5. 10). Taking into account the dose-response data of 10-10^6 CFU/serving (Oscar, 2004), this risk on illness per year dropped from 2.754% to 1.732% in young consumers age (0-14 years) and from 1.570% to 1.120% in adult consumers (>14 years).

Table 5. 7 : Cheese consumption data, obtained from interviews with cheese buyers in supermarkets in the Musanze region of Rwanda (Modes indicated in bold)

	Number of respondents (families) :90						
	Number of individual consumers : 370						
Gender	*Male*			*Female*			
	38.9 % (144/370)			**61.1% (226/370)**			
Age (Years)	*0-14*	*15-18*	*19-29*	*30-65*	*>65*		
	22.7% (84/370)	16.2% (60/370)	**38.6% (143/370)**	18.9% (70/370)	3.5% (13/370)		
Portion size	*250 gr*	*125gr*	*62.5gr*				
	2.4% (9/370)	17.6% (65/370)	**80% (298/370)**				
Frequency	*Monthly*	*Bi-monthly*	*Weekly*	*2-3 times a week*	*4-6 times a week*	*Daily*	*2-times daily*
	21.6% (80/370)	23.5% (87/370)	**38.1% (141/370)**	13.2% (49/370)	3.2% (12/370)	0.3% (1/370)	0.0% (0/370)
Refrigeration at home	*Refrigerated*			*Not refrigerated*			
	47.5% (176/370)			**52.5% (194/370)**			
Mixed with other foods	*Mixed*			*Not mixed*			
	1.3% (5/370)			**98.7% (365/370)**			

Table 5.8 : Risk on illness per serving and per year (including the distribution of frequency of consumption) by consumption of boiled milk directly in milk shops in Musanze region in Rwanda.

	Fraction of population		
	Dose-response 10^5-10^{10}CFU/serving (Blaser and Newman 1989)	**Dose-response 10-10^6CFU/serving (Oscar 2004)**	**Dose-response 1-10^3 CFU/serving (Blaser et al.,1982)**
Risk on illness per serving	0.00%	0.10%	11.08%
Risk on illness per year	0.004%	8.40%	11.40%

Table 5.9 : Risk on illness of Salmonella in households by consumption of boiled milk, purchased in milk shops and in % of the households re-boiled before consumption

	Fraction of population		
	Dose-response 10^5-10^{10} CFU/serving (Blaser and Newman 1989)	**Dose-response 10-10^6CFU/serving (Oscar 2004)**	**Dose-response 1-10^3 CFU/serving (Blaser et al.,1982)**
Risk on illness per serving-YOUNG	0.03%	4.27%	4.75%
Risk on illness per year-YOUNG	2.32%	4.98%	4.75%
Risk on illness per serving -ADULTS	0.04%	4.35%	4.75%
Risk on illness per year-ADULTS	2.47%	4.69%	4.75%

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Table 5.10 : Risk on illness for *Salmonella* due to cheese consumption, purchased in Musanze region in Rwanda, before 2012 in supermarkets and milk shops, after 2012 (intervention of Rwandan government) only restricted in supermarkets to re-control the informal market of Gouda cheese

	Fraction of population exposed		
Dose-response	**10^5-10^{10} CFU/serving (Blaser and Newman 1989)**	**10-10^6CFU/serving (Oscar 2004)**	**1-10^3 CFU/serving (Blaser et al.,1982)**
Risk on illness per serving-YOUNG ***(Before 2012)***	0.000%	0.024%	16.078%
Risk on illness per serving-YOUNG ***(After 2012)***	0.000%	0.016%	10.608%
Risk on illness per serving-ADULTS ***(Before 2012)***	0.000%	0.028%	16.334%
Risk on illness per serving-ADULTS ***(After 2012)***	0.000%	0.018%	10.806%
Risk on illness per year-YOUNG ***(Before 2012)***	0.002%	2.754%	21.156%
Risk on illness per year-YOUNG ***(After 2012)***	0.000%	1.732%	13.946%
Risk on illness per year-ADULTS ***(Before 2012)***	0.000%	1.570%	21.094%
Risk on illness per year-ADULTS ***(After 2012)***	0.000%	1.120%	13.932%

5.6 DISCUSSION

Given the location of the milk shops involved in this study i.e. the center of the town of Musanze, the majority of consumers are young people, present on a daily basis in the town for occupational reasons like commerce and transport. The predominance of male consumers might be linked with the Rwandan culture, in which ladies are less subjected to habits of eating and drinking in public. However, for home consumption both genders are represented almost equally. It appears that milk is purchased by neighboring retail points, covering short distances every day, to buy milk for direct consumption. Most of Rwandese believe that milk is a "pure" drink, not to be mixed with other foods, and in case it is mixed, it is mostly for making tea. It was observed that milk is transported in cleaned plastic gallons or small stainless steel containers, well cleaned as it is a habit in the Rwandan culture. Based also on those short distances generally not exceeding 500 metres, covered in a time around 5 minutes, the growth of *Salmonella* was assumed to be negligible, given that in a fluctuating temperature comprised between 10-30°C, the lag phase of *Salmonella* takes more than 5 hours (Bovill et al., 2000), and the daily consumption frequency observed led us to assume that milk is bought just before consumption. For the absence of cross-contamination in household (for both cheese and milk), the best case of sufficient level of hygiene was considered, based on the fact that the inhabitants of towns are more aware of basic hygienic food handling practices than rural citizens. The statistically significant differences observed among children and adults in frequency and volumes for milk, might be attributed to the higher frequency of consumption by the young and their lower volumes consumed. Cheese is consumed less frequently than milk, in families. The observed differences in the age categories are linked with the consumed portions and the frequency of consumption for cheese. A highlighted earlier no statistical significant difference was observed among the genders (p=0.725).

The inhabitants of wide Musanze district were estimated at 377 904 in 2013 (Republic of Rwanda, 2014). According to the World Bank (2015), the whole Rwandan urban population was 27% in 2013, what leads to an estimate of 102 034 inhabitants of Musanze town, including all the age categories.

The informal market being large with different sources of milk like mobile traders and direct purchase of milk from farms neighboring the town, an assumption of 25% based on personal observations can be made to have an idea of the number of inhabitants who purchase milk in milk shops. By taking this in consideration, 25 508 people buy milk in milk shops and are represented by the 190 milk consumers interviewed in this study (Figure 5.2). Thus, considering that 54 of them (28.4%) consume milk directly in milk shops and the rest (namely 136 consumers in households, who are 71.6%), 7 244 persons consume milk in milk shop directly, and 18 264 are consumers in households. For cheese consumption, the number of consumers from supermarkets can be stimated to 2% of the wide district inhabitants (7 558 consumers represented by the 370 interviewees in this study, with 1 716 young and 5 842 adults, based on percentages presented in Table 5.7) taking into account the personal observation that cheese is consumed by people with high incomes, who are estimated at this percentage (Republic of Rwanda, 2014).

