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FUNGAL AND AFLATOXIN CONTAMINATION OF RAW COW'S MILK MARKETED IN ABIDJAN, CÔTE D'IVOIRE

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ABSTRACT

Raw cow's milk is a highly nutritious food but also constitutes a favorable substrate for the growth of microscopic fungi, some of which are capable of producing mycotoxins hazardous to human health. The present study aimed to characterize fungal contamination in raw cow's milk produced and marketed in Abidjan (Côte d'Ivoire). A total of twenty-six (26) raw milk samples were collected from vendors and producers identified in five (5) municipalities: Port-Bouet, Yopougon (Lievre-rouge), N'Dotre, Abobo (Derrière-rails), and Songon. Mycological analyses, performed according to ISO standard 21527-1 (2008) on SDA and PDA media, revealed significant and widespread fungal contamination. Mean yeast counts ranged from $1,5 \cdot 10^4$ CFU/mL in Port-Bouet and Abobo (Derrière-rails) to $1,7 \cdot 10^7$ CFU/mL in Yopougon (Lievre-rouge). Mold counts ranged from $6,6 \cdot 10^2$ CFU/mL in Port-Bouet to $9,1 \cdot 10^4$ CFU/mL in Yopougon (Lievre-rouge). Yeasts exhibited a 100% occurrence frequency across all samples regardless of the sampling site, while molds were detected in 76,9% of samples with variable frequencies across sites (50% to 100%). Morphological identification of isolates revealed three mold genera: *Aspergillus*, *Penicillium* and *Rhizopus*. The results highlight milks poor microbiological quality and underscore the need to strengthen hygienic practices and sanitary surveillance across the dairy supply chain in Abidjan.

The qualitative investigation of aflatoxins B1, B2, G1 and G2 by thin-layer chromatography (TLC) revealed no contamination in all analyzed samples. Indeed, the presence in raw milk of the genera *Aspergillus* and *Penicillium*, known for their mycotoxigenic potential, could represent a health risk for all consumers.

Keywords: Raw Milk, Fungal, Mycotoxins, Contamination, Abidjan

1 INTRODUCTION

Milk plays an important role in the human diet due to its nutritional richness and high biological value. It is an essential source of protein, fat, carbohydrate, vitamins and minerals needed for the growth and maintenance of physiological functions in the human body [1]. In Côte d'Ivoire, particularly in the district of Abidjan, raw cow's milk is widely consumed by the population in different forms, notably for its nutritional qualities, its accessibility and its use in several artisanal food preparations.

However, raw milk is also a highly perishable food and is an excellent growth medium for many microorganisms due to its high water and nutrient content. Although milk is generally sterile in the mammary gland of a healthy animal, it can be contaminated during milking, transport, marketing or storage by various microbial agents from the environment, milking equipment, manipulators or even livestock feed [2].

Among the microbiological contaminants found in raw milk, microscopic fungi occupy an important place. Yeasts and especially molds are frequently isolated in dairy products marketed under poor hygiene conditions. Certain fungal genera such as *Aspergillus*, *Penicillium* and *Fusarium* are of major health interest due to their ability to produce toxic secondary metabolites called mycotoxins [3]. Mycotoxins are now a global public health concern because of their toxic effects on humans and animals. Among them, aflatoxins are the most feared because of their hepatotoxic, immunosuppressive, mutagenic and carcinogenic power. Aflatoxin M₁, derived from aflatoxin B₁ ingested by animals, including cows, through contaminated feed, can be excreted into milk and persist even after certain technological treatments [4]. The regular consumption of milk contaminated by toxigenic fungi or mycotoxins thus represents a significant health risk, particularly among children, the elderly and immunocompromised individuals.

In several African countries, the conditions of production, storage and marketing of raw milk are mostly artisanal and are often characterized by insufficient hygiene measures and sanitary control. In Abidjan, raw milk is generally marketed under conditions likely to promote microbial contamination, notably repeated operations of transfilling and splitting into small units, the lack of an effective cold chain as well as the numerous manipulations carried out during marketing.

Despite the importance of this issue, data on fungal and bacteriological contamination of raw milk marketed in Abidjan are still limited. Indeed, scientific studies concerning raw milk have generally focused on the presence of pathogenic bacteria in this food matrix: *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus* spp. or *Bacillus cereus* [5].

