Original Research Article

Quantitative Risk Assessment of Aflatoxin M1 Related to the Consumption of Raw Cow's Milk in Daloa (Côte d'Ivoire)

ABSTRACT

Cow's milk represents a potential health risk for consumers.The objective of this work is to assess the sanitary quality of raw cow's milk produced and consumed by the populations of Daloa. For this purpose, a total of 30 raw milk samples were collected from 3 raw cow's milk extraction points in the city of Daloa. Mycotoxins were determined using gas chromatography. The results showed that 100% of the analyzed samples were contaminated with aflatoxin M1 (AFLM1). These results indicated that the aflatoxin M1 levels in the analyzed samples exceeded the maximum limit (ML) set by European regulations. To assess the risk of contamination for the population of Daloa, a survey was conducted among consumers of raw cow's milk. A total of 388 individuals were interviewed to gather their opinions. The survey involved a form with questionnaires related to the objectives of the study. This form provided information on identity, marital status, consumption patterns, frequency, quantity, and the socio-economic characteristics of the consumers. The results showed that the risk of liver cancer related to the consumption of raw cow's milk in the city of Daloa is approximately 36.08% to 36.16%; representing 36,080 to 36,150 cases per 100,000 inhabitants.Thus, to ensure consumer safety, good hygiene practices in the preservation of feed for cattle and the construction of enclosures for them must be implemented.

Keywords: Contamination, aflatoxin M1, raw milk, risk assessment.

1. INTRODUCTION

Milk is a food of high nutritional value, very rich in proteins, lipids, carbohydrates, and especially trace elements such as calcium [1]. As such, it holds an indisputable place in the human diet in many countries. In Africa, and particularly in Côte d'Ivoire, raw cow's milk is part of the dietary habits of many communities. Thus, in pastoral systems, it represents social, nutritional, and cultural value [2]. It is essential for good nutritional balance, especially for children, and serves as a solid basis for the intake of animal proteins necessary for each individual [3]. The local raw cow's milk market is extremely narrow, and its consumption is mostly limited to certain segments of the population [4].The production of raw cow's milk in Côte d'Ivoire is low, covering only 17% of total consumption [5]. Additionally, 92% of Ivorian producers are small-scale farmers, and the majority of the milk produced is sold directly to consumers by farmers and small traders in uncontrolled informal markets [6]. It is noteworthy that most farmers, milkers, collectors, and sellers have little knowledge of cow's milk safety throughout the production and sales chain. Consequently, the quality of milk from these small producers is constantly questioned due to the precarious hygiene conditions observed in the dairy sector [7, 8]. It is important to note that in Africa, very little attention is given to this sector, as evidenced by the few studies on biological or chemical risk assessments conducted [9].With societal changes and the food safety risks that can disrupt food security, consumers have become more concerned about the sanitary quality of food, particularly cow's milk. They demand better protection against contamination risks. Among the multitude of known contaminants, mycotoxins (toxic substances produced by molds) and their metabolites, notably aflatoxin M1 (AFM1), pose serious public health problems. Of the 300 secondary metabolites identified internationally, about thirty have concerning toxic properties that pose contamination risks to foods such as raw cow's milk [10]. This risk could be evaluated through a scientific and modern approach, which is risk assessment [11]. Microbiological risk assessment is a new tool for evaluating the food and water safety [12]. This method has been used in several studies to provide solutions to food safety problems. Some authors, like [13], have assessed the risk level associated with *Clostridium perfringens* in the consumption of *attiéké*. Similarly, the work of [14] characterized microbial risks related to the consumption of roasted beef (*Choukouya*) in Côte d'Ivoire. Moreover, recent studies conducted by [15] have quantitatively assessed the risk associated with *Bacillus cereus* for *attiéké* consumers in the cities of Yamoussoukro and Daloa. These studies represent significant advances in improving food safety. This study is therefore highly relevant.Given the potential health risks posed by cow's milk to consumers, it is appropriate to conduct this study. The objective of this work is to assess the sanitary quality of raw cow's milk produced and consumed by the population of Daloa.

2. MATERIAL AND METHODS

2.1. Materials

2.1.1. <u>Study Area</u>

The study area selected for this work is the city of Daloa (**Fig. 1**). It is a very important region for cattle farming, resulting in high production and consumption of raw cow's milk. Additionally, with a population of 245,361 inhabitants, the city of Daloa represents a good market for raw cow's milk consumption [16].