This information gives a baseline for quantification of probable cases of salmonellosis resulting from boiled milk, purchased in informal milk shops, and cheese, purchased in various retail shops before 2012 and in supermarkets after 2012; all thus consumption in Musanze town. As example, taking into account the most recent dose-response model available (Oscar, 2004), 7 persons among 7 244 people are at risk of getting infected at serving, and 608 are infected in one year by the consumption of boiled milk directly in the milk shops (mostly adult men, with age 19-29 years), considering the risk encountered presented in Table 5.8. For milk consumption in households and the risk of illness by *Salmonella*, 535 adults and 341 children are presumed to be infected in one year from boiled milk purchased at milk shops, reference made to obtained risk calculations in Table 5.9. For cheese consumption, the risk for the adults is lower than for milk (Table 10). This might be attributed the lower consumption frequency. In that case, 19 adults and 29 children are likely to get infected per year, in the current situation, after 2012. After continuous reports on illnesses incriminating cheeses, as confirmed by the competent authority namely the Rwanda Bureau of Standards, a ban was put on all cheese in 2013. All cheese makers received trainings on good hygienic practices before official authorization of activities, and henceforth the commercialization of cheese is no longer allowed in milk shops.

The interventions on behalf of the government and its partners led to improved cheese quality and safety (LANDOLAKES, 2015). Before the interventions, the risk was indeed higher, being 27 infections in adults and 29 in children in a year. This is indeed a best case, seeing that before the interventions more people were consuming cheese from the informal market. This means that the interventions on behalf of the government and its development partners allowed to reduce the risk of illness by 30% in adults and 38% in children. Thus, it appears clearly that the commercialization of cheese in the formal market led to tangible results. Meanwhile, there is still a gap in milk shops, which are operating under insufficient hygienic conditions. It would have been more effective to conduct, in the same time, food safety interventions in the milk shops, since the situation is more critical than in cheese. A previous study by Kamana et al. (2015) indicated that the compliance with good hygienic practices in milk shops was 23.0%. Despite the efforts on behalf of the government which suppressed milk kiosks previously present in open areas of the towns and cities (personal observations), the official registration of milk shops was conducted mainly for tax collections, and this should go in parallel with hygiene and safety of consumers. The same situation prevails also in Mali, where milk shops were identified as less sustainable structures hampering the implementation of good hygienic practices (Bonfoh et al., 2003). The inspections on behalf of the environmental health officers from the Ministry of Health at this level should be reinforced. To our knowledge, no previous studies were performed on the exposure assessment on *Salmonella* in boiled milk. *S. aureus*, *E. coli* and *Enterococcus* spp. were estimated to cause illnesses in 652 raw milk consumers in Ivory Coast on a daily basis (Kouame-Sina et al., 2012). In Ethiopia, Makita et al. (2012) estimated the annual incidence of staphylococcal poisoning at 20.0% per 1000 people consuming informally marketed home-made yoghurt. Kilango et al. (2012) estimated the probability of purchasing milk contaminated with *S. aureus* at 22.7% with 217 people likely to purchase contaminated boiled milk informally marketed in Tanzania. This situation emphasizes on the necessity of implementation of good hygienic practices in the African informal dairy chains, since it is the most predominant, thus involving high number of consumers (Fokou et al., 2010; Omore et al., 2001; Sinja et al., 2006).

5.7 CONCLUSION

This study aimed to calculate the risk of salmonellosis faced by boiled milk and cheese consumers in Rwanda, exemplified by the Musanze region. Milk is consumed directly in milk shops or in households, by different categories of consumers with both genders and different ages including children, adolescents, adults and the elderly. In milk shops, milk is directly consumed after boiling, and is taken for home consumption by different consumers in households, and some of them re-boil it before consumption. Although milk is boiled in different steps of the informal chain, it does not guarantee milk and dairy products safety due to possible recontaminations. In the Rwandan case, it can be suggested that milk should be re-boiled once in households in other to mitigate the risk of infection. In milk shops, good practices especially hygiene of the personnel and cleanliness of used utensils should be enhanced. The professionalization of the cheese sector in Rwanda contributed to the mitigation of the risk, but also there still improvements could be done for safer commercialized cheeses.

However, these calculations demonstrate clearly the efforts made (or to be made) reduce the occurrence of *Salmonella* in the Rwandan dairy chain and will benefit the Rwandan population and decrease the burden of food borne disease. The present data on exposure assessment, in a country like Rwanda where there is no accurate way of reporting food-borne diseases can serve as an indicator on the actual situation on the number of infected people per year and inform the decisions makers for further interventions to be put in place for safer milk and dairy products.

ANNEXES

Annex1: Detailed results of compliance to good practices at collection, small scale cheese processing, wholesale, supermarkets and milk shop levels.

A. Milk collection centers (n = 8)

No.	Question	Yes	NO
1	Are personnel and vehicles have adequate access to the place of collection for the suitable hygienic handling of milk?		1,2,3,4,5,6,7,8
2	In particular, is access to the place of collection clear of manure, silage, etc.?		1,2,3,4,5,6,7,8
3	Does the milk hauler, prior to collection, check the individual producer's milk to ensure that the milk does not present obvious indications of spoilage and deterioration?		1,2,3,4,5,6,7,8
4	Are collection and chilling centre, designed and operated in such a manner that minimizes or prevents the contamination of milk.?	1,2,3,4,5,6,7,8	
5	Is Milk collected under hygienic conditions to avoid contamination ?	1,2,3,4,5,6,7,8	
6	In particular, does the milk hauler take samples in such a way to avoid contamination and ensure that milk has the adequate storage/in-take temperature prior to collection?		1,2,3,4,5,6,7,8
7	Does the milk hauler receive adequate training in the hygienic handling of raw milk?		1,2,3,4,5,6,7,8
8	Do milk haulers wear clean clothing?		1,2,3,4,5,6,7,8
9	Are milk hauling operations performed by persons without risk of transferring pathogens to milk?		1,2,3,4,5,6,7,8
10	Is appropriate medical follow-up done in the case of an infected worker?		1,2,3,4,5,6,7,8
11	Do milk haulers perform their duties in a hygienic manner so that their activities do not result in Contamination of milk?		1,2,3,4,5,6,7,8
12	Is the access to the clean area restricted to workers only ?		1,2,3,4,5,6,7,8
13	In case the driver clothing and footwear contaminated with manure, is there a possibility to change or clean the soiled clothes and footwear?		1,2,3,4,5,6,7,8
14	Do the drivers/milk supplier receive training on basic hygiene?		1,2,3,4,5,6,7,8
15	Is there an effective cleaning and disinfection plan?	1,2,3,4,5,6,7,8	
16	Is there a pest control plan?		1,2,3,4,5,6,7,8
17	Is the temperature controlled to prevent milk adulteration before delivery?	1,2,3,4,5,6,7,8	
18	Is there an approved maintenance and calibration plan?		1,2,3,4,5,6,7,8
19	Is there an energy source available, full time?	1,2,3,4,5,6,7,8	

