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REGULAR ARTICLE

HYGIENIC QUALITY ASSESSMENT OF FRESH BEEF MEAT IN BUKAVU URBAN SLAUGHTERHOUSES, SOUTH KIVU PROVINCE OF THE LONG SALE CHAIN: POTENTIAL HEALTH RISKS FOR CONSUMERS EASTERN D.R. CONGO

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ABSTRACT

Meat is generally subject to multiple sources of microbial contamination related to the length and complexity of their journey from farm to consumer's table. The purpose of this study is to assess the current hygienic quality level of fresh beef slaughtered in Bukavu urban slaughterhouses, South Kivu to identify the health dangers to consumers. The meat samples were taken from 78 carcasses into three periods: at the slaughterhouse, to the market at the end of the transport position of sale and butchery. Microorganisms were sought following appropriate ISO standards. Total Aerobic *Mesophilic* Flora (FAMT), total *coliforms*, *staphylococcus* and other *enterobacteria* were counted more ($p<0.001$) at the slaughterhouse and the market at the end of transportation to butchery Chi^2: 64.90; 82.91 and 176.5, respectively; $p<0.001$). Hygienic quality of beef meat is poor, this study revealed a very high level of contamination of the collar and shoulder of beef carcass analyzed from slaughterhouse to distribution location ($p<0.001$). The very high bacterial load of these products is observed at the slaughterhouse and the public market during carcasses transport, the lesser butchery. This charge varies as well according to the slaughterhouse where the sampling took place, site and date of collection, including public slaughterhouse, most visited by distributors and that of suburban is the most famous. Beef carcasses are contaminated almost at the end of the week (thursday) by pathogenic bacteria such as; *Staphylococcus aureus*, *Salmonella* ssp., *E. coli*, *coliforms* and other *Enterobacteriaceae* represent a great danger of food poisoning to consumers, hence the need to implement an effective program against beef contamination, veal and respect for hygiene breeding farm, slaughterhouses, slaughter procedures, method of handling meat, and transport to the sale to consumer.

Keywords: consumption, meat, contamination, bacteria, human health risk, Bukavu

INTRODUCTION

The recent crises that have shaken and still shake food sector and particularly meat, suggest that the quality of it is constantly deteriorating and that it is better to turn to alternative sources of protein **(Dennaï *et al.*, 2001)**. Meat is the muscle of the transformation products after animal death. It is traditionally considered to be the vehicle for many food borne diseases in humans because of health defects **(Dennaï *et al.*, 2001; Fosse, 2003; Vaillant *et al.*, 2004; Fosse *et al.*, 2006)**. It is a highly perishable foodstuff and whose hygienic quality depends, first of contamination during slaughtering and cutting operations and secondly, the development and growth of flora contaminants during cooling, storage and distribution **(Dennaï *et al.*, 2001; El Hadef *et al.*, 2005)**. If meats are subject to multiple sources of contamination linked to the length and complexity of their journey from the farm to the consumer's table, these potential hazards need to be considered in terms of real health risk.
To do this, we must weigh each of these hazards in terms of frequency or probability of occurrence, and in terms of severity. This dimension called risk analysis has long been neglected, but it is the basis of all public health recent policy **(Fosse, 2003; El-Hadef *et al.*, 2005)**.
While many studies have been made on hygienic and microbiological quality of meat in most continents **(Collobert *et al.*, 2002, 2007; Herau *et al.*, 2007)**, few studies are listed in the countries of the Sub-Saharan Africa **(Wade, 1992)**. In DR Congo, the little work done on the meat quality cover microbiology and the bacteriological quality of meat, internal organs of pigs and cattle for human consumption **(Krubwa, 2002)**. From these studies, it appears that the analysis of the microbiological quality of meat has revealed that the internal organs of the animal are the busiest in bacteria and were the source of *salmonella* infections more dangerous. They exhibit more than pork liver is five times more contaminated than beef liver **(Krubwa, 2002)**. Similarly, current production practices carcasses can cause contamination of carcasses with pathogens such as pathogenic E. *coli*, *Salmonella enterica*, *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Mycobacterium bovis*, *Mycobacterium tuberculosis* **(Fosse *et al.*, 2006; Salifou *et al.*, 2010; Laila *et al.*, 2016)**. These microbiological germs are mostly responsible for food poisoning in consumers **(Fosse *et al.*, 2006, Vaillant *et al.*, 2004; El ham and Nahla, 2011)**.
In South Kivu between 2012 and 2014, tons of meats were destroyed by the state veterinary service as suspected to be unfit for human consumption. Formally, it was announced that the meat were destroyed as obtained by applying bad felling techniques (bad bleeding) and/or diseases such as tuberculosis, local tuberculosis, fluke, liver abscess, tapeworms and localized tuberculosis. These few statistics provide information instead of providing pleasure and joy; the meats available in the markets of Bukavu city constitute a health risk for South Kivu consumers. Hence the need to identify pathogenic bacteria in beef and to estimate the risk to consumers often persists in the consumption of meat, without checking the source and mode production or retention by the seller. To do this, it is necessary to continue work to better assess the hygienic quality of the beef carcasses meat in the distribution chain.
The main purpose of this study is to contribute to the knowledge of hygienic quality of food of animal origin to assess their risk to the health of consumers, by i) assessing the bacteriological quality of cattle meat of slaughterhouse to the point of sale and the change in the amount of microbiological germs of beef carcasses meat after transport to sales positions, 2i) counting the total load aerobic mesophilic flora from the same carcasses and 3i) the identification and isolation of pathogenic bacteria of beef and identifying potential health risks to humans eating them.

MATERIALS AND METHODS

South-Kivu province and study sites

South Kivu is located in the eastern Democratic Republic of Congo, approximately 1°36' and 5°51' South latitude on the one hand and 26°47' and 29°20' longitude East of somewhere else. The province is limited to the East by the Republic of Rwanda, which it is separated by Ruzizi River and Lake Kivu, Burundi and Tanzania, which it is separated by Lake Tanganyika. The study has been carried out in the provincial capital of Bukavu, specifically in each slaughterhouse three

communes to the city, including the commune of Bagira, Kadutu and Ibanda (Figure 1).
Meat samples were collected in slaughterhouses, butcher shops and other places of distributions available in the middle.
The public slaughterhouse of Bukavu/Elakat located in Ibanda commune, precisely to Ruzizi II border to the east, separating province of the Republic of Rwanda; is the most slaughterhouse visited by the sellers. CIRIRI suburban abattoir is built opposite the first road to Walungu territory, on the hill overlooking Bukavu town between Mulwa and Ciriri neighborhoods in Bagira town. The Brasserie slaughterhouse built in front of Brasserie Bralima on leading road to Pharmakina society, commune Kadutu. And Bagira slaughterhouse is located in the area A just below Bagira central market in Bagira town are of beef production reference sites before sampling.

Materials

The appreciation of hygienic quality of fresh beef along the sales chain was conducted from January 1st to June 28, 2015 on 78 meat samples from 26 animals (i.e. 3 sites sampling per carcass/animal) slaughtered in Bukavu abattoirs, South Kivu province. After weighing and stamping, half carcasses were packed in jute bags before being transported outside the slaughterhouse, on foot, by bike or in a taxi to sales positions. Sometimes, some butchers (sellers) carried the naked carcasses and does not packed up. Once the position of sale, carcasses were unpacked and placed on tables where they were fragmented bone and gradually to room temperature, to be sold by the kilogram in Butchery shops or sold by estimating the amount in others local urban markets. They were well kept at that temperature all day or until their complete sale. The investigation of hygienic quality appreciation of meat was designed according to the approaches described by **Abbey Avery and Borlaug-Ruan (2004)**.

Methodology

Sampling of beef carcass meat

The samples were taken two days a week for 6 weeks because of the slaughter by slaughterhouse days per week. The day of sampling varied every Monday and Thursday of the week so that the results are representative of the whole week. The sampling periods were: (1) to slaughterhouse after the post mortem inspection, (2) the sales positions just after the transportation and installation of meat on the stalls, (3) and at mid-day selling around 15 hours, eight hours after slaughter. The sampling technique was identical for all samples and was made according to **ISO 17604 (2003)**. Many sample copies were made per site each day on field visit.
Four samples of 5 cm and a maximum thickness of 1 cm each were made by half-carcass. The destructive method was performed using a punch of 2.5 cm in diameter to define the surface to be sampled. This sampling was done using a clamp and a blade mounted single use of a scalpel handle. The day before, the sampling equipment was sterilized pastor oven and the day of sampling, the punch was sterilized with the flame from the alcohol burnt before each operation. The samples at the slaughterhouse were carried out after post-mortem inspection on five different half-carcasses. The carcasses were randomly selected (beginning, middle and end of slaughter chain) and alternated so as to have a carcass right and a left half carcass. The sampling sites were respectively: the collar, the shoulder and the side (Figure 1).
Once installed on the table in butcher shops or sales positions, new samples were immediately carried out on the same carcasses after transport; but this time on neighborhoods (1/4 carcass) carcasses previously taken to slaughterhouses. The samples were taken right next to the previous locations. A third sample was taken on the remains of neighborhoods that made the second sample objects, time after 8 hours am sales operations and handling of meat. This time, the levy sites varied from one district to another made of the latter are gradually broken up for sale. A total of four samples were collected by half-carcass including two by neighborhood. Samples taken by carcasses were deposited together aseptically in a pre-identified bag, closed and placed in a cooler where the temperature was maintained between 0 and 4 °C. After the samples at slaughterhouses and those just after the transportation, the samples collected were immediately brought back to the microbiology laboratory of Bukavu Institute of Higher Education in Medical Techniques “ISTM-Bukavu" for possible bacteriological analysis. The last samples were also reduced in the same laboratory at about 15 hours. Before analysis, the samples were kept at 4°C in the laboratory. Bacteriological analyzes were performed within 24 hours after collection. For these analyzes, a volume of 100 ml of previously sterilized peptone water was introduced into each stomacher bag containing the total sample size of 20 cm^2. The whole was milled for 2 to 3 minutes in the Stomacher. The supernatant was collected in a sterile bottle and was the stock solution 100. The different dilutions were made from the stock solution. Counting the isolation and identification of germs sought were well done and in accordance with ISO 6887-2 **(ISO, 2004)**.
The germs were sought total mesophilic aerobic flora (FAMT), *Enterobacteriaceae* and *salmonella* that are the three indicators of the health of the slaughter process **(European Commission, 2005)**, *Pseudomonas* which are indicators of psychotropic spoilage of meat and organisms that can grow on the meat stored at room temperature (0°C to 30°C), *Escherichia coli* providing information on the conditions of slaughter **(Cartier, 1990)** and the enumeration of pathogens such as *Staphylococcus* and *Clostridium perfringens*. Samples taken in the morning were always stocked in the afternoon of the collection day. The FMAT was seeded, incubated at 30°C and refined in accordance with ISO 4833 **(ISO, 2003)**; the *Enterobacteriaceae* sought in accordance with ISO 21528-2 **(ISO, 2004)**; *Salmonella* sought in accordance with ISO 6579 **(ISO, 2002)**; Suspected pathogenic staphylococci in accordance with ISO 6888-1 **(ISO, 2003)**, *Clostridium perfringens* in accordance with the ISO 7937 standard **(ISO, 2005)** and finally *Escherichia coli* in accordance with ISO 7251 **(ISO, 2005)**. These different methods of microbiological analysis of bovine animals’ meat presented above are already used by **Salifou *et al.* (2013), Laila *et al.* (2016) and Dennai *et al.* (2001)**.
For each microorganism being sought, the results were expressed as colony forming unit (CFU) to 10g of carcasses and sampled in accordance with specific ISO standard every germ for quantitative research and in the absence or presence of germs for qualitative research. The average costs were calculated daily, sampling period and each germ.

Statistical analysis

The procedure of Generalized Linear Models (Proc GLM) of SAS (Statistical Analysis System, version11 (2013) USA was used for analysis of variance. The sampling period (abattoir, transport and butchery shop), the sampling day (Monday and Thursday) and the position of the carcasses (beginning, middle and end) were used as a source of variation. The significance of the effect sampling period or day effect sampling position or effect slaughter chain was determined by *F*-test. The position of the carcasses on the slaughter chain was not significant and therefore was not considered in the analysis model. The mean and standard deviations of counted nuclei were calculated and compared in pairs by the Student *t*-test. The chi-square, X^2-test was used to know the difference in the distribution of different frequencies or bacterial loads in different sampled carcasses.
To this end, the chi-square test (in MINITAB ver.17) was applied in order to want to know if there was a significant association between the frequency of observations (counts) of microorganisms and different sampling sites samples of meat. The *Z*-tailed test was used to compare the frequencies in pairs.

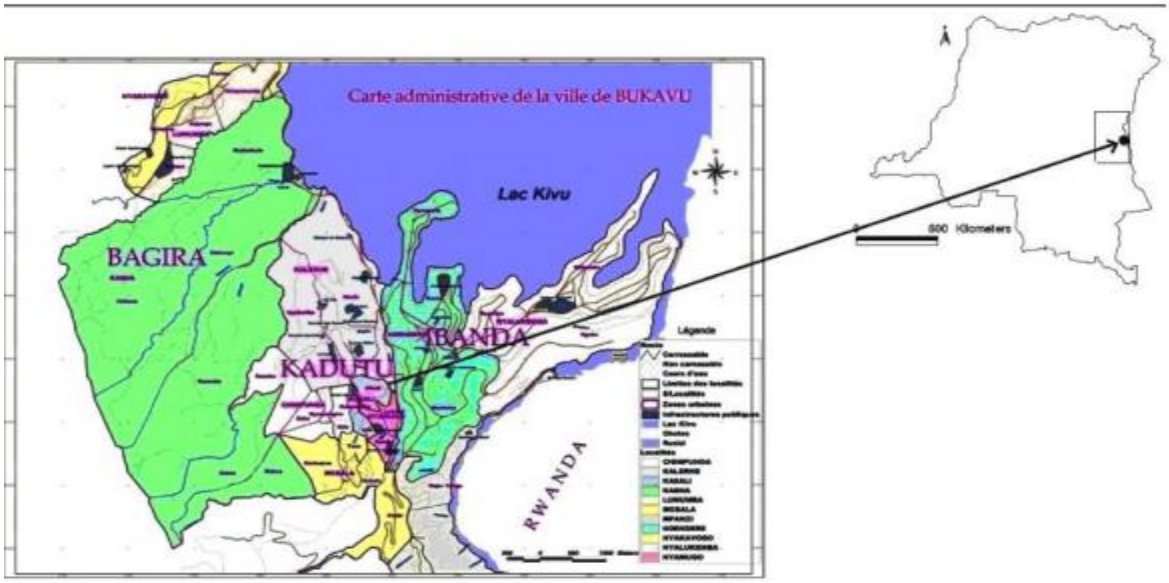

Figure 1 Map showing the administrative division of Bukavu town: the study sites are successively represented

RESULTS AND DISCUSSION

The results of this investigation show off the sanitary quality of fresh meat from bovine animals slaughtered in Bukavu urban slaughterhouses, South Kivu along sales chain is poor and the degree of contamination by pathogenic bacteria is a potential risk of exposure to diseases among consumers.

At the end of bacteriological analyzes of 78 meat samples from 26 animals (carcasses) slaughtered in the slaughterhouses of Bukavu town, South Kivu province, various pathogenic bacteria were identified present in half-carcasses of fresh meat from cattle collected by slaughterhouse, where the variation of bacterial load is a slaughterhouse function of where took sample place in the distribution and sampling day.

Bacteriological quality of beef carcass meat sold in slaughterhouses, butchery shops and on the shelves of Bukavu markets

At the end of bacteriological analyzes performed on the half-carcasses of fresh cattle meats, different pathogenic bacteria have been identified in samples of beef collected by slaughterhouse, where the variation of bacterial load is a function of the slaughterhouse and from where took place the sample in the distribution ($p<0.001$).

It is apparent five groups of bacteria isolated in beef carcass meat, distributed according to slaughterhouse and the sampling period, including: Flora Mesophilic Aerobic Total (FMAT), total coliforms with the species identified and isolated *Escherichia coli* and other Enterobacteriaceae, Pseudomonas with the species *Pseudomonas aeruginosa*, staphylococci (micrococcaceae) with *Staphylococcus aureus*, Negative Staphylococcus Coagulase (NSC) and Salmonella (Enterobacteriaceae) with two important pathogenic species identified; *Salmonella typhimurium* and *Salmonella enterica*. There is also the presence of other unspecified Salmonella and Enterobacteriaceae. Table 1-4 shows the different identified bacteria and charge along the three periods of samples.

The charge in bacterial load periods following meat samples in urban slaughterhouses Bukavu South Kivu are not surprising; they have been highlighted repeatedly in different African slaughterhouses by **Kebede in (1986)** and **Ibrahim (1992)** for Dakar, Senegal; **Krubwa (2002)** to DR Congo; **Bouchra *et al.* (1998)** for Rabat, Morocco; **Dennaï *et al.* (2001)** for Kenitra in Morocco; **Sallam and Samejima (2004)** for Egypt; **El Hadef *et al.* (2005)** for Algerian urban slaughterhouses; **ANSSA, (2007)** for Mali; **Mbawala et al. (2010)** for Ngaoundere city slaughterhouses in Cameroon**; Salifou *et al.* (2010, 2013)** and **Agossa (2010)** for Cotonou-Porto-Novo slaughterhouses in Benin; **Arua Odwar *et al.* (2014)** in their microbiological quality study of chicken meat in Nairobi, Kenya and recently **Laila *et al.* (2016)** for Fez city of Morocco slaughterhouses.

After counting, isolation and identification of germs, it is clear from these study five groups of bacteria contaminating the beef urban slaughterhouses in the sales chain. These bacteria are distributed according to the slaughterhouse, the sampling period and the sampling days are: Flora Mesophilic Aerobic Total (FMAT), total coliforms with the species identified and isolated *Escherichia coli* and other Enterobacteriaceae, *Pseudomonas* with *Pseudomonas aeruginosa* species, staphylococci *(micrococcaceae)* with *Staphylococcus aureus* and other Negative Staphylococci Coagulase (NSC) and Salmonella *(Enterobacteriaceae)* with two important pathogenic species identified; *Salmonella typhimurium* and *Salmonella enterica*. There is also the presence of other unspecified Salmonella and Enterobacteriaceae in this study. Table 1-4 shows various identified bacteria and charge along the three periods of samples.

In the following paragraphs, we will consider successively these various pathogenic microbial species affecting the hygienic quality (microbiological) meat and assess their potential health risks to consumers, by comparison of our results with that of the available literature.