The present study aims to characterize the fungal and bacteriological contamination of raw cow's milk produced and marketed in Abidjan. This would help to better manage the risks associated with the consumption of this food and contribute significantly to the protection of consumer health.

2 MATERIALS AND METHODS

2.1 Biological material



Figure 1: Samples of raw cow milk as sold on the market

The biological material consisted of samples of raw milk intended for human consumption (Figure 1), collected from different farms and sales outlets (markets) in the district of Abidjan.

3 METHODS

3.1 Sampling

3.1.1 Sample size and distribution

A total of thirteen (13) raw milk sellers/producers were identified in the different study areas. Given the small population, a comprehensive sampling was adopted. Thus, all the sellers identified were included in the study and raw milk samples were taken from each of them.

Finally, the samples were distributed as follows: eight (8) samples in Port-Bouet, six (6) samples in Yopougon (Lievre-rouge), six (6) samples in N'Dotre, four (4) samples in Abobo (Derriere-rails) and two (2) samples in Songon.

3.1.2 Sampling technique and transport of samples

The samples were taken directly from the sellers and producers, between 6 a.m. and 10 a.m., in accordance with local consumption practices. A quantity of 0,5 L sample of raw milk, contained in non-food-grade plastic packaging as sold on the market was buy, immediately placed in an isothermal cooler containing dry ice and transported to the laboratory of microbiology. Once at the laboratory, the samples were immediately subjected to mycological analyses.

3.2 Yeast and mold research

3.2.1 Preparation of the stock suspension and serial dilutions

A quantity of 25 mL aliquot of each sample of raw milk was taken to prepare the initial suspension (10^0). From this initial suspension, the 10^{-1} dilution was done by taken 1 mL of the initial suspension and adding into 9 mL of tryptone salt. The same procedure was applied to obtain subsequent dilutions (up to 10^{-7}) from each previous dilution.

3.2.2 Enumeration of Yeasts

The yeast count was performed according to ISO [6]. The method used was surface seeding on Sabouraud Dextrose Agar (SDA) medium supplemented with chloramphenicol. For this purpose, a volume of 0.1 mL of the stock suspension and each decimal dilution were deposited on the surface of the SDA medium contained into Petri dishes. The spreading was carried out homogeneously using a sterile spreader, under aseptic conditions near a Bunsen burner flame. The Petri dishes were then incubated at a temperature of 25-28 °C for 3–5 days. After incubation, the characteristic colonies of yeasts such as their creamy, smooth, domed and white to cream appearance, were counted. Each experiment was repeated 3 times, and Petri dishes containing between 15 and 150 colonies were retained. Results were expressed in colony forming units per milliliter (CFU/mL) of milk according to the standard used.

3.2.3 Enumeration of molds

The enumeration of molds was carried out according to ISO [6], by the surface sowing method on medium Potato Dextrose Agar (PDA), adapted to the development of filamentous molds. Thus, a volume of 0,1 mL of the stock suspension and each decimal dilution was deposited on the surface of the sterilized PDA medium, then homogeneously distributed using a sterile spreader. The operations were carried out under aseptic conditions near a Bunsen burner flame. The Petri dishes were then incubated at a temperature of 25-28 °C for 3–5 days. At the end of incubation, the characteristic colonies of molds, identified by their fluffy or cottony appearance, their filamentous growth and their varied colors (white, green, black or gray), were enumerated. Each experiment was repeated 3 times, and Petri dishes containing between 15 and 150 colonies were retained. The results were expressed in colony forming units per milliliter (CFU/mL) of milk, in accordance with the standard used.

3.2.4 Expression of Enumeration Results

The average fungal load was calculated using the formula ISO [7]. The enumeration results were expressed in CFU/ mL using the formula:

$$N = \frac{\sum c}{(n_1 + 0.1 \times n_2) \times V \times d}$$

- N = number of microorganisms in CFU per mL
- ΣC = sum of all colonies on all retained plates.
- n1 = number of plates retained at the first retained dilution.
- n2 = number of plates retained at the second retained dilution.
- d = dilution ratio of the first retained dilution.
- V = volume of inoculum applied to each Petri dish.

