Original Research Article

An overview of food safety and bacterial and fungi foodborne zoonoses in food production pig meat in the slaughterhouses of Kinshasa, Democratic Republic of the Congo

Abstract

Food-producing animals are the major reservoirs for many foodborne pathogens such as Campylobacter species, non-Typhi serotypes of *Salmonella enterica*, Shiga toxin producing strains of *Escherichia coli*, and *Listeria monocytogenes*. The zoonotic potential of foodborne pathogens and their ability to produce toxins causing diseases or even death are sufficient to recognize the seriousness of the situation. This research the evidence that links animals as vehicles of the foodborne pathogens *Candida albicans, Citrobacter freundii, Escherichia coli, Enterobacter agglomerrans, Klebsiella oxytoca, Proteus mirabilis, Salmonella spp, Shigella dysenteriae, Staphylococcus aureus* et *Yersinia enterocolitica* their current status. We conclude that these pathogenic bacteria will continue causing outbreaks and deaths throughout the world, because no effective interventions have eliminated them from animals and food. This study contributes to improving the health of the meat consumed by the population Kinoise and popularization of national and international opinion about the danger posed by the consumption of this meat if there is infection. Matete market with 42.8% of samples contaminated with *Staphylococcus aureus*, three out of six samples shall be declared unfit for human consumption and samples from other sites should also be declared unfit for consummation because the count reveals the threshold recommended by the FAO. This is 33.3 % (case 2/6) of sample contaminated with *Staphylococcus aureus*, Central Market 33.3% *Staphylococcus aureus* and *Salmonella spp*), Gambela 33.3% (*Staphylococcus aureus a*nd *Salmonella spp*), Liberté market 20% contaminated with *Salmonella* spp samples.

Keywords: Pork, *Enterobacteriaceae*, Mushrooms, slaughterhouse, FAO

INTRODUCTION

Pathogens shared with wild or domestic animals cause more than 60% of infectious diseases in man (Taylor et al., 2001). Such pathogens and diseases include leptospirosis, cysticer cosis and echinococcosis, toxoplasmosis, anthrax, brucellosis, rabies, Q fever, Chagas disease, type A infl uenzas, Rift Valley fever, severe acute respiratory syndrome (SARS), Ebola haemorrhagic fever, and the original emergence of HIV (IRLI, 2012; Grace et al., 2011; Molyneux et al., 2011; WHO, 2006; Karesh et al. 2005; Daszak et al., 2000). Zoonotic diseases are often categorized according to their route of transmission (vector-borne or foodborne), pathogen type (micro parasites, macro parasites, viruses, bacteria, protozoa, worms, ticks, or fleas), or degree of person-to-person transmissibility (Lloyd et al., 2009).

Food-producing animals (chickens, pigs, and turkeys) are the major reservoirs for many foodborne pathogens such as Campylobacter species, non-Typhi serotypes of Salmonella enterica, Shiga toxin-producing strains of *Escherichia coli*, and *Listeria monocytogenes*. The zoonotic potential of foodborne pathogens and their ability to produce toxins causing diseases or even death are sufficient to recognize the seriousness of the situation. Foodborne pathogens cause millions of cases of sporadic illness and chronic complications, as well as large and challenging outbreaks in many countries and between countries. The magnitude of this problem is demonstrated by the significant proportion of the 1.5 billion annual diarrheal episodes in children less than 3 years of age that are caused by enteropathogenic microorganisms, which results in more than 3 million deaths per year (EFSA-ECDC, 2016).

However, it is estimated that the reported incidence of food-borne disease represents less than 1% to 10% of the real incidence (Scallan et al., 2011). The importance of food-producing animals as carriers of pathogenic bacteria is real; for example, beef is reported to be the vector of 7% of the 1.7 million cases of foodborne disease that was recorded during 1996 to 2000 in England and Wales (Anderson et al., 2009).