The charge in *Pseudomonas* (*Pseudomonas aeruginosa*) is important in meat produced at Ciriri and Bagira slaughterhouse. This charge amounted to the market at the end of transportation to butchery shop ($p<0.001$). Furthermore, this bacterial species was not identified in the meat produced in Elakat slaughterhouse, reason for which daily variation of the analysis of their office in carcasses slaughterhouse was not performed. But it is present in beef meat of Brasserie slaughterhouse and of local butcheries selling during transport of the carcass Chi^{2}: 300; $p<0.001$).

This lack of *Pseudomonas* in the samples is explained by the made the slaughter of an animal in good conditions (absence of stress in animals) causes glycogenolysis which subsequently produced lactic acid inhibitory effect on the development of spoilage bacteria *(Clostridium* and *Pseudomonas)* **(Mbawala *et al.*, 2010;. Varnam and Sutherland, 1995)**. The *Pseudomonas* is a Gram-negative bacilli localized to the skin of the animal and facilitating the surface putrefaction of fresh meat stored in a humid atmosphere **(Laila *et al.*, 2016)**. According **Sallam and Samejima (2004)**, P. *aeruginosa* are loads of carcasses at slaughterhouses in Egypt and during transport have been low compared to that enumerated in the butchery. *Pseudomonas* observed carcasses to the butchery denotes a secondary contamination that may be disfavor good preservation of meat in time and therefore to changes in organoleptic characteristics.

Table 1 Quantity variation of microbiological germs carcasses according to the sampling period of Elakat slaughterhouse

	Bacteria (CFU/10g of meat)	**Movement levied meat**					***X²*-test**
		After slaughter	**Transport**		**Mi-sales**		
		Collection sites					
Groups	**Species**	**Abat.**	**Buch.**	**Markt.**	**Buch.**	**Markt.**	***X²***
FMAT	-	192	90	102	92	100	64.90**
Total Coliforms	*Escherichia coli*	134	66	68	60	74	45.91**
	Other *enterobacteria*	134	66	68	60	74	45.91**
Staphylococci (Micrococaceaea)	NSC	210	92	100	141	51	121.87**
	Staphylococcus aureus	91	61	164	132	141	57.88**
Salmonella (Enterobacteriaceae)	*Salmonella typhimurium*	240	100	140	160	168	64.67**
	Salmonella enterica	283	159	138	162	182	70.49**
	Others *Salmonella*	216	264	210	199	170	21.98**

Legend: *FMAT: Flora Mesophilic Aerobic Total; NSC: Negative Staphylococci Coagulase; CFU: Colony Forming Units, DF= 4; different significance levels of the X²-test variable association *p<0.05; **p<0.001; otherwise it is not significant when the value obtained in X² is followed by no asterisk (p>0.05). Other: undetermined species; Abat. : Abattoir/slaughterhouse; Buch. : Butchery; Markt. : Market*

Table 2 Quantity variation of microbiological germs carcasses according to the sampling period of Ciriri slaughterhouse

	Bacteria (CFU/10g meat)	**Movement levied meat**					***x²*-test**
		After slaughter	**Transport**		**Mi-sales**		
		Collection sites					
Groups	**Species**	**Abat.**	**Buch.**	**Markt.**	**Buch.**	**Markt.**	***x²***
FMAT	-	160	142	118	136	130	7032
	Coliforms	582	144	138	140	142	678.9**
Total coliforms	*Escherichia coli*	160	66	166	138	124	48.84**
	Other *enterobacteria*	222	82	132	120	118	80.87**
Pseudomonas	*Pseudomonas aeruginosa*	220	100	120	60	120	112.3**
Staphylococci	NSC	140	130	144	98	30	82.91**
(Micrococaceaea)	*Staphylococcus aureus*	240	180	92	130	47	164.2**
Salmonella	*Salmonella enterica*	120	128	1136	144	148	2393**
(Enterobacteriaceae)	Other *Salmonella*	800	162	226	442	226	740.3**

Table 3 Quantity variation of microbiological germs carcasses according to the sampling period of Brasserie slaughterhouse

	Bacteria (CFU/10g of meat)	**Movement levied meat**					***x²*-test**
		After slaughter	**Transport**		**Mi-sales**		
		Collection sites					
Groups	**Species**	**Abat.**	**Buch.**	**Markt.**	**Buch.**	**Markt.**	***x²***
FMAT	-	186	70	116	189	197	82.91**
	Coliforms	200	199	101	164	136	44.96**
Total coliforms	*Escherichia coli*	100	144	182	134	192	37.1**
	Other *enterobacteria*	100	58	70	68	60	16.02*
Pseudomonas	*Pseudomonas aeruginosa*	100	100	0	0	0	300**
Staphylacocci	NSC	343	166	194	152	70	214.7**
(Micrococaceaea)	*Staphylococcus aureus*	120	56	154	152	170	63.15**
Salmonella	*Salmonella enterica*	160	210	150	120	140	28.97**
(Enterobacteriaceae)	Other *Salmonella*	100	140	160	150	150	15.71*

Table 4 Quantity variation of microbiological germs carcasses according to the sampling period of Bagira slaughterhouse

	Bacteria (CFU/10 g of meat)	**Movement levied meat**					**X²-test**
		After slaughter	**Transport**		**Mi-sales**		
		Collection sites					
Groups	**Species**	**Abat.**	**Buch.**	**Markt.**	**Buch.**	**Markt.**	***X²***
FMAT	-	403	152	251	150	253	176.5**
	Coliforms	245	100	145	99	146	96.07**
Total coliforms	*Escherichia coli*	100	60	140	142	158	52.73**
	Other *enterobacteria*	214	60	140	58	56	186.9**
Pseudomonas	*Pseudomonas aeruginosa*	140	91	93	62	182	78.99**
Staphylococci	NSC	252	101	142	126	42	177.9**
(Micrococaceaea)	*Staphylococcus aureus*	188	130	58	133	55	112.6**
Salmonella	*Salmonella enterica*	160	120	140	50	110	59.66**
(Enterobacteriaceae)	Other *Salmonella*	146	142	142	126	144	1829**

Level contamination of beef depending on the sampling day, type of identified bacteria and samples from the sites on carcass distributed in slaughterhouses

In the sample set, the charge in Flora Mesophilic Aerobic Total (FMAT) has varied according to the slaughterhouse and periods of samples (p<0.001). The meat taken to the slaughterhouse, immediately after slaughter to the point of sale is much more loaded in aerobic mesophilic flora in the slaughterhouse and the market at the end of transportation to butchery shop Chi²: 64.90; 82.91 and 176.5; p<0.001) for meat produced at Elakat, Brasserie and Bagira slaughterhouse respectively. By cons, to Ciriri slaughterhouse the FMAT load amounted is more to the slaughterhouse and butchery shop that market, but this change is not significant in terms of sampling period's Chi²: 7.032, p>0.05). Charge in FMAT also varied depending on the sampling date, sampling sites on the carcass of cattle and the slaughterhouse where the removal occurred (p<0.0001). In all slaughterhouses, the highest contamination were obtained on Thursday with a significant variation as slaughterhouses and analyzed portions (p<0.001). Except for the collar when the low contamination level was obtained on Monday, but no significant association with the slaughterhouse where the sampling took place Chi²: 8.78; p=0.032) (Table 5).

The **Salifou *et al.* (2013)** study on bacteriological quality of fresh beef meat from slaughtered in Cotonou-Porto-Novo abattoirs, Benin during distribution chain exhibited as compared to the sampling period, FAM identified in half-carcasses of fresh beef meat was counted more butchery shop and end transportation than to the slaughterhouse. Its results expressed as log CFU/g or log CFU/cm², indicate that the average values found in the slaughterhouse together spread a fairly high degree of contamination of the sampled carcasses. Loads Carcass aerobic mesophilic flora found in this research are slightly higher than that found in the municipal slaughterhouse of Kenitra in Morocco on 32 sampled carcasses (5.15 log CFU/g) by **Dennaï *et al.* (2001)** and lower than that registered by **Oumokhtar *et al.* (1998)** on 20 samples of meat from the slaughterhouse Rabat (8.109 CFU/g). The work of **Salifou *et al.* (2012)** on the hygiene of slaughter process in the Cotonou-Porto-Novo slaughterhouses gave an average disburden of 3.0 ± 0.12 log CFU/cm² November-December 2009 and 5.09 ± 0.16 log CFU/cm² November-December 2010 for mesophilic aerobic flora. The results obtained by **Salifou *et al.* (2012)** are lower than those obtained in this study.

According to Regulation No. 2073/2005 of the European Union **(Commission EU, 2005)**, these results are not satisfactory and sign of poor hygiene of sampled cattle carcasses. The high load FMA observed at slaughterhouse, during transport and slaughter indicates both a defective general hygiene carcass involving non-compliance and secondly the effectiveness of sanitary measures seem not satisfactory in the slaughterhouse and in the distribution chain.

In fact, the slaughterhouse is one of the major critical points of Meat Hygiene and slaughter is considered the stage where the greatest opportunities for contamination exist (80 to 90% of micro-flora meat reaching the consumers result of contamination occurring at the slaughterhouse) **(Jouve, 1990)**. FMAT among the microorganisms that can affect the health of consumers by causing food borne poisoning and may alter the organoleptic characteristics of the carcasses **(valiant *et al.*, 2004; Fosse *et al.*, 2006; Laila *et al.*, 2016)**.

Gradual increase in average microbial load observed by sampling period shows the effectiveness of further contamination of carcasses at their exit from the slaughterhouse on the one hand and contamination related to transportation, the conservation mode (no cold) and manipulations (cuts, etc.) on the other. The significant difference observed between the charge raised for slaughterhouse and that recorded the butchery shop shows that the sanitary quality of meat available to consumers, even if it is not already acceptable to the release of slaughterhouse is much influenced by the steps downstream of the carcass production chain. This increased load microorganism is due to the operations of cutting and sales that contribute (through the tools used, the type of packaging and labor) to the contamination of new surfaces such as bare. These findings are consistent with observations made by other authors in their old notes **(Bryan *et al.*, 1988; Ekanem , 1985)**, they have noticed that in developing countries, the lack of hygiene producers and distributors of meat, the exposure of food to dust and flies promotes contamination by pathogenic microorganisms. Moreover, the first food material can be contaminated from the start or during processing by pathogenic bacteria and thus present a risk to the consumers health **(Bryan, 1988).**

A high content FMAT may be accompanied by an early spoilage of the meat. However, unlike **Letouze *et al.*, (1986)**, which notes the lack of relationship between the load FMAT and probable time of occurrence of alteration phenomenon, by an assumption that it is the result of specific bacterial proliferation germs showing a part of the total flora.

The high loads FMAT in urban slaughterhouse cattle meat Bukavu-South Kivu can also be explained by the fact that the slaughterhouse of Bukavu are part of non-mechanized slaughterhouses and fixed phones, where the realization of bleeding and all animal carcass transformations and fifth district are in the same location following a handcrafted model. It is likely that corrective/preventive measures such as the introduction of chain and rigorous cleaning work system of the equipment used will lower sensibly the total bacterial load.

The charge in load FMAT depending on the day of sampling shows instability in the working method. The highest rates recorded almost on weekends (Thursday) confirm the hypothesis of **Salifou *et al.* (2010)**; assumption that the hygienic quality of slaughter process on the last day of the week is influenced by the number of animals slaughtered that day and that is almost double the usual slaughter capacity of slaughterhouses; these are not functional on Sundays and other days of the week not presented in this study.

Table 5 Charge in Aerobic Mesophilic Flora (CFU/10g) of carcasses slaughterhouse where sampling took place

Day and websites taken	Names of slaughterhouse where sampling took place				Statistics *Chi*²-test)		
	Elakat	Ciriri	Brasserie	Bagira	*DF*	X^2	*P*
1. Flank	Total Aerobic Mesophilic per abat./day						
Monday	100	148	140	100	3	16131	0.001
Thursday	103	166	230	267	3	81802	<0.0001
2. Shoulder							
Monday	194	242	306	280	3	27847	<0.0002
Thursday	298	220	230	184	3	29202	<0.0003
3. Necklace							
Monday	191	250	230	240	3	8.7849	0.032
Thursday	290	160	226	232	3	37374	<0.0001

Loads of total coliforms in meat (*Escherichia coli* and other Enterobacteriaceae) are as high as for FMAT to abattoir and the market at the end of transportation to butchery (p<0.001). E. *coli* is the most dominant of all slaughterhouses and significantly varied in all periods of samples (p<0.001; Table 1-4 above). For meat taken at shoulder and collar of the carcass of cattle slaughter, the high contamination was obtained on Monday and is associated with the four slaughterhouses samples (p<0.0001). By cons, there is a contamination of carcasses taken sides, but there is no association between variation in the bacterial load of the carcass flanks and sampling slaughterhouse (Monday X^2: 3.351, p=0.341 and Thursday X^2: 9.047; p=0.029) (Table 6).

Like FMAT, loads of total coliforms in meat (*Escherichia coli* and other *Enterobacteriaceae*) are as high and vary from period to period (p<0.001). Total *coliforms* and others *enterobacteria* were counted more for slaughterhouse and the market at the end of transmission and the lowest load were obtained at butchery shop. These germs respectively provide information about the state of freshness of meat and on the conditions of slaughter **(Cartier, 1990)**. Fecal coliforms other categories of coliform live in the intestines of humans and animals, their presence result of bad conditions during the slaughter process **(Collobert *et al.*, 2002)**. In four abattoirs in the Calvados department, **Collobert *et al.* (2002)** reported an average contamination of 1.42 logs CFU/cm² for *Enterobacteriaceae* of 233 slaughtered cattle carcasses. Similarly, **Vallotton (2004)** reports that 70% of carcasses have load total coliforms below 1.5 log CFU/cm² and 30% have a load of between 1.5 and 4 log CFU/cm².

Variation charges in total coliform fillers and other enterobacteria obtained in this study according to the sampling periods is different from that obtained by **Salifou *et al.* (2013)** who discovers in slaughterhouses Cotonou-Porto-Novo, Benin that the charges in enterobacteria were least counted for slaughterhouse than at the end of transport, while the load in the highest enterobacteria was obtained at the butcher shop.

The charges in enterobacteria observed in this study are largely above those obtained by **Collobert *et al.* (2002)** and **Vallotton (2004)** after conversion of CFU/10g values in log CFU/g or log CFU/cm². According to Regulation No. 2073/2005 of the European Union **(European Commission, 2005)**, charges coliform and other enterobacteria exceed the maximum threshold allowed (2.5 log CFU/cm²) for that quality is satisfactory. Although the majority of these germs are considered non-pathogenic, they can in some cases be responsible for gastroenteritis disorders in humans, such as E. *coli* 0157: H7 **(Levine *et al.*, 1991)**.

Escherichia coli (coliform) specie is the most dominant of all slaughterhouses and studied varied significantly in all periods of samples (p<0.001). These results are contradictory to those of **Salifou *et al.* (2013)**, which indicate in his study that the average load of E. *coli* has not changed a sampling period the other, however, it notes a trend, the charge in E. *coli* increases to abattoir for butchery without, however, significant differences.

Presence of coliform bacteria in all samples taken at the slaughterhouse shows a poor condition slaughter. The mean values obtained in this study are much higher than that found by **Sumner *et al.* (2003)** in South Australia. In 1268 carcasses examined, E. *coli* is observed in 10% in Australia **(Phillips *et al.*, 2001)**. In the United States, 44% of beef carcasses meat examined were positive for E. *coli* **(Siragusa et al., 1998)**.

For meat taken at shoulder and necklace carcass cattle slaughterhouse where sampling has taken place, the high contamination was obtained on Monday and is associated with the four slaughterhouses samples (p<0.0001). By cons, there is a contamination of carcasses taken sides, but there is no association between variation in the bacterial load of the carcass flanks and sampling slaughterhouse (Monday $X^{2:}$ 3.3515, p=0.341 and Thursday $X^{2:}$ 9.0473; p=0.029). For cons, the outcomes of the study **Salifou *et al.* (2013)** reveal daily loads *Enterobacteriaceae* that have not varied from one day to the other at the slaughterhouse and during transport.

If at butchery, charging in total *coliforms* varied significantly from one day to another, it is because hygiene conditions fluctuate from day to day. According **Collobert *et al.* (2007)**, high loads in mesophilic aerobic flora and total *coliforms* and others *enterobacteria* are due to a failure of cleaning and disinfection of equipment cutting cycle. In most of our butchers, hardware is just flushed at the end of the day. If we should note the absence of period effect coupled with the presence of day effect to the butchery would prove that the biggest in total *coliform* contaminations are brought to slaughterhouse. Their presence attests contamination from the poor condition of slaughter definitely associated with poor post-slaughter handling. The hygiene risks associated with the presence of *Escherichia coli* in the meat and meat products are a public health problem with serious **(Cohen and Karib, 2006; Dennaï *et al.*, 2001)**. Coliforms are a considerable portion in the FMAT in this study.

Table 6 Load Variation total coliforms and other enterobacteria (CFU/10g) of carcasses by slaughterhouse which took place the taking

Day and websites taken	Names of slaughterhouse where sampling took place				Statistics Chi²-test		
	Elakat	Ciriri	Brasserie	Bagira	*DF*	X^2	*P*
1. Flank	Total Aerobic Mesophilic per abat./day						
Monday	136	152	136	162	3	3.3515	0.341
Thursday	130	147	130	100	3	9.0473	0.029
2. Shoulder							
Monday	140	148	128	300	3	110.19	<0.0001
Thursday	130	150	124	200	3	23,656	<0.0001
3. Necklace							
Monday	230	143	240	250	3	33,635	<0.0001
Thursday	164	242	242	110	3	65873	<0.0001

Staphylococci also exhibit great variability in the slaughterhouse that in mid-sale (p<0.001). The distribution is significantly varied according to the three periods of samples. Negative Staphylococci Coagulase dominate position in the contamination of cattle carcasses meat followed by *Staphylococcus aureus*, ranging according to the slaughterhouse and periods of samples (p<0.001). Loads counted staphylococci isolated and identified in each of the parts analyzed and the sampling date have varied from the slaughterhouse where the sample held in the other (p<0.0001). Null charge in staphylococci were obtained daily samples and are based on sampling sites on the carcass at Elakat slaughterhouse and the highest load was obtained on Monday and Thursday during transport (Table 7).

These samples could be contaminated with *Staphylococcus aureus* carriers in the various manipulations by distributors. Added to this is the contamination by the animal. The muscle superficially soiled, lets indeed easily penetrate deeply by these microorganisms during cutting. If storage at room temperature is extended, meat and meat products can promote the proliferation of S. *aureus* toxin production then causing poisoning that can be sometimes serious **(Dannai *et al.*, 2001)**.