B. Small cheese plants (n = 3)

No.	Question	Yes	NO	NA
1	Is there an effective cleaning and desinfection plan?		1,2,3	
2	Is there an effective pest control plan?		1,2,3	
3	Is water quality managed in a proper way?		1,2,3	
4	Is the temperature controlled in order to keep the cold chain along the Entire production process?		1,2,3	
5	Are all workers trained on FSMS?		1,2,3	
6	Is personnel hygiene checked and monitored?		1,2,3	
7	Is there an effective maintenance and calibration plan?		1,2,3	
8	Is there a waste management plan?		1,2,3	
9	Is any milk control carried out prior to processing?		1,2,3	
10	Is there a traceability /recall plan?		1,2,3	
11	Are measures taken to avoid physical and chemical contaminations?		1,2,3	
12	Is there a precised work methodology?		1,2,3	
13	Is HACCP implemented?		1,2,3	
14	Does the plant have any certification?		1,2,3	
15	Is there a person (or group of persons) in charge of quality management?		1,2,3	
16	Are the products enough known and specified?		1,2,3	
17	Is the intended use of products taken in account?		1,2,3	
18	Are flow diagrams available for all products?		1,2,3	
19	Are flowdiagrams followed as they are set up?		1,2,3	
20	Are all dangers identified and documented?		1,2,3	
21	Are CCPs known and documented?		1,2,3	
22	Are critical limits for each CCP available?		1,2,3	
23	Is there a monitoring system for each CCP?		1,2,3	
24	Is there a corrective action plan?		1,2,3	
25	Are procedures of verification and validation available?		1,2,3	
26	Are documentation and record keeping carried out?		1,2,3	
27	Are products stored in a manner that avoids contamination and adulteration?		1,2,3	

C. Wholesale markets (n = 2)

No.	Question	Yes	NO
1	Is there an effective cleaning and disinfection plan?		1,2
2	Is there an effective pest control plan?	1,2	
3	Is the temperature controlled in order to keep the cold chain along the entire production process?	1,2,	
4	Are all workers trained on FSMS?		1,2
5	Do workers have facilities to achieve requirements in hygiene?	1,2	
6	Is there an effective maintenance and calibration plan?		1,2
7	Is there a traceability /recall plan?	1,2	
8	Is there a precised work methodology?	1,2	
9	Are the premises designed in a manner that minimizes contamination?		1,2

D. Supermarkets (n = 6)

No.	Question	Yes	NO
1	Is there an effective cleaning and desinfecrtion plan?	1,2,3,4,5,6	
2	Is there an effective pest control plan?	1,2,3,4,5,6	
3	Is the temperature controlled in order to keep the cold chain along the Entire production process?	1,2,3,4,5,6	
4	Are all workers trained on FSMS?		1,2,3,4,5,6
5	Do workers have facilities to achieve requirements in hygiene?	1,2,3,4,5,6	
6	Is there an effective maintenance and calibration plan?		1,2,3,4,5,6
7	Is there a traceability /recall plan?		1,2,3,4,5,6
8	Is there a precized work methodology?	1,2,3,4,5,6	

E. Milk shops (n = 6)

No.	Question	Yes	NO
1	Is there an effective cleaning and disinfection plan?		1,2,3,4,5,6
2	Is there an effective pest control plan?		1,2,3,4,5,6
3	Is the temperature controlled in order to keep the cold chain ?	1,2,3,4,5,6	
4	Are all workers trained on FSMS?		1,2,3,4,5,6
5	Do workers have facilities to achieve requirements in hygiene?		1,2,3,4,5,6
6	Is there an effective maintenance and calibration plan?		1,2,3,4,5,6
7	Is there a waste management plan?		1,2,3,4,5,6
8	Is any milk control carried out prior to processing?	1,2,3,4,5,6	
9	Is there a traceability /recall plan?		
10	Are measures taken to avoid physical and chemical contaminations?	1,2,3,4,5,6	
11	Is there a precised work methodology?		1,2,3,4,5,6
12	Is personnel hygiene checked?		1,2,3,4,5,6
13	Is water quality checked?		1,2,3,4,5,6

Annex 2 : Questionnaire on cheese consumption and consumer behaviour

College of Agriculture, Animal Science and Veterinary Medicine

Department of Animal Production

Faculty of Bioscience Engineering

Department of Food Safety and Food Quality

QUESTIONNAIRE ON CHEESE CONSUMPTION

- Filled by :
- Date :
- Supermarket :

Section 1. : Consumers*

1.1. Age : **a**. 0-14 years (Children) **b**.15-18 years (Adolescents) **c**.19-29 years (Adult Group 1) **d**.30-65 years (Adults Group 2) **e**.> 70 years (Elderly)

1.2. Gender: **a.** Male **b.** Female

1.3. Type of portion : **a.** 1/4 (250gr) **b.** 1/8 (125gr) **. c** 1/16 (62.5gr)

1.4. Frequency : **a**. Once a month **b**. Once in two weeks **c**. Weekly **d.** 2-3 times a week **e.** 4-6 times a week **f.** Daily **g.** Twice daily

*** : LEXIQUE FOR SECTION 2.**

Section 2. Home consumption

4.1. Home consumers, quantity and frequency :

Age category(years)		Gender	Number	Portion			Frequency						
				a	b	c	Once a month	Once in 2 weeks	Once a week	2-3 times a week	4-6 times a week	Daily	2 times daily
A	0-14												
B	15-18												
C	19-29												
D	30-65												
E	>65												

