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Retrospective study of the contamination of exported sesame by *Salmonella* species from 2007 to 2017 in Burkina Faso

Muller K. A. Compaoré[1, 2]*, Virginie Marie Yougbaré[2], René Dembélé[1, 3], Fulbert Nikièma[2], Kabré Elie[2] and Nicolas Barro[1]

[1]Laboratoire de Biologie moléculaire, d'Epidémiologie et de Surveillance des agents Transmissibles par les Aliments (LaBESTA), Centre de Recherche en Sciences Biologiques Alimentaires et Nutritionnelles (CRSBAN), École Doctorale Sciences et Technologies, Université Joseph KI- ZERBO, 03 BP 7021 Ouagadougou 03, Burkina Faso
[2]Laboratoire National de Santé Publique (LNSP), 09 BP 24 Ouagadougou 09, Burkina Faso.
[3]Unité de Formation et de Recherche en Sciences Appliquées et Technologies (UFR/SAT), Université de Dédougou, BP 176 Dédougou Burkina Faso.

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Sesame (*Sesamum indicum*) is one of the oldest and most traditional oilseed crops, valued for its high-quality seed oil. This culture was introduced in Burkina Faso at the beginning of the 20th century where it occupies a place of cash crop. The majority of sesame produced in Burkina Faso is export oriented. However, the contamination by *Salmonella* remains an unsolved problem. This retrospective study was carried out in order to assess the microbiological safety of sesame samples received at the Laboratoire National de Santé Publique between 2007 and 2017. Out of 359 samples unevenly distributed according to months, 26.46% showed the presence of *Salmonella* species. The persistence of this food borne pathogen in sesame is a strong signal that new strategies of growing, harvesting and postharvest and special attention and emphasis on control measures must be given to the chain of production of this commodity in a view of its economic and medical impact.

Key words: *Salmonella*, *Sesamum indicum*, Burkina Faso.

INTRODUCTION

The worldwide food poisoning pathogen, *Salmonella* first came into prominence in the 1880s, soon after the isolation of the "hog cholera bacillus" by Salmon and Smith (Steele, 1969). Since, this pathogen has been recognized as responsible for a wide range of outbreak (Sir William, 1956; Puglisi and Maida, 1969; Small and Sharp, 1979; Unicomb et al., 2005; Angulo et al., 2006; Kunwar et al., 2013). While *Salmonella* remains the leading cause of bacterial gastroenteritis, it is also one of the most extensively studied and well characterized bacterial species (Chami and Bao, 2009). However, despite a vastly greater understanding of their structure, relationships and natural history, *Salmonella* remains what they were in 1900: an unresolved conundrum in microbiology, epidemiology, and public health (Hardy, 2004). *Salmonella* acts as primary reservoir for foods

*Corresponding author. E-mail: mullercompaore@yahoo.fr. Tel: 0022670227311.

such as chicken meat, milk and milk products, eggs and meat products (Birol Özkalp, 2012). Nowadays, *Salmonella* has been detected in a wide range of agricultural products such as sesame and *Salmonella* outbreaks associated with sesame seed-based products have been reported (Unicomb et al., 2005). In Burkina Faso, studies have shown that *Salmonella enterica* is circulating species. Bonkoungou et al. (2013) find out 15 different serotypes of *S. enterica* subsp. enterica in childhood diarrhea samples: *Salmonella* Typhimurium, *Salmonella* Cubana*, Salmonella* Muenster*, Salmonella* Angers*, Salmonella* Banana*, Salmonella* Dublin*, Salmonella* Kentucky*, Salmonella* Montevideo*, Salmonella* Ouakam*, Salmonella* Soerenga*, Salmonella* Poona*, Salmonella* Stanley*, Salmonella* Tamberma*, Salmonella* Vilvoorde*,* and *Salmonella* Typhi*.* On the other hand, some other published papers report the presence of *S. enterica* in raw meat (Kagambèga et al., 2011), in milk (Touwendsida et al., 2014) and water, fish, lettuce (Traoré et al., 2015).