However, an increasing trend (0.66 germs/g at the slaughterhouse, 5.0 germs/g after transport and 5.66 germs/g butchery) was obtained by **Agossa (2010)** reflecting contamination by man whenever the carcass in contact with the latter in particular during transport and skinning. Research *Staphylococcus aureus* on local beef at retail outlets and Dakar consumption showed that 42% of carcasses were positive **(Wade, 1992)**. But the contamination is often secondary as *Staphylococcus aureus* is a germ of human contamination to the poor hygiene on. **Laila *et al.* (2016)** in his study on the evaluation of the hygienic quality of meat and certain meat products taken from the city of Fez, Morocco concerning *Staphylococcus aureus*, the results show an absence of this bacteria in the pieces of meat beef, liver, poultry and meats. While poultry meat, poultry products and beef liver were contaminated with S. *aureus* and non-compliance rates vary from one category to another and the high percentage is observed in poultry sausages.

Table 7 Load Variation staphylococci (CFU/10g) of carcasses slaughterhouse where sampling took place

Day and websites taken	Names slaughterhouse where sampling took place				Statistics Chi²-test)		
	Elakat	Ciriri	Brasserie	Bagira	*DF*	X^2	*P*
1. Flank	Total Aerobic Mesophilic per abat./day						
Monday	0	253	268	250	3	257.97	<0.0001
Thursday	0	256	300	238	3	274.92	<0.0001
2. Shoulder							
Monday	0	364	254	215	3	335.01	<0.0001
Thursday	0	254	242	99	3	298.32	<0.0001
3. Necklace							
Monday	0	200	243	300	3	274.76	<0.0001
Thursday	0	210	344	236	3	314.47	<0.0001

Loads counted staphylococci isolated and identified in each of the parts analyzed and the sampling date have varied from the slaughterhouse where the sample held in the other (P<0.0001). Null loads staphylococci were obtained daily samples in Elakat slaughterhouse specifically, they are based on samples from the carcass and the highest load sites were obtained on Monday and Thursday during transport in other slaughterhouses. **Salifou *et al.* (2013)** by cons, when it discovers to in his study that the average load staph has not changed a sampling location to another. He adds that no significant difference was observed between the different average loads observed the slaughterhouse staphylococci and at the butchery shop.

Staphylococcus aureus among the microorganisms which can touch consumer health by causing poisoning food-borne and those that can alter organoleptic characteristics of the carcasses. Among bacterial pathogens include *Salmonella spp, Staphylococcus aureus, Listeria monocytogenes, Yersinia enterocolitica* **(Cottin, 1988; Fournaud and Jouve, 200; Dickson and Anderson, 1992)**.

Cases of *Salmonella* have been enumerated, identified and isolated in the beef carcasses meat along periods of samples with a significant variation slaughterhouses during transport in butcheries or for mid-sale (p<0.001). Loads salmonella in parts of the considered frame (flank, shoulder and necklace) are fatal and vary daily sampling according to the slaughterhouse. Although the trend contamination degree of carcass shoulder is increasing day by sampling and varies along the sales chain based on each slaughterhouse, no significant difference was observed between these frequencies (p>0.05; Table 8). Salmonella species

identified were: *Salmonella typhimurium*, *Salmonella enterica* and *Salmonella* spp.
These results demonstrate, contrary to **Salifou et al. (2010)** results, *Salmonella* spp. is common in the beef carcasses meat to Bukavu-South Kivu slaughterhouses. The research results of Laila al., 2016 spread *salmonella* presence in all categories of raw meat. According to **Ghafir and Daube, (2007)**, poultry and especially eggs and beef carcasses meat, is the main source of human cases of salmonellosis. This is in agreement with our results.
In member countries of the **European Union (2004)** and as part of monitoring the product contamination meat with salmonella, many studies have been conducted for the detection of *Salmonella* in beef. The prevalence varied by country: 0.8% in Greece (n=516), 2% in Ireland (n=2176), 3% in Spain (n=233), 3.86% in Hungary (n=1558) and 0.3% in Italy (n=153) **(EFSA, 2006)**.
Loads salmonella in parts of the considered frame (flank, shoulder and necklace) are fatal and vary daily sampling according to the slaughterhouse. Although the trend degree of shoulder carcass contamination is increasing day by sampling and varies along the sales chain based on each slaughterhouse, no significant difference was observed between these frequencies ($p>0.05$). Salmonella species identified were: *Salmonella typhimurium*, *Salmonella enterica* and *Salmonella* spp. These results show that the surface of carcasses does contain salmonella, which can vary depending on the contamination site **(Hinton *et al.*, 1998)** or sampling. **Phillip *et al.* (2001)** detected salmonella in 0.2% of sampled carcasses and 0.1% of boneless beef in Australia.
In another study, **Van *et al.* (2005)** have highlighted the emergence of *Salmonella enteritidis* in industry poultry and the danger for the consumer it may cause. Indeed, All salmonella serotypes may in theory cause a systemic infection in humans to decreased immune status, while most will generate feverish diarrhea, vomiting, abdominal pain and in the elderly or immuno-défiscients bacteraemia, septicemia and extraintestinal maps, in particular vascular **(Baumler *et al.*, 2000)** .The lack of hygiene on farms and in slaughterhouses and the use of broad-spectrum antibiotics are the most important factors contamination **(Korsac *et al.*, 2004)**.
The lack of significant difference between the sampling day the number of *Salmonella* spp. isolated by period and sampling sites in this study does not rule on the effectiveness of further contamination to that recorded at the slaughterhouse despite the trend. However, the health risk is for consumers.
These observations show how many times the hygienic quality freshly slaughtered beef meat to urban slaughterhouses Bukavu, South Kivu and sold in markets and butcheries from Bukavu prone to bacterial contamination in disturbing thresholds for the health of consumers, from the slaughterhouse to the place of sale.

Daily absence of certain bacteria such as *Pseudomonas* and *Staphylococcus* our abattoir samples does not necessarily imply the absence of the surface of carcasses tested, but would especially at a sampling problem as their distribution can be so punctual that we were able to miss them by taking some tattered and not others; some authors believe that it is not desirable to use them as hygienic quality indicator slaughterhouse meat **(Stolle, 1988)**.
The impact of this research to the population of Bukavu is that it permeates to the community of the dangers to the development of bacteria on beef and state veterinary service to know the hygienic quality of meat produced in slaughterhouses and sold to urban markets.
Finally, this study which was limited to a category of animal food products should continue to lead to the evaluation of the full microbiological quality of other meat and meat products consumed by the population of Bukavu city, South Kivu province in eastern Congo. We have reviewed the estimation of potential health risks spanned by the consumption of beef contaminated the population of Bukavu, but the specifics of these hazards for humans were identified by **Fosse *et al.* (2006)** cattle slaughterhouse of the Great West region of France who discovers twenty-five biological hazards that can be transmitted to humans by the consumption of beef.

Table 8 Salmonella expense of Charge (UFC/10g) of carcasses slaughterhouse where sampling took place

Day and websites taken	Names slaughterhouse where sampling took place				Statistics Chi²-test)		
	Elakat	Ciriri	Brasserie	Bagira	*DF*	X^2	*P*
1. Flank	Total Aerobic Mesophilic per abat./day						
Monday	140	170	200	124	3	21,369	<0.0001
Thursday	132	100	184	139	3	25908	<0.0001
2. Shoulder							
Monday	150	180	162	145	3	4.5636	0207
Thursday	144	182	159	158	3	4.6205	0202
3. Necklace							
Monday	240	160	250	132	3	52394	<0.0001
Thursday	340	152	256	146	3	115.2	<0.0001

CONCLUSION

This study discovers the hygienic quality of fresh beef meat slaughtered in urban slaughterhouses along the sales chain that can be beneficial for limit contamination of meat and reduce health hazards transmitted to humans by the consumption of beef. This study will help the researcher to uncover the critical areas of risk analysis dimension among consumers that many researchers were not able to explore. Thus a new theory about the dangers transmitted to humans by the consumption of other contaminated meat, may be arrived at.
Worry about contributing to hygienic quality perception (microbiology) food of animal origin, in order to identify potential hazards transmitted to humans by beef consumption, by assessing the bacteriological quality of fresh beef meat from slaughterhouse to the point of sale and the amount variation of microbiological germs of beef carcasses meat after transport to sales positions (meat injurious to health); enumeration of total aerobic mesophilic flora load from the same carcasses; and by identification and isolation of beef pathogenic bacteria.
Microbiological study assessing the hygienic quality of fresh beef meat has revealed a very high degree of contamination in most samples of beef carcass meat analyzed from slaughterhouse to distribution location. The very high bacterial load of these products is observed at the slaughterhouse and the public market during carcasses transport, it is lesser at butchery shop. This charge varies as well according to slaughterhouse, site and date of collection, including public Bukavu/Elakat slaughterhouse, most visited by distributors and that of pre-urban Ciriri is the most famous. The necklace and shoulder are most contaminated sampling sites at the end of the week (Thursday) by pathogenic bacteria such as *Staphylococcus aureus*, *Salmonella* ssp., *E. coli*, *Coliforms* and other *Enterobacteria* represents a great food poisoning infection danger for consumer, hence the need to implement an effective program against the contamination of beef meat and observe hygiene from the breeding farm, abattoirs, slaughtering procedures, the method of handling meat, transportation to sale to consumers.
As for the microbial charge just after slaughter, certain conditions such as the cleanliness of the animals, respecting the water diet, hygienic condition of the slaughter premises, cleanliness knives used in the bleeding and evisceration, have an effect on the nature and number of microorganisms present in the carcasses. Hence, the decrease in fresh meat microbial load of fresh beef meat along the chain of sale would be possible by improving the hygienic conditions of the slaughterhouse, the place of sale and method of handling meat by actors (butchers and sellers) including the replacement of display and cutting taking place in the open air by the use of cold storage and packaging in bags in the refrigerator pieces of meat ready for sale. It would be important that the public be able to invest in the maintenance of good quality instead of sales to try to reduce the rate and microbial diversity on meats intended for human consumption. The control of transport conditions through the application of good hygiene practices and the temperature of respect would allow owners to avoid contamination and microbial growth in local sales outlets.

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REFERENCES

Abbey, A. & Borlaug-Ruan (2004). Red Meat and Poultry Production and Consumption and Distribution in Ethiopia in Addis Ababa (Ethiopia), *ILRI, Ethiopia*, 64 pp.
https://www.worldfoodprize.org/documents/filelibrary/images/youth_programs/2004_interns/averyabbey_abb75f25dedec.pdf

Agossa, A. (2010). Evaluation of the hygienic quality of fresh meat from cattle slaughtered at abattoirs Cotonou, Porto-Novo. *Memory Master in Production and Animal Health EPAC UAC,* 61pp.

ANSSA-NationalAgency of Food Safety (2007). Health Quality Assessment red meat after slaughter controled Mali and health risk. Ministry of Health, *Republic of Mali*, 78 pp. http://mail.cnom.sante.gov.ml/docs/Viande%20Rouge.pdf

Arua, J., Odwar, G., Kikuvi, J., Kariuki. N. & Kariuki S. (2014). A cross-sectional study on the microbiological quality and safety of raw chicken meats sold in Nairobi, Kenya. *BMC Research Notes 2014(7):* 627.

Bäumler, A., Tsolis, R. & Heffron, F. (2000). Virulence Mechanisms of Salmonella and Their genetic basis. In: C Wray, Wray A. (Eds.), Salmonella in Domestic Animals. *CABI Publishing: Oxon,* 57-72. http://dl.dairycattlecenter.com/book/Salmonella%20in%20Domestic%20Animals.pdf

Bryan, F. L., Michanie, S. C., Alvarez, P. & Paniagua, A. (1988). Critical Control Points of street vended foods in Dominican Republic. *Journal of Food Protection, 51:*373-383.

Cartier, P. (1990). Hygienic quality control methodology for a before cattle. *Meat & Prod. Meat, 1990(11):*215-216.

Cohen, N. & Karib, H. (2006). Hygienic risk related to the presence of Escherichia coli in Meat and Meat Products: A real public health issue? *Technologies Laboratory, 1:* 4-9.

Collobert, J. F., Dorey, F., Dieuleveux, V. & Quillien, N. (2002). Bacteriological surface quality of carcasses of cattle. *Science food 22 (2):* 327-334.

Collobert, J. F., Dieuleveux, V., Theze, S. & Dorey, F. (2007). Evaluation of the effectiveness of cleaning and disinfecting a beef cutting plant. *Food Science 27(1*): 47-57.

Commission Europeenne (2005). Regulation (EC) No 2073/2005 of the Commission of 15 November 2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union European, L* 338/24. https://eur-lex.europa.eu/legal-content/FR/ALL/?uri=CELEX:32005R2073

Cottin, J. H., Bizon, C. & Carbonelle, B. (1988). Study of Listeria monocytogenes in meat taken from 415 cattle. *Sci. Foods, 5, Series IV:* 145-149.

Dennaï, N., Kharrati, B. & Elyachioui, M. (2001). Assessment of the microbiological quality of carcasses freshly slaughtered cattle. *Ann. Med. Vet. 145:* 270-274.

Dickson, J. & Anderson M. E. (1992). Microbiological decontamination of food animal carcasses by washing and sanitizing systems.J. *Food Prot., (55),* 133-140.

EFSA (2006). Trends and Sources of zoonoses, zoonotic agents and antimicrobial resistance in the european union in 2004; *EFSA Journal (European Food Authority safty), (310),* 23-41; 128-132; 201-203; 205.

Ekanem, E. O. (1985). The street food trade in Africa: safety and socio-environmental issues. *Food Control, 9:*211-215.

El Hadef, El., Okki, S., ElGroud, R., Kenana, H. & Quessy, S. (2005). Evaluation of superficille contamination of beef and sheep carcasses from the municipal slaughterhouse Constantine in Algeria. *Canadian veterinary journal, 46 (7):* 638-640.

El ham, I. A., & Nahla A. R. (2011). Incidence of Clostridium perfringens in Meat Products at Some Egyptian Governorates. *International Journal of Microbiological Research 2 (3):* 196-203.

Fosse, J. (2003). The dangers for humans transmitted through the consumption of meat. Evaluation of the use of control means to slaughter. *Th. Med. Vet., Nantes,* 304 pp.

Fosse, J., Cappelier, J. M., Laroche, M., Fradin, N., Giraudet, K., Magras, C. (2006). Meat beef: an analysis of the hazards biological for the consumer applied to the slaughterhouse. *Renc. Rech. Ruminants 13:* 411-414.

Fournaud, J. & Jouve, J. L. (2000). Meat. Microbiological challenge. *Die meats,* 133-141. http://bibfac.univ-tlemcen.dz/snvstu/opac_css/doc_num.php?explnum_id=2168

Ghafir, Y. & Daube, G. (2007). Update on methods for monitoring microbial contamination of food of animal origin. *Ann Medvet. 151:*79-100.

Girma Kebede, (1986). Contribution to the study of the surface contamination of carcasses of cattle to slaughterhouses in Dakar (Senegal). *PhD thesis, Faculty of Medicine and Pharmacy to obtain the rank of Doctor of Veterinary Medicine of the University of Dakar. TD 86.17,* 115 pp. http://www.beep.ird.fr/collect/eismv/index/assoc/TD94-25.dir/TD94-25.pdf

Herau, V., Loukiadis, E., Sandrin, Gabriel, Robez, E., Kerourédan, M. & Brugere, H. (2007). Potential microbiological hazards effluent slaughterhouse. *Meet Research Ruminants 1:* 203-206.

Hinton, M. H., Hudsonw, R. & Mead, G. C. (1998). The bacteriological quality of British beef. Sampled carcasses prior to chilling. *Meat Science, 50 (2):* 265-271.

Ibrahima, Wade., (1992). Contribution to the study of the bacteriological quality of local beef at the retail outlets and Dakar consumption. *PhD thesis, Faculty of Medicine and Pharmacy to obtain the rank of Veterinary Doctor of Cheikh Anta Diop University, Dakar.* TD 92.17, 97 pp. https://www.memoireonline.com/08/09/2554/m_Contribution--letude-de-la-qualite-bacteriologique-des-aliments-vendus-sur-la-voie-publique-de0.html

ISO 6579, (2002). Horizontal method for the detection of Salmonella spp. *V08-013:* 1-26. https://www.boutique.afnor.org/norme/nf-en-iso-6579/microbiologie-des-aliments-methode-horizontale-pour-la-recherche-des-salmonella-spp/article/705190/fa107313

ISO 4833 (2003). Horizontal method for the enumeration of microorganisms. *V08-011:* 1-9. https://www.boutique.afnor.org/norme/nf-en-iso-4833-1/microbiologie-des-aliments-methode-horizontale-pour-le-denombrement-des-micro-organismes-partie-1-comptage-des-colonies-a-/article/798129/fa163727

ISO 6887-2 (2004). Preparation of samples, initial suspension and decimal dilutions for microbiological examination. *V 08- 010-2:* 16pp.

ISO 7251 (2005). Microbiology of food. Horizontal method for the detection and enumeration of presumptive Escherichia coli. *Third Edition*: 13pp.

ISO7937 (2005). Microbiology of food horizontal-method for the enumeration of Clostridium perfringens: 16pp. https://www.boutique.afnor.org/norme/nf-en-iso-4833-1/microbiologie-des-aliments-methode-horizontale-pour-le-denombrement-des-micro-organismes-partie-1-comptage-des-colonies-a-/article/798129/fa163727

Jouve, J. L. (1990). Food Microbiology and meat industry. *Meat & Prod. Meat, 11(6):*207-213.

Korsak, N., Clinquart, A. & Daube, G. (2004). Salmonella spp. in foodstuffs of animal origin: a real public health problem*? Ann. Med. Vet, 148:*174-193.

Krubwa, L. (2002). Microbiology and bacteriological quality of pig meat for human consumption. 134 pp. http://remvt.cirad.fr/CD/EMVT82_3.PDF

Laila, B., Sanae, B., Bouchra, S., Mohamed, A. & Abdelhakim E. O. L. (2016). Evaluation of the hygienic quality of meat and certain meat products taken from the city of Fez, Morocco. *International Journal of Innovation and Applied Studies, 15(3):*547-554.

LeTouze, J. C., Vendeuvre, J. L. & Rozier, J. (1986). Method Microbiological quality assessment of products of the primary cut pork. *Meat & Prod. Meat, 1985(6):*236-244.

Levine, W. C., Craun, W. T. & Stephenson, G. F. (1991). Waterborne disease outbreaks, *1986-1989.J. Food Proto, 54:* 71-78.

Mbawala, A., Daoudou, B. & Ngassoum, M. B. (2010). Microbiological quality of kilishi (dried meat product) produced in the town of Ngaoundere (Cameroon). *Tropicultura, 28(3):*153-160.

Oumokhtar, B., Karib, H., Bouchriti, N. & Araba, A. (1998). Assessment of the bacteriological quality of the meat and offal bulls freshly slaughtered at slaughterhouses in Rabat. *Acts Inst. Agro.Vét. 3:* 169-176.

Philippi, D., Sumner, S., Alexander, J. F. & Dutton, K. M. (2001). The microbiological quality of Australian beef. *J Food Prot. 64 (5):* 692-696.