4.5. Is cheese refrigerated once home? : **a.** Yes **b**. No

4.3. Way of consumption : **a**. Without mixing with foods **b.** Mixed with foods (if b. go to 4.4.)

4.4. Other foods : **a**. Pasta **b**. Other to specify...

Annex 3 : Questionnaire for milk consumption and consumer behaviour

College of Agriculture, Animal Science and Veterinary Medicine

Department of Animal Production

Faculty of Bioscience Engineering

Department of Food Safety and Food Quality

QUESTIONNAIRE ON MILK CONSUMPTION IN MILK SHOPS

- Filled by :
- Date :
- Milk shop :

Section 1. : Consumers in MS

1.1. Age : **a**. 0-14 years (Children) **b**.15-18 years (Adolescents) **c**.19-29 years (Adult Group 1) **d**.30-65 years (Adults Group 2) **e**.> 70 years (Elderly)

1.2. Gender: **a.** Male **b.** Female

1.3. Type of glass : **a.** Glass 1 (50cl) **b.** Glass 2 (40 cl)

1.4. Frequency : **a**. Once a month **b**. Once in two weeks **c**. Weekly **d.** 2-3 times a week **e.** 4-6 times a week **f.** Daily **g.** Twice daily

Section 2. Home consumption

4.1. Home consumers, quantity and frequency :

Age category(years)		Gender	Number	Glass/ Cup (1)			Frequency						
				a	b	c	Once a month	Once in 2 weeks	Once a week	2-3 times a week	4-6 times a week	Daily	2 times daily
A	0-14												
B	15-18												
C	19-29												
D	30-65												
E	>65												

(1) : specify the type of cup and measure the content

4.2. Way of transport from MS to home : **a**. in plastic container **b**. in inox can **c**.Other(Specify)..

4.3. Distance covered from MS to home **a.** <100m **b**.100-500m **c**.600m-1km **d.** >1 km

4.4. Is milk refrigerated once home? : **a.** Yes **b**. No

4.5. Time from MS to home : **a** <5 min **b**.5-30 min **c**.> 30 min

4.6. Way of consumption : **a**. Without boiling **b.** After boiling (if b, go to 4.4.)

4.7. Way of boiling : **a**. Electricity **b**. Fire wood **c.** Charcoal

4.8. Is milk mixed with other foods? **a**. Yes (If yes, go to 4.6) **b**. No

4.9. Foods mixed with milk : **a**. Tea b. Corn flakes **c.** Other to specify...

CHAPTER 6 : General discussion

CHAPTER 6 : General discussion

In developing countries, efforts have been concentrated in milk production increase for food security purposes (Bonfoh et al., 2006; Rhone et al., 2008; Sraïri et al., 2006). It has been noticed that several development programs put in place prioritize the yield increase by means of animal health improvement, pasture management, genetic improvement, etc. In Rwanda, the result of those interventions carried out for decades are highlighted by poverty alleviation and malnutrition decrease (Rawlins et al., 2014). The identified programs, listed in Table 2 (Chapter 1) contributed significantly to food security and improved the livelihoods of Rwandans. The Rwandan livestock sector is one of the pillars which led to the spectacular achievement in 2005-2010 with approximately one million people moved up the poverty line (World Bank, 2012). In order to make those tangible results more sustainable, supplementary programs regarding food safety should be put in place, seeing that food safety is the ultimate goal of food security. Globally, the focus is nowadays shifting to food safety (Unnevehr, 2015), and as a recent example the theme of this year's world health day was "food safety" (WHO, 2015). The presented research in this PhD, on the safety and hygiene status of the Rwandan milk and dairy chain can substantially contribute to this necessity of shifting from food security to food safety in the interventions carried out in the Rwandan milk and dairy chain, by keeping sustainable the available achievements and re-orient the upcoming ones. Indeed the efforts to reach the targeted goals of transforming the agriculture sector by increasing production and make it market oriented by 2020 (as indicated in Chapter 1) should be continued, but it is time to start focusing on food safety along the chain. The national dairy strategy (MINAGRI, 2013) highlighted the fact that the production is expected to be increasing to reach 810 million Liters by 2017.

6.1 Structure of the milk and dairy chain and role of informal market

As presented in Chapter 1, the Rwandan milk and dairy chain is characterized by two routes, namely the formal market and the informal one. The informal market, which takes the lion's share to the detriment of the formal one, finds its origin in the **lack of processing facilities** in Rwanda (Karenzi et al., 2013; LANDOLAKES, 2015b; MINAGRI, 2005). Various reasons lead to this situation.

Based on examples for the four milk basins involved in this study, it appears that in the **Umutara milk basin** (Figure 1.2), the farms are scattered in the savannah region, with less rain than the rest of the country. Despite the government's efforts to abandon the open-grazing system, it still persists in this region due to empirical beliefs that cows should be raised without confinement and graze in a "natural" pasture for good quality milk. This milk basin is very large with an area estimated to 4 225 km^2 (MINAGRI, 2008) and the scattered localization of farms contributes to the introduction of milk in informal vending circuits. Only one dairy plant is present for this whole region, with a processing capacity estimated at 5 000 liters/ day in 2008, which especially shows that there is a huge need in processing facilities, since the daily milk production was approximately 79 000 liters in 2008 (MINAGRI, 2008).

Meanwhile, the recent reorganization of this dairy plant with an intention to double its processing capacity is a good initiative but there is still room for other initiatives in milk processing. The installed milk collection centers contributed significantly to the chain organization, but as mentioned in chapters 2 and 3, the interconnection between the two routes (formal and informal) is troubling and this appears typically in all basins, where milk is regularly supplied to collection centers and is purchased from there by vendors who resell it informally in most of cases. Especially for Umutara milk basin, informal resellers from Kigali city prefer to purchase milk from there because of the existence of flat road infrastructures (not hilly as in the other regions of the country) making easy the transport to Kigali city, the capital and the most populated city of Rwanda. The introduced stainless steel cans facilitate milk transport and contribute to hygienic requirements fulfillment as explained in Chapter 3.

In **Nyanza milk basin** where the production of milk was estimated to be 75 000 liters/ day in 2007 (MINAGRI, 2008), there is also one milk plant, the most ancient in the country, facing challenges of old fashioned equipment, causing frequent stoppages (MINAGRI, 2009). This plant processes mostly for the national program of "Inkongoro ku mwana" (literally meaning one cup per child) which is a campaign to provide milk on a daily basis to children in primary schools in order to fight malnutrition. Milk processed in this plant comes mainly from the neighboring Songa research station, a branch of the Rwanda Agriculture Board carrying out research on cattle genetic improvements. This situation contributes significantly to the predominance of the informal milk in this region.