Sesamum indicum is an extraordinary plant that can be grown on almost all types of land. It is a survivor crop known around the world. For 5,000 years, it has been planted by subsistence farmers in areas that will not support the growth of other crops or under very difficult conditions with drought and/or high heat (Langham, 2007). *S. indicum* is an erect annual plant of variable size, with mature plants ranging from 40 to over 200 cm in height. Its stems are obtusely square with grooves on their faces, yellow-green but often splashed with varying amounts of a striking deep eggplant-purple color (Bedigian, 2004). This plant is adaptable to a range of soil types, although it performs well in well-drained, fertile soils of medium texture (typically sandy loam) at neutral pH (Islam et al., 2016). Sesame is thought to have originated in Africa and later taken to India at a very early date (Alegbejo et al., 2003). Its origin is still controverted and requires further deep genetics research.

Although, dried fruits such as sesame are too dry to allow bacterial growth, pathogens such as *Salmonella* can become sheltered and cause diseases. There are few reports on the presence of *Salmonella* in dry products, and most of these studies were focused on pre- and postharvest practices for processing of ready-to-eat products. Sesame contamination by *Salmonella* contributes to negative economic impacts due to the cost of surveillance investigation, treatment, prevention of illness and worse, contribute to an international pathogen's exchanges.

Burkina Faso is a land lock country located in West Africa and neighbored by six countries (Mali, Côte d'Ivoire, Ghana, Togo, Benin and Niger). Its economy remains essentially agricultural. More than 86% of the active population derives its income from agriculture (MAAH, 2010). Sesame has been cultivated since the beginning of the 20th century in Burkina Faso and entered crop rotations before the development of the industrial cotton crop. Burkina Faso produces mostly mixed (mixed) sesame sold mainly in China, Japan and Turkey (Rongead, 2013). The majority of the national production is export oriented. Besides, the international trade command sesame to be in a best quality as possible that mean free of bacteria such as *Salmonella* and other chemicals such as pesticides. However, in Burkina Faso, the international sesame demand is far higher than global supply, particularly in the conventional market. This exacerbates the pressure on producers and traders throughout the sesame commodity chain and has fostered some structural changes over the last decade (Glin et al., 2013).

Performing sesame quality in Burkina Faso supposes casting a glance at the sesame quality background. In response, a retrospective study was undertaken on sesame samples, received at the Laboratoire National de Santé Publique from 2007 to 2017 with the aim of assessing the microbiological safety of sesame submitted to exportation, with a particular focus on the detection of *Salmonella* species.

MATERIALS AND METHODS

Study site and sampling

This study was conducted in Burkina Faso, West African country which covers 273,800 km^2 of land and 400 km^2 of water. Its economy is closely linked to agriculture. These sesame samples submitted to this study (359 sesame samples) were obtained by the National Public Health Laboratory for foods quality control before exportation. The locality of the country from which these seed samples were harvested remains unknown but their common point is that they have been cultivated in Burkina Faso and are all intended for export.

Salmonella detection

Conventional cultivation technique was used for the isolation of *Salmonella* spp. Detection of the presence of *Salmonella* is carried out according to the ISO 6579:2002 (updated in 2007) standard - Horizontal method for detection of *Salmonella* spp. This includes four stages of the detection process as described by Fricker (1987) and Zadernowska and Chaj (2012).

Pre-enrichment in non-selective liquid medium broth

For the first stage, 25 g of each sesame sample were preenriched in 225 ml of non-selective buffered peptone water (Liofilchem diagnostic, Italy) and incubated for 18 to 20 h at 37°C. Pre-enrichment culture allows the number of cells of interest to increase and to repair any lesions of damaged cells and thus regain their resistance to selective agents, prior to enrichment.

Selective enrichment in liquid media

After the non-selective pre-enrichment stage, 1 and 0.1 ml of each sample suspension was transferred into 10 ml of selective media Tetrathionate broth Müller-Kauffman (Liofilchem diagnostic, Italy)

Table 1. Sesame distribution over years and *Salmonella* presence.