The increase of human population and urbanization, the per capita income, the globalization, the changes on consumer trends (more protein in the diet) have increased the consumption of animal products (Dhama et al., 2013). Estimations suggest that consumption of these products will rise to 376 million tons by 2030 (Dhama et al., 2013).

This high demand of animal products provokes intensive animal production and processing of products, with an increased movement of foods globally. This situation could conduce to defective processing practices and an augment of the risk of contamination by foodborne pathogens at any point of the farm to fork chain. Animal and animal products contamination is a serious concern because it is difficult to control. Many factors could be involved in contamination, including these from the environment (associated fauna, water from different sources, and animal manure disposal, etc.), and human related animal handling (slaughtering and processing practices, and storage procedures, etc.) (Sofos, 2008).

In the Democratic Republic of the Congo (DRC), suspect outbreaks of foodborne disease are reported in different report. In most cases, food-borne zoonoses aetiologies remain undetermined, and the relative disease burden compared to other infectious diseases cannot be readily.

Some characteristics of animal production and food consumption habits in DRC that may promote zoonotic disease transmission include: (1) high density of both human and animal populations living in close proximity;(2) a predominance of smallholder production systems with mixed species and little/no biosecurity; (3) the presence of abattoirs and wet markets operating with rudimentary hygiene, limited cold chain for distribution and low levels of meat inspection; (3) widespread consumption of raw/undercooked blood, meat, fish, organ tissues, raw leaf vegetables and wild animal products and (4) use of untreated wastewater and sewage for agriculture.

Pig production and slaughtering systems, however, have not evolved at the same pace as pig population growth. Pigs are typically raised extensively or semi-extensively, although more and more professional pig farmers are converting to a complete intensive system. Due to the absence of professional pig slaughterhouses, pigs are generally slaughtered on slaughter slabs or in the streets. According to the Animal Slaughterhouse and Meat Inspection Act, all slaughtered animals and meat should be examined by a meat inspector. However, the Government of DRC has so far been unable to implement these regulations.

Pigs are known to be a source of several zoonotic agents, which may be transmitted to humans by direct contact or by indirect transmission pathways. Some of the agents are transmitted by the

food-borne route through faecal contamination of meat during the slaughtering process. Besides bacteria and parasites, pigs harbour a variety of viruses in their gastrointestinal tract, which do not necessarily cause disease in the animals (CDC, 2015; Kreuzer et al., 2012). Some of the viruses are closely related to human viruses and are therefore suspected to have a zoonotic potential. For a part of them, zoonotic transmission has already been proven.

In the USA, the pathogen-food category pairs responsible for most outbreak-associated illnesses are Salmonella in chicken, Salmonella in pork, and Salmonella in seeded vegetables (Sachsenroder et al., 2012). Evidence of food-borne diseases in low- and middle-income countries is still limited, but recent studies suggest that the most significant also comes from biological hazards (Grace, 2015), with an estimated 20 % of all human illness and death associated with endemic zoonoses (Grace et al., 2011).

Given the increased demand for pork and the disproportionately slow progress of production systems, the importance of pork as a carrier of zoonotic agents is likely to increase. The consumption of raw or poorly cooked pork can be a source of various zoonotic diseases, including parasitic zoonoses such as trichinellosis, taeniosis and toxoplasmosis (Dorny et al., 2009). Assessing and monitoring the potential threat emerging from this growing market will therefore become of increasing importance for safeguarding public health. To this aim, we performed a cross-sectional study in slaughterhouses of the Kinshasa Province, in which furthermore, the results should help to estimate the risk of zoonotic bacterial and fungi transmissions through the pig food chain and to highly exposed persons such as slaughterers, veterinarians and consumers.