Still, the Nyanza town is known to be a place for fermented milk (ikivuguto) informally processed, commercialized especially in the shops on the road linking Kigali to Bujumbura (Burundi), and is an automatic stop point for milk purchase.

The situation is more critical in **Gishwati milk basin**, in the North West of the country, where the 70 000 liters produced daily are not formally processed (MINAGRI, 2009). Till recently, there was no processing plant in this region, after the two large companies formerly operating in this area, namely "Laiterie de Gishwati" and " Laiterie Nkamira" were damaged during the tragic civil war in 1994.

Milk produced passes informally the border to the Democratic Republic of the Congo, and the rest is sold informally in Rubavu and Musanze towns (Karenzi et al., 2013; SNV, 2008). The abundance of cheese processing plants in this region finds its origin in this, since cheese is locally processed as an alternative of formal milk processing.

In **Kigali milk basin**, where the zero-grazing is predominant, there is no exception to overall rule of dominance of informal market. Even though the largest dairy processing company of the country is situated in this region, it does not process all the 956 254 tons annually produced (MINAGRI, 2013). Therefore, there is also in this region a challenge of having highly producing farms but these farms not supplying to this dairy company but de facto delivering to the informal market. In addition to this, milk collected in all the other regions is supplied mostly to towns and even to Kigali city and often sold via milk shops.

Another factor, apart from enough milk processing capacity towards dairy products, that influences the predominance of the informal market is the **attractive prices** offered by mobile traders to farmers, in comparison to processors who pay less and are more demanding in terms of quality (Karenzi et al., 2013; Swai and Schoonman, 2011). Thus, farmers prefer quick and easy money from mobile traders who don't even take care of the quality of milk. For that reason, even the installed milk collection centers are not fully using their operational capacity as it was highlighted in the national dairy strategy which aims to reverse the trend (MINAGRI, 2013). In addition to that, some households prefer to buy milk directly from farmers, and are reluctant to buy milk from mobile traders who are often suspected of frauds (addition of water, adulterated milk, etc).

As presented in chapter 2 and 3 the milk is then further distributed to informal milk shops and cheese factories. Cheese processing was an informal activity till 2013, when interventions from the government led to its professionalization (LANDOLAKES, 2015b). Milk shops are scattered in towns and cities, and constitute a source of milk consumption by residents (Karenzi et al., 2013; Modderman, 2010; SNV, 2008). In this research it was demonstrated that both milk shops and small scale cheese factories need further professionalization and capacity building in order to be able to guarantee basic requirements in avoiding cross-contamination, post-contamination and compliance to good practices.

The government and its development partners have concentrated remarkable efforts in the modernization of the dairy chain. The implementation of more than 120 milk collection centers all over the country, was profitable to farmers who have an opportunity of market, and has a positive impact in food safety and quality since milk is chilled just after milking in collection centers (short covered distances from farms to collection centers allow quick cooling).

As presented in Chapter 3, the implementation of those centers contributed significantly but there are still efforts to be concentrated at this level concerning trainings of the personnel, maintenance of available equipment and pest control. As mentioned earlier, their operational capacity is not fully exploited in some cases. This is thus only part of the way to improve but the government in its national dairy strategy expects the implementation of a new processing plant to cover the Gishwati milk basin (MINAGRI, 2013). The encouragement of processing companies is thus now the following step. After the milk collection centers are now operating, this also achieved for farmers to be grouped in cooperatives and as such there is already a platform ready for implementation of processing companies all over the country. The government should facilitate private investors in this domain. It is in this framework that the government has facilitated an implementation of the Mukamira dairy plant which is intended to process milk from the Gishwati basin. Some other small companies have started working i.e. Kivu dairy in 2014 and The Blessed Dairies in 2012. Similar initiatives should be encouraged, and the government should promote investments in this sector in order to fully make profitable the installed collection centers (Figure 6.1.)