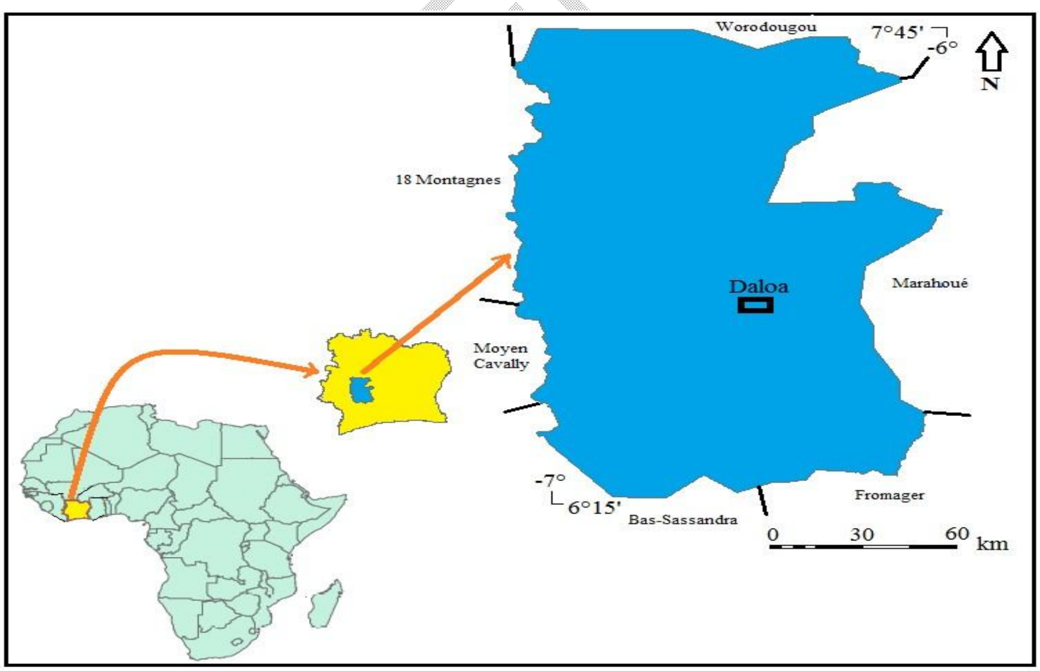

Fig. 1. Location of the study area

2.1.2. <u>Raw Material</u>

The raw cow's milk samples collected in the city of Daloa constituted the raw material (**Fig. 2**). These raw cow's milk samples were gathered from farmer-producers in three major cattle farming areas in the city of Daloa.

Fig. 2. Sample of raw cow's milk

2.2. Methods

2.2.1. <u>Sampling</u>

2.2.1.1. Determination of the Sample Size or Number of People to Survey

The population surveyed for the risk assessment of aflatoxin M1 contamination related to the consumption of raw cow's milk consisted of raw cow's milk consumers. For this survey, 388 people were investigated across the city in different neighborhoods (**Table 1**), following the method proposed by [17]. This method recommends calculating the sample size for a survey using the following formula:

$$n = \frac{t^2 p\,(1-p)}{e^2}$$

With:
n: Sample size
t: Margin coefficient (1.96)
e: Margin of error (0.05)
p: Proportion of elements in the parent population (p = 0.5)

Table 1. Distribution of surveyed individuals by neighborhood

Neighborhoods	People surveyed
Cafop	62
Lobia	36
Kennedy	27
Abattoir	59
Tazibo	25
Marin	52
Orly	36
Soleil	31
Manioc	34
Texas	26
TOTAL	388

2.2.1.2. Collection of Samples for Analysis

The raw cow's milk samples were collected from herdsmen in the three major cattle farming areas of the city of Daloa: the Abattoir 2, Cafop, and Kennedy 1 neighborhoods. A total of thirty (30) composite samples of raw cow's milk were collected. These raw cow's milk samples were randomly taken from different cows and placed in jars covered with aluminum foil and sealed tightly to limit oxidation. Additionally, the collected samples were labeled and transported directly to the National Agricultural Development Support Laboratory in Abidjan in coolers containing dry ice (aseptic conditions and under a cold regime, refrigerated cooler) at 4°C for analysis.

2.2.2. Survey Procedure

Before the survey, the questions formulated based on the study's objectives were tested on a small number of consumers. This test ensured the proper formulation of the questions. The investigations were conducted randomly throughout the city to carry out the survey. For this purpose, the adopted procedure was a structured interview based on a pre-prepared questionnaire. The questions were asked in French and focused on the consumption pattern of raw cow's milk (quantity consumed, mode of consumption, and consumer characteristics). Whenever necessary, a translator guide was included to communicate with respondents who did not speak French. The surveys were conducted in ten (10) neighborhoods of the city of Daloa. The respondents, with a minimum age requirement of 13, were surveyed without distinction of ethnicity or gender. At the end of this survey, three hundred and eighty-eight (388) people were investigated, following the method proposed by [18].