Salifou, C. F. A., Salifou, S., Tugan, P. U., Ahounou, G. S., & Youssao, A. K. I. (2010). Evaluation of the hygiene of the slaughter process the Cotonou-Porto Novo slaughterhouses using bacteriological examination surface. 13th *Days Muscle Science and Technology of Meat*, 19 and 20 October 2010 in Clermont Ferrand, France, 175-176.

Salifou, C. F. A., Boco, K. C., Ahounou, G. S., Tougan, P. U., Salifou, S., Kpodekon, T. M., Farougou, S., Mensah, G. A., Clinquart, A. & Youssao, A. K. I. (2012). Evaluation of the slaughter process of cattle to the Cotonou-Porto Novo slaughterhouses in southern Benin. *International Journal of Biological and Chemical Science, 6 (6):* 6049-6061.

Salifou, C. F. A., Boko, K. C., Attakpa Y. E., Agossa, R., Ogbankotan, I., Farougou, S., Mensah, G. A., Salifou, S., Clinquart, A. & Youssao, A. K. I. (2013). Evaluation of the bacteriological quality of fresh meat from cattle slaughtered in Cotonou, Porto-Novo slaughterhouses in the distribution chain. *Journal of Animal & Plant Sciences, 17(2):* 2567-2579.

Sallam, Kh. I. & Samejima, K. (2004). Microbiological and chemical quality of ground beef Treated with sodium lactate and sodium chloride During refrigerated storage. *Lebenson Wiss Technol. 37 (8):* 865-871.

Siragusa, G., Dorsa, W., Cutter, C. G., Bennett, J. & Keen Koohmaraie, M. (1998). The incidence of Escherichia coli are beef carcasses and Its Association with mesophilic aerobic plate count categories During the slaughter process. *Journal of Food Protection 61:*1269-1274.

Statistical Analysis System (1996). - SAS / STAT User's Guide, to, 6, 4th ed, *Cary, NC, USA, SAS Institute*. https://support.sas.com/documentation/onlinedoc/91pdf/sasdoc_91/stat_ug_7313.pdf

Stolle, E. F. A. (1988). Microbiogical monitoring program at slaughterlines- A new concept of meat hygiene. *Meat Sci., 1988(22):*203-211.

Sumner, J., Petrenas, E., Dean, P., Dowsett, P., West, G., Wiering, R. G. & Raven (2003). Microbial contamination is beef and sheep carcasses in South Australia. *International Journal of Food Microbiology, 81:*255-260.

Vaillant, V., De Valk, H. & Baron, E. (2004). Morbidity and mortality due to infectious diseases foodborne France, 1st ed. Saint-Maurice: *Institut de Veille*

Sanitaire, 192 pp. https://sites.anses.fr/fr/system/files/private/Morbidite_mortalite_INVS_2004.pdf
Vallontton, F. M. (2004). Hygiene assessment on a cattle slaughter line with bacteriology surface. *Thesis of Veterinary Medicine, National School Veterinary Toulouse:* 74 pp. http://oatao.univ-toulouse.fr/1296/1/celdran_1296.pdf
Van, F., Immerseel, Buck, D., Boyen, F., Pasmans, F., Bertrand, S. & Collard, J. M. (2005). Salmonella in poultry and eggs: a danger for consumers who demand the establishment of an effective control program. *Ann. Med. Vet, (149):* 34-48.
Varnam, A. H. & Sutherland, J. P. (1995). Meat and meat products: technology, chemistry and microbiology, *Chapman & Hall Ltd, London*, 430pp. https://www.cabdirect.org/cabdirect/abstract/19961408238
Wade, I. (1992). Contribution to the Study of bacteriological quality of the local Beef at points of retail and Dakar consumption. *Thesis DVM, School of Inter-State Veterinary Sciences and Medicine of Dakar:* 97pp. http://www.beep.ird.fr/collect/eismv/index/assoc/TD92-17.dir/TD92-17.pdf

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SCICELL

REGULAR ARTICLE

BIOTHERAPEUTIC POTENTIAL OF A COMERCIAL DAIRY PROBIOTIC CONTAINING *Lactobacillus casei* SHIROTA FOR THE CONTROL OF CANINE HOOKWORM

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ABSTRACT

Domestic dogs (*Canis familiaris*) are present in the homes of several families around the world, and are one of the animals with greater contact with humans, either as a guard dog or just as a pet. With this, they can transmit parasites to their owners, since dogs are natural hosts of several parasites, among them the helminths of Ancylostomidae family. Ancylostomiasis presents as main symptoms abdominal pain and anemia. The treatment is performed with anthelmintics, however, it was found that these parasites are acquiring resistance to current drugs, which makes necessary the development of alternative therapies. Thus, this study aims to evaluate the therapeutic effect of a probiotic preparation containing 1x1010 CFU of *Lactobacillus casei* Shirota, administering 80 mL of this, for a group of four naturally infected dogs during 40 alternate days and, in parallel, administering a same volume during 30 consecutive days for a similar group of dogs. The protective effect was also evaluated by administering 80 ml of the preparation for a group of 5 healthy dogs for 30 consecutive days, which were maintained along with other infected animals. The number of eggs per gram of feces (EPG) was determined every 7 days, noting possible changes. As results, it was observed the potential use of probiotics in the treatment of hookworm in dogs as an inexpensive alternative therapy, since the assessed preparation induced significant reduction of the parasitic load of infected animals ($p < 0.005$) in comparison with the control group, but in the other hand it was not observed protective effect, since the parasitosis have established in the healthy dogs which were pretreated with *L. casei* Shirota.

Keywords: Probiotics; intestinal parasites; hookworm, *Lactobacillus casei* Shirota

INTRODUCTION

Dogs are domestic animals present in the homes of several families, being the animal with which the most people develop an affective bond, even going so far as to consider them as "part of the family". However, this close proximity between people and dogs generates risks of development of zoonotic diseases, since dogs are hosts of several parasites, among them the helminths of the Ancylostomidae family (**Coêlho *et al.*, 2013**).

These parasites belong to the Animalia kingdom, filo Nemathelmintes, sub-filo Nematoda, class Secernentea, order Strongylida, family Ancylostomidae. There are three species that are etiological agents of human hookworms, being *Necator americanus*, *Ancylostoma duodenale* and *Ancylostoma ceylanicum*, and among them the first two species are those that parasitize humans more frequently (**Mitreva *et al.*, 2005; Seguel and Gottdenker, 2017**).

These parasites can cause intestinal disorders in dogs, leading to the manifestation of symptoms such as anemia, weight loss and abdominal pain, and may even lead to death, and are capable of triggering pathological processes in humans, among them: Larva Migrans Syndrome (LMS) and Eosinophilic Enteritis. They are also causes of inflammatory edema, itching, redness and hemorrhage, being *Ancylostoma braziliense* and *A. caninum* immature larvae the main etiological agents, parasites of the small intestine of dogs and cats (**Moro *et al.*, 2008**).

To prevent this parasitic disease, it is important to adopt the antiparasitic treatment in dogs. There is a great availability of antiparasitic drugs, however, there is difficulty in controlling the infections caused by Ancylostomidae in dogs, since the available treatments require the administration of several doses throughout the life of the animal, which leads to the abandonment or neglect of the therapy, in addition to the risk of induction of resistance and inherent toxicity to some allopathic antiparasitics (**Coêlho *et al.*, 2013**).

Another important factor in the spread of this parasite is the large number of abandoned dogs that are not treated. Studies carried out in different Brazilian cities have shown that a large part of dog stool samples collected on the streets was contaminated with hookworms, consisting a great risk of transmition to other animals, such as stray dogs and cats and, consequently, to humans who come into contact with these infected animals (**Alves *et al.*, 2016; Moreira *et al.*, 2014; Castro *et al.*, 2005**).

Thus, it is necessary to develop new strategies to control this parasitosis, being one of the alternatives the use of probiotic foods, taking into account their benefits and low cost.

Probiotics are products composed of living and non-pathogenic microorganisms that can promote a favorable balance of the gastrointestinal tract microbiota as well as a positive modulation of the immune response, thus contributing to the prevention and treatment of pathologies (**Coêlho *et al.*, 2013)**.

Bacteria present in probiotic foods are gram-positive, usually catalytic and producing lactic acid. Such microorganisms are tolerable to the pH of the gastrointestinal tract, and can compete in different ways with other microorganisms, including those that are considered pathogenic (**Badaró *et al.*, 2008; Flesch *et al.*, 2014; Siqueira et al., 2008; Varavallo *et al.*, 2008)**.

Bacteria of *Lactobacillus* genus are able to tolerate the acidic pH, surviving the passage through the gastrointestinal tract. These microorganisms are producers of lactic acid and, unlike most obligate anaerobes, are able to grow in the presence of oxygen. The production of lactic acid, as result of carbohydrates fermentation, is capable of inhibiting the growth of competing microorganisms, and can control several infectious processes. Such microorganisms may also enhance the immune response, and these properties may be particularly useful in assisting in the control of various infectious processes, including hookworm in dogs (**Coêlho *et al.*, 2013; Loukas *et al.*, 2001**).

Some species of *Lactobacillus* belonging to the "*casei* group" are considered probiotic, that is, capable of positively affecting the health of the individual when administered in correct amounts. These microrganisms are highly used in the food industry in the manufacture of fermented milks or in the improvement of the quality of cheeses acting as initiators of the fermentation (**Buriti *et al.*, 2007**). According to **Moal *et al.* (2014)**, such a beneficial effect may be due to competition for mucosal and nutrient binding sites, the production of antimicrobial compounds or the reduction of intestinal pH regarding the production of lactic acid and other short chain organic acids, a factor that also may provide a reduction in the incidence of pathogens.

The aim of this study was to evaluate the beneficial effects of the ingestion of a commercially available probiotic food containing $1x10^{10}$ colony forming units (CFU) of *Lactobacillus casei* Shirota as a mean of preventing in healthy dogs or treating hookworm infection in naturally infected dogs.

MATERIAL AND METHODS

This study was approved by the Animal Research Ethics Committee of FUNVIC - Faculty of Pindamonhangaba, protocol number 009/2009.

For the selection of the animals fecal samples were collected from a total of 150 dogs from residences or kennels, of which 24 were selected to compose the study.

The fecal samples were collected and sent under refrigeration to the Laboratory of Parasitology and Malacology - LAPAM of the Christian Life University Foundation/Faculty of Pindamonhangaba - FUNVIC, and analyzed according to the Willis method (flotation in NaCl d=1.2). After initial diagnosis, quantitative analysis was performed to determine the number of eggs per gram of feces (EPG) using the Macmaster method.

To perform the experiment, the animals were distributed into four groups, as follow:

Group 1 **-** Six animals naturally infected with hookworm, which were treated on alternate days, during 30 days, with 80 mL of daity probiotic containing $1x10^{10}$ CFU of *Lactobacillus casei* Shirota.

Group 2 **-** Six animals naturally infected with hookworm, treated continuously during 30 days, with 80 mL of of daity probiotic containing $1x10^{10}$ CFU of *Lactobacillus casei* Shirota.

Group 3 **-** Six healthy animals, which were submitted to the same treatment of group 2.

Group 4 **-** Six infected animals, which were not submitted to any treatment (control).,

The evaluation of the treatments was done by performing fecal sample collection every 7 days during the period of time of each experiment. To determine the therapeutic efficacy of the fermented milk in study, the samples were analyzed regarding the determination of EPG (Eggs Per Gram of fezes). The reduction of the parasite load of the animals was calculated according to the following formula **(Pereira-Junior *et al.*, 2017)**:

$$\textbf{Reduction (\%):} \frac{\text{EPG average of initial day} - \text{EPG average of day n} \times 100}{\text{EPG average of initial day}}$$

Statistical methods were used to evaluate the results, been chosen according to the characteristics of the sample distribution. The Kruskall-Wallis test was used at a significance level of 5% and Student-Newman-Keuls test to evaluate differences between means, using BIO ESTAT software 5.O.

RESULTS AND DISCUSSION

As observed in Table 1, the evaluation of the mean EPG values of dogs belonging to group 1 (animals infected with hookworm which were treated on alternate days with the probiotic drink evaluated) showed that there was no significant reduction ($p < 0.005$) of these values during the four week treatment and the followed two weeks experiment extension. It is observed that after the fifth and sixth week of treatment the values related to EPG reduction were significant, when compared to the values obtained at the initial day (one day before the beginning of the experiment).

The efficacy of the probiotoic dairy consumption was also observed after evaluating the mean EPG values of dogs infected with hookworm belonging to Group 2 (which were treated with *Lactobacillus casei* Shirota daily). It was observed that there was a significant reduction ($p < 0.0001$) of this parameter in the third and fourth week of treatment when compared to values obtained at the initial day (one day before the beginning of the experiment).

These results ara according to those presented by **Coêlho *et al.* (2013)** that demonstrated the potential use of probiotic preparations in the control of hookworm in dogs belonging to a group of 10 naturally infected animals which were treated with 5 mL of a probiotic preparation with three different strains of *Lactobacillus* containing $1x10^6$ CFU / mL for 28 days. At the end of the treatment, it was observed a parasite load reduction of 88,83%, which was statistically significant ($p < 0.05$) regarding the control group.

The benefits of using probiotics in dogs parasitized with hookworm were also demonstrated in a study by **Mussi *et al.* (2008)**, in which 19 dogs infected with this parasite were treated with anthelmintics such as febantel, pirantel pamoate and praziquantel concomitantly with a commercial probiotic food, the preparation being administered for 15 days on alternate days. The results showed that the mentioned probiotic promoted a beneficial effect influencing positively in the health of the animals.

It should be noted, however, that the species of *Lactobacillus* evaluated in the present study had already shown promise for the control of other parasitic diseases, among which giardiasis and triquinellosis. **Coêlho *et al.* (2016)** evaluated the use of a commercially available dairy drink containing $1,6x10^6$ live *Lactobacillus*, *L. casei* Shirota species for the control of giardiasis in naturally infected children, and observed that after 21 days of treatment, evolutionary forms of this parasite were not detected, with improvement of the clinical condition and the consistency of the feces.

According to **Flesch *et al.* (2014)**, the use of probiotics stimulates phagocytosis, since such microrganisms induce an increase in the count of circulating lymphocytes and cytokines. Thus, the intestinal immune system is optimized, decreasing the incidence of infections and favoring the intestinal microbiota, promoting the local immune response and at a systemic level.

Regarding the immune humoral response, **Martinez-Gómez *et al.* (2009)** carried out a study that evaluated the therapeutic effect of a probiotic preparation containing $1x10^8$ CFU of *L. casei* Shirota on 60 CD1 mice infected with *Trichinella spiralis*, a parasite belonging to the Nematoda class. Therefore, it was observed that intraperitoneal administration of such preparation, which was administered once a week for 3 weeks, caused a significant ($p<0.05$) decrease of adult parasites found in the intestines of the animals. In addition, is was shown a statistically significant ($p<0.05$) increase in IgA levels in the intestinal mucosa of the animals, in relation to the control group.

Table 1 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in dogs infected with hookworm and treated with a probiotic preparation containing *Lactobacillus casei* Shirota administered on alternate days (Group 1)

	Initial Day	**1st week**	**2nd week**	**3rd week**	**4th week**	**5th week**	**6th week**
EPG Average	36825	3612,5	2437,5	1525	1112,5	862,5*	375*
% EPG reduction	-	90,19%	93,38%	95,86%	96,98%	97,66%	98,98%

* = significant reduction in parasite load ($p < 0.005$) compared to day 0.

Table 2 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in dogs infected with hookworm and treated with a probiotic preparation containing *Lactobacillus casei* Shirota daily (Group 2)

	Initial Day	**1st week**	**2nd week**	**3rd week**	**4th week**
Average	13130	9465	6050	740*	390*
% reduction	-	27,91%	53,92%	94,36%	97,03%

* = significant reduction of parasite load ($p < 0.0001$) in relation to day 0.

After evaluating the mean EPG values of the uninfected dogs belonging to Group 3 which were treated with *Lactobacillus casei* Shirota, it was observed that there was a significant increase ($p < 0.005$) in EPG in the fourth week of treatment when compared to the initial day values (one day before the start of the experiment), as shown in Table 3.

It´s important to highlight that this group was designed to evaluate the protective effect of the probiotic preparation, in order to prevent the animals to the risk of natural infection.

These results showed that the use of *L. casei* Shirota in the experimental conditions evaluated in the present study, did not induce protective activity for uninfected dogs, since despite the previous treatment with the probiotic preparation they subsequently developed the parasite in question. In this context, it should be emphasized that the frequency of probiotic preparation consumption is relevant.

The lack of ability of probiotics to induce protection against the establishment of a hookworm infection may be related to immunity mechanisms involved in both the colonization process of the intestinal mucosa and in the process of inducing an effective immune response against the above mentioned parasite.

It is known that probiotics exert a significant influence on the modulation of the function of dendritic cells in the intestinal mucosa. Although dendritic cells are components of the innate immune system, being able to phagocytose pathogens, their most important function is to process antigenic material and present it to specialized cells of the immune system. Due to its great plasticity and maturation capacity in response to local danger signals derived from innate immunity,

dendritic cells are a key element in the connection between innate immunity and responses of adaptive immunity (**You *et al.*, 2014; Bernardo, 2013).**
Thus, it is possible to infer that the time elapsed between colonization of the intestine by the parasite and the development of an effective immune response induced by *L. casei* Shirota and controlled by immune cells, such as dendritic cells, was not sufficient to determine the control of the infectious process in this group, unlike the groups in which the animals were infected before starting treatment.

Table 3 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in uninfected dogs treated with a probiotic preparation containing *Lactobacillus casei* Shirota daily (Group 3)

	Initial Day	**1ft week**	**2nd week**	**3rd week**	**4th week**
Average	0	0	0	0	730*

* = significant reduction in parasite load (p <0.005) compared to initial day

Finally, regarding the results of the mean EPG values of the naturally infected animals that did not undergo any treatment (group 4 - control) shown in Table 4, it is observed that there was no significant reduction during the period of the experiment. Note that this group was performed aiming to evaluate the progression of the disease, in the absence of any treatment, particularly with regard to the number of EPG. The persistence of hookworm eggs in the feaces of the animals belonging to group 4 reinforces the evidence that the significant reduction of EPG observed in groups 1 and 2 was due to the action of *Lactobacillus* present in the milk beverage evaluated, not being a reduction that could happen naturally.

Table 4 Mean values of eggs per gram of feces (EPG) and percentage of EPG reduction in dogs infected with hookworm and not treated (Group 4)

	Initial Day	**1ft week**	**2nd week**	**3rd week**	**4th week**
EPG Average	5630	5950	5500	5650	5580
% EPGreduction	-	-5,7	2,3	-0,3	0,8

Therefore, it was demonstrated the feasibility of using a commercially available probiotic food containing *Lactobacillus casei* Shirota for the control of such parasitosis, since the consumption of the evaluated probiotic food induced a significant reduction of the parasite load in feaces of dogs naturally infected with hookworm.