3.3 Identification of isolated yeasts and molds

After incubation, molds (on PDA medium) and yeasts (on SDA medium) obtained were observed based on their macroscopic characteristics. Distinct colonies were selected and then subcultured onto a new medium to obtain pure cultures [8, 9].

3.3.1 Macroscopic identification

Macroscopic identification of yeasts and molds was performed using pure cultures. For each culture, the colonies obtained were observed visually to assess the color of the surface and underside of the colonies, their texture (cottony, powdery, velvety, or granular), their topography (flat, pleated, on the ground, or raised), and the appearance of their borders [10].

3.3.2 Microscopic identification

Microscopic identification of yeasts and molds was performed using the slide culture technique described by Riddel [11]. A drop of PDA agar for molds and SDA agar for yeasts was placed on a sterile slide and then inoculated with an aseptically collected colony fragment. A sterile coverslip was carefully placed on the agar block. The entire assembly was placed in a container of distilled water and covered with aluminum foil to maintain a humid atmosphere conducive to fungal growth (**Figure 2**).

After incubation for 3 to 7 days at room temperature, the coverslips were removed, and the slide preparations were observed under a light microscope at 10X and 40X magnification. The microscopic examination focused on observing hyphae, conidiophores, phialides, vesicles, spores, and conidia, as well as their morphology. The identification of the different genera was carried out by comparing the observed morphological characteristics with the taxonomic descriptions available in specialized mycology books and identification keys [12].



Figure 2: Slide culture technique of yeasts and molds

3.4 Frequency of sample contamination by yeasts and molds

The frequency of yeast and mold contamination was determined to assess the proportion of raw milk samples contaminated by these different fungal groups.

The contamination frequency was calculated using the following formula:

$$F (\%) = \frac{n}{N} \times 100$$

- F = Frequency of occurrence;
- n = Number of positive samples for the fungal group considered;

- **N** = Total number of samples analyzed.

3.5 Aflatoxins research

Aflatoxins B1, B2, G1 and G2 were detected by thin-layer chromatography (TLC) according to the method described by Konate[13]. This method includes successive steps of extraction, purification, delipidation, re-extraction of aflatoxins, then separation and identification on silica plates.

3.5.1 First extraction

This first step involves extracting aflatoxins and any other compounds present in the samples. For each sample, 20 mL of raw milk was placed in an Erlenmeyer flask with 100 mL of extraction solvent composed of 80 mL of methanol and 20 mL of distilled water. The mixture was stirred for 15 minutes using a magnetic stirrer and then filtered through filter paper. The resulting filtrate (filtrate 1) was retained for the purification step.

3.5.2 Purification (protein extraction)

Filtrate 2 was obtained by adding 45 mL of precipitation reagent to 50 mL of filtrate 1. After homogenization, the mixture was left to stand for 15 minutes, then filtered.

Before the delipidation step, the silica gel plate was prepared in an oven at 105°C for one hour, as was the saturation cell, which contained 2 mL of isopropanol, 93 mL of chloroform, and 5 mL of acetonitrile.

3.5.3 Delipidation

Filtrate 2 was subjected to liquid-liquid extraction using petroleum ether in a separating funnel to remove the lipid fraction. Three successive extractions were carried out with 10 mL of petroleum ether each time. The recovered organic phases were eliminated after each extraction.

3.5.4 Extraction of aflatoxins

From the delipided filtrate 2, aflatoxins were extracted with 3 times 10 mL of chloroform in a separating funnel. The collected chloroformic extract was collected in a clean capsule, then evaporated to dryness in a water bath or on a hot plate.

3.5.5 Separation and identification

The silica plate was graduated as follows: a first horizontal line was drawn 1 cm from the bottom of the plate, delimiting the deposition line, and a second horizontal line 13 cm from the bottom, corresponding to the elution front (i.e. a useful migration of 12 cm for a 7 cm wide plate).

The deposits of controls (aflatoxin standards) and extracts to be analyzed were carried out on the deposit line. The plate was then introduced into the previously saturated migration tank, which was immediately and carefully closed again. The migration was stopped when the solvent front reached about 1 cm from the top line (elution front). The plate was then removed and dried at room temperature.

The observation was carried out under ultraviolet lamp, allowing the visualization of fluorescent spots characteristic of aflatoxins. The identification was carried out by comparing migration distances with those of the standards.