Microbial pathogens can cause disease by consumption of the animal products contaminated with microorganisms or their toxins. This research the evidence that links animals as vehicles of the foodborne pathogens *Candida albicans, Citrobacter freundii, Escherichia coli, Enterobacter agglomerrans, Klebsiella oxytoca, Proteus mirabilis, Salmonella spp, Shigella dysenteriae, Staphylococcus aureus* and *Yersinia enterocolitica*, their impact, and their current status.

2. Material and method

2.1 Study area

Slaughter points were selected according to the different districts of the city of Kinshasa.

(a) **Slaughterhouse of the Liberté market**

Located within the Marché de la Liberté on Lumumba Boulevard, in the Commune of Masina, Bitabe District, Tshangu District, in the Eastern part of the city of Kinshasa.

(b) **Slaughterhouse of the Matete market**

Located in the market of the same name, Quartier Mutoto, next to the Maison Communale and the Tribunal de Grande Instance of Matete, District of Mont-Amba, in the South-eastern part of the city of Kinshasa.

(c) **Slaughterhouse at the Bumbu market**

Located in the market of the revolution at the crossroads of the Liberation and Revolution avenues, Lubudi district, Bumbu Commune, Funa District, in the western part of Kinshasa. Here, there are nearly 1,000 pig slaughters per year.

(d) **Slaughterhouse of the Gambela market**

Located within the Gambela Market at the intersection of Gambela and Sport Avenues in the Commune of Kasa-Vubu, Funa District, in the heart of the city centre. The market serves part of the Commune of Kalamu, Limete, Ngiri-Ngiri, Bumbu, Kinshasa, Bandalungwa and Kintambo.

(e) **Slaughterhouse of the** Marché Central

Located in the central market of the city of Kinshasa at pavilion 6, Commune de la Gombe, District of Lukunga, in the northern part of the city of Kinshasa, this slaughterhouse provides meat to the inhabitants of the surrounding neighbourhoods and those coming from various backgrounds across the country.

2.2. MATERIALS

2.2.1 Biological material

The study material consists of 30 pork samples taken from five slaughterhouses in a few markets in Kinshasa. In each site, 6 samples are taken at peak times (1:00 pm to 3:00 pm) depending on the availability of meat.

Sampling equipment

They were used to collect, store and transport the samples under aseptic conditions (without influence of the external environment) from the sampling sites to the laboratory for further analyses.

Table 1: Different types of culture media by purpose

Enrichment	Isolation	Identification	Enumeration
Selenite broth Alkaline peptone water	Mac Conkey Mannitol Salt Agar (M.S.A) Sabouraud Chloramphenicol (S.C) *Salmonella-Shigella* agar (S.S.A) Hektoene Methylene Blue Eosin Agar (E.M.B)	PCA on surface	Candida ID Trycase sulfite Neomycin agar (TSN) Sulfure-Indole Mobilité (SIM) Sodium Citrate (Media Simmons) Urée-indole-milieu Kligler

2.3 METHODS

2.3.1 Sample collection

After identification of the different killings, the meat samples are taken (200 gr per sample) and placed in sterile glass jars and then in the isothermal tank charged with cold accumulator.

Thirty samples of pork meat are taken and then divided by killings into six (6) pieces and sent to the laboratory for analysis.

2.3.2 Macroscopic and microscopic analyses

(a) Macroscopic analyses

It is based on the observation of the conditions under which the meat is spread in the killings and the physical appearance of the meat.

(b) Microscopic analyses

We used two sub-methods: quantitative and qualitative.

- ***Quantitative analysis***

This sub-method uses two techniques, dilution and enumeration. Dilution is carried out after inoculation of the sample in selenite broth and peptone water; using a dropper, a 0.001 ml sample of each sample is placed in 1 ml of physiological water which represents a dilution of 10-3.

Enumeration is performed from the diluted solution after incubation at 37°C for 24 hours. One drop or 0.001 ml of each sample is taken from the platinum loop and stained in a zig-zag pattern on the surface of the medium for enumeration. These boxes are incubated at 37° C for 24, 48 or 72 hours depending on the strain to be isolated. This is how the counting of colonies that have grown can be carried out.