2.2.3. Assessment of the Quantity of Raw Cow's Milk Consumed Per Person

The assessment of the quantity of raw cow's milk consumed per person, per meal, and per day was conducted during an interview. This involved a discussion in which participants were asked to describe the quantities of raw cow's milk they purchased and consumed daily. These quantities, as described by the consumers, were then purchased and measured to estimate the actual amounts. The average masses obtained served as the database for risk calculation.

2.2.4. Determination of Aflatoxin M1 in Raw Cow's Milk Samples by GasChromatography

2.2.4.1. Preparation of Raw Cow's Milk Samples

Aflatoxins M1 are detected in the fat (F) of raw cow's milk. For this purpose, the raw cow's milk samples contained in jars were stored at room temperature (25°C) for six days. This technique allowed the decantation of the raw cow's milk. After decantation, two phases were obtained: a hydrophilic phase (containing water) and a lipophilic phase (containing fat) (**Fig. 3**). Subsequently, a quantity of 10 g of the lipophilic phase from each decanted raw cow's milk sample was collected and dried in an oven (AC GRACIOTTI) at 55°C for 24 hours. To each dried sample, 10 g of anhydrous sodium sulfate

(Na_2SO_4) was added. The mixture was homogenized by manual stirring until a fine powder was obtained. This fine powder constituted the fat of the raw cow's milk.

Photograph b. Extracted fat

Photograph a. Decanted raw milk

Fig. 3. Decantation and extraction of fat from raw cow's milk

2.2.4.2. Extraction and Purification of Aflatoxin M1

A quantity of 5 g of each fine powder sample of raw cow's milk was taken and ground in a mortar. The ground sample was mixed with 10 mL of distilled water and 50 mL of chloroform, then homogenized with a mixer for 2 minutes. The resulting solution was filtered using a press filter. A quantity of 25 mL of the filtrate was recovered and filtered through a bed of anhydrous sodium sulfate (Na_2SO_4). The obtained extract was concentrated to 2.5 mL by vacuum evaporation. Purification was carried out using a florisil cartridge. Specifically, the 2.5 mL filtrate obtained previously through vacuum evaporation was poured into the cartridge containing 20 mL of hexane for elution. The mixture was manually stirred. After discarding the eluate, 20 mL of ether was added to the resulting solution to remove the fat. To elute aflatoxin M1, 20 mL of chloroform-acetone (15/5; v/v) was added to the final solution. The eluate was vacuum evaporated at 35°C until a concentration of 25 µL of the final solution was reached. This new solution was used for injection into the chromatograph.

2.2.4.3. Quantification of Aflatoxin M1 by Gas Chromatography

The quantification of aflatoxin M1 was performed using a gas chromatograph (SHIMADZU GC-14A split/splitless) equipped with a 63Ni electron capture detector and a SHIMADZU CR6A CHROMATOPAC integrator. A quantity of 5 µL of each purified raw cow's milk sample solution was injected into the gas chromatograph, which was connected to a computer.

2.2.5. <u>Risk Assessment</u>

The risk assessment related to aflatoxin M1 in cow's milk was conducted following the method of the Codex Alimentarius Commission [19]. It was carried out in four steps: hazard identification, hazard characterization, exposure assessment, and risk characterization. The exposure assessment step provides a mathematical link (model) between the quantity of microorganisms ingested and the fraction of the consumer population that would become ill. As for the risk characterization step, it establishes the risk of disease occurrence for a given population [20].

2.2.5.1. Hazard Identification

Hazard identification involved a literature review of the risk that could cause adverse health effects and that may be present in a food group or a specific food [11]. The mycotoxin studied in this work is aflatoxin M1.

2.2.5.2. Hazard Characterization

A description of the severity of the effects resulting from the ingestion of the hazard in food was carried out during the hazard characterization step. Establishing the dose-response relationship or the relationship between the level of risk and the level of toxic effect should allow for determining the infectious dose or the disease [13, 21].

2.2.5.3. Exposure Assessment
2.2.5.3.1. General Overview of the Evaluation Model
The exposure assessment is based on scenarios of consuming contaminated food [22]. For this study, it was assumed that raw cow's milk is consumed immediately after purchase or production without being heated. This scenario suggests that contamination by aflatoxin M1 in raw cow's milk indicates that the feed consumed by the dairy cows is contaminated by *Aspergillus flavus*, which produces aflatoxins B1.