CONCLUSION

These results showed hat the evaluated probiotic containing $1x10^{10}$ CFU of *Lactobacillus casei* Shirota, exerted therapeutic effect in dogs naturally infected with hookworm. This treatment resulted in a significant reduction of EPG in the faeces of the animals when administered daily, as weel as on alternate days. The results also revealed that the administration of the studied dairy probiotic in healthy dogs, kept in the same environment as parasitized dogs, did not promote a protective action, since the infection prevention was not observed. Therefore, the use of probiotics in the control of intestinal parasitoses can be seen as a potential alternative treatment, taking into account its low cost and the health benefits provided.

REFERENCES

Alves, A.P.S.M., Silva-Coêlho, F.A., Coêlho, M.D.G., 2014. Frequência de enteroparasitos em fezes de cães coletadas em praças públicas do município de Pindamonhangaba, SP, Brasil. *Revista de Patologia Tropical*, 43, 341-350. https://doi.org/10.5216/rpt.v43i3.32204

Badaró, A.C.L., Guttierres, A.P.M., Rezende, A.C.V., Stringheta, P.C., 2008. Alimentos Probióticos: Aplicações como promotores da saúde humana – Parte 1. *Revista Digital de Nutrição,* 2, 1-29.
(No Doi)

Bernardo, D. 2013. Human intestinal dentritic cells as controllers ofmucosal immunity. *Revista española de enfermidades digestivas*, 105(5), 279-290. http://dx.doi.org/10.4321/S1130-01082013000500006

Buriti, F.C.A., Saad, S.M.I., 2007. Bactérias do grupo *Lactobacillus casei*: caracterização, viabilidade como probióticos em alimentos e sua importância para a saúde humana. *Archivos Latinoamericanos de Nutricion*, 57, 373-380.
(No Doi)

Coêlho, M.D.G., Silva-Coêlho, F.A., Mancilha, I.M, 2013. Probiotic Therapy: A Promising Strategy for the Control of Canine Hookworm. *Journal of Parasitology Research*, 2013, 1-6. http://dx.doi.org/10.1155/2013/430413

Coêlho, M. D. G., Ramos, L.L., Pereira, R.B., Rocha, L.O., Lino, F.P.S., Silva-Coêlho, F.A., 2016. Avaliação do consumo de *Lactobacillus casei* Shirota para o controle de giardíase em crianças. *Revista de Patologia Tropical*, 45, 169-178. https://doi.org/10.5216/rpt.v45i2.41772

Castro, J.M., Santos, S.V., Monteiro, N.A., 2005. Contaminação de canteiros da orla marítima do Município de Praia Grande, São Paulo, por ovos de *Ancylostoma* e *Toxocara* em fezes de cães. *Revista da Sociedade Brasileira de Medicina Tropical*, 38, 199-201. http://dx.doi.org/10.1590/S0037-86822005000200017

Flesch, A.G.T., Poziomyck, A.K., Damin, D.C., 2014. O uso terapêutico de simbióticos. *Arquivo Brasileiro Cirurgia Digestiva*, 27, 206-209.

Loukas, A., Prociv, P., 2001. Immune Responses in Hookworm Infections. *Clinical Microbiology Reviews*, 14, 689-703. http://dx.doi.org/10.1128/CMR.14.4.689-703.2001

Martínez-Gomez, F., Santiago-Rosales, R., Bautista-Garfias, C.R., 2009. Effect of *Lactobacillus casei* Shirota strain intraperitoneal administration in CD1 mice in the establishment of *Trichinella spiralis* adult worms and on IgA anti-T spiralis production. *Veterinary Parasitology*, 162, 171-175. http://dx.doi.org/10.1016/j.vetpar.2009.02.010

Mitreva, M., McCarter, J.P., Arasu, P., Hawdon, J., Martin, J., Dante, M., Wylie, T., Xu, J., Stajich, J.E., Kapulkin, W., Clifton, S.W., Waterston, R.H., Wilson R.K., 2005. Investigating hookworm genomes by comparative analysis of two *Ancylostoma* species. *BMC Genomics*, 6, 58. http://dx.doi.org/10.1186/1471-2164-6-58

Moal, V.L.L., Servin, A.L., 2014. Anti-infective activities of *Lactobacillus* strains in the human intestinal microbiota: from probiotics to gastrointestinal anti-infectious biotherapeutic agents. *Clinical Microbiolology Reviews*, 27, 167-199. http://dx.doi.org/10.1128/CMR.00080-13

Moreira, A.P.S., Coêlho, M.D.G., Santos, I.A., Bozo, L.S.O., Maciel, L.T.R., 2016. Contaminação em logradouros do município de Pindamonhangaba - SP por parasitos potencialmente zoonóticos em fezes caninas. *Revista Ciência e Saúde On-line*, 1, 45-50.
(No Doi)

Moro, F.C.B., Pradebon, J.B., Santos, H.T., Querol, E., 2008. Ocorrência de *Ancylostoma* spp. E Toxocara spp. em praças e parques públicos dos municípios de Itaqui e Uruguaiana, fronteira oeste de Rio Grande do Sul. *Biodiversidade Pampeana*, 6, 25-29.
(No Doi)

Mussi, E.M., Mattos-Júnior, D.G., Balthazar, L.M.C., 2008. Associação de febantel, pamoato de pirantel, praziquantel e probiótico em cães (*Canis familiaris*) naturalmente infectados por ancilostomídeos. *PubVet*, 2, 1-11.
(No Doi)

Pereira-Júnior, R.A., Sousa, S.A.P., Valadares, A.A., Valadares, R.R., Rosa, F.C., Sato, M.O., 2017. Eficácia de Ivermectina e Albendazol contra nematoides gastrintestinais em rebanho ovino naturalmente infectado no município de Palmas, Tocantis, Brasil. *Revista Científica de Medicina Veterinária,* 28, 1-10.
(No Doi)

Seguel, M., Gottdenker N., 2017. The diversity and impact of hookworm infections in wildlife. *International Journal for Parasitology: Parasites and Wildlife*, 6, 177-194. http://dx.doi.org/10.1016/j.ijppaw.2017.03.007

Sequeira, T.C.G.O., Ribeiro, C.M., Gomes, M.I.F.V., 2008. Potencial bioterapêutico dos probióticos nas parasitoses intestinais. *Ciência Rural*, 38, 2670-2679. http://dx.doi.org/10.1590/S0103-84782008005000001

Varavallo, M.A., Thomé, J.N., Teshima, E., 2008. Aplicação de bactérias probióticas para profilaxia e tratamento de doenças gastrointestinais. *Semina: Ciências Biológicas e da Saúde,* 29, 83-104.

(No Doi) You, J., Dong, H., Mann, E. R., Knight, S. C., Yaqoob, P. 2014. Probiotic modulation of dendritic cell functionis influenced by ageing. *Immunobiology*, 219(2), 138-148. http://dx.doi.org/10.1016/j.imbio

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REGULAR ARTICLE

BACTERIOLOGICAL QUALITY OF STREET FOODS VENDED IN BUKAVU CITY: POTENTIAL HEALTH RISKS TO CONSUMERS OF SOUTH KIVU PROVINCE, EASTERN D.R. CONGO

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ABSTRACT

Foods vended in roadsides of our African cities is a reality and constitute a major problem of public health starting the multiplicity and diversity of microbial flora that they carry. To address these challenges, this study was performed to control the hygienic quality of street foods vended in urban zones of Bukavu city in South Kivu province, DR Congo and assess the potential health risks to consumers. This prospective study was conducted among street vending food from vendors in three urban zone of Bukavu city. A total of 80 food samples compressing boiled meat (16), roast fish (18), sausages (21), fresh milk (13) and loaf (12) from 320 vendors were purchased and analyzed. Standard microbiological methods NF ISO 7218: 1996 were used for isolation, enumeration and identification of bacteria. Investigations into the point of sale and microbiological test results revealed the presence of a perpetual contamination risk by vendor categories. All street food samples tested are contaminated to varying degrees by bacteria, including: FMAT, total coliforms with *Escherichia coli*, *Staphylococcus* sp. with *Negative Staphylococci Coagulase* and *Staphylococcus aureus* and *salmonella* with species *Salmonella enterica*, represent a great risk of street food poisoning for over 350 consumers per month. The mean bacterial counts in these foods expressed to CFU/10g of each food collected exceed the standards set by the Codex Alimentarius, significant and highly statistically significant according different categories of vendors and sampling sites ($p<0.0001$). Samples collected from vendors in Kadutu urban zone (the most popular and unhealthy in the city) are more contaminated. Dishes that are not subjected to heating during preparation have the highest microbial load. This is the case of fresh milk where the total mesophilic flora is of order of 10^6 CFU/10g. This is also the case of street food which, after cooking are exposed for a long time at room temperature: boiled meat and sausages contain an uncountable amount of bacteria. Total coliforms, and *Salmonella* sp. are more loaded in boiled meat, fresh milk and sausages. Many *Staphylococcus* sp. are in the loaf. Much (77%) contaminated dishes are from ambulant vendors than other distributors, followed by semi-stationary and stationary vendors respectively ($p<0.001$). Contamination of street food in Bukavu is multifactorial and hygiene vendors contribute significantly to contamination factor, including unhygienic managers, dirty environment and poor water quality. Hence, sustainable development of communities through good hygiene practices in street foods handling. The government should thus strengthen health checks at street food and ensure their hygienic quality before consumption by the population in order to prevent these diseases and improve health of consumers.

Keywords: street foods, bacteriological quality, consumers health risk, Bukavu city

INTRODUCTION

Street foods are foods and beverages ready to eat prepared and/or sold by ambulant or stationary vendors, especially in streets and other similar places. They are an important part of the daily urban food consumption of million consumers in low or middle income **(FAO, 1989; OIT, 1972).** For many people with limited resources, street food is often the cheapest and most accessible to get a meal nutritionally balanced out of the house **(FAO, 1997; 2007; 2009).**
According to some researchers, this phenomenon affects all layers of the large cities of black African population: students, civil servants, married, single, etc. easy to eat and easily outside the home and relatively low cost **(Canet and Ndiaye, 1996; Chauliac *et al.*, 1998; Barro and Traore, 2002).** But unfortunately these foods undergo in the process of manufacturing and selling unhygienic operations resulting mostly to microbial contamination and/or toxigenic **(Manzilima, 2011; Kama, 2014; Baba-Moussa *et al.*, 2012).**
Indeed, preparing and selling food on the streets can cause big problems for consumer health. Various studies on food streets in Africa argue that hypothesis, such is the case for Kenya by **Gitahi (2012)**, Burundi by **Noel (2013),** Nigeria by **(Okojie and Isah, 2014; Chibundu *et al.*, 2012; Okonko *et al.* , 2009),** Ghana by **(Mensah *et al.*, 2012, 1999, 2002; Feglo and Nkansah, 2010),** Benin by **Baba-Moussa *et al.* (2012),** Burkina Faso **(Barro *et al.*, 2002),** Dakar **(Diallo, 2010; Dione, 2000; Soumare, 1997),** Cameroon **(Ngabet Njassap 2001),** Madagascar **(Rakotondramanana 1998; Ravaonindriana *et al.*, 1999)** and also in countries around the world: India **(Chirag *et al.*, 2013),** Philippines **(Dexter *et al.*, 2014);** etc. to not only mention it. These works showed cases of food-borne infections such as microbial agents. Which constitutes a major risk to public health **(WHO, 1996).**
In fact, Bukavu city is not safe by this scourge, like all cities of the Democratic Republic of Congo it is under pressure due to rapid urbanization, high population due to rural migration, but also knows a degrading situation of employment, housing and nutrition, but also new profitable activities **(FAO, 1998).** However, to deal with these challenges, another phenomenon is dispersal in our streets, the central market where utilities: this is street food, known as "Malewa" in Congolese language; which is a social reality in Bukavu. It is found everywhere in the vicinity of the streets and in markets where the food safety status is not guaranteed **(Ranaivoarimanana, 2006).** This appears to meet the food needs of Bukavu population as was the case for Kisangani and Kinshasa city **(Manzilima, 2011; Kama, 2014).**
The safety of street food depends on several factors such as the quality of different materials to use and good practice of preparation. In most cases this security is not guaranteed and street food often becomes epidemic sources and gastrointestinal diseases such as gastroenteritis and diarrhea of microbial origin **(Adams and Motarjemi, 1999; Barro and Traore 2002; Tjoa *et al.*, 1977; Owhe-Ureghe *et al.*, 1993; Umoh and Odoba, 1999).**
Several studies have shown that street food is exposed to severe environmental conditions such as the presence of insects, flies and air pollution **(Sobel *et al.*, 1998).** Until today, most street vendors ignore good food hygiene practices. They expose the food in poor conditions creating cross-contamination and failures in food preservation **(Ekanem, 1998).**
It is in this context that we propose to control the bacteriological quality of some consumer street food in Bukavu city urban zones and health risks to consumers from Bukavu where cases of food poisoning caused by street foods have been observed and reported in the past three years. In addition, cholera, also called disease of dirty hands, which caused the death of hundreds of people in urban

health zones of South Kivu and also some deaths in Bukavu between 2000 and 2015, is worrying consumers food vended on the street. Our study investigate the microbiological quality (hygienic) and assessment of potential health risks to consumers of these foods by discovering the risk of infection by study site according to vendors' categories.

MATERIALS AND METHODS

Study areas and types of vendors

The food samples were collected in public procurement and in crowded areas of three urban zones constituting Bukavu city (including Bagira, Kadutu and Ibanda). Vendors were grouped into three categories according to the study sites and methods of sale: ambulant vendors cooking at home and not having a fixed point of sale; semi-stationary vendors preparing and selling outdoors along the road or under trees in the street and stationary vendors with dining facilities. During the collection, special attention was paid to the immediate environment of vendors and their hygiene practices. Thus, attention has been paid to the presence of solid or liquid waste, to the nearby waste water drains, unpleasant odor, utensils, food handling and dishwater.

Sampling

The range of street food available in Bukavu reflects ethnic and cultural richness of various inhabitants of South Kivu province. Collection was carried out for 4 months from April to July 2015. A minimum of 320 study participants were required for the study. However, in this study a total of 330 respondents were interviewed to provide the 320 required for the research. The response rate in the study was therefore 97 %.
A pre-selection of dishes was made in advance because of the higher risk or lower contamination and their high consumption frequency. Food samples were collected at different times and according to their shelf life. The samples were taken immediately from a total of 80 food samples compressing boiled meat (16), roast fish (18), sausages (21), fresh milk (13) and loaf (12) from 320 vendors, whose one food sample for 5 vendors (sampling interval k=5) were evaluated: after cooking, when vendors have tidy utensils and more or less clean; at the time of the service and by the end of sale. The same food samples were selected from the same vendor categories. They are placed in sterile plastic bags containing ice and sent to the Microbiological Laboratory of Bukavu Institute of Higher Education in Medical Techniques "ISTM-Bukavu" for analysis within 2 hours. Many sample copies were taken by site and vendor category each day of field visit.
The materials and equipment used for these analyzes are standard and comply with the standard **NF ISO 7218: 1996** on general rules for microbiological examinations **(AFNOR, 1996).** Standard microbiological methods were used for isolation, enumeration and identification of bacteria.

Search of microorganisms

Among microorganisms responsible of food poisoning, *enterobacteria* coliforms, *Staphylococcus aureus, Yersinia enterocolitica, Clostridium difficile, Salmonella, Shigella*, yeasts and fungi as well as *streptococci* prominently. The bacterial count was made from different foods mentioned above by the method described by Speck **(Speck, 1976).** It consists in the stock solution 1:10 by taking 10g of the sample which will be ground and dissolved in 90 ml of buffered peptone water (BPW). Different seeds were determined on specific media by morphological criteria. Biochemical criteria complement the morphological observations. So:
- *Staphylococci* were identified on Chapman manity medium after 48 hours of incubation at 37°C. The yellow colonies underwent tests of coagulase and catalase.
- Spores and clostridia have been identified in the Bryan and Burkey broth after heating for 5 min at 80°C to destroy vegetative forms.
- *Coliform enterobacteriaceae* are identified on eosin methylene blue medium. The presumptive identification of *Escherichia coli* is made by Mackenzie test. For gas producing coliforms is lactose bile broth brilliant green that was used.
- Incubation lasts 48 hours at 37°C.
- *Salmonella* and *Shigella* are sought on MacConkey medium after incubation at 37°C for 24 hours.
- The total mesophilic flora FMAT was determined after plating 0.1 ml of the 10-1 and 10-3 dilutions of the samples on petri dishes containing nutrient agar and incubated for 48h at 37°C.

These steps were repeated two times to calculate the average number to consider. Results of bacterial load are presented in CFU/10 g of food tested.

Statistical data analysis

The procedure of Generalized Linear Models (Proc GLM) of SAS (Statistical Analysis System, version11 (2013) USA, which is well suited to analyzing data with variables complex in distribution terms was used for analysis of variance. Cross-tabulations were made of the levels of the various bacteria tested and the responses obtained in the interviews. The significance of any observed differences was determined using χ^2 test and Student's t-test. The comparison of means was made through the ANOVA One-way test. Statistical significance was set at $P \leq 0.05$. In order to determine the effect of the knowledge and practices of vendors on the microbial quality of street foods, we calculated confidence intervals after factors and levels of contamination were cross-tabulated.

