

Original Research Article

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Microbiological Investigation of Braised Meat

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ABSTRACT

Meat is an important part of human diet with strong implications in health, economy, and culture worldwide. Even if eating meat has recently become subject to criticism for health-related, environmental or humanitarian reasons, it still contribute to the gastronomic pleasure of urban and rural consumers across continents. In West Africa, meat is eaten in several forms, included braised meat which is often sale on street. The possible contamination of this prized foodstuff by the pathogen *Bacillus cereus* (*B. cereus*) and in particular by the emetic *B. cereus* was investigated. For this purpose, a survey to assess the braised meat production conditions was carried out from thirty three (33) vendors in Korhogo city, by using a structured questionnaire, whereas 99 braised meat samples were analyzed for the enumeration of total mesophilic bacteria, enterobacteria as well as the pathogen *B. cereus*. *B. cereus* strains isolated were then screened by polymerase chain reaction (PCR), for emetic strains detection. The results revealed the presence of total bacteria count ($2,51 \pm 0,18$ to $5,04 \pm 0,26$ log cfu/g); Enterobacteriaceae ($8,89 \pm 0,55$ to $9,23 \pm 0,15$ log cfu/g) and *B. cereus* ($5,66 \pm 0,17$ to $6,99 \pm 0,08$ log cfu/g) in Braised meat. However, none emetic strain was detected among *B. cereus* isolated. The poor body and work environment hygiene of the braised meat vendors could explain this foodstuff contamination.

Keywords

Bacillus cereus,
Emetic strains,
Braised meat,
Hygienic

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Introduction

Meat is one of the most important sources of animal protein available in the tropics. It is often associated with wealth and a highly nutritional diet (Paredi *et al.*, 2013; Modlinska *et al.*, 2018). Even if eating meat has recently become subject to criticism for health-related,

environmental or humanitarian reasons, this foodstuff provides important amount of proteins, vitamins as well as minerals, that keep our body healthy. Besides, meat is one of the primary dietary sources of creatine, which has been suggested to have positive impact on the immune and neurological system (Pereira and Vicente, 2013; Ribas-Agustí *et al.*, 2019).

Not only does meat production contribute considerably to food security and sustainable land use, but also to the socioeconomic well-being of rural communities, and to the gastronomic pleasure of urban and rural consumers across continents (Metzger *et al.*, 2018; Santoze and Gicheha, 2018).

In Ivory Coast, meat is eaten in several forms included the locally called « braised meat » (Roesel, and Grace, 2016). This foodstuff is a barbecue type, cooked outdoors by using live coals or woods. Even if being part of street foods, this dish is very prized by the most of Ivory Coast's population. Therefore, ensuring microbial safety of this foodstuff is an important issue in this context of consumption and production, all the more so as meat constitutes a favorable medium for the proliferation of spoilage and pathogenic microorganisms. Indeed, the high meat protein content provides nutrients for microbial proliferation whereas its neutral pH and high water activity are favorable conditions for the growth of a wide range of microorganisms. Thus, meat constitutes a potential vehicle for microbial pathogens and was reportedly associated to various bacterial food borne diseases in humans (Fegan and Jenson, 2018; Niyonzima *et al.*, 2018; Kang *et al.*, 2019).

Foods associated with outbreaks of *B. cereus* diarrhoea frequently include protein-rich foods and researchers have emphasised the importance of the presence of this bacteria or its spores in meat and meat products for the occurrence of such outbreaks (Soares *et al.*, 2012).

In meat production there is a focus on potential faecal pathogens like *Salmonella* and *Escherichia coli*, and considerable resources are used to sample the environments for these potential pathogenic bacteria. More other, in central Africa, specially in Rwanda, the

microbiological quality of meat consumed within the households, as well as meat-based meals consumed outside the home, was thoroughly investigated (Niyonzima *et al.*, 2016, 2017). However, to the best of our knowledge, no study has yet assessed the occurrence of pathogen such as *B. cereus* in Ivory Coast braised meat. Yet, the incidence of *B. cereus* food poisonings is steadily increasing and lethal cases of *B. cereus* emetic intoxications have been described worldwide (Dierick *et al.*, 2005; Mahler *et al.*, 1997; Naranjo *et al.*, 2011; Shiota *et al.*, 2010; Delbrassinne *et al.*, 2011).