The total number of colonies for a sample is calculated by multiplying the number of colonies after counting by the inverse of the dilution or inoculum as shown in the formula below [19]:

$$\mathbf{F = N \times f \times d}$$

Where, **F** is the formula to calculate the total number of colonies for a sample, **N** is the number of colonies; **f** is the factor and **d** as dilution to use.

Under aseptic conditions, we incorporate one drop of each dilution into the Plate Count Agar medium and incubate at 30 to 37°c for 72 hours to count total mesophilic germs;

Table 2: Culture conditions and strains to be enumerated from the media used

Media	Incubation	Strains to be counted
Mac Conkey cristal purple	37° to 44°C ; 24 to 48 hours	Thermo tolerant coliforms
Sabouraud chloramphenicol	37°c ; 5 days	Yeasts and moulds
Mannitol Salt Agar (PSA)	37°C ; 4 to 48 hours	*Staphylococcus aureus*
Trycase Sulfite Neomycin	46°C in aerobics; 72 hours	*Clostridium sulfato* reducer
Hektoene and Eosine blue methylene	37°c ; 24 to 48 hours	*Salmonella* and *Shigella*
Pyocyanosis	37°C ; 24 to 48 hours	*Pseudomonas aegunosa*

- ***Qualitative analysis***

This sub-method uses three techniques: Seeding, Isolation and Identification.

3. Results

3.1 Socio-demographic parameters

We were interested in the level of education of the vendors, the types of transport of the slaughtered meat, the way the meat sold at the markets is displayed and the personal opinion of the vendors on the quality of the meat offered for sale at the markets.

We conducted our survey among 114 meat vendors in the five slaughterhouses that we divided into different markets:

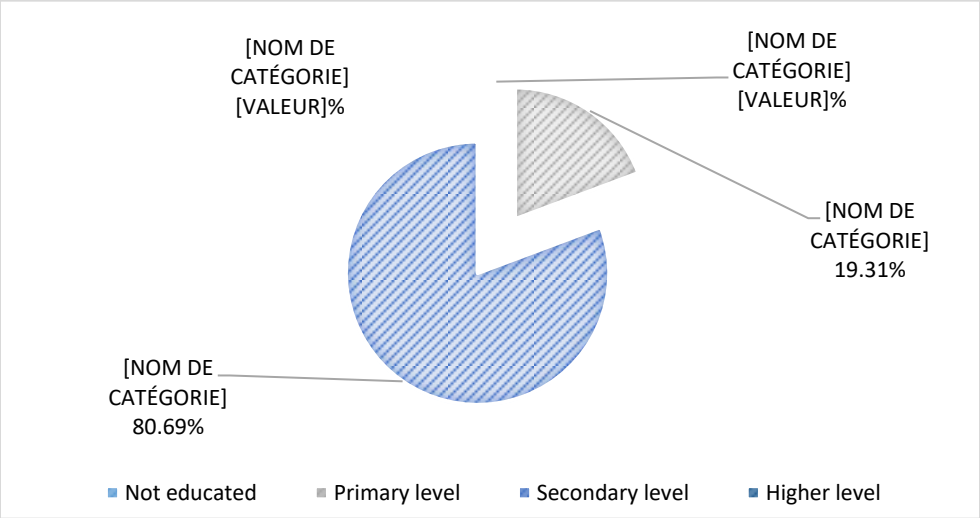

Figure 1: Education level of vendors at sampling sites

Secondary school students make up the highest percentage of vendors in the various markets at 80.69 per cent, followed by primary school students at 19.31 per cent. It should be noted that no cases of primary level and uneducated people were revealed (**Figure 1**).