2.2.5.3.2. Modeling of Exposure Assessment
The amount of aflatoxin M1 ingested was obtained by multiplying the distribution of aflatoxin M1 concentration (C) by the distribution of the quantity (Q) of raw cow's milk consumed per meal per person. The method used to perform this operation was Monte Carlo simulation. The equation for calculating the quantity of aflatoxin M1 (expressed in µg) ingested per person is:

$$\mathbf{I = Q \times C}$$

The use of this relationship required experimental data for aflatoxin M1 concentrations (expressed in µg/kg) in the raw cow's milk sold and a survey of households on the quantities of raw cow's milk consumed per meal. The data collected on these two parameters provided their distributions. The uncertainty regarding the parameters of the evaluation model was quantified using the bootstrap method [13].

2.2.5.3.3. Risk Characterization

The characterization integrates the results of hazard identification, hazard characterization, and exposure assessment to estimate risks by performing a quantitative assessment of the likelihood and severity of adverse effects that may occur in the population following the consumption of cow's milk. In the probabilistic risk estimation method used in this study, the consumption distribution and the contamination distribution of the population are considered. Then, through Monte Carlo simulations, the distribution of contaminant ingestion by the population is determined. By comparing the value of the toxic dose lower than 0.20 ng/kg [10] with the contaminant ingestion distribution, the risk value range for 100,000 inhabitants is determined.

2.2.3. <u>Statistical Analyses</u>
2.2.3.1. Descriptive Statistics
The data collected from the survey and the quantification of aflatoxin M1 in the raw cow's milk samples were subjected to statistical analyses. Recoding was performed to allow for data processing. These data were entered into a computer equipped with Excel 2010 software. The results were presented in the form of tables and graphs.

2.2.3.2. **Monte Carlo Simulation**
MATLAB R 2015b software was used to determine the risk of intoxication associated with the consumption of raw cow's milk. Based on the amount of aflatoxin M1 found in raw cow's milk and the consumption data, and applying the mathematical model established during the exposure assessment step, a cumulative probability curve was obtained using Monte Carlo simulation.

3. Results and Discussion
3.1. Characteristics of Consumers of Raw Cow's Milk
In preparation for the characterization of the risk associated with aflatoxin M1 for consumers of raw cow's milk, a survey was conducted. This survey helped identify the general consumption pattern of raw cow's milk (**Table 2**). The results revealed that raw cow's milk is consumed by both Ivorians (72%) and foreigners (28%) across almost all age groups within the city of Daloa. Thirty-one percent of consumers are under 13 years old, while 34% of raw cow's milk consumers are between 13 and 30 years old, 19% are between 30 and 40 years old, and 16% are over 40 years old. These results are similar to those reported by [4]. Despite the low production of raw cow's milk, it remains an available food accessible to all income levels, likely due to its low cost and its nature as a ready-to-consume food. The consumption of raw milk has been recorded across all social classes, including among youth, adolescents, and adults, particularly the uneducated (50%). These results may be explained by the fact that raw cow's milk is a food that contains numerous nutrients and is ready to consume [3].

Table 2. Characteristics of Cow's Milk Consumers

Characteristics		**Percentages (%)**
Nationalities	Ivorian	72
	Foreign	28
Age Groups	<13 years	31
	13-30 years	34
	30-40 years	19
	> 40 years	16
Education Level	Primary	18
	Secondary	25
	Superior	10
	Uneducated	50