The present study aims to investigate the hygienic conditions of braised meat production as well as the prevalence of *B. cereus* emetic strains in this foodstuff.

Materials and Methods

Hygiene related to braised meat production

A survey was conducted to investigate the hygienic conditions related to the production of braised meat, from braised meat vendors of Korhogo, the north of Ivory Coast. For this purpose, thirty three (33) braised meat production places were studied.

The survey was carried out through an interview with the vendors. We remained on the premises while the meat was being prepared, stored and/or sold and we observed the procedure employed by the braised meat handlers.

Informations regarding the braised meat procedures, equipment and hygiene, cleaning and sanitation procedures were collected through a structured questionnaire. The responses from the interview were verified on the site and when necessary corrections were made.

Braised meat sampling

A total of ninety-nine thousand (99) braised meat samples were collected from 11 vendors, during four (04) month, at the rate of once a week, from February to May 2019.

Samples were aseptically collected from each seller, placed in sterile stomacher bags (Biorad, Belgium) and held at 4°C in a cooling box with freeze packs. Samples were then transported to the laboratory within approximately 1 h, for analysis within the following 24 hours.

Microbiological analysis

Microbiological analyses were carried out in accordance with the European Regulation EC 2073/2005. Enumeration of bacteria was performed for all samples. Reference methods were used for enumeration of hygiene indicator bacteria namely total bacteria count and *Enterobacteriaceae*.

The analyses were performed in solid samples, as described ISO 4833-2003 and ISO 21528-2004 respectively, has a detection limit of 1 log cfu/g (ISO 2007).

Bacillus cereus was qualitatively detected in the braised meat samples by following the ISO7932:2004 standard protocol. The non-selective enrichment step was performed in buffered peptone water (VWR, Belgium), whereas Mannitol Egg Yolk Polymyxin Agar (MYP) (Biorad, Belgium) was used for the isolation of presumptive *B. cereus* colonies. For *B. cereus* confirmation, the characteristic strains isolated were grown overnight on Luria Bertani agar (Biorad, Belgium).

Each strain was then diluted in 40 ml of saline water (0,85%) before spotting (3 µl) on different media: Columbia-based blood agar (containing 5% sheep blood), erythrocyte agar

(Oxoid) and Mannitol-egg-Yolk-Polymixin (MYP) plates (BioRad). Haemolysis and lecithinase activities were read after 24 h.

Emetic strains detection

Bacillus cereus strains isolated from braised meat were screened by PCR polymerase chain reaction, for the presence of emetic strains.

For this purpose, Two distinct primer pairs (Em1F/R and CesF1/R2) targeting conserved sequence motifs of the cesB NRPS (non-ribosomal peptides synthetase) gene were used (Ehling-Schulz *et al.*, 2004; 2005) in this study.

Statistical analysis

One-way analysis of variance (ANOVA) was used to test the equality of means for normally distributed variables.

Results and Discussion

Braised meat production and sales conditions in Korhogo city

Table 1 describes the conditions of production and sale of braised meat in the municipality of Korhogo.

Most braised meat sellers (87.87%) are illiterate and have poor body and work environment hygiene (84.84%).

Public slaughterhouses were found to be the main procurement place for meat (78,78 %).

Furthermore, all the investigated sellers reported to be exposing meat to ambient temperature, before and after cooking process. The average duration of meat exposition remains variable, in function of the meat sales flow.