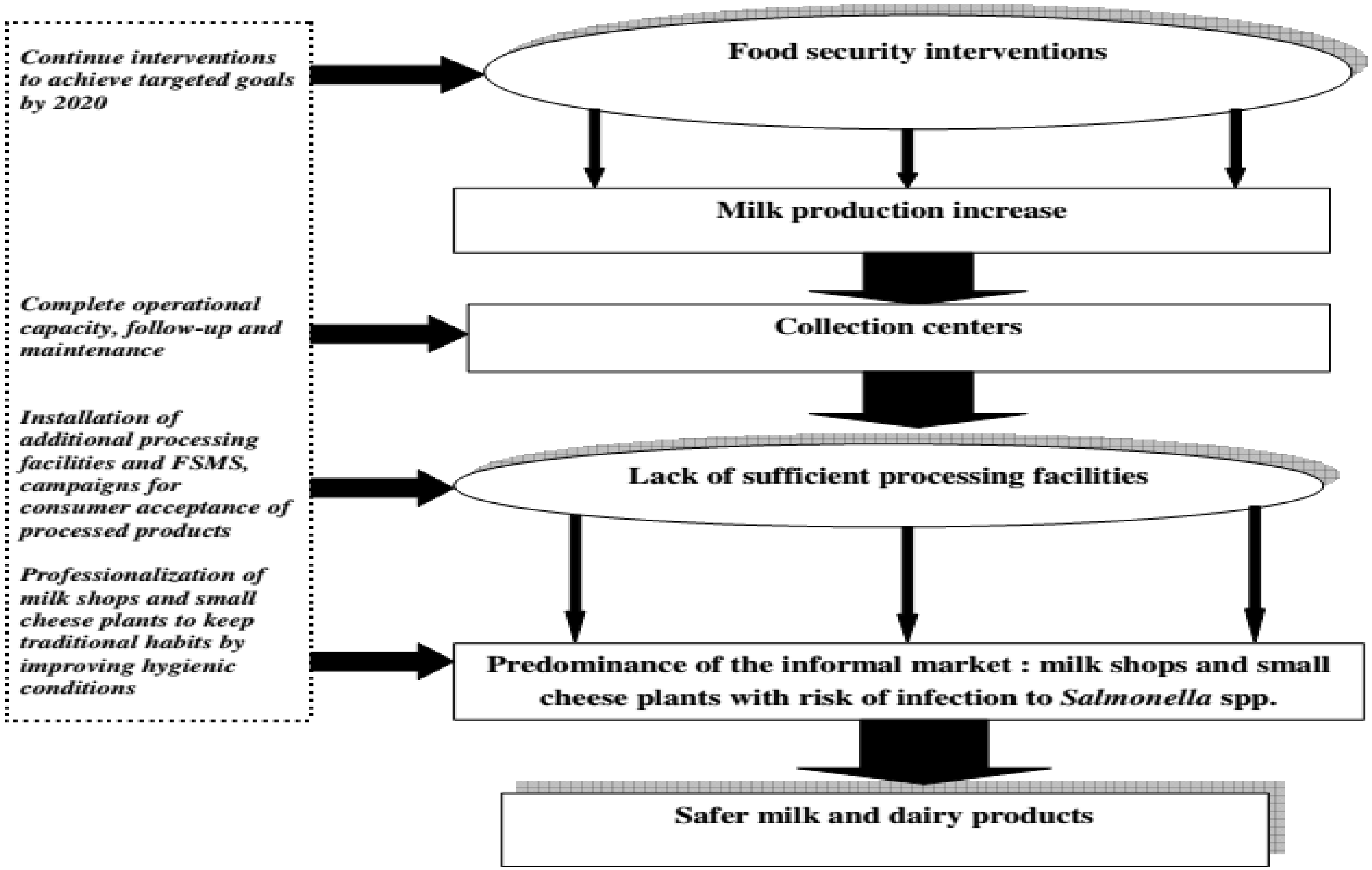

Figure 6.1. : Proposed way forward to modernize the Rwandan milk and dairy chain

Consumers are also playing an important role in the importance of the informal milk. First, many of Rwandans believe that milk is a "pure" drink, and once it is processed it loses its originality. Their beliefs are due to the fact that pasteurization denaturizes some milk volatile compounds (Varnam and Sutherland, 1994) which imply a different odor and taste, and most of consumers are reluctant to consume processed milk. This is valid for both boiled milk and fermented milk : it is commonly believed that pasteurized fermented milk is not tasty, which makes it unpopular. The effect of pasteurization and the use of imported lactic starters confer to it a different taste, and consumers are reluctant to it. A recent study by Karenzi et al. (2015), characterized "*ikivuguto*" and a starter culture from "*ikivuguto*" was set up and this should be vulgarized and enable local processors to process "*modern ikivuguto*" satisfying consumers by its improved taste. Therefore, when the government is stimulating milk processing and formalization of the milk chain, the perception and preferences by the Rwandan consumer may not be neglected. In the same time, the current campaign recently launched by the Government to encourage people to drink more milk (literarly called "*shisha wumva*" what means "*feel the goodness*") should be coupled with a sensitization for more consumption of processed products.

6. 2 Microbiological status and hygiene status along the milk and dairy chain in Rwanda

The **microbiological quality of milk at farm level** showed the impact of farming intensification and related quality improvements. By linking the observed level of hygienic parameters fulfilling and the microbiological quality, in chapter 3, it appears that the traditional farming with lack of basic infrastructures and hygienic requirement contribute to the low quality of milk in those farms. It is important to emphasize on the success of animal health monitoring at farm level, but improvements to be done in this area are still huge. The farmers' cooperatives should be used to provide trainings to farmers on basic (good) hygienic practices and the foreseen total abandonment of the open-grazing system should be achieved in due time. This governmental decision was not respected entirely, and many of open-grazing farmers were reluctant to change their traditions, but nowadays they are encouraged to modernize, as the country is facing land scarcity and is densely populated (Bigagaza et al., 2002).

The high number of **collection centers** and the introduction of **stainless steel cans** for milk transport and the short distances covered by farmers supplying milk are at the basis of the nearly acceptable microbiological quality at collection level, as shown in Chapter 2.

The MCCs complied with only 26 % of the hygienic requirements (Chapter 3). This intervention was a typical success story to be vulgarized to other developing countries where it does not exist. In case of farmers without cooling facilities, the implementation of collection centers was proven to be effective for milk quality and market purposes in different developing countries i.e. Uganda (Grillet et al., 2005), Ethiopia (Godefay and Molla, 1999), Trinidad (Adesiyun et al., 1995) and Zimbabwe (Mutukumira et al., 1996). But it also demonstrates the continuous effort in investment in training of the farmers and personnel of the MCC in hygiene, importance of temperature and time, cleaning of the cans, etc. as concluded in Chapter 3.

The implementation of HACCP and certification against ISO 22000 : 2005 in one **processing company** highlighted the importance of effective food safety management systems (FSMS) in meeting required microbiological quality requirements. The companies without installed FSMS showed direct implication of this on microbiological quality and this was confirmed by lower scores on hygienic parameters fulfillment. The FSMS implementation in developing countries implies a huge economic burden (Jirathana, 1998; Muchangos et al., 2012; Sarter et al., 2010). In the Rwandan food industry, HACCP is being progressively introduced (RSB, 2015b), and one other dairy company got its certification this year after benefiting from support on behalf of the second phase of RDCP by Land O'Lakes (Landolakes, 2015a). The rest of dairy companies are focusing on the fulfillment of the requirements for the national "S" quality mark certification, and plan for HACCP in the near future as well. The challenge will be the effectiveness of HACCP to be implemented, since it has been proven that small companies are more likely to fail in making effective HACCP (Opiyo et al., 2013; Sampers et al., 2012). Therefore, this issue should be considerably taken into account by processors and the competent authority. The measured microbiological quality of pasteurized products in Chapter 2 slightly exceeded legal limits and this indicated ineffective pasteurization and/or post-pasteurization contamination. The expected successful implementation of FSMS and included monitoring of CCPs should contribute in solving this.

Cheese processing sector and the **milk shops** were found to be the critical steps of the chain in terms of hygiene and safety.

The first findings of this study (presented in Chapter 2) provided the first reliable scientific information on microbiological quality and safety in Rwanda in 2012 and contributed in the implementation of interventions.

The interventions by the Government and its partners especially the second phase of RDCP led to tangible results. Trainings of the cheese processors on basic hygiene requirements, provision of modern equipment and introduction of other types of cheese (Cheddar, Saint Paulin, Gruyère, and Ricotta), and in the same time good hygienic practices were implemented. A national cheese exhibition was organized in May 2014 (Figure 6.2) and cheese were ranked based on microbiological quality and safety. This initiative should be maintained and inspire a benchmarking culture which can contribute in the increase of quality. In our third round of sampling, in 2014, the situation improved as now *Salmonella* was absent from all cheeses (n=36) sampled in supermarkets (Chapter 5).