3.2. Mycotoxicological Characteristics (Aflatoxin M1) of Raw Cow's Milk

To assess the risk associated with aflatoxin M1 for consumers of raw cow's milk, a mycotoxicological characterization was performed on various samples of raw cow's milk. This involved determining the aflatoxin M1 content in each of the samples. The analysis results show that the raw cow's milk samples are heavily contaminated with aflatoxin M1 (**Fig. 4**). This contamination was observed at the three raw cow's milk extraction sites: Abattoir 2, Kennedy 1, and Cafop. The quantities of aflatoxin M1 found in the raw cow's milk samples ranged from 0.071 to 0.322 µg/kg (**Fig. 4**). The analysis indicated that 46.66% of the samples had aflatoxin M1 levels ranging from 0.071 to 0.100 µg/kg. Furthermore, 6.66% of the raw cow's milk samples had aflatoxin M1 levels between 0.100 and 0.143 µg/kg; 16.66% between 0.143 and 0.190 µg/kg; and finally, 30% of the raw cow's milk samples had aflatoxin M1 levels between 0.190 and 0.322 µg/kg.After the mycotoxicological analyses, the results revealed that all samples from the three sites (Cafop, Kennedy, and Abattoir 2) were heavily contaminated with aflatoxin M1. The quantities of aflatoxin M1 found in the raw cow's milk samples ranged from 0.071 to 0.322 µg/kg (**Fig. 4**). The analysis showed that 46.66% of the samples had aflatoxin M1 levels ranging from 0.071 to 0.100 µg/kg. All raw cow's milk samples exhibited contamination levels exceeding the maximum allowable limit (ML) of 0.050 µg/kg [23]. The values obtained are significantly higher than the Estimated Daily Intake (EDI) of raw milk in Ethiopia (0.145×10^{-3} µg/Kg bw/day) [24] and in Iran ($0.145 - 0.300 \times 10^{-3}$ µg/Kg bw/day) [25]. These results differ from those of [26] and [27], who found aflatoxin M1 levels in raw cow's milk samples below the maximum allowable limit. Moreover, the aflatoxin M1 levels in the raw cow's milk samples were relatively close to those obtained by [28] in Kuwait, which were 59%. [29], who worked on 163 raw cow's milk samples, reported aflatoxin M1 levels close to those found in the studied samples (56.59%). Authors such as [30] in Pakistan, [31] in Iran, and [32] in Syria also found aflatoxin M1 in the raw milk samples they analyzed, with levels of 20%, 36%, and 7.20%, respectively. It is also important to highlight that high aflatoxin M1 levels in raw cow's milk samples (0.11 µg/kg) have already been reported in studies by [33] in Morocco, [34] in Libya, and [35] in Egypt. In comparison, the incidence of contamination by AFM1 in raw cow's milk in Europe is much lower (~0.010 µg/kg, below 0.050 µg/kg) [36–38] than that obtained in our study. This is justified by stricter regulatory measures in force in the European Union compared to those applied in African countries. Rigorous monitoring and control plans should be implemented at the Ivorian level to reduce the risk of aflatoxin M1 contamination in raw cow's milk.