RESULTS AND DISCUSSION

Bacteriological quality of street foods vended in Bukavu city

The enumeration of total flora (mesophilic aerobic bacteria) and specific flora (all pathogens and toxigenic) samples collected in Bukavu city gave the results shown in Tables 1 and 2. All street food samples collected are contaminated to varying degrees by isolated and identified bacteria including: FMAT, total coliforms with *Escherichia coli*, *Staphylococcus* species with Negative Staphylococci Coagulase and *Staphylococcus aureus* and *salmonella* with the species *Salmonella enterica.* The quantities of bacteria obtained by CFU/10g of each food exceed the standards established by Codex Alimentarius, significant and highly statistically significant according to vendors categories and sampling sites ($p<0.0001$). This is consistent with the study of **Feglo and Sakyi (2012), Feglo and Nkansah (2010)** in Kumasi, Ghana, which reveals that most ready-to-eat foods in Kumasi were contaminated with enteric bacteria and other potential food poisoning organisms (*Coagulate negative staphylococci, Staphylococcus aureus, Escherichia coli, Salmonella enterica, Enterobacter cloacae*, etc.) with bacterial counts higher than the acceptable levels. In his study, **Mensah *et al.* (2012, 2002, and 1999)** exhibits the presence of mesophilic bacteria in the majority of samples (69.7%): *Bacillus cereus* was isolated in 28 of them (5.5%), and *Staphylococcus aureus* in 163 (31.9%). He said, hundred seventy-two samples (33.7%) contained *enterobacteria*.
The following tables of this study show that dishes that are not subjected to heating during preparation have highest microbial load. This is the case of fresh milk where the total mesophilic flora is of the order of 10^5 CFU/10g and varies to sampling sites. Is 102.10^4 CFU/10g in fresh milk collected in Bagira urban zone, 11.10^5 in Kadutu and 12.10^4 in Ibanda urban zone. But also more interesting, it is 10^5 CFU/10g in fresh milk collected at Brasserie market and 2.10^5 CFU/10g at Muhanzi, markets of Kadutu urban zone. These outcomes are similar to those obtained by **Baba-Moussa *et al.*, (2006),** which noted a high level of contamination in the street food that are not subjected to heating during preparation sold in Benin, Cotonou city.
This is also the case of foods selected in this investigation that, after preparation are exposed for a long time at room temperature: boiled meat and sausages contain an uncountable amount of FMAT bacteria in dishes taken from Brasserie market and roasted fish which have a total mesophilic flora 2.10^5 CFU/10g in Bagira and order of 10^6 CFU/10g roasted fish collected in Kadutu urban zone, the most popular and unhealthy city. The presence of unacceptable levels of mesophilic flora in meat and fish may suggest inadequate handling during the display (**Babe *et al.*, 2018**) of fish before sale by the vendors. In this Nairobi location 50% of stalls were dusty and had houseflies suggesting inadequate sanitation **(Gitahi, 2012)**.
But street foods like loaf has a total mesophilic flora equal to 657.10^2, 435.10^3 and 765.10^2 CFU/10g in Bagira, Kadutu and Ibanda urban zone respectively. Total coliforms, *Staphylococcus* sp and *Salmonella* sp are more numerous in boiled meat, fresh milk and sausages. The presence of *Staphylococcus aureus* and *Salmonella* sp. was also reported in previous studies on street food by **Okonko *et al.* (2008b, c)** and in sausages vended in Abeokuta and Benin-city, Nigeria in a study by **Oluwafemi and Simisaye (2005).** According **Oluwafemi and Simisaye (2005)** most of the sausage being sold as ready-to-food pose health risk to consumers, making it imperative to institute not only sanitary measures during its production and sales bit for retailers selling raw of preprocessed foods to have a steady source of power supply.
Note that, all analyzed foods contain salmonella *(Salmonella enterica* and other *Salmonella)* in the order of 10^2 and 10^4 CFU/10 g of food. *Escherichia coli* and

Staphylococcus aureus are present at relatively high rates. The presence of *Staphylococcus auerus*, a pathogenic organism of public health concern and significance in these street foods might have contaminated from source as a result of handling by processors. Improper handling and improper hygiene might lead to the contamination of ready-to-eat food and this might eventually affects the health of the consumers **(Dunn *et al.*, 1995; Adebolu and Ifesan, 2001, Omemu and Bankole, 2005; Okonko *et al.*, 2008b, c).** It was reported that counts of 10^7 cells/g for B. *cereus* **(ICMSF, 1974)**, and 10^6 cells/g for enterotoxigenic S. *aureus* **(Bergdoll, 1979)** are required to present a risk of intoxication. It is therefore suggested that street food vendors should be educated on the adverse effect of using untreated or polluted water for processing as these could serve as sources of fecal contamination. However, the vendors/handlers should observe strict hygienic measures so that they will not serve as source of chance inoculation of microorganisms and contamination of these street foods. Incidences of *E. coli, Enterobacter aerogenes* and other index of poor sanitary quality found in this study are in agreement with those of **Trevett *et al.* (2005)** and **Hogue *et al.* (2006)**.

As for staphylococci they are present in significant numbers in roast fish and loaf, but virtually low in fresh milk collected, but worrying in Bagira, Kadutu and Ibanda urban zone (Table 2). **Gitahi (2012)** in his study on the microbial quality, strain and distribution of selected food enterotoxigenicity terminal pathogens in relation to the hygienic practices in industrial area, Nairobi, Kenya discovered that the presence of *Escherichia coli* and *Staphylococcus aureus* in street food samples selected was qualitatively isolated in 3 food samples. *Escherichia coli* and *Staphylococcus aureus* in street food samples selected was qualitatively isolated in 3 food samples.

Table 1 Quantity variation of bacteria in some street foods collected from different categories of Bukavu vendors

Groups	Species	Samples collected					χ^2-test
		Boiled meat	Roasted fish	Sausages	Fresh milk	Loaf	
Ambulant vendors							
Global contamination (FMAT)	-	344.10^2	455.10^2	344.10^4	605.10^3	5.10^5	8837963**
Total coliforms	*Escherichia coli*	324.10^2	176.10^3	238.10^3	288.10^3	198.10^3	198111**
	Others *Enterobacteria*	234.10^3	189.10^3	365.10^3	237.10^2	343.10^2	486389**
Staphylococci	Negative Staphylococci coagulase	321.10^2	31.10^3	15.10^4	166.10^3	21.10^4	226953**
	Staphylococcus aureus	554.10^3	620.10^3	163.10^2	219.10^3	2.10^5	812693**
Salmonella	*Salmonella enterica*	866.10^3	543.10^3	435.10^3	321.10^3	34.10^3	843808**
	Others *Salmonella*	134.10^3	343.10^3	324.10^3	534.10^4	244.10^4	126789**
Semi-stationary vendors							
Global contamination (MTAF)	-	354.10^3	657.10^3	876.10^3	659.10^3	9.10^5	280962**
Total coliforms	*Escherichia coli*	546.10^2	6.10^3	209.10^3	43.10^4	237.10^3	599633**
	Others *Enterobacteria*	548.10^3	779.10^3	329.10^3	93.10^4	856.10^3	353790**
Staphylococci	Negative Staphylococci coagulase	324.10^2	3.10^5	543.10^3	86.10^4	23.10^4	1032460**
	Staphylococcus aureus	327.10^3	356.10^3	234.10^3	886.10^3	54.10^4	569968**
Salmonella	*Salmonella enterica*	546.10^3	768.10^3	657.10^3	869.10^2	998.10^3	745181**
	Others *Salmonella*	435.10^3	756.10^2	553.10^3	867.10^2	657.10^2	891659**
Stationary vendors							
Global contamination (FMAT)	-	987.10^3	10^4	354.10^3	953.10^2	765.10^2	2136641**
Total coliforms	*Escherichia coli*	546.10^3	435.10^3	867.10^2	657.10^2	888.10^3	1163814**
	Others *Enterobacteria*	876.10^2	987.10^2	132.10^2	2.10^4	897.10^2	111818**
Staphylococci	Negative Staphylococci coagulase	645.10^2	443.10^2	129.10^3	10^5	987.10^4	37525141**
	Staphylococcus aureus	234.10^2	325.10^2	657.10^2	276.10^2	879.10^2	66748**
Salmonella	*Salmonella enterica*	325.10^2	$154.6.10^2$	768.10^2	$.10^4$	324.10^2	82399.2**
	Others *Salmonella*	657.10^2	567.10^3	876.10^2	435.10^2	768.10^3	1488162**

Legend: *FMAT: Flora Mesophilic Aerobic Total; NSC: Negative Staphylococci Coagulase; CFU: Colony Forming Units, DF= 4; different significance levels of the X^2- test variable association *p<0.05; **p<0.001; otherwise it is not significant when the value obtained in X^2 is followed by no asterisk (p>0.05). Others: undetermined species.*

He adds that vegetables had unacceptable contamination levels of coliforms and *Staphylococcus aureus,* while meat (fish) from Nanyuki road had unacceptable levels of *coliforms.*

When an analysis of contamination vendor category, majority of products sampled and analyzed (Table 1), we note that ambulant vendors have mostly much contaminated dishes than other distributors (p<0.001). Among these walking the load in total mesophilic flora is very high in loaf 5.10^5 CFU/10g, sausages 344.10^4 followed by fresh milk 605.10^3 and roast fish 455.10^2 CFU/10g as well as total coliform bacteria (*Escherichia coli* and others), staphylococci (Negative Staphylococci Coagulase NSC and *Staphylococcus aureus*) and Salmonella *(Salmonella enterica* and others Salmonella) which are of the order of 10^2 and 10^3, in others street foods analyzed. But *Staphylococcus aureus* and NSC 2.10^5 and 21.10^4 and CFU/10g respectively are many in bread. Milk have an important microbial load in Salmonella 534.10^4 CFU/10g (Table 1). Mesophilic aerobic bacteria are present in foods in favor of a time/temperature favorable to their growth. All food (foodstuff) ready to eat may have been kept in too high temperature and/or too long **(SCAV, 2007).** According to the hygienic order, this also involves the cooked food (tolerance: 1 million per gram) than the mixed products (tolerance 10 million per g). From 100 million cells/g of food, it is considered corrupted and unhealthy for consumption.

Pathogens account for 77% of ambulant vendors against 33% and 26% respectively for semi-stationary and stationary sellers. Almost 61% contain coliforms. As for pathogenic E. *coli*, it is present to 83% in foods collected from the street. Similarly sulphite-reducing anaerobes are also found in these foods to 66% (Table 3). Note that, pathogenic E. *coli* bacteria normally found in the intestines of livestock. It is transmitted to humans through direct contact with infected feces, or by indirect contact, such as when we eat meat or fresh milk that came into contact with the bacteria during slaughter or treatment.

In semi-stationary vendors, only fresh milk has a large load in FMAT and coliforms, *Salmonella* sp. and *Staphylococcus* sp. exceeding the standards established by the Codex Alimentarius. The comparison of the three vendor categories shows that as microbial loads sample selected among stationary vendors contain anaerobic mesophilic aerobes and sulphite-reducing exceeding significant prescribed standards. This is in agreement with those of **Baba-Moussa *et al.* (2006)** in Benin, Cotonou city who notes in his study that street vendors have mostly much contaminated dishes than other distributors. This can be explained by the fact that food usually transported on the head are poorly covered and are subject to air pollution, given the density of car and motorcycle taxis in the city of Bukavu. These street vendors are usually forced to walk to the edge and along the roads that still resemble most aerosol products for mobile devices. More street vendors very often lack of water to rinse utensils with which they provide service. This is an aggravating factor of contamination. Also we note that in stationary vendors all samples contain mesophilic aerobic microorganisms and anaerobic sulphite-reducing exceeding the prescribed standards. This is explained by the presence of sidewalks,

sewage and garbage discarded in nearby streets pollutant of sale and the environment sellers with pathogens.

The heavy load foods *Salmonella* ssp. analyzed in all vendor categories exposes consumers to food poisoning. The presence of *Salmonella* sp. in street food threatens food security of the population. However, microbial loads observed for aerobic mesophilic flora, beyond June 10^6 germs set by **AFNOR** (French Association for Standardization), shows a significant level of contamination of street food vended in Bukavu city.

Table 2 Quantity determination of bacteria (CFU/10g) in some street foods collected by sampling sites

Bacteria (CFU/10g of food)	**Samples collected**	**Names of sampling sites**					**χ^2-test**
FMAT		**Bagira**	**Brasserie**	**Muhanzi**	**Kadutu**	**Ibanda**	**χ^2**
	Boiled meat	12.10^4	uncountable	348.10^2	28.10^4	265.10^3	61991306**
	Roasted fish	2.10^5	58.10^3	219.10^2	10^6	5.10^4	2604018**
	Sausages	349.10^4	uncountable	32.10^4	9.10^4	90880	267332207**
	Fresh milk	102.10^4	10^5	2.10^5	11.10^5	12.10^4	2016693**
	Loaf	657.10^2	768.10^3	987.10^2	435.10^3	765.10^2	1322772**
Total coliforms							
	Boiled meat	10^5	29.10^4	109.10^4	19.10^4	256.10^3	153503**
	Roast fish	17.10^4	2.10^4	10^3	29.10^4	265.10^3	484737**
	Sausages	19.10^4	129.10^3	176.10^3	165.10^3	176.10^3	12791.9**
	Fresh milk	123.10^3	209.10^3	10^5	176.10^3	234.10^3	75707.8**
	Loaf	657.10^3	987.10^3	546.10^3	866.10^2	908.10^3	796901**
Staphylococci							
	Boiled meat	234.10^2	128.10^3	287.10^3	29980	276.10^2	521814**
	Roast fish	17.10^4	1739.10^2	165.10^3	67.10^4	574.10^3	713705**
	Sausages	187.10^3	276.10^3	286.10^3	269.10^3	248.10^3	24703**
	Fresh milk	2554	5477	$37.6.10^2$	4538	7688	3121.45**
	Loaf	768.10^3	879.10^3	987.10^3	87.10^3	546.10^2	1452646**
Salmonella							
	Boiled meat	18.10^4	10^5	9807	6579	9870	389843**
	Roasted fish	98.10^3	298.10^3	254.10^2	435.10^2	29.10^3	536535**
	Sausages	23.10^3	287.10^3	38.10^4	254.10^3	65.10^3	457992**
	Fresh milk	76.10^3	64.10^3	69.10^3	98.10^3	231.10^2	45098.6**
	Loaf	987.10^2	876.10^4	98.10^4	547.10^2	987.10^3	1533501**

Table 3 Percentage of bacteria affecting consumer health for different vendor categories of Bukavu city

Vendor categories	**Pathogens bacteria (%)**	**Toxicogenic bacteria (%)**
Ambulant	77	75
Semi-stationary	33	60
Stationary	26	20

Epidemiological profile on the consumption of street food in Bukavu city

The figure 1 results exhibits the number of disease cases in people who have eaten street food register in Bukavu General Reference Provincial Hospital (HPGR-Bukavu) between 1992 and 2015. The last three years (2013- 2015) prevalence of people having eaten street food in Bukavu city is growing, it was 300 cases in September 2013, 350 cases in May 2014 and more than 380 cases in April 2015. This prevalence was almost the same between 2004 and 2006 but a decreasing manner (Figure 1), the worrying numbers for the city and public health.

Household surveys in developing countries have shown that the householder takes on average a meal out of the household. As indicated in the above tables that the quality of street food eaten outside the home is questionable, the consequences in terms of poverty are enormous. Indeed, the head of household, main breadwinner for the family has to take meals outside the home for various reasons. If the head of household was infected with a pathogen, consequences are immediate and harmful to his family. Thus, the quality of food consumed should be a priority for the fight against poverty **(Gohou, 2005).**

Most people who consumed these foods contaminated with Salmonella for example, show up at the hospital with symptoms of diarrhea, sometimes with blood in the stool; abdominal cramps; nausea; vomiting (sometimes); fever; chills; headache. Symptoms usually appear 6 to 72 hours after consumption of contaminated food. The symptoms usually last 3 to 7 days. There are several strains of E. *coli* bacteria and while most are harmless, some of them can lead to health problems more often, we talk of food poisoning (severe gastroenteritis), but also urinary tract infections observed in Bukavu city. Staphylococcus also causes a range of diseases mild skin infections such as boils, pimples, impetigo and cellulitis, more serious illnesses such as food poisoning, bacteremia (blood infection), Syndrome toxic shock and pneumonia (microbiologist **Claude Wakeka,** personal communication). Prevalence of 22% in *Staphylococcus aureus* mastitis were also detected in isolates in Turkey by **Murat *et al.* (2009).**

As noted in the document by **Mensah *et al.* (2012)**, well-coordinated surveillance data on foodborne diseases in Africa after consumption of street food is lacking. There are, however, data on a number of homes, as well as epidemiological studies in infants and the youth children suffering of diarrhea.

An outbreak of bloody diarrhea caused by *Escherichia coli* 0157 infections occurred in southern Africa with a prevalence of 42% among 778 residents who were studied. The consumption of meat, juice and fresh milk and untreated water were predisposing factors. According **Mensah *et al.* (1999)** in Accra, vendors were carriers of a variety of bacterial enteropathogens, including *Salmonella*. Defective personal hygiene can facilitate the transmission of these pathogens via food to humans. The serving stage is a critical point in the street food industry. Enteropathogens can survive on the hands for three hours or longer. Diarrhea pathogens on the hands of mothers can be transmitted to infants **(Mensah, 1997)**.

E. coli 0157: NM was recovered seven affected households having consumed street food vended in Swaziland and South Africa. Isolates from 27 of the 31 samples from patients and the environment was indistinguishable from gel electrophoresis patterns in pulsed field. Important factors that contributed to the outbreak have been droughts and wind causing the increased transport *E. coli* O157 by food sold on the street, heavy rain and contaminated surface water **(Effler *et al.*, 2001).**

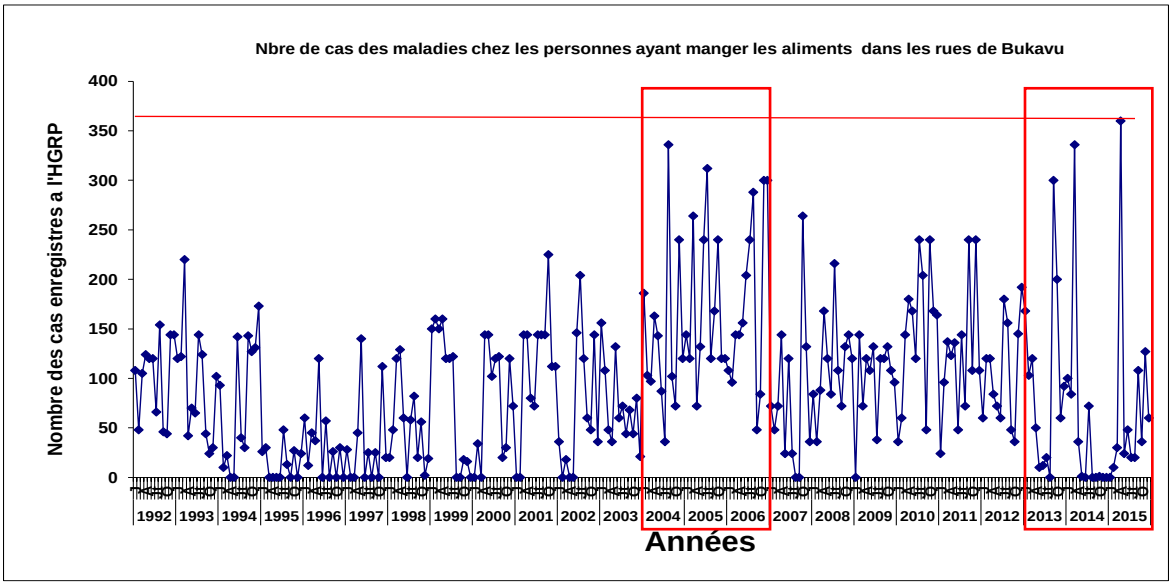

Figure 1 Number of illness cases recorded in Bukavu General Hospital between 1992 and 2015 for people who have eaten street foods in Bukavu city

Two other outbreaks occurred in Ghana in 2001. In the first forty-six Ghanaians who attended a funeral eaten apapransa (toasted corn flour dish with soup palm, fish and crabs) were admitted to hospital with diarrhea and vomiting. in the second home, about 20 people who ate rice dumplings with soup dumplings palm or corn with okra stew were admitted to hospital with diarrhea and vomiting. The likely cause of both outbreaks was not identified **(Ghanaweb, 2001).** E. *coli* was detected also in hand washings of high-income and low-income mothers in India at levels of 7.0 + 4.2 log10 CFU/ml and 9.0 + 5.7 log10 CFU/ml, respectively **(Mathur and Reddy, 1983)**. In Peru, E. *coli* was detected in 11 of 78 mothers' hand washings **(Black *et al.*, 1989)**. In Thailand, enterotoxigenic E. *coli* (ETEC) was detected in 6 of 42 mothers' hands and in 50 of 37 children's hands. The samples were from homes where children were suffering from ETEC diarrhea. In most instances the type isolated from diarrhea cases corresponded to that isolated from hands **(Echeverria *et al.*, 1987)**. During cholera epidemic in Mali in 1984, there were 1793 cases and 406 deaths. Case-control studies have identified three transmission routes: consumption of street food, drinking water the biggest village well and eat millet left in the drought-affected area in Mali **(Tauxe *et al.*, 1988).** Cholera is endemic in most countries, its transmission through food is appreciated, there is evidence to show that preparation and food handling by infected persons and physicochemical characteristics of growth of the food aid *Vibrio* sp. - high moisture content, a neutral/alkaline pH, the absence of competing bacteria allow transmission of *Vibrio cholerae.* There were 578 cases of *Shigella flexneri* in 2001, which have been associated with corn flour vended on public roads in South Africa. In 2002, botulism type A cause of canned fish contaminated tomato sauce is also two deaths in South Africa. A large outbreak of acute *aflatoxicosis* due to contaminated maize consumption took place in 2004 **(Nyikal *et al.*, 2004).** This affected more than 317 people and has a fatality rate is estimated at 39% around.