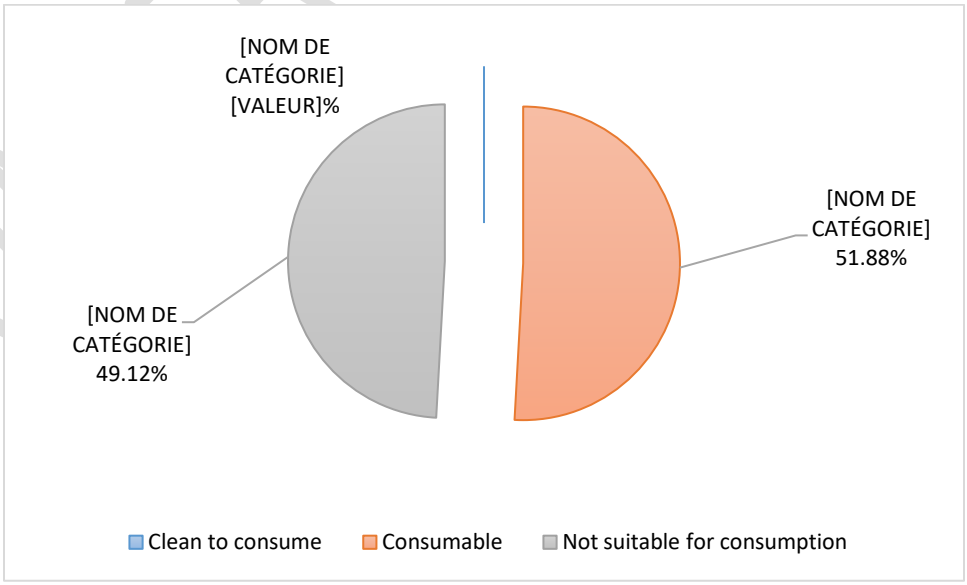

Figure 2: Sellers' personal opinion on meat quality

51.88% of the vendors believe that in a state of slow degradation, the meat offered for sale is still edible, 49.12% acknowledge that it is unhealthy and none of them find it safe (**Figure 2**).

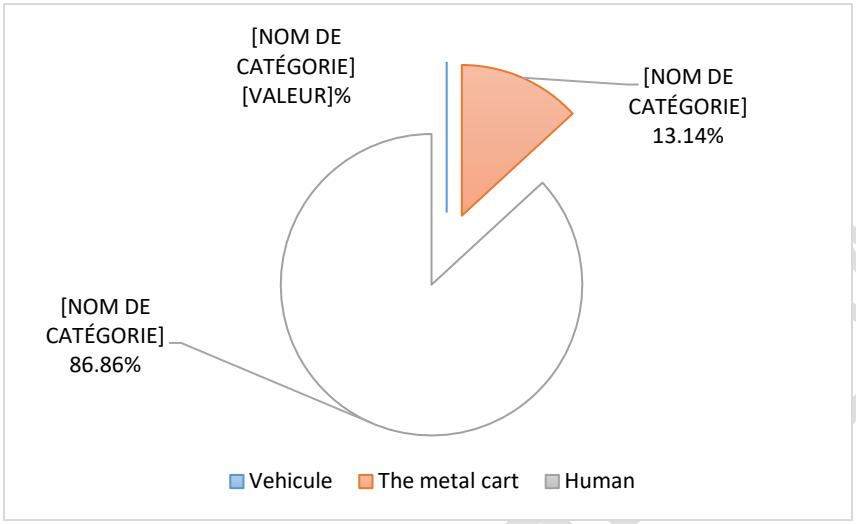

Figure 3: Means of transporting meat from slaughterhouses to markets

None of the vendors use the vehicle to transport the meat to the market, from where the most commonly used means of transport is still human with 86.84%; only 13.16% use metal carts.