3.6 Data processing and statistical analysis

The data collected during this study was captured, organized and processed using Microsoft Excel. Descriptive statistical analyses were carried out to determine the frequencies of contamination, the means and the standard deviations of the various variables studied. The 95% confidence intervals were also calculated for some variables to estimate the accuracy of the averages obtained.

4 RESULTS

4.1 Fungal loads of the raw milk samples

4.1.1 Average Yeast Loads

According to Table 1, the average yeast loads observed in the samples varied depending on the sampling site. The highest average yeast load was recorded in the raw milk samples collected in Yopougon (Lievre-rouge). However, the lowest loads were observed in the samples from Port-Bouët and Abobo (Derrière-rails).

Table 1: Average yeast loads in raw milk samples by sampling site

Sampling sites	Average loads in CFU/mL	IC 95% (CFU/mL)
Port- Bouët	$1,5.10^4$	$1,6.10^2 - 1,5.10^6$
Yopougon (Lievre-rouge)	$1,7.10^7$	$5,8.10^2 - 5,3.10^{11}$
N'Dotre	$8,0.10^5$	$1,2.10^{-2} - 5,0.10^{13}$
Abobo (Derrière-rails)	$1,5.10^4$	$4,0.10^1 - 6,0.10^6$
Songon	$8,1.10^6$	$2,0.10^1 - 3,0.10^4$

4.1.2 Average Mold load

The average mold load varied from one commune to another. The highest average mold load was observed in raw milk from Yopougon (Lievre-rouge), while the lowest load was observed in milk collected in Port-Bouët (Table 2).

Table 2: Average mold loads in raw milk samples by sampling site

Sampling sites	Average fungal loads in CFU/mL	IC 95% (CFU/mL)
Port-Bouët	$6,6.10^2$	$9,3.10^0 - 4,7.10^4$
Yopougon Lièvre-rouge	$9,1.10^4$	$4,1.10^{-5} - 2,0.10^{14}$
N'Dotre	$7,3.10^3$	$1,4.10^{-8} - 3,6.10^{15}$
Abobo Derrière-rails)	$1,1.10^3$	$1,8.10^{-9} - 7,5.10^{14}$
Songon	$1,2.10^3$	$1,4.10^{-5} - 7,5.10^{11}$

4.2 Frequency of sample contamination by yeasts and molds

4.2.1 Overall frequency of sample contamination

The overall frequency of yeast and mold contamination in raw milk samples is presented in Table 3. According to this table, yeast was detected in all samples analyzed, representing a contamination rate of 100%, while the overall frequency of mold contamination was 76.9%.

Table 3: Overall frequency of sample contamination by yeasts and molds

Fungus	Number of samples contaminated (n=26)	Frequency of contamination (%)
Yeast	26	100
Mold	20	76,9

(n= total number of raw milk samples analyzed)

4.3 Frequency of contamination of milk samples by yeasts and molds

The frequency of yeast and mold contamination in the samples varied across the municipalities studied.

Yeast contaminated 100% of the samples in all municipalities, indicating its systematic presence in all analyzed samples. This high occurrence suggests a wide distribution of yeast in commercially available raw milk, regardless of the sampling site.

Regarding mold, variations were observed between municipalities. The highest contamination rate was recorded in N'Dotre and Songon, where all analyzed samples were contaminated. Relatively high rates were also observed in Port-Bouet (75%) and Yopougon (Lièvre-rouge) (66.7%), while the lowest rate was noted in Abobo (Derrière-rails) (50%).

The presence of yeast and mold in all samples from several municipalities reflects significant fungal contamination of commercially available raw milk in the studied areas (Figure 3).