In **milk shops**, the microbiological quality was found to be highly variable, given the prevailing situation with the vending environment which is not fit for safe commercialization of milk (Chapter 3 and 4). Milk shops are not exclusively designed for milk sale only, other items such as cakes, cheese (before 2013), eggs, tea, sodas, even cooked foods in some cases are also commercialized there. It is common in Rwanda to see those numerous places with a notice in local language "*Amata na fanta bikonje*" (meaning "fresh milk and soda"). The turnover of those milk shops is very high, resulting in start up and failures, reselling of businesses and also many temporarily employees, and therefore, it is very difficult to monitor them. Refrigeration also is not adequate and permanent (defect refrigerators, switching off for power saving, power cuts and lack of alternative power source), design and layout allowing easy cross-contaminations, and low level of personnel hygiene ,making this level of the chain particularly vulnerable. The government has put significant efforts so far in the case of cheese factories, more important efforts should be concentrated to the milk shops in the future, given the higher consumption frequency as demonstrated in Chapter 5. The exposure and risk assessment calculations in this work (Chapter 5) demonstrated that the risk on illness per serving was estimated to 11.08% in milk shops.

Rwanda achieved significant results in terms of environmental policy, and its capital is among the cleanest cities in Africa (Manirakiza, 2012) and this should be reflected also in the milk shops. There is a huge need to set for basic requirements for all places in which milk is commercialized.

Mozarella

Feta

Gruyère

Halloumi and Saint Paulin

Figure 6.2 : Some of the newly introduced cheeses presented in the cheese exhibition in Kigali, Rwanda (June 2014)

Therefore, milk shops should be owned by professionals and we suggest that jointly with training institutions short trainings on basic hygiene requirements should be regularly organized and attendees should receive a certificate allowing them to open a milk shop for a given period. In addition to this, the premises should be equipped with water facilities, designed in order to be easy to clean and disinfect, refrigerators should be calibrated and all workers should undergo a regular health monitoring (Figure 6.1). In the case of meat, such interventions were conducted in Rwanda and there are minimal requirements for small butcheries e.g. insect catching lamps, the design of butcheries, and health monitoring of workers. It was proven that even though those interventions were put in place, commercialized meat was still not complying with microbiological standards (Niyonzima et al., 2013). More stringent interventions were conducted in catering places, but despite this different cases of food poisoning are frequently reported in newspapers.

6.3 Role of government in further enforcement of hygiene rules and good practices

From the conducted research both by sampling (Chapter 2), observations (Chapter 3 and 4) and calculations (Chapter 5) it can be concluded that there is a general problem of food safety interventions which do not meet the ultimate goal of their purpose and for that reason, the interventions suggested in this work should be followed by the improvement of the **inspections on behalf of the competent authority**. Enforcement of good hygienic practices and agricultural practices by governments by means of inspections, has been demonstrated to be effective at different steps of the dairy chain following and implementing good practices and improve hygiene status as demonstrated in several countries e.g. at farm level in The Netherlands by Noordhuizen and Metz (2005) and in Brazil by Costa et al. (2013), at farm and processing levels in England and Wales by Bailey and Garforth (2014) and in Peru by Fuentes et al. (2014). A study conducted in Tanzania at processing level by Kussaga et al. (2014b) confirmed the positive role of governmental inspections in enhancing food safety and quality in the African context.

At farm level, the inspections from the competent authorities are not yet in place in Rwanda. In Rwanda, the Rwanda Standards Board (Formerly Rwanda Bureau of Standards) is the competent authority in charge of standardization, research and inspection for all food commodities (RSB, 2013).

In the dairy chain, inspections are conducted at processing, wholesale and retail levels and in parallel the Ministry of Agriculture delivers animal products certification services including enforcement of sanitary laws, monitoring and surveillance of animal diseases (MINAGRI, 2014). Besides those two bodies, environmental health officers under the Ministry of Health operate hygiene inspections at retail level (personal observations and in detail described in chapter 3).

Once those inspections on compliance with good practices and food safety rules are reinforced in farms, milk collection centers, cheese and dairy processing companies, supermarkets and milk shops, the microbiological quality and safety of milk and dairy products will continue to be improved. This might contribute significantly in eliminating *Salmonella* spp. along the whole chain.

The prevalence of *Salmonella* spp. in ready-to-eat products indicates that for sure many cases of salmonellosis happen in Rwanda but are not correctly reported, as **the official way of reporting food-borne diseases is still in its inception** in Rwanda. In the exposure and risk assessment calculations (Chapter 5) it was estimated that 214 200 people are likely to get infected on a year due to consumption of boiled milk in milk shops, and 11 960 adults and 11 902 children in home consumption. Infected people get treatments in hospitals and health centers but there is not yet coordination of all information on food-borne diseases all over the country. Cases of food poisoning are indicated only in case of massive infection for example in meetings, marriages and other events but still are just classified as food-borne diseases without further investigations to identify the exact incriminated pathogens. Information is frequently given in newspapers but there is still no public awareness for food safety issues, as the public opinion is still focusing on food security issues and other diseases like malaria and HIV/AIDS.

6. 4 Microbiological safety of milk and dairy products in African context

In the set-up of this research, a focus was made both on *Salmonella* spp., as zoonotic pathogen, finding its contamination source with animals and also ***Listeria monocytogenes***, an environmental pathogen, reported to be associated with environmental contamination routes.

This study revealed the absence of *Listeria monocytogenes* in all the analyzed samples (chapter 2, 3 and 5), it was not found, not on product samples nor on environmental swabs.

This may find its origin in the behavior of *Listeria* spp. which is genuinely psychotropic and is a particular threat in chilled products with extended shelf live and also in refrigerated foods.

Its absence in milk and dairy products was also noticed in other (African) countries as Zimbabwe (Gran et al., 2003), Tanzania (Schoder et al., 2013), Niger (Pistocchini et al., 2009) and India (Grace et al., 2010), whereas it poses a tremendous challenge in industrialized countries, particularly in cheese (Kousta et al., 2010). This might be linked to the environmental conditions (ambient temperature and deficiencies in the cold chain) which causes its presence in milk but in very low numbers . It is still debated to which extent the possible presence of competition from indigenous microbiota in the milk or the cheese might be inhibitory to the outgrowth of *L. monocytogenes* to levels that cause illness. However, from the present study it seems that *Salmonella* rather than *L. monocytogenes* is priority in ensuring food safety of milk and dairy products in Rwanda. .