In 2008, an unprecedented number of street food-related events were reported to the Regional Office. These included anthrax in Zimbabwe; Typhoid fever and botulism in Uganda; beans and maize seed contaminated by chemicals in Kenya and Nigeria; poisoning by pesticides cabbage and other vegetables in Senegal; and *salmonellosis* due to the mouse fish in Mauritius. Others are poisonous mushrooms in Algeria; associated diarrhea Gala Dinner Meals in Nigeria; bromide poisoning in Angola and food poisoning in Nigeria, Madagascar, Angola, Kenya, Mauritius, Côte d'Ivoire, Benin, Congo, Ethiopia, Burkina Faso and Botswana. There were outbreaks of diarrhea in the Congo, Kenya, Madagascar, Burundi, Comoros, Uganda, Kenya, Botswana and Mozambique due to the consumption of street food. Fewer cases of foodborne outbreaks occurred in 2009. These keys were *shigellosis* in Malawi, Kenya and acute aflatoxicosis konzo DRC and Angola **(Mensah *et al.*, 2012)**.

Environmental and social characteristics of street food vendors in Bukavu city

Environment vendors

The results of estimating linear model (GLM, Table 4) exhibit there twelve variables statistically significant influent on the quality of the immediate vendors environment and their hygiene practices on microbiological quality of street food, it results in the exposure of food to houseflies (GLM: Z=1.27, p<0.05), the presence of stalls and improvised structures located along the sidewalks being the place of sale of street food (GLM: Z=7.22; p<0.001). The food vendors share the sidewalk with many other street vendors selling clothes, toys and especially sewage and garbage is discharged into the nearby streets of sale (GLM: Z=-5.35, p< 0.001). The presence of houseflies implies probable lack of adequate sanitation. This agreed with **(Muinde and Kuria, 2005)** who found houseflies in most of the street food stalls in Nairobi. **Mwadime, 2001** noted houseflies in 54.8% of the vending stalls. This implies that food contamination is most likely to occur despite efforts to keep the vending area clean. In a study conducted in Ghana by **Annan-Prah *et al.* (2011)**, food items were sold in the open-air which was dusty, near drainage gutters and some near garbage bins. **Paulson (1994)** reported that outbreaks are generally caused by foods due to poor personal hygiene of the vendors and that have been mishandled or mistreated during preparation or storage. Unhygienic surroundings like sewage, improper waste disposal system, and inadequate water supply attract flies and houseflies which further increases food contamination **(Chumber *et al.*, 2007).**

After cooking, foods are arranged on tables often even the floor (GLM: Z=-2.47, p<0.05) and briefly covered close to busy streets (GLM: Z=-1.91, p<0.05) and are reheated before being served for most of the time (GLM: Z=-2.99, p=0.003). The food cooking ahead of time, exposure to flies, and makes handling food on the floor and hand were identified as potential contamination factors of street food in Ghana **(Mensah *et al.*, 2002). Muinde and Kuria (2005)** reported about 85 % of the vendors prepared their foods in unhygienic conditions given that garbage and dirty waste were close to the vending stalls. Majority of the vendors left the food cool naturally which could lead to multiplication of microorganisms present in the food at the time of storage (**Gitahi, 2012)**.

Table 4 Generalized Linear Model (GLM) testing influence of independent factors (vendors quality and their environment hygienic practices) on the microbiological quality of street food vended (dependent variable) in urban zone of Bukavu city

GLM: *Gaussian identity model*						
Environmental quality	**Coef.**	**OIM Std. Err.**	**Z**	**P>|z|**	**[95% Conf. Interval]**	
Presence of sidewalks	.2917397	.0404132	7.22	**0.000**	.2125312	.3709481
Food wrapping nature	-.0821282	.0410271	-2.00	**0.045**	-.1625398	-.0017167
Selling food along street	-.0409156	.0428148	-0.96	0.339	-.1248311	.0429998
Presence of sewage and garbage	-.5120828	.0957703	-5.35	**0.000**	-.6997892	-.3243764
Spread food	-.0105502	.0042713	-2.47	**0.014**	-.0189217	-.0021787
Cooking foods before serving	-1.667467	.5585333	-2.99	**0.003**	-2.762172	-.5727623
Urban zone	-.0603794	.0316646	-1.91	**0.037**	-.1224409	.001682
Year to work	-.0241399	.0136762	-1.77	0.078	-.0509447	.0026649
Food proxy place vended	-.0115558	.0388798	-0.30	0.766	-.0877589	.0646473
Packaging type	-.0590675	.0434543	-1.36	**0.017**	-.1442362	.0261013
Food protection against insects	.000962	.0515662	0.02	0.985	-.1001058	.1020298
Work-related problem	.0322824	.015205	2.12	**0.034**	.0024811	.0620838
Hygiene service passage	.0004151	.0520125	0.01	0.994	-.1015275	.1023576
Exposing food to insects	.0868986	.0682821	1.27	**0.020**	-.0469319	.2207291
Handling food	.0107749	.0687665	0.16	**0.005**	-.124005	.1455548
Provision dishwater	-.0963449	.0594317	-1.62	**0.015**	-.212829	.0201391
Tools for serving food	.0010743	.0114172	0.09	**0.025**	-.0213029	.0234516
Constant	.2378941	.192922	1.23	0.021	-.1402261	.6160143

Other statistics : *Number of observations=333; Log likelihood = -377.7840824; AIC (Akaike's Information Criterion)= 1.37425 ; BIC (Schwarz's Bayesian Criterion) = -3421.5*

We see when tracking sales transactions that ambulant vendors and semi-stationary do not have enough water for dishes (GLM: Z=-1.62, p<0.05). The dish washing waters were sometimes the contamination source of street food vended with unacceptable levels (above 10^2) of different bacteria such as, coliforms and *Staphylococcus aureus* (P<0.05) **(Barro *et al.*, 2006)**. In addition the status and type of packaging used are poor (GLM: Z=-1.36, p<0.05) and are made of waste paper, moldy, inadequate plastic bags, cement bags of paper.

According **Gitahi (2012)** in industrial area of Nairobi, Kenya a total of 69% of vendors dump their wastes into Nairobi city council waste bins, while 79% use the Nairobi city council sanitary facilities. 87% of vendors used polythene bags for packaging take away rations. Majority of these vendors serve food with their hands (GLM: Z=0.09, p=0.025). **Carl *et al.* (2013)** states that some production and handling practices expose consumers to substantial health risks in Nairobi. The passage of health service on sale point has a positive influence on improving hygienic quality of the street food sellers environment, but not statically significant (p>0.05) (Table 4).

I terms of medical certificates none vendors (ambulant, semi-stationary and stationary) had food handlers' medical certificate. These results are very different from those obtained by **Gitahi (2012)** in Nairobi, were 24% (7/29) vendors had food handlers' medical certificate. And the levels noted in the streets of Accra Ghana (40%) by **Ackah *et al.* (2011)**. **Annan-Prah *et al.* (2011)** observed that 45% of street food vendors in Cape coast Ghana were not certified medically to handle food. As highlighted in the standard newspaper, Kenya of September 13, 2011, there is a need to ensure food handlers are immunized or treated against typhoid and other food borne illnesses. According **Mensah *et al.* (2012)** emphasizes that in the WHO African region, human factors including: unhygienic practices and deliberate contamination, environmental factors, such as unsafe water, unsafe waste disposal and exposure of food to insects and dust, undercooked food, and prolonged storage of cooked food without refrigeration are the main predisposing factors for street foods contamination.

Social characteristics of street food vendors

Table 5 summarizes results obtained in the survey on the social characteristics of vendors. We can see that the vast majority of street vendors (84%) are women, a Congolese national, South Kivu Province which generally comes from the outskirts of Bukavu. This result is not surprising for an African country like DRC in general and the South Kivu province in particular where sociologically food cooking is reserved for women. It is similar to the 71.22% obtained in Burkina by **Barro *et al.*, (2002),** similar to that of **Baba-Moussa *et al.* (2006)** in Benin, Cotonou city, but different from that obtained by **Khairuzzaman *et al.* (2014)** in Bangladesh, which states in its study both males and females, married and unmarried operate as street food vendors.

Indeed, it is true that women play a very vital role in the street food sector through their direct and/or indirect involvement in the business. Additionally a significant number of street vendors are woman-headed households **(Ackah *et al.*, 2011; WIEGO, 2014)**. The women engage in the sale of food because it is an activity that does not require at least in our African countries to have a high intellectual level or even be literate. It is not possible to suit the absolute number of street food vendors in Bukavu city, as was also the case in Dhaka by **Benjamin (2011),** India by **Bhowmik (2010).** 44% of these vendors are ambulant, 30% are semi-stationary, and 26% stationary. Nearly 78% are illiterate and most (60%) received no training for sale. By cons, in Ouagadougou city only 50% are illiterate, 3.57% achieved the top level **(Barro *et al.*, 2002).** The level of education achieved by the street food vendors in Bangladesh is comparatively low and in the case of a majority, education levels varied between grades 5 and 8 **(Khairuzzaman *et al.*, 2014)**. This lack of education has consequences for the ignorance of the basic rules of hygiene and sanitation in these sellers. Our study shows that only 8% of the saleswomen wear caps and aprons but many of them are clean (73%). Unfortunately (80%) of them serve food with their hands. Also, at the same time they serve food they handle currency money which are for the dirtiest and germ carriers. This practice can lead to bacterial contamination inherent to the safety of food. Hands carry most of the fecal contamination of germs time (E. *coli*, other heat resistant) which are often responsible for diarrheal diseases and gastroenteritis.

This has already been proven in several studies: **FAO/WHO (1990),** in Lebanon, **Harakeh *et al.*, (2005)** results showed that the meat-based fast food were contaminated with *Salmonella paratyphi* (serogroup A) and *Shiga Toxin* (Stx)-producing E. *coli* (STX-EC) and in Nigeria **Tjoa *et al.*, (1977)**, **Owhe-Ureghe *et al.*, (1993)** report the case. The selling part is very safe. Sewage and garbage is discarded in nearby streets selling places, attracting so as disease vectors. The consumption service and Veterinary **(SCAV, 2007)**-Neuchâtel, **Khairuzzaman *et al.*, (2014)** for Bangladesh and **Okonko *et al* (2009)** for Nigeria, confirmed that contact with equipment and utensils not washed or disinfected; the use of multi-use hand towels; handling of foodstuffs with unwashed hands; inadequate cooking; too slow cooling before storage; too high temperature or too long conservation are street food contamination modes aerobic mesophilic bacteria (FMAT).

In South Kivu, Bukavu city as in most African cities sub-Saharan informal food sector is an important source of employment in urban areas, particularly for people who do not have a high level of education and which would have had trouble finding a skilled job. This sector generates annual daily business of several million Congolese francs in a country like the DRC. This street food is cheaper and allows households to save on their daily income, for the same meal would cost 3 to 5 times more than in a traditional restaurants (formal sector), or even at home. But can we say that these people really are saving? The question needs to be asked because this power sometimes has adverse effects on the economy arising from medical expenses caused by food toxi-infections, work absences and can even generate casualties.

An international workshop was held in Ouagadougou on 22-24 November, 1999 on the theme, "the food industry for a healthy nutrition in West Africa." One of the sub theme was "street/new foods to food production, marketing summons, quality and health effects." Several papers under this theme showed that the street food sector is growing while the products offered are not always of good

quality **(Umoh and Odoba, 1999).** And one of the recommendations of this workshop was to improve the nutritional, hygienic and organoleptic quality of food produced by small units of transformations to provide the populations of urban centers healthy and balanced products.

Table 5 Social characteristics of vendors

Characteristics of vendors	N=320	Proportion (%)
	Gender	
Men		16
Women		84
	Vendor categories	
Ambulant		44
Semi-stationary		30
Stationary		26
	Level of education	
Illiterate		78.67
Primary/Literate		18.67
Secondary/Higher		2.67
	Types of training	
Technical		3.33
On the job		36
Without level		60.67

CONCLUSION

This study aimed to control the hygienic quality of street foods vended in urban zones of Bukavu city and assess the potential health risks to consumers in South Kivu province, DR Congo in general. This prospective study was conducted among street vending food from 320 vendors in three urban zones of Bukavu city. From April to July, 2015, a total of 80 food samples compressing boiled meat (16), roast fish (18), sausages (21), fresh milk (13) and loaf (12) from 320 vendors were purchased and analyzed. Standard microbiological methods were used for isolation, enumeration and identification of bacteria.

Investigations on the sales premises and microbiological test results revealed the presence of a perpetual risk of contamination as study sites and vendors 'categories. The results of this study indicate that all street food samples tested are contaminated to varying degrees by isolated and identified bacteria including: FMAT, Total coliforms with *Escherichia coli, Staphylococcus* species with Negative Staphylococci coagulase and *Staphylococcus aureus* and *Salmonella* species with *Salmonella enterica.*

The quantities of bacteria obtained by CFU/10g of each food taken exceed the standards established by the Codex Alimentarius, very important and significant for different vendors categories and sampling sites ($p<0.0001$). Food samples collected from vendors in Kadutu urban zone (the most popular and unhealthy city) are more contaminated. The foods that are not subjected to heating during the preparation have the highest microbial load. This is the case where fresh milk the total mesophilic flora is of the order of 10^5 and varies the sampling sites. This is also the case of the foodstuff after cooking are exposed for a long time at room temperature: boiled meat and sausages contain an uncountable number of bacteria. But products like loaf has a total mesophilic flora equal to 657.10^2, 435.10^3 and 765.10^2 CFU/10g in Bagira, Kadutu and Ibanda urban zones respectively. Total coliforms, *Staphylococcus* sp, and *Salmonella* sp. are more numerous in the boiled meat, fresh milk and sausages.

When had a contamination analysis by vendor category, the majority of products sampled and analyzed, we see that ambulant vendors have mostly dishes much more contaminated than other distributors ($p<0.001$). They are followed by semi-stationary and stationary vendors respectively. It was found also that the contamination of street food is multifactorial and vendors' hygiene contribute significantly to contamination factor, including unhygienic managers, dirty surroundings and poor water quality.

Epidemiological findings show that the prevalence of diseases recorded in Bukavu General Hospital "HPGR", people having eaten street food in Bukavu city is growing for the last three years (2013-2015), it was 300 cases in September 2013, 350 cases in May 2014 and more than 380 cases in April 2015. This prevalence was almost the same between 2004 and 2006 but a decreasing manner. These results are part of an approach similar to those for other cities in black Africa and including Kisangani by (**Kama, 2014; Manzilima, 2011)** for Nigeria by **Oranusi *et al.* (2003), Okonko *et al.* (2008a, b, c),** and a few years ago **Mensah *et al.* (2012).**

In general, interventions and programmes can only be successful if they do not focus on one aspect alone. Tackling only food quality, for instance, cannot ensure that street food vendors play the most positive role in realizing food security of the urban population. It is important not to forget that the street foods constitute a very heterogeneous sector and the interventions need to be carefully planned by keeping different aspects such as gender, secondary audience, and local customs into consideration. It is also necessary to differentiate between vendors selling freshly prepared food on the spot or hawking dishes prepared earlier at home, with the second practice being much more risky in terms of foodborne pathogen and spores. Needless to say, general education levels also play an important role in ensuring safe street foods. The more both vendors and patrons will be educated and the more they will know about issues such as nutrition and food safety, the more they will be interested in having the business as clean and the products as healthy as possible.

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REFERENCES

Ackah, M. E. T., Gyamfi, A. K. Anim, J., Osei, J. K., Hansen & Agyemang, O. (2011). Socio-Economic Profile, Knowledge of Hygiene and Food Safety Practices among Street-Food Vendors in some parts of Accra-Ghana. *Internat. Journal of Food Safety, 13:* 191-197.

Adams, M. & Motarjemi, Y. (1999). Basic food safety for health workers. *WHO*, Geneva, 121pp. http://apps.who.int/iris/handle/10665/65992

Adebolu, T. T. & Ifesan, B. O. (2001). Bacteriological quality of vegetables used in salads. *Niger. J. Microbiol. 15 (1):* 81-85.

AFNOR (1996). Analyse microbiologique : Méthodes horizontales. Paris : Association Française de Normalisation (AFNOR) : 1, 521 pp.

Annan-Prah, A. D. H., Amewowor, A. K., Osei-Kofi, J., Amoono, S. E., Akorli, S. Y., Saka, E. & Ndadi, H. A. (2011). Street foods: Handling, hygiene and client expectations in a World Heritage Site Town, Cape Coast, Ghana. *African Journal of Microbiology Research, 5(13):* 1629-1634.