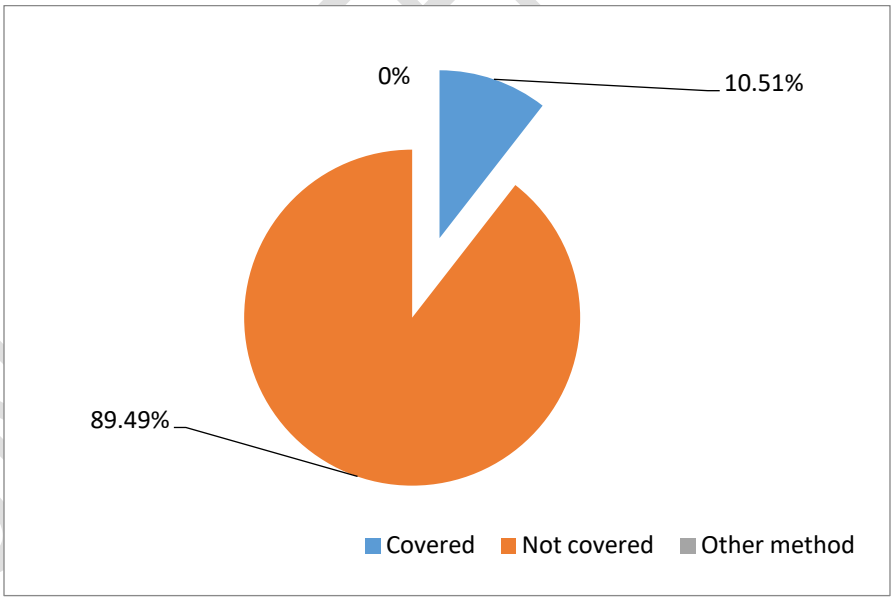

Figure 4: Method of displaying the meat sold at the different markets

The majority of vendors, 89.47%, expose their meat to the open air, which is the real source of meat contamination, and only 10.53% cover their products.

3.2. Microbiological analyses of pig meat

The results obtained during the microbiological analyses of samples of meat produced in the five slaughterhouses focused on the microbial load involved in various food poisonings and contamination germs (diseases of dirty hands).

Table 3: Isolation and Enumeration of Germs by Sampling Site

		Slaughterhouses				
		Gambela	**Liberté**	**Marché Centrale**	**Matete**	**Bumbu**
GERMS	***Candida albicans***	6.10^3	Sterile	36.10^4	48.10^4	Sterile
	Citrobacter freundii	20.10^4	12.10^4	Sterile	Sterile	Sterile
	Escherichia coli	Sterile	Sterile	135.10^4	Sterile	12.10^4
	Enterobacter agglomerrans	Sterile	Sterile	Sterile	45.10^4	Sterile
	Klebsiella oxytoca	Stérile	Sterile	81.10^4	Sterile	Sterile
	Proteus mirabilis	24.10^4	135.10^3	Sterile	Sterile	Sterile
	Salmonella spp	95.10^4	45.10^5	8.10^4	5.10^4	Sterile
	Shigella dysenteriae	Sterile	Sterile	Sterile	35.10^4	Sterile
	Staphylococcus aureus	7.10^4	Sterile	85.10^4	Sterile	9.10^4
	Yersinia enterocolitica	Stérile	45.10^3	Sterile	Sterile	Stérile

Of all the samples examined, two had a higher number of germs. One of the Liberté market samples with 135.10^3 germs of *Proteus mirabilis* and one of the Marché central market samples with 135.10^4 germs of *Escherichia coli*.

One of the Gambela samples has the lowest number of *Candida albicans* sprouts with 6.10^3. The other samples had more than 6.10^3.