This study demonstrates also the importance of **traditional heating process** and their effectiveness by completely eliminating *Salmonella* spp. While pasteurization was suspected to be ineffective in formally processing companies, a certitude was obtained in the informal chain where the heating treatments are totally effective. This brings a paradox of formally products not correctly heated on one hand and informal products properly heated. Here comes the importance of decriminalization of informally marketed products. In fact, even though this study suggests the increase of processing facilities it also emphasize on the fact that traditional habits should not be ignored. By strengthening the professionalization of the milk shops, traditional products preferred by consumers will still be available on the market, with improved quality and safety. Seeing the heat treatments are not the cause, the observed contaminations in informally marketed milk and dairy products find their origin in post-contaminations exclusively (Chapter 2 and 3). This finding also refers to the enforcement of international standards and guidelines, as stated by Codex Alimentarius Commission (Codex Alimentarius, 2011) by the Rwanda Standards Board, "as such" translated in the Rwandan legislation and guidance documents. A translation towards the particular Rwandan situation and context need to be made before enforcing them towards the Rwandan dairy chain, and surely for those more traditional processing as milk shops and small scale cheese factories.

For instance, Codex Alimentarius suggests that pasteurization should be carried out at 72°C for 15 seconds in case of continuous flow and 63°C for 30 minutes in case of batch pasteurization (Codex Alimentarius, 2011), and this should not appear as such in a standard regarding the Rwandan context where informal traders are not even aware of what is pasteurization. The translation should be simply indicating that milk should be heated until it foams as this study in Chapter 4 confirmed that the heating practices in the Rwandan informal dairy chain allow to eliminate *Salmonella* spp. thus eliminating also other pathogens killed in the same conditions.

6.5 Microbiological risk assessment in African context

Microbiological risk assessment is a useful tool to provide information on the probability of infections by pathogens in the food chain. After collecting prevalence information (Chapter 2, 4 and 5), and insight of the dairy chain (Chapter 2 and 3), data on consumption behavior and consumption frequency/volume (Chapter 5) exposure can be modeled and linked with dose-response data to build a model of probability of food-borne illnesses. This tool can be successfully applied to the developing countries facing the lack of reports on food-borne diseases. Most of developing countries are equipped with laboratory facilities that can allow the investigation on the prevalence of food-borne diseases and then, this can be used to get insight in the number of people who are infected per serving and per year, to allow adapted interventions to mitigate the encountered risks. The present study allowed us to get insight in the probability of infection of salmonellosis per serving and per year, and will be used by competent authority to identify the intervention strategies. This approach requires skills in advanced mathematical modeling, and thus trainings for food safety authorities officers are necessary.

CONCLUSION

By having an overall view of the status of the microbiological hygiene and safety of milk and dairy products in Rwanda, a conclusion can be made that although microbiological limits are exceeded in some cases, in general the microbiological quality and safety was acceptable. Thanks to multiple interventions for safety along the chain, remarkable results were achieved especially in the cheese sector. The sector of milk shops are seeking for additional professionalism and capacity to enhance the safety and avoid cross-contaminations by *Salmonella*. Other African countries like Tanzania (Kussaga et al., 2014b), Senegal (Breurec et al., 2010) and Zimbabwe (Gran et al., 2003) showed less satisfactory results with pathogens isolated from formally processed products.

Although this study was the first to be conducted on microbiological quality and safety of milk and dairy products in Rwanda, it does not pretend to be the only one and further research is warranted concerning the different topics addressed in this PhD study to contribute on the improvement of quality and safety in the Rwandan food sector. We recommend that the cause of the absence of *Listeria monocytogenes* would be further investigated, also in particular in cheese processing. The newly processed types of cheese also should be investigated for their microbiological quality and safety as in many other countries cheeses have been notified as an at risk product for listeriosis. Furthermore the recent initiatives and incentives given for implementation of food safety management systems should be made sustainable and therefore a study on their effectiveness should be conducted by using adapted tools such as the microbial assessment scheme (Jacxsens et al., 2009)..

In addition, this study should inspire researchers to perform exposure assessment in other areas of food safety ranging from microbial hazards to chemical contaminants in some of the suspected food products in Rwanda that lack control measures and may pose a risk to public health such as grilled meat, maize flour, and served foods in caterings and the numerous boarding school canteens.

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CURRICULUM VITAE

Olivier KAMANA was born at Ruliba (Rwanda) on October 15th 1982. After his secondary school in 2000 at Saint John junior seminary he was granted an excellence scholarship on behalf of the Government of Rwanda and pursued his Veterinary studies at Cheikh Anta Diop University at Dakar, Senegal. Upon completion of his DVM he was employed as a graduate assistant in the department of veterinary parasitology and a year later continued his studies in the same university and obtained an MSc in Animal Production with specialization in animal product quality and safety. From 2008 to 2014 he worked as an assistant lecturer in the Higher Institute of Agriculture and Animal Husbandry, in Rwanda. He was in charge of teaching Food Safety Management Systems and Animal Product Inspection. In 2009 he joined the University of Ghent for the International Training Program on Food Safety, Quality Assurance Systems and Risk Analysis. In September 2010 he was granted a research scholarship by the Belgian Development Agency and started his PhD at Ghent University in March 2011. In September 2014 he was appointed to be the Head of Department of Food Safety and Quality Management in the newly established University of Rwanda.

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- **Kamana, O**., Ceuppens, S., Jacxsens, L., Kimonyo, A., & Uyttendaele, M. (2014). Microbiological quality and safety assessment of the Rwandan milk and dairy chain. *Journal of Food Protection*, *77*(2), 299-307.
- **Kamana, O**., Jacxsens, L., Kimonyo, A., & Uyttendaele, M. (Submitted). Insights in hygienic practices in the Rwandan milk and dairy chain. *International Journal of Dairy Technology*.
- **Kamana, O**., Ceuppens S., Jacxsens, L., Kimonyo, A., & Uyttendaele, M. (Submitted). Microbiological quality and safety in milk shops and small cheese units in Rwanda. *International Dairy Journal*
- **Kamana, O**., Seydi M. (2008). A study on the microbiological quality and safety of hen eggs according to different storage conditions. *Proceedings of the International conference on comptetitiveness of the poultry sector in Africa*. Dakar, Senegal, 4th-9th May 2008.
- **Kamana, O** (2014). A study on the non-conformities in HACCP implementation. A case study in senegalese fisheries (*Etude des non-conformités dans l'application du système HACCP. Cas des industries de transformation des produits de la pêche au Sénégal*). *PAF-ISBN : 978-3-84-16-2506-9.*