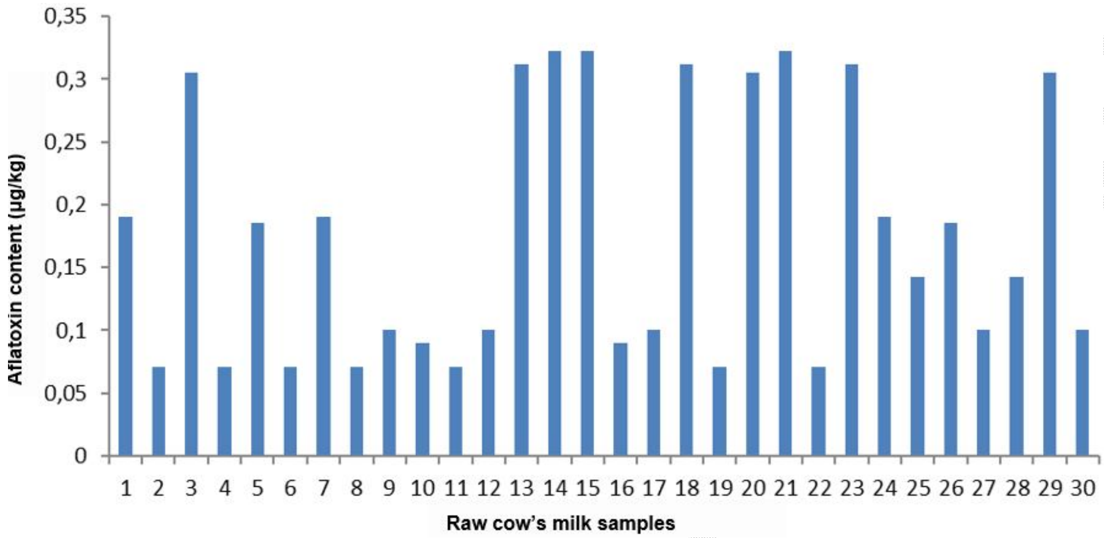

Fig. 4.Distribution of Aflatoxins in Raw Cow's Milk Samples

3.3. Exposure Assessment and Risk Characterization

Although cattle often serve as an effective filter against fungal toxins, the presence of aflatoxin M1 in raw cow's milk could pose a risk to human health [39]. Based on the distribution of aflatoxin M1 content and the consumption of raw cow's milk samples, a mathematical model was established to assess exposure. Monte Carlo simulation was performed through a cumulative probability curve. Thus, the distribution of aflatoxin M1 ingestion by the population is determined. In the context of quantitative mycotoxicological risk assessment (in accordance with the Codex Alimentarius), which requires the selection of a matrix/hazard pair [40], aflatoxin M1 was chosen as a hazard due to its potential to cause toxic infection. The results of the risk assessment for aflatoxin M1 related to the consumption of raw cow's milk revealed that the risk ranges from 36.08% to 36.16% (**Fig. 5**). This represents 36,080 to 36,150 cases of aflatoxin M1 intoxication per 100,000 inhabitants. The risk due to the presence of aflatoxin M1 in raw cow's milk is real and significant. The risk from aflatoxin M1 would be exacerbated by cross-contamination. Indeed, observations made in the field revealed that some consumers store raw cow's milk in refrigerators, in contact with other foods and various products that generally do not undergo any disinfestation. Retailers or street vendors handle multiple goods and serve raw cow's milk to consumers without washing their hands. The consumer exchanges currency for raw cow's milk and consumes it directly without cleaning the packaging (plastic bag, container). If the raw cow's milk is packaged in a jar, only the contents are consumed, either directly or with bowls. Additionally, the cleaning of jars after use does not always adhere to good hygiene practices. These containers may thus serve as reservoirs for potentially harmful germs, including molds. Furthermore, raw cow's milk is extracted in a artisanal manner by herders. Additionally, cows generally consume food waste such as cassava peels, yam peels, plantain peels, and other foods collected and deposited in landfills. These landfills constitute a potential source of microorganisms responsible for toxin secretion. It should be noted that inadequate hygiene in the storage facilities for cow feed could contribute to the contamination of these feeds by molds responsible for the secretion of aflatoxin B1, whose metabolism leads to the production of aflatoxin M1. All these practices could be sources of contamination of raw cow's milk by molds.

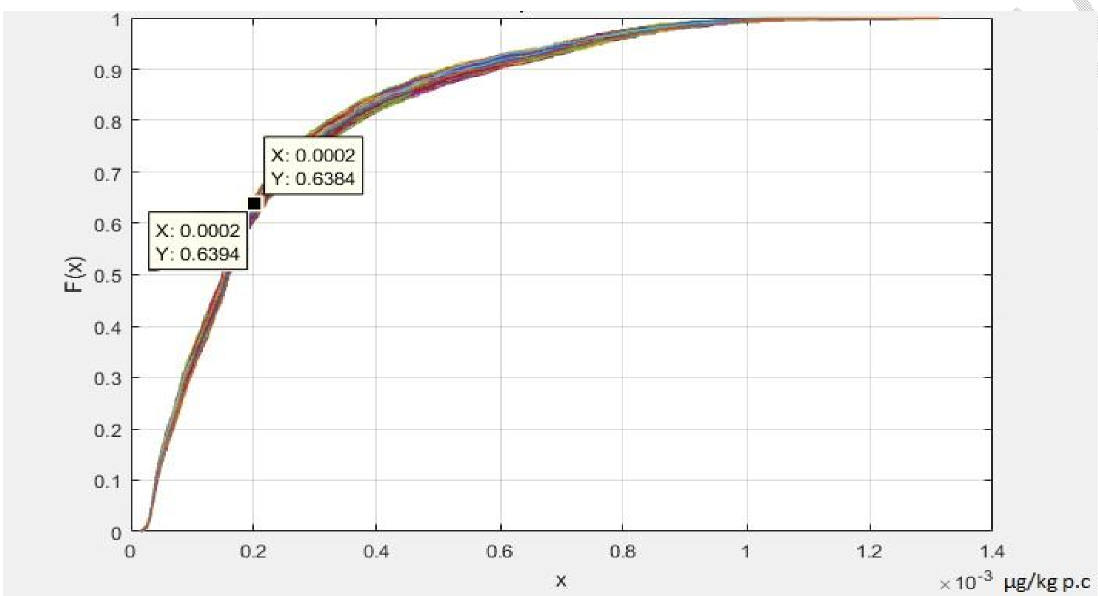

Fig. 5. Risk of toxic infection associated with the consumption of raw cow's milk according to Monte Carlo simulation.