Year	Number of analyzed samples	*Salmonella* detected cases [n (%)]
2007	83	22 (26.5)
2008	113	34 (30)
2009	27	6 (22.2)
2010	26	6 (23)
2011	8	3 (37.5)
2012	2	1 (50)
2013	1	1 (100)
2014	2	1 (50)
2015[a]	31	12 (38.7)
2016	35	7 (20)
2017[b]	31	2 (6.5)
Total	359	95 (26.46)

[a]Year of the largest percentage of contaminated probes. [b]Year of the smallest percentage of contaminated probes. Years 2011 to 2014 were not consider.

and 10 ml of selective media Rappaport Vassiliadis Soy (Difco laboratories), respectively. A brilliant green at 0.95% was added to the selective media Tetrathionate broth in order to inhibit the growth of Gram-positive bacteria and then incubated for 18 to 20 h at 37 ± 1°C. The selective media Rappaport Vassiliadis was incubated for 18 to 20 h at 42 ± 1°C. Selective enrichment procedures involve inhibitory substances or procedures to impede the growth of most organisms but permit, though not necessarily encourage, the growth of the desired organisms (Fricker, 1987).

Plating on selective media

During the third stage, a loopful of cultures suspension from selective media was placed on two selective media, so as to receive individual colonies. The first of them was the Xylose Lysine Deoxycholate (HiMedia Laboaratories, India) agar and the second was *Salmonella-Shigella* (HiMedia Laboaratories, India) agar. The choice of selective plating media must receive special attention. They must support the growth of a very wide range of strains of the particular type required and should, wherever possible, inhibit the growth of other bacteria. Typical *Salmonella* colonies onto Xylose Lysine Deoxycholate agar can be colorless, very light, slightly shiny and transparent (color of the medium) with a dark tinted center, surrounded by a light red area and yellow edge, or of pink to red color, with or without a black center. Onto the *Salmonella-Shigella* agar, typical *Salmonella* colonies are colorless or very light pink, opaque or semi-transparent with a black center or not.

Biochemical and serological identification of characteristic colonies

At least five colonies suspicious for *Salmonella* were picked per plate and purified onto nutrient agar for 24 h. Then, colonies were sowed onto triple sugar iron agar (Difco laboratories) to access sugar utilization, MR-VP broth for Voges Proskauer reaction, Christensen agar for urea utilization and peptone water broth for indole production. *Salmonella typhimurium* (ATCC 14028) and *Salmonella Enteritidis* (ATCC 13076) were used as positive control. Suspected colonies were purified on nutrient agar and then submitted to API 20E (BioMérieux) test for biochemical confirmation. The key biochemical tests including the fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H_2S production, and fermentation of dulcitol (Odumeru and León-velarde, 2000).

RESULTS

The results showed that *Salmonella* was detected in 95 (26.46%) sesame samples out of a total of 359 samples examined. The annual distribution of *Salmonella* from 2007 to 2017 varies from 6.5 to 100% (Table 1).

The distribution of sesame samples was not uniform over the years, the number varied from 01 sample in 2013 to 113 sample in 2008. Surprisingly, *Salmonella* was still detected in each year even if sample number were low. The highest number of samples was 113 in 2008 with a contamination rate of 30%.

From the year 2011 to 2014 (4 years), few samples (13 samples) were received with the lowest number in 2013 where only one sample was received but shown the presence of *Salmonella.*

Figure 1 shows the percentage of *Salmonella* detected cases compare to the number of samples received per year for statistical matter, we expressly remove the sample of the years where the number is below 30% of average samples taken per year (2011, 2012, 2013 and 2014).

While trying to understand if there is a link between the number of sesames analyzed at the National Public Health Laboratory and the national sesame exportation, we used the INSD data stat (2010 and 2018). Figure 2 shows the evolution of sesame exportation in tons and the income it generates in CFA franc during the study period.