Table 4: Degree of contamination of samples per slaughterhouse

Slaughterhouses	*Candida albicans*	*Citrobacter freundii*	*Escherichia coli*	*Enterobacter agglomerrans*	*Klebsiella oxytoca*	*Proteus mirabilis*	*Salmonella spp*	*Shigella dysenteriae*	*Staphylococcus aureus*	*Yersinia enterocolitica*	Total
Gambela	1	1	-	-	1	1	-	1	-	-	5
Liberté	-	1	-	-	2	1	-	-	1	-	5
Marché Central	1	-	1	1	-	1	-	1	-	-	5
Matete	1	-	-	-	-	-	1	3	-	1	6
Bumbu	-	-	2	-	-	-	-	2	-		4
Total	3	2	3	1	3	3	1	7	1	1	25

The slaughterhouse in Matete has the highest level of contamination with 6 contaminations out of 6 samples, the slaughterhouse in Bumbu is the least contaminated with 4 contaminations out of 6 samples. The others have 5 out of 6.

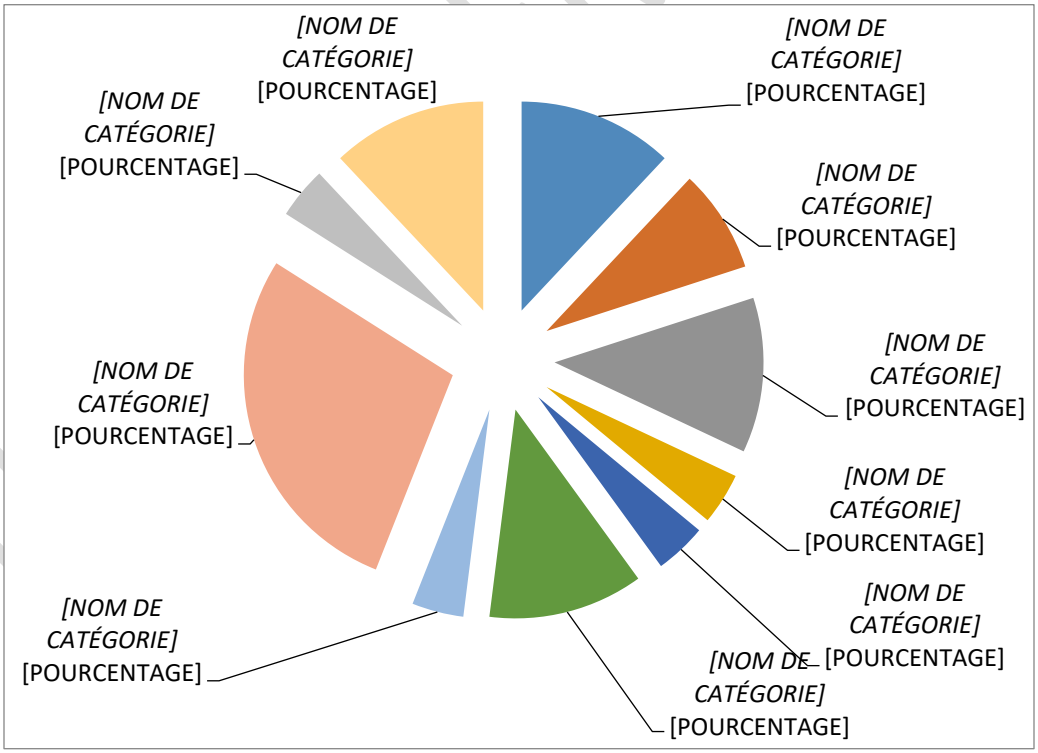

Figure 5: Frequency of germs isolated and identified in 5 markets of study

Of all the germs isolated and identified, Staphylococcus aureus presents the highest frequency (7) or 28% contamination, *Enterobacter agglomerans*, *Kbebsiella xytoca*, *Shigella dysenteriae* and *Yersinia enterocolitica* are the least frequent with 4% contamination; Citrobacter occurs twice, or 8%, and the others 3 times out of 25 or 12% contamination (**Figure 5**).

DISCUSSION

The vendors surveyed were of average educational level, with 80.69% at secondary level and 18.42% at primary level.