Baba-Moussa, L., Bokossa, Y. I., Baba-Moussa, F., Ahissou, H., Adeoti, Z., Yehouenou, B., Mamadou, A., Toukourou, F. & Sanni, A. (2006). Etude des possibilités de contamination des aliments de rues au Benin : Cas de la ville de Cotonou. *J. Rech. Sci. Univ. Lomé (Togo), 8(2):* 149-156.

Babe, T., Munyuli B. M., Ombeni, B. J., Kashosi M. T., & Mwangi, T. B. (2018). Hygienic quality assessment of fresh beef meat in Bukavu urban slaughterhouses, south Kivu province of the long sale chain: potential health risks for consumers eastern D.R. Congo. *Bacterial Empire, SciCell, 1(1) :* 1-9.

Barro, N. & Traore, S. A. (2002). Aliments de rue au Burkina Faso : caractéristique des vendeurs et de consommateurs, salubrité des aliments de rues et santé des consommateurs. *Rapport CRSBANSADAOC sur l'alimentation de rue au Burkina-Faso*, 49 pp. http://www.pathexo.fr/documents/articles-bull/T102-1-3267-5p.pdf

Barro, N., Bello, A. R., Savadogo, A., Ouattara, C. A. T., Ilboudo A. J. & Traoré, A. S. (2006). Hygienic status assessment of dish washing waters, utensils, hands and pieces of money from street food processing sites in Ouagadougou (Burkina Faso). *African Journal of Biotechnology, 5 (11):*1107-1112.

Barro, N., Cheik, A. T. O., Nikiema, P. A., Ouattara, A. S. & Traore, A. S. (2002). Evaluation de la qualité microbiologique de quelques aliments de rue dans la ville d'Ouagadougou au Burkina-Faso. *Cahiers Santé, 12(4):* 369-374.

Benjamin, E. (2011). Street Food Governance in Dhaka (Bangladesh): the appropriation of street vending spaces and the informal politics of exploitation,

in *Proceedings of the International RC21 Conference, Session 27, Amsterdam, The Netherland, July 2011.* http://www.bip.org.bd/SharingFiles/journal_book/20140427160039.pdf
Bergdoll, M. S. (1979). Staphylococcal intoxications. In: Riemann H, Bryan F (eds.). Food-Borne infections and intoxication. *Academic press, New York,* 444-490.
Bhowmik, S. (2010). Street Vendors in the Global Urban Economy, Routledge: *Taylor & Francis, New Delhi, India*, 2010. https://www.taylorfrancis.com/books/9780203150542
Black, R. E., Lopez de Romana, G., Brown, K. H., Bravo, N., Bazalar, O. G. & Kanashiro, H. C. (1989). The incidence and etiology of infantile diarrhea and major routes of transmission in Huascar, Peru. *American Journal of Epidemiology; 189:*785-99.
Canet, C. & N'diaye, C. (1996). L'alimentation de rue en Afrique. *FNA/ANA, 17(18):* 4-13.
Carl, J. L., Hess, S., Okello, J., Hansson, H. & Karanja N. (2013). Food health risk perceptions among consumers, farmers, and traders of leafy vegetables in Nairobi. *Food Policy* 38 (2013): 92–104.
Chauliac, M., Bricas, N., Ategbo, E., Amoussa, H. W. & Zohoun, I. (1998). L'alimentation hors du domicile des écoliers de Cotonou (Bénin). *Cahiers Santé, 8* : 101-108.
Chibundu, N. E., Funmi, O., Kayode, Stephen, O., Fapohunda, Momodu, F., Olorunfemi & Barinaada T. Kponi1 (2012). Aflatoxigenic moulds and aflatoxins in street-vended snacks in Lagos, Nigeria. *Internet Journal of Food Safety, 14(2014):* 83-88.
Chirag, G. K., Lakshmi, B. & Avanish, K. (2013). Study of Hygienic practices of street food vendors in Allahabad city, India and Determination of Critical control points for safe street food. *The Allahabad Farmer, LXVIII (2):*125-132.
Chumber, S. K., Kaushik, K. & Savy, S. (2007). Bacteriological analysis of street foods in Pune. *Indian J. Public Health, 51(2):* 114-6.
Dexter D. R., Buted, R. & Alex, P. & Ylagan (2014). Street Food Preparation Practices. *Asia Pacific Journal of Education, Arts and Sciences, 1(2):*53-60.
Diallo, M. L. (2010). Contribution à l'étude de la qualité bactériologique des repas servis par Dakar catering selon les critères du groupe servir. Thèse de doctorat en vétérinaire (diplôme d'Etat), Faculté de Médecine, de Pharmacie et d'Odonto-Stomatologie de Dakar (grand Amphi), 101pp.
Dione, A. (2000). Contribution à l'étude de la qualité bactériologique de quelques denrées alimentaires d'origine animale commercialisées sur le marché dakarois. *Dakar : Thèse : Med. Vét., (3),* 120 pp.
Dunn, R. A., Hall, W. N., Altamirano, J. V., Dietrich, S. E., Robinson-Dunn, B. & Johnson, D. R. (1995). Outbreak of *Shiegella flexneri* linked to salad prepared at a central commissary in Michigan. *Public Health Reports. 110 (5):* 580-586.
Echeverria, P., Taylor, D. N., Seriwatana, J., Leksomboon, U., Chaicumpa, W., Tirapat, C. et al. (1987). Potential sources of enterotoxigenic Escherichia coli in homes of children in Thailand. *Bulletin of the World Health Organization, 65:*207-15.
Effler, E., Isaäcson, M., Arntzen, L., Heenan, R., Canter, P., Barrett, T., Lee, L., Mambo, C., Levine, W., Zaidi, A., & Griffin, P. M. (2001). Factors contributing to the emergence of *Escherichia coli* O157 in Africa. *Emerg Infect Dis, 7(5):* 812–819,
Ekanem, E. O. (1998). The street food trade in Africa: Safety and socio environmental issues. *Food Control. 914:* 211-215.
FAO (1989). Les aliments vendus sur la voie publique. Rapport d'une consultation d'experts FAO : *Alimentation et Nutrition, Indonésie Jakarta, (46)*, 96 pp.
FAO (1997). Street foods (FAO food and nutrition paper) - Alimentation de rue (Étude FAO alimentation et nutrition) - Alimentos que se venden en la vía pública (Estudio FAO alimentación y nutrición), http://www.fao.org/docrep/W4128T/W4128T00.HTM
FAO (1998). Food and Nutrition. Paper n° 46, street foods, FAO Report, Yogiakarta, Indonesia. www.fao.org/docrep/W4128T/w4128t14.htm
FAO (2007). Promesses et défis du secteur alimentaire informel dans les pays en développement, http://www.fao.org/docrep/011/a1124f/a1124f00.htm
FAO (2009). Les bonnes pratiques d'hygiène dans la préparation et la vente des aliments de rue en Afrique. Outils pour la formation, http://www.fao.org/docrep/010/a0740f/a0740f00.htm
FAO/OMS (1990). Draft code of hygienic practice for the preparation and sale of street foods. Programme mixte FAO/OMS sur les norms alimentaires *ALINORM, 91/15, Appendix II*, Rome. 113pp. http://www.scirp.org/(S(351jmbntvnsjt1aadkposzje))/reference/ReferencesPapers.aspx?ReferenceID=1169260
Feglo, P. & Nkansah, M. (2010). Bacterial load on Ghanaian currency notes. *African Journal of Microbiology Research, 4(22)*:2375-2380.
Feglo, P. & Sakyi, K. (2012). Bacterial contamination of street vending food in Kumasi, Ghana. *Journal of Medical and Biomedical Sciences, 1(1):* 1-8.
Ghanaweb, (2001). http://www.ghanaweb.com/GhanaHomePage/NewsArchive/artikel.php?ID=17141 (Accessed 11 March 2011).
Gitahi, M. G. (2012). Microbial quality, strain distribution and enterotoxigenicity of selected food borne pathogens in relation to the hygienic practices in industrial area, Nairobi, Kenya. MSc thesis, department of Food Science, Nutrition and Technology, Faculty of Agriculture, College of Agriculture and Veterinary Sciences, *University of Nairobi*, 92pp.
Gohou, G. (2005). Hygiène des aliments et développement soutenable : impact du monde invisible (microscopique) sur la réduction de la pauvreté ; CESS Institute, Québec, Canada.
Harakeha, S., Hadi, Y., Maya, G., Elie, B., Shadi, H., El-Fadeld, M., Imad, T. & Raja, T. (2005). Isolation, molecular characterization and antimicrobial resistance patterns of Salmonella and Escherichia coli isolates from meat-based fast food in Lebanon. *Science of the Total Environment, 341(2005):*33– 44.
Hogue, B. A., Hallman, K., Levy, J., Bouis, H., Ali, N., Khan, F., Khanan, S., Kabir, M., Hossain, S. & Alam, M. S. (2006). Rural drinking water at supply and household levels: Quality and management. *Int. J. Hyg. Environ. Health. 209:* 451- 460.
ICMSF-International Commission on Microbiological Specification for Food (1974). Sampling for Microbiological Analysis Principles and Specific Application. *University of Toronto Press, Toronto*, 1-18.
Kama, K. C. (2014). Qualité hygiénique des aliments vendus sur la voie publique à Kisangani : Indicateur de contamination : Flore Mésophile Aérobie Totale (FMAT) et Spore de *Bacillus* (Cas du marché central de Kisangani). 22 pp. http://www.cd.chm-cbd.net/aliments/kisangani/Introductio-conclusion.pdf
Khairuzzaman, Md., Fatema M. C., Sharmin Z., Arafat, A., Mamun & Latiful, Md. B. (2014). Food Safety Challenges towards Safe, Healthy, and Nutritious Street Foods in Bangladesh. *International Journal of Food Science, vol. 2014,* 9 pp.
Manzilima, P. A. (2011). Les alimentations de rue à Kisangani, mémoire, Faculté de médecine, *UNIKIS*, 22pp. http://www.cd.chm-cbd.net/aliments/kisangani/Introductio-conclusion.pdf
Murat, K., Mehmet, N., Burhan, A. & Etinkaya, C. (2009). Investigation of Toxin Genes by Polymerase Chain Reaction in Staphylococcus aureus Strains Isolated from Bovine Mastitis in Turkey. *Food borne Pathogens and Disease, 6(8):* 1029-1035.
Mathur, R. & Reddy, V. (1983). Bacterial contamination of infant foods. *Indian Journal of Medical Research; 77:*342-6.
Mensah P. (1997). Persistent diarrhoea in Ghana. (Report submitted to Japan International Cooperation Agency; 1997.
Mensah, P., Mwamakamba, L., Mohamed, C. & Nsue-Milang, D. (2012). Public health and food safety in the WHO African region. *African Journal of Food, Agriculture, Nutrition and Development, 12(4):* 6317-6335.
Mensah, P., Owusu-Darko, K., Yeboah-Manu, D., Ablordey, A., Nkrumah, F. K. & Kamiya, H. (1999). The role of street food vendors in the transmission of enteric pathogens. *Ghana Medical Journal;* 33:19-29.

Mensah, P., Yeboah-Manu, D., Kwaku O-D. & Ablordey, A. (2002). Street foods in Accra, Ghana: how safe are they?. *Bulletin of the World Health Organization, 80 (7):* 546-554
Muinde, O. K. & Kuria, E. (2005). Hygienic and sanitary practices of vendors of street foods in Nairobi, Kenya. AJFAND online www.ajfand.net *5 (1):* 1-1.
Mwadime, E. N. M. (2001). Nutritional and Safety Quality of street foods in Korogocho and Industrial area of Nairobi, Kenya. *Msc. Thesis.* pp 39-54.
Ngabet, N. H. V. (2001). Contribution à l'étude de la qualité microbiologique du lait fermenté « KOSSAM » commercialisé dans les rues de Yaoundé (Cameroun). *Dakar : Thèse : Med. Vét., (11),* 70 pp.
Noel, J. M. (2013). Insalubrité: la marque de fabrique de nombreux restaurants à bas prix à Gitega, Bujumbura (Gitega). *IWACU.* http://www.iwacu-burundi.org/insalubrite-nombreux-restaurants-a-bas-prix-gitega/
Nyikal, J., Misore, A., Nzioka, C., Njuguna, C., Muchiri, E., Njau, J., Maingi, S., Njoroge, J., Mutiso, J., Onteri, J., Langat, A., Kilei, I. K., et all. (2004). Outbreak of Aflatoxin Poisoning, Eastern and Central Provinces, Kenya, January-July 2004, *MMWR Weekl, 53(34):*790-793.
OIT (1972). Employment, incomes and equality: A strategy for increasing productive employment in Kenya. *Genève*. http://www.wiego.org/publications/employment-incomes-and-equality-strategy-increasing-productive-employment-kenya
Okojie, P. W. & Isah, E. C. (2014). Sanitary Conditions of Food Vending Sites and Food Handling Practices of Street Food Vendors in Benin City, Nigeria: Implication for Food Hygiene and Safety. *Journal of Environmental and Public Health, 2014, Article ID 701316,* 6 pp.
Okonko, I. O., Adejoye, O. D., Ogun, A. A., Ogunjobi A. A., Nkang, A. O. & Adebayo-Tayo, B. C. (2009). Hazards analysis critical control points (HACCP)

and microbiology qualities of sea-foods as affected by handler's hygience in Ibadan and Lagos, Nigeria. *African Journal of Food Science, 3(2):*035-050.
Okonko, I. O., Adejoye, O. D., Ogunnusi, T. A., Fajobi, E. A. & Shittu, O. B. (2008a). Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria. *Afr. J. Biotechnol. 7 (3):* 617-621.
Okonko, I. O., Ogunjobi, A. A., Fajobi, E. A., Onoja, B. A., Babalola, E. T. & Adedeji, A. O. (2008b). Comparative studies and microbial risk assessment of different Ready-to-Eat (RTE) frozen sea-foods processed in Ijoraolopa, Lagos State, Nigeria. *Afr. J. Biotechnol. 7 (16):* 2898-2901.
Okonko, I. O., Ogunjobi, A. A., Adejoye, O. D., Ogunnusi, T. A. & Olasogba, M. C. (2008c). Comparative studies and Microbial risk assessment of different water samples used for processing frozen sea-foods in Ijoraolopa, Lagos State, Nigeria. *Afr. J. Biotechnol. 7 (16):* 2902-2907.
Oluwafemi, F. & Simisaye, M. T. (2005). Extent of microbial contamination of sausages sold in two Nigerian cities. In: the Book of Abstract of the 29th Annual Conference and General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by Nigerian Society for Microbiology (NSM), *University of Agriculture, Abeokuta, from 6-10th November, 2005.* 28 pp.
Omemu, A. M. & Bankole, M. O. (2005). Ready-to-eat (RTE) vegetable salad: effect of washing and storage temperature on the microbial quality and shel-life. In: the Book of Abstract of the 29th Annual Conference and General Meeting (Abeokuta 2005) on Microbes As Agents of Sustainable Development, organized by *Nigerian Society for Microbiology (NSM), UNAAB, from 6-10th Nov, 2005*. 22pp.
Oranusi, S. U., Umoh, V. J. & Kwaga, J. K. P. (2003). Hazards and critical control points of kunun-zaki, a non-alcoholic beverage in Northern Nigeria. *Food Microbiol. 20:* 127-132.
Owhe-Ureghe, O. E., Ekundayo, A. O., Agboniahor, D. E., Oboh, P. A. & Orhue, P. (1993). Bacterial examination of Somme ready-to-eat foods marketed in Ekpoma, Edo state of Nigeria. *Nigeria Food J., 11:* 45-52.
Paulson, D. S. (1994). A comparative evaluation of different hand cleansers. *Dairy Food Environ. Sanit., 14:* 524-528.
Rakotondramanana, N. H. (1998). Aperçu sur la qualité bactériologique des aliments préparés et vendus sur la voie publique à Antananarivo. Université d'Antananarivo, *ESSA, dép. Elevage : Mémoire de fin d'étude*. www.beep.ird.fr/collect/eismv/index/assoc/TD06.../TD06-35.pdf
Ranaivoarimanana, R. L. O. (2006). Contribution à l'étude de la qualité microbiologique d'un aliment de rue dans la ville de talatan'ny volonondry (Madagascar) : cas du "koba ravina". *Thèse de Docteur vétérinaire (diplôme d'Etat), Faculté de Médecine, de Pharmacie et d'Odonto-Stomatologie de Dakar*. 100 pp. www.beep.ird.fr/collect/eismv/index/assoc/TD06.../TD06-35.pdf
Ravaonindriana, N. (1999). Qualité bactériologique d'un aliment de rue commercialisé dans la ville d'Antananarivo : Cas des glaces et crèmes glacées. *Archives Institut Pasteur de Madagascar, 65(1) :* 39 pp.
SCAV (2007)-Service de la consommation et des affaires vétérinaires-Neuchâtel. Les germes aérobies mésophiles. J-Martin Ducommun/fichepca.doc/2007.
https://www.ne.ch/autorites/DDTE/SCAV/denrees-alimentaires/Documents/FicheAerobies.pdf
Sobel, J., Mahon, B., Mencluz, C. E., Passaro, D., Cano, I., Baier, K., Raciopp, I., Hutwagner, L. & Mintz, E. (1998). Reduction of fecal contamination of street-vended bereages in Guatemala by a simple system for water purification and storage, handwashing, and barrage storage. *American Journal of tropical Medicine Hygiene, 59 (3):* 380-387.
Soumare, I. G. (1997). Contribution à l'étude de la qualité hygiénique des eaux de boissons vendues sur la voie publique à Dakar. *Dakar : Thèse : Med. Vét., (10),* 84 pp.
Speck, M. L. (1976). Compedium of methods for examination of food microbiological. *American Pub Health Assoc (Washington DC):* 417-23.
Tauxe, R. V., Holmberg, S. D., Dodin, A., Wells, J. V. & Blake, P. A. (1988). Epidemic cholera in Mali: high mortality and multiple routes of transmission in a famine area. *Epidemiol. Infect., 100(2):*279-89.
Tjoa, W. S., Dupont, H. L. & Sullivan, P. (1977). Location of food. Consumption and travelless diarrhea. *Am. J. Epidemiol., 106:* 61-66.
Trevett, A. F., Carter, R. C. & Tyrrel, S. F. (2005). The importance of domestic water quality management in the context of faecal- oral disease transmission. *J. Water Health. 3:* 259- 270.
Umoh, V. J. & Odoba, M. B. (1999). Safety and quality evaluation of street foods sold in Zaria (Nigeria). *Food Contr., 10:* 9-14.
WHO (1996). Essential Safety Requirements for Street vended foods. OMS, 2001. Salubrité des aliments. Organisation Mondiale de la santé. Food Safety Unit, Genève, 41 pp. www.who.int/foodsafety/publications/street-vended-food/en/
WIEGO (2014). Street Vendors in Women in Informal Employment: Globalizing and Organizing (WIEGO), http://wiego.org/informaleconomy/occupational-groups/street-vendors