DISCUSSION

This was the first and unique retrospective study about

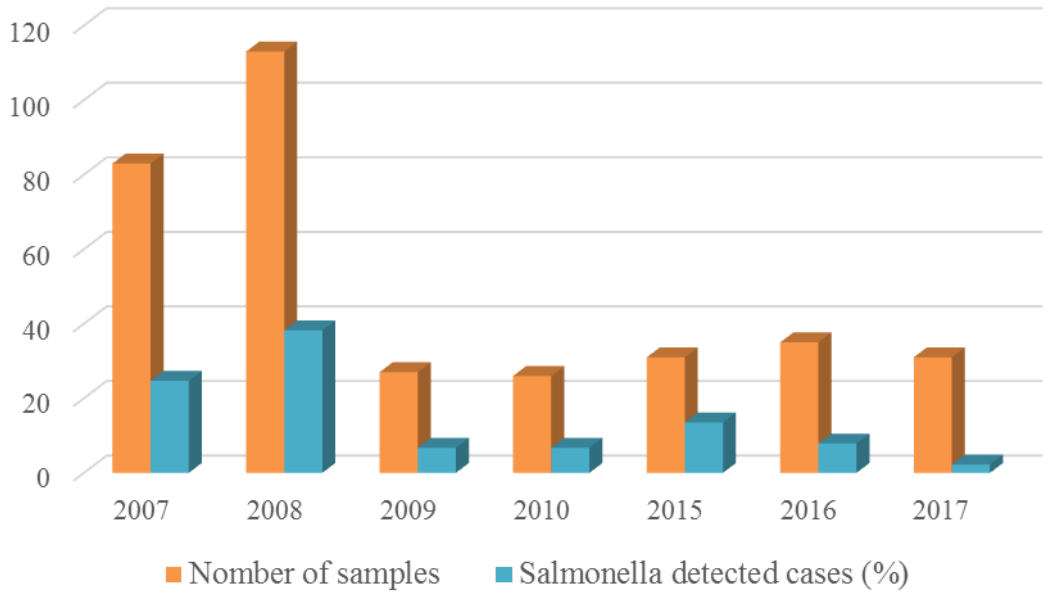

Figure 1. Distribution of samples and the presence of *Salmonella* per year (years 2011 to 2014 were removed).