A very small proportion (0.88%) of the vendors surveyed consider the meat offered for sale in private slaughterhouses in Kinshasa to be fit for consumption. But for 48.15% of the sellers, the meat put on sale at the market is unfit for consumption; for 50.88% it is edible.

Almost all the meat put on the market is transported by humans (86.86%). Human contact with food is said to be a source of contamination. The metal carts used (13.14%) are not exclusive for the transport of meat.

89.49% of the vendors expose the meat to the open air, hence the high risk of microbial contamination by different vectors; only 10.53% of the vendors cover their products.

The results of the microbiological analyses carried out throughout our research corroborate with those of Samba (2004), Masungi (2003), Kunga (2004) and Tshibanda (2006), which allowed the successive identification and isolation of bacteria of the *Enterobacteriaceae* family : *Citrobacter freundii*, *Escherichia coli*, *Enterobacter agglomerans*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Salmonella spp*, *Shigella dysenteriae*, *Yersinia enterocolitica*; the family *Micrococcaceae*: *Staphylococcus aureus*, and the fungus *Candida albicans*. These germs are responsible for food poisoning resulting in diseases such as diarrhoeal, respiratory, urogenital, etc.

Our results show that 12 % of the contaminated samples are due to Salmonella spp whose presence makes the sample declared unfit for human consumption, 28 % of the contaminated samples are due to Staphylococcus aureus whose number of samples is well above 102 and therefore considered unfit for human consumption. All other samples contaminated by other Enterobacteriaceae representing 48% are below the threshold required by the FAO to declare the

sample clean or unfit for human consumption, three samples are contaminated by Candida albicans representing 12%.

In addition to the Matete market where 42.8% of the samples are contaminated with Staphylococci aureus, three out of six samples could be declared unfit for consumption; the samples from the other sites could also be declared unfit for consumption because the count reveals the threshold recommended by the FAO. These are: 33.3% (2 cases/6) of samples contaminated with Staphylococcus aureus; Central Market 33.3% (Staphylococcus and Salmonella); Gambela 33.3% (*Staphylococcus* and *Salmonella*), Liberté Market 20% of samples contaminated with *Salmonella spp*.

CONCLUSION

The results of our study may complement the scarce public health data on pork-borne zoonoses in Kinshasa (DR of the Congo) toward a better understanding of their relative impact and importance. Of the work carried out and found on the search for microorganisms from fresh meat sold in Kinshasa's markets, most was based on beef.

Given the importance and the number of slaughterings of small ruminants and pigs in slaughterhouses as well as the eating habits of consumers with regard to these species, it requires special attention from experts in the field as well as from the competent authorities so that there can be significant improvements in order to help slaughterers to do better and make consumers safer. A survey of 114 meat sellers in the targeted slaughterhouses shows that the meat offered for sale does not meet the required standards.

Indeed, the quality of the meat to be consumed is not only limited to its organoleptic characteristics, but also to the conditions under which it is preserved, transported and sold at the various distribution points. The organoleptic and nutritional potential of pigmeat in particular means that it is both attractive (appealing) and perishable, which means that it must be handled and stored with care so as not to damage the health of consumers. Research on the enumeration, isolation and identification of microbes on meat from pigs slaughtered and sold on the markets in Kinshasa has enabled us to discover the germs responsible for meat spoilage and the various diseases caused by them in consumers of spoilt meat.

This analysis gave us two groups of germs: bacteria and fungi. Bacteria are made up of two families: *Enterobacteriaceae* and *Micrococcaceae*, the second group, that of fungi, precisely *Candida albicans* which is a yeast.

We are not responsible for stating that the germs identified are the only ones that can be found in samples taken from private slaughterhouses in Kinshasa, the media used are mostly selective and other techniques or methods could certainly reveal other germs.

Given the clear relevance of pork as a possible source of human illness in Kinshasa (DR of the Congo), further efforts should be undertaken to monitor the related animal and human disease burdens and to strengthen food safety throughout the pork production chain.

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