Microbiological analyses

Analytical methods

The braised meat sold in the municipality of Korhogo were analysed for their bacteria content. Ninety-nine thousand (99) braised meat samples were collected from 11 vendors and investigated. Table 2 describes the levels of hygiene indicator bacteria, namely the total bacteria count and Enterobacteriaceae, as well as the occurrence of *B. cereus* in braised meat sold indifferent area of Korhogo city. The mean total bacteria count ranged from $8,89 \pm 0,55$ to $9,23 \pm 0,15$ log cfu/g and the mean Enterobacteriaceae ranged from $2,51 \pm 0,18$ to $5,04 \pm 0,26$ log cfu/g, whereas significant *B. cereus* counts ($5,66 \pm 0,17$ to $6,99 \pm 0,08$ log cfu/g) were found in braised meat samples.

Whereas the contamination of braised meat has been carry out though the reaserch of pathogen like Salmonella, *Clostridium perfringens* (Dibi *et al.*, 2017), there is a lack of information on the contamination of this foodstuff by *B. cereus*. Yet, the incidence of this pathogen food poisonings is steadily increasing and lethal cases of *B. cereus* emetic intoxications have been described worldwide (Delbrassinne *et al.*, 2011; Schulz *et al.*, 2015; Glasset *et al.*, 2016).

The braised meat sold in different areas of Korhogo city contained significant *B. cereus*, Enterobacteriaceae and aerobic mesophilic bacteria counts (Table 2). The contamination of braised meat probably occurred during handling and preparation or post-processing contamination. In 2017, Tewari *et al.*, have isolated enterotoxinogenic *B. cereus*, harboring enterotoxin genes hblDAC complex, nheABC complex, cytK and entFM, from cooked meat. They suggested that, a relatively higher incidence of these pathogens in meat products, can pose a potential public health threat. The presence of *B. cereus* in Japan meat products (18.3%) and raw meat

(6.6%) was also reported in a previous study. According to the authors, the main source of *B. cereus* contamination in meat products is contaminated meat product additives (Konuma *et al.*, 1988). Furthermore, *B. cereus* enterotoxigenic genes were directly detected in Meat and Meat Products, by multiplex Polymerase Chain Reaction in India (Rather *et al.*, 2011; Sánchez *et al.*, 2014).

In the present study, the braised meat contamination may be explained by the poor body and work environment hygiene of the braised meat vendors. Indeed, the wearing of clean coats and hair covers by braised meat workers were observed in neither sampling area. Masks and gloves were also not worn by the producers. There is also no hand washing between braised meat handling and money handling. Furthermore, The lack of waste containers with covers in the premises of production resulted in braised meat scraps being thrown away by vendors, attracting a variety of insect pests. Recently, different points of braised meat sale located in poorly maintained environments are been highlighted (Dibi *et al.*, 2017). The levels of total mesophilic bacteria and Enterobacteria are in accordance with the lack of hygiene of both meat handling personnel and production premises. Several authors have reportedly associated the microbiological quality of meat to the hygiene of meat handlers and the processing environment (Abdalla *et al.*, 2009; Çalicioğlu *et al.*, 2010; Wheatley *et al.*, 2014). Indeed, the lack of knowledge about good hygienic practices could be linked to the insufficient education of braised meat producers who were mostly illiterate (Table 1).

This low level of schooling witch is probably linked to the poverty could explained the very poor body and work environment hygiene. Another reason of braised meat contamination by *B. cereus* would be the storage conditions.

Table.1 Braised meat production and sales conditions in Korhogo city

Vendors	Vendors educational level (%)			Meat storage (%)				Workplace and personal hygiene (%)		Meat procurement place (%)		
	Analphabets	Primary level	Secondary level	Before cooking		After cooking		Unhygienic	Hygienic	Farmers	Slaughterhouse	Market
				Ambient temperature	Cold storage	Ambient temperature	Cold storage					
33	29 (87, 88 %)	4 (12,12 %)	0 (100,0)	33 (100,0)	0 (100,0)	33 (100,0)	0 (100,0)	28 (84,84 %)	15,15 (%)	2 (6,06 %)	26(78,79 %)	5 (15,15 %)

Values are numbers (percentage) of braised meat production and sales conditions in Korhogo city. 33 (thirty three)vendors were interviewed on their working conditions.