4. Conclusion

This study on the risk assessment of toxic infection related to aflatoxin M1 for consumers of raw cow's milk was conducted in the city of Daloa. It demonstrated that raw cow's milk is consumed by all social classes and age groups. Moreover, this widely consumed product poses a health risk to consumers and represents a public health issue in the city of Daloa. Additionally, the risk assessment of aflatoxin M1 related to the consumption of raw cow's milk revealed that it is real and exists at very high levels (0.071 - 0.322 µg/kg), exceeding the recommended maximum limit (0.050 µg/kg) in cow's milk. This study is just a contribution to the assessment of aflatoxin M1 in raw cow's milk. These results should draw the attention of the authorities and serve as a basis for conducting awareness campaigns for the general public and cattle breeders about the reality of foodborne mycotoxicological risks. Therefore, to ensure consumer safety, measures such as the implementation of good hygiene practices in the preservation of cattle feed and the construction of enclosures for cattle to control their diet and environment should be taken to reduce primary contamination of cows and protect consumers from toxic infections related to raw cow's milk.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

REFERENCES

[1] FAO. Milk and dairy products in human nutrition. Rome, Italy, 2009.

[2] Fokou G, Koné BV BB. My milk is pure and cannot make you sick: motivations of informal sector actors and quality of local milk in Mali. Rev Afr Santé Prod Anim 2010; 8: 75–86.

[3] Amiot J., Fournier S., Lebeuf Y., Paquin P. SR. Composition, physicochemical properties, nutritional value, technological quality and analysis techniques of milk. In: Montréal É polytechnique de (ed) Vignola C.L., Science and technology of milk: transformation of milk (2nd Eds). Montréal, 2002.

[4] Kouamé-Sina S.M., Bassa A., Dadié A., Makita K., Grace D., Djè M. BB. Analysis of microbial risks of local raw milk in Abidjan (Côte d'Ivoire). Rev Africaine Santé Prod Anim 2010; 8 (S): 35–42.

[5] MIRAH-DPP. Yearbook of animal and fishery resources statistics. Direction. Abidjan, Côte d'Ivoire, 2012.

[6] A. Y-B. Managerial efficiency of cattle breeders in Côte d'Ivoire: case of the departments of Toumodi and Korhogo. University of Cocody, Côte d'Ivoire, 2008.

[7] AfDB (African Development Bank). Livestock development phase II. Mid-term evaluation. Final report. Abidjan, Côte d'Ivoire, 2002.

[8] Essan Z, Goran BN, Aw S, et al. Study of the influence of osmotic dehydration parameters on water loss of tropical fruits: trials with papaya (Carica papaya) and mango (Mangifera indica). 2012; 4330–4339.

[9] Grace D., Makita K. EEKK et BB. Safe food, fair food: Participatory risk analysis for improving the safety of informally produced and marketed food in sub–Saharan Africa. *Rev Africaine Santé Prod Anim* 2010; 8(S): 3R11.

[10] AFSSA. Risk assessment of mycotoxins in the human and animal food chains. Agreste strain, Journal «Le Monde», 2009, p. 24.

[11] Assidjo, E., Aw, S., Akmel, C., Akaki, D., Elleingand, E., & Yao B. Risk analysis: An innovative tool for improving food safety. Rev Africaine Santé Prod Anim 2013; 11: 3–13.

[12] FAO/WHO. Characterization of hazards related to the presence of pathogens in food and water - guidelines. Assessment. 2004.

[13] Akmel D.C., Aw S., Montet D., Assidjo N., Degni M.L., Akaki D., Moretti C., Elleingand E., Brabet C., Baud G., Mens F., Yao B., Michel T., Durand N., Assin H. B. Microbiological risk associated with the consumption of attieke in Cote d'Ivoire. Food Control 2017; 81: 65–73.

[14] Dibi, E. A. D. B., N'Goran-Aw, Z. E. B., Akmel, D. C., Tano. K., & Assidjo EN. Microbial risks associated with the consumption of braised beef "Choukouya" in Côte d'Ivoire. Int J Innov Appl Stud 2017; 19: 496–507.

[15] Koko A. C., Kouamé K. B., Diomandé M., N'Goran M. F. ADC an. ANE. Quantitative Assessment of the Risk Associated with Bacillus cereus Group for the Attieke Consumer in Daloa City (Côte d'Ivoire). Asian Food Sci J 2018; 1: 1–13.

[16] INS. General census of population and housing., http://www.ins.ci/n/documents/RGPH2014_expo_dg.pdf. (2014).

[17] Giezendanner F.D. Random sample size and margin of error, http://icp.ge.ch/sem/cms-spip/spip.php?article1641 (2012).

[18] Watts, B.M. Ylimaki, G.L. Jeffery, L.E. Elias LG. Basic methods for sensory evaluation of foods. Ont., IDRC. Ottawa, 1991.