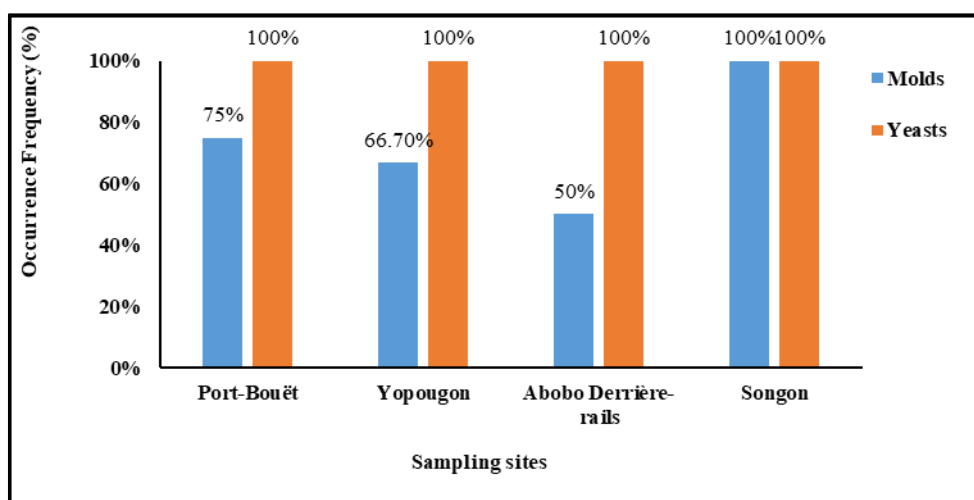
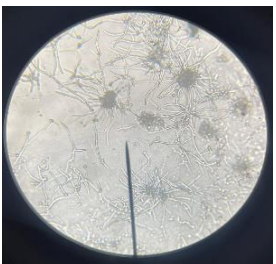
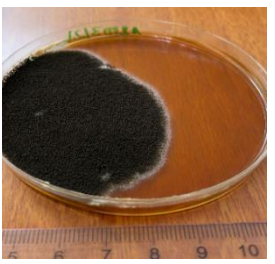


Figure 3: Frequency of yeast and mold contamination in raw milk samples by sampling site.

4.4 Phenotypic profile and characteristics of some molds and yeasts isolated from analyzed raw milks

Figure 4 shows the phenotypic profiles of some isolated fungi as well as their macroscopic and microscopic aspects which can be used to identify them.

Macroscopic aspect	Microscopic aspect	Probable genera
 Small, aligned, whitish to greenish-white colonies with a slightly powdery texture and high relief.	 Thin mycelial filaments, radiating star-shaped structures with clearly visible multiple branches, resembling a paintbrush	<i>Penicillium sp.</i>
 Completely black colonies, very dense, powdery texture, flat relief	 Long, straight conidiophores with globose terminal vesicles and black conidia arranged in an aspergillus head	<i>Aspergillus sp.</i>

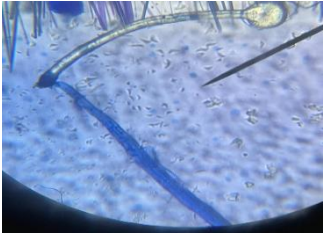
 A completely white colony, cottony texture, extending over the entire box, with a typical fluffy appearance.	 Broad, non-septate hyphae	<i>Rhizopus sp.</i>
 Creamy white, glossy, domed colonies	 Oval cells, multilateral budding, nascent pseudohyphae	<i>Saccharomyces sp.</i>

Figure 4: Phenotypic profile and characteristics of some molds and yeasts

4.5 Types of Molds and Yeasts isolated in raw milk samples analyzed

Based on the phenotypic profiles and characteristics of the isolated fungal strains, three main types of molds and one yeast were identified in the analyzed raw milk samples. These were the genera *Aspergillus*, *Penicillium*, and *Rhizopus* for the molds, and *Saccharomyces* for the yeasts. Table 4 presents the different types of molds and yeasts identified according to the raw milk sampling locations.

Table 4: Presence in raw milk of the different types of molds identified according to the sampling locations.

Types of molds	Port-Bouët	Yopougon Lièvre-rouge	N'dotre	Abobo Derrière-rails	Songon
<i>Aspergillus</i>	+	+	+	+	+
<i>Penicillium</i>	+	+	+	+	+
<i>Rhizopus</i>	+	+	+	+	-
<i>Saccharomyces</i>	+	+	+	+	+

(+) presence of gender; (-) absence of gender

4.6 Mycotoxin profile of raw milk samples

The search for aflatoxins B1, B2, G1 and G2 was carried out on the 26 raw milk samples collected. The results showed that none of these aflatoxins was detected in the analyzed samples. Thus, no contamination profile by the aflatoxins sought was highlighted in the raw milk samples studied.