Table.2 Bacteria counts of braised meat from eleven area in Korhogo city

	Charge moyenne en log cfu/g		
	Enterobacteriaceae	Mesophilic aerobic bacteria	<i>Bacillus cereus</i>
Natio kobadara	4,68 ± 0,08	9,13 ± 0,11	6,42 ± 0,36
Banaforo	5,22 ± 0,06	9,11 ± 0,07	5,66 ± 0,17
Cocody	4,65 ± 0,07	9,22 ± 0,03	6,99 ± 0,08
Haousabougou	4,91 ± 0,21	9,01 ± 0,16	6,90 ± 0,26
Dem	4,96 ± 0,06	9,11 ± 0,04	6,97 ± 0,07
Koko	4,57 ± 0,34	9,16 ± 0,18	6,97 ± 0,22
Soba	4,71 ± 0,26	8,89 ± 0,55	6,10 ± 0,68
Sinistre	4,39 ± 0,32	9,15 ± 0,02	6,79 ± 0,08
Quartier 14	2,51 ± 0,18	9,11 ± 0,05	6,07 ± 0,20
Tegueré	5,04 ± 0,26	9,23 ± 0,15	6,91 ± 0,15
Petit Paris	4,82 ± 0,37	9,00 ± 0,14	6,26 ± 0,74
Air France	4,59 ± 0,91	9,13 ± 0,11	6,42 ± 0,36

Values are mean (±standard deviation) of triplicate bacterial loads (in log cfu/g) measurements.

The findings from this study indicate that all vendors stored meat at ambient temperature, before and after cooking process. Even worse, the average duration of meat storage remains variable, in function of the meat sales flow. Since fresh meat is a highly perishable product due to its biological composition, an inadequate storage temperature can favour bacteria development and affect shelf life and freshness. The meat storage conditions can be explained by the low financial capacity of a number of braised meat vendors, which cannot afford the purchase of refrigerator for meat transportation and storage. Our study is in accordance with some authors who reported the transportation of meat under non-refrigerated temperature conditions in developing countries (Adzitey *et al.*, 2011; Roesel *et al.*, 2014; Nyonzima *et al.*, 2017).

Emetic strain detection

B. cereus strains isolated were investigated by PCR for the presence of emetic strains. Among the 54 *B. cereus* strains tested, none emetic strain was identified by PCR. Our findings agree with the results from previous study conducted in 2015 by Ehling-Schulz *et al.*, where the scarcity of emetic strains in meat product was reported. According to these authors, it might be speculated that certain food niches provide an advantageous environment for growth/and or antibiotic production, allowing a selective substrate occupancy by emetic strains. Meat dishes rather seems to be the niche of diarrhea bacteria.

There are thus most frequently the vehicles for transmission of the diarrheal form of the illness, caused by *B. cereus*. In addition, after tested a panel of foods regarding the potential of cereulide formation by *B. cereus*, Agata and collaborators (2002), have observed a very low toxin production in meat despite a good bacterial growth. To our knowledge, this is the

first study on prevalence of *B. cereus* emetic strains in the braised meat. While previous studies had mainly focus on potential faecal pathogens like Salmonella and Escherichia coli in meat production. Although heat is mentioned for bacteria destruction or number reduction, significant *B. cereus* was isolated from braised meat. These results suggest that the prevalence of *B. cereus* is probably higher than previously thought. Although emetic strains were not found in these randomly sampled braised meat, *B. cereus* may cause severe food poisoning as indicated by previous articles. Therefore, additional studies on a large size of sample are needed. On account of the poor body and work environment hygiene of the braised meat vendors reported in the present study, it seems essential to sensitize them on good hygienic practices along the braised meat chain and on the risk of disease transmission.

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