[19] FAO/WHO. Principles and guidelines for the conduct of microbiological risk assessment.

[20] R. CK. Assessment of the risk of salmonellosis associated with the consumption of curdled milk (artisanal fermented milk) produced in Yamoussoukro. Nangui Abrogoua University, 2015.

[21] Cornu, M., Bergis, H., Miconnet, N., Delignette-Muller, M. L., & Beaufort A. Assessment of microbiological risks: general presentation and application. Générale du FROID 2003; 1032: 33–42.

[22] Alimentarius C. Principles and guidelines for the management of microbiological risks. In: Food and Agriculture Organization of the United Nations, World Health Organization, Geneva. 2007, p. 12.

[23] Codex-stan. general standard for contaminants and toxins in food and beverages. 1995; 193: 34.

[24] Zebib H, Abate D, Woldegiorgis AZ. Exposure and Health Risk Assessment of Aflatoxin M1 in Raw Milk and Cottage Cheese in Adults in Ethiopia. *Foods* 2023; 12: 1–11.

[25] Mokhtari, S.A., Nemati, A., Fazlzadeh M et al. Aflatoxin M1 in distributed milks in northwestern Iran: occurrence, seasonal variation, and risk assessment. *Env Sci Pollut Res* 2022; 29: 41429–41438.

[26] A-J. S. Sources and levels of human exposure to aflatoxins in nandi county, KENYA. Université de Nairobi, 2013.

[27] Nuryono N., Agus A., Wedhastri S., Maryudani Y-B., Sigit Setyabudi F-M-C., Böhm J. R-FE. A limited survey of aflatoxin M1 in milk from Indonesia by ELISA. Food Control 2009; 20: 721–724.

[28] Dashti B., Al-HamLi S., Alomirah H., Al-Zenki S., Bu-Abbas A. SW. Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of total aflatoxin in local and imported animal feed. Food Control 2009; 20: 686–690.

[29] Mahmoudi R. NR. Aflatoxin B1 and M1 contamination in cow feeds and milk from Iran. Food Agric Immunol 2014; 26: 131–137.

[30] Hussain M, Choudhry MA. Functional properties of maize flour and its blends with wheat flour : optimization of preparation conditions by response surface methodology. 2013; 45: 2027–2035.

[31] Rahimi E., Bonyadian M., Rafei M. KH-R. Occurrence of aflatoxin M1 in raw milk of five dairy species in Ahvaz, Iran. Food Chem Toxicol 2010; 48: 129–131.

[32] Ghanem I. OM. Aflatoxin M1 in raw, pasteurized and powdered milk available in the Syrian market. Food Control 2009; 20: 603–605.

[33] El Marnissi B., Belkhou R., Morgavi D-P., Bennani L. BH. Occurrence of aflatoxin M1 in raw milk collected from traditional dairies in Morocco. Food Chem Toxicol 2012; 50: 2819–2821.

[34] El Gerbi A-M., Aidoo K-E., Candlish A-A. TR-F. Occurrence of aflatoxin M1 in randomLy selected North African milk and cheese samples. Food Addit Contam 2004; 21: 592–597.

[35] Ghareeb K., Elmalt L-M., Awad W-A. BJ. Prevalence OF Aflatoxin M1 in Raw Milk Produced in Tropical State (Qena, Egypt) and Imported Milk Powder. J Vet Anim HSci 2013; 3: 1–4.

[36] Velasco M-L-R., Delso M-M-C. ED-O. ELISA and HPLC determination of the occurrence of aflatoxin M-1 in raw cow's milk. Food Addit Contam 2003; 20: 276–280.

[37] Boudra H., Barnouin J., dragacci s. & MDP. Aflatoxin M1 and Ochratoxin A in Raw Bulk Milk from French Dairy Herds. American Dairy Science Association. J Dairy Sci 2007; 90: 3197–3201.

[38] Bellio A., Bianchi D. M., Gramaglia M., Loria A., Nucera D., Gallina S., Gili M. DL. Aflatoxin M1 in Cow's Milk: Method Validation for Milk Sampled in Northern Italy. Toxins (Basel); 8. Epub ahead of print 2016. DOI: 10.3390/toxins8030057.

[39] Fangeat L. Les mycotoxines chez les bovins. l'Université Claude- Bernard - Lyon I, 2008.

[40] Mégraud F., Denis J.-B., Ermel G., Federichi M., Gallay A. KI& FA. Appréciation des risques alimentaires liés aux campylobacters. Application au couple poulet / Campylobacter jejuni. Agence Fra. France, 2003.