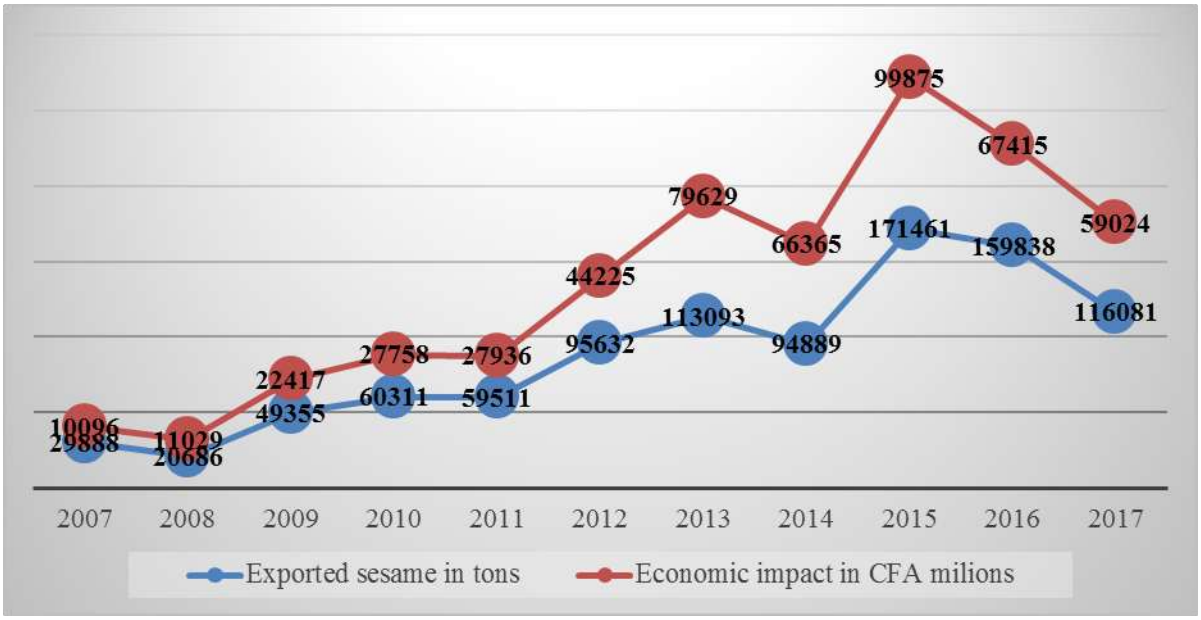

Figure 2. Sesame exportation from 2007 to 2017.
Source: INSD (2010, 2018).

sesame contamination by the worldwide food poisoning pathogen *Salmonella* in Burkina Faso. A compilation of the results of sesame analyzed from 2007 to 2017 were analyzed. These sesame samples were received at the National Public Health Laboratory for *Salmonella* research. Out of 359 samples received unevenly according to months, the results showed the mean prevalence of 26.4% sesame probes contaminations by *Salmonella* between 2007 and 2017. This rate is high but seems to have its origins in the traditional cultural practices of sesame post-harvest treatment. Alaouie et al. (2017) find out a 17% prevalence of *Salmonella* spp. while assessing the microbial quality of Tahini (Sesame Paste) in Lebanon. In fact, sesame harvesting involves a very important step, namely drying. Sesame is thus exposed to all kinds of physical, chemical and biological

contaminations. Sesame seeds are sometimes known to harbor various molds, fungi and bacteria relatively (Angulo et al., 2006) because postharvest treatments are usually done at room temperature in open. Contamination can occur due to poor hygiene around the space reserved for sesame drying. Most of the time, the feces of poultry and birds helped by the wind are responsible. Good agricultural practices on the farm and protection of the product from animals and birds during the drying process will help mitigate the risk of *Salmonella* contamination of sesame seeds (FSANZ, 2013). *Salmonella* spp. can remain viable for months in soil. The organism may also be dispersed in dust and aerosols generated during the handling and processing of animals (Lake et al., 2010). According to Brockmann et al. (2004), sesame seed can be contaminated with *Salmonella* during growth, further storage, or processing and irrigation and fertilization with manure and sludge, plant irrigation with surface water from streams, and animal droppings are potential sources of contamination. Further studies are needed to specify the circulating species in Burkina Faso sesame.

The sesame of Burkina Faso at the rate of 86% is exported oriented (Compaore, 2014). Local use of sesame is mainly for traditional dishes and transformation into sweet backers and salted or natural seed. So, even the low rate of local use of sesame, in regard of *Salmonella* prevalence can cause a public health issue if adequate treatments are not done. Sesame seed are consumed worldwide and has been linked to some international outbreaks (Sir William, 1956; Puglisi and Maida, 1969; Small and Sharp, 1979; Unicomb et al., 2005; Angulo et al., 2006; Kunwar et al., 2013). This increases the surveillance level in imported countries. More, *Salmonella* outbreaks linked to sesame products have heightened alerts and product control for the EU and Asian countries. We suppose that the high number of samples received at the National Public Health Laboratory for *Salmonella* research in 2007 and 2008 might be linked to the international trade control and some posted outbreak alerts due to *Salmonella* in sesame products (Unicomb et al., 2005). In February 2006, organic sesame seed from Burkina Faso were blocked at the entrance of the European Union (EU) because of *Salmonella* contaminated (Ouedraogo, 2006). That was a very important issue for sesame exporters. The operators concerned, with the support of NGOs organized a round table with all stakeholders in the sesame sector, with a view to implement a series of measures to face the risks of contamination. One of the measures is the systematic quality control upstream before export.

Therefore, the quality control was required in the exported country to avoid important lost from exporters by setting up a pretreatment. Sesame contamination by *Salmonella* spp. might be linked to collection system that consisted, sometimes to mix different sesame seed received from elsewhere. Seeds are mixed together without any precaution or treatment. The consequence is that a quality of an entire stock can be easily compromised causing an important lost for exporters. Sesame seeds collected elsewhere by, posed a serious problem of its heterogeneity and at the same time setup difficulties of sampling for *Salmonella* detection. Besides, the standard method of *Salmonella* spp. detections in food products (ISO 6579:2003) are still in use by many labs, especially by regulatory agencies and well accepted. Assessing to the microbiological quality of the entire batch by examining only one 25-g sample seems unacceptable. In general, the larger the sample examined, the greater the chance of isolating the desired organisms (Fricker, 1987). The sampling system will have to be reviewed to better guide and protect producers and exporters.

Sesame is more an important source of income and economic factor growth for Burkina Faso. From 2007 to 20013, sesame exportation jumped from 29887.5 to 113092.9 tons, respectively (Figure 2) (INSD, 2010, 2018). This generated a very important income for all the sesame actors. The drastic drop of sesame exportation in 2014 might be linked to the socio-political conditions that the country was facing. It was notably the popular insurrection which stunned the economy of the country especially the flow of imports and export. The year 2015 was the highest year that a very important quantity of sesame seeds was exported. It was believed that this large quantity of sesame exported is the sum of the sesame not exported from the 2014 campaign and that of 2015. Unfortunately, the following two years registered a decrease of exportation. We did not find any relation between the quantity of exported sesame seeds and the quantity of sesame sample brought at the laboratory for control. *Salmonella* presence in sesame seeds is a very big issue, specific methods for the prevention and control of salmonellosis have been undertaken with the support of several associations and farmers' cooperatives, government and NGOs. In order to break the contamination chain specifically, the way *Salmonella* came to be carried into sesame seed. We believed that it is necessary for all sesame growers, collectors and exporters, at different levels, to be trained in food safety and postharvest treatment. Training is essential to ensure that actors understand their responsibilities in terms of sesame seed handling. Fortunately, over the past decades, several associations and farmers' cooperatives supported by the government and NGOs have been working to this end.

From 2011 to 2014, 8, 2, 1 and 2 sesames samples were received successively in four months. Surprisingly, all samples showed at least a positive *Salmonella* cases even if the sample number were low. It is then, imperative to setup an easier and clear traceability that can be understood by all sesame actors in order to solve the issue at the root. In many cases, bacteria contamination

could be avoided with the proper food safety procedures put into place. Interestingly, the *Salmonella* cases decreased notably the last three years and dropped from 38.7 to 6.5%, giving an evidence that a background work were done. On the other hand, while quality is improving these last three years, exported sesame quantities are decreasing because of the important fluctuation of sesame seed kilogram price in the local and international market.

The market for sesame in Asia and Europe is growing at a very high rate in the last decade because the products from sesame readily meets the health requirements for food in the developed world and the popular cuisine in the oriental world (Olowe et al., 2005). This makes the overall international demand of sesame seed steadily growing. In Burkina Faso, this demand is far higher than global supply despite the increase of arable land. From 2007 to 2017, exports of Burkina Faso sesame rose from 29887.5 to 116081.2 tons, with a peak of 171461.1 in 2015, and generated at the same period around 1941.5 USD billion (INSD, 2010, 2018). This production ranked the country among the top ten worldwide countries' producing sesame (Gamené, 2016). On the contrary and according to our knowledge, the increase in production is linked to an improvement of quality as the contamination rate dropped from 26.5 to 6.5%.

Conclusion

In view of this strong growth and the economic income it generates, new strategies of quality improvement should be developed to setup the country in the best place in the international market. This retrospective study about the sesame contamination by *Salmonella* spp. over eleven years showed that contamination rate is high but it also decreases drastically over years. It is believed that our study would contribute and serve as a starting point for more accurate knowledge of the quality of the sesame of Burkina Faso.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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