5 DISCUSSION

The results obtained highlight a global fungal contamination of raw cow milks marketed in the district of Abidjan, characterized by both high yeast and mold loads. The average yeast loads ranged from $1.5 \cdot 10^4$ UFC/mL in raw milk samples collected at Port-Bouët and Abobo (Derrière-rails) to $1.7 \cdot 10^7$ UFC/mL in those from Yopougon (Lièvre-rouge). At the same time, mold loads ranged from $6.6 \cdot 10^2$ UFC/mL in samples from Port-Bouët to $9.1 \cdot 10^4$ UFC/mL in those from Yopougon (Lièvre-rouge). On the microbiological level, these results reflect a significant deterioration in the hygienic quality of the raw milks analyzed. The simultaneous presence of yeasts and molds at high concentrations indicates a favorable environment for the development of these microscopic fungi, which can be attributed to several factors. Raw milk, rich in nutrients and with high water activity, is a particularly suitable substrate for fungal growth. In the absence of adequate refrigeration and heat treatment, microorganisms initially present or introduced during handling can multiply rapidly. In addition, insufficient hygiene conditions during milking, transport and marketing promote

continuous exogenous contamination of the product. Lavoie *et al.* [14], working on raw milk mentioned that 67% of the raw milk isolates were yeasts, which were assigned to 37 species across 11 genera, while 33% were molds, which were assigned to 33 species across 25 genera. Moreover, the composition and diversity of the fungal microflora were totally different for each farm, each of which had a unique species. Furthermore, Hamdi *et al.* [15] isolated different mold and yeast (73.33%) from 55 milk samples with ten samples (18.18%) demonstrating mixed fungal infection. Among these isolates, *Candida albicans* (43.64 %), *Penicillium spp* (38.18 %), and *Aspergillus fumigatus* (25.45 %) were shown to be the most common fungi linked with the examined milk samples, while low percent was identified for yeast *Rhodotorula* and *Aspergillus flavus* 7.27% and 1.81%, respectively.

It should also be noted that these raw milks are marketed in plastic bottles and, for a large part, preserved in barrels or cans. This type of packaging and storage, often not airtight and poorly adapted to the requirements of milk hygiene, can promote contamination and proliferation of microscopic fungi. Indeed, these containers are frequently reused without proper sterilization and exposed to the environment, which increases the risk of introducing microorganisms throughout the marketing chain.

In view of the yeast loads, and referring to the indicative threshold of 10^4 CFU/mL proposed by Jiang *et al.* [16], all samples show an unsatisfactory microbiological quality, with particularly high levels in certain samples derived from Yopougon (Lievre-rouge), reflecting a more marked degradation.

These results are consistent with those reported by Freitas *et al.* [17], who reported yeast leaven loads between 10^5 and 10^7 CFU/mL in raw milks from informal marketing channels. Regarding molds, the levels observed are comparable to those reported by Iqbal *et al.* [18], which highlighted loadings ranging from 10^2 to 10^5 CFU/mL in informal dairy production systems.

Moreover, the frequency of appearance of yeasts was 100% in all milks from the municipalities studied, indicating their systematic presence in all samples analyzed. This result is consistent with the findings of Jiang *et al.* [16], who reported frequencies of yeast occurrence in raw milk ranging from 88% to 100% in low-and middle-income countries.

This almost systematic presence of yeasts in the raw milks analyzed can be explained by their wide distribution in the environment, their ability to multiply rapidly in a nutrient-rich medium, as is the case with milk, and their ability to develop under unfavourable storage conditions [9]. Regarding molds, variable frequencies of milk contamination (50% to 100%) were observed depending on the sampling sites. These frequencies, although lower than that of yeasts, confirm the existence of a significant fungal contamination of the raw milks studied. Similar results were reported by Freitas *et al.* [17] who reported mildew occurrence rates ranging from 62% to 100% in milk in extensive farming systems. The differences in frequency of occurrence observed between milks from different sampling sites could be associated with variations in hygiene conditions, handling and environmental exposure of milks.

The morphological identification of mold isolates highlighted three genera, namely *Aspergillus*, *Penicillium* and *Rhizopus*. This diversity of genera reflects a fungal contamination of multiple origins, mainly related to the production environment, raw milk handling conditions and marketing practices. It indicates that milk is exposed to various sources of contamination throughout the chain, particularly during milking, storage and transport. That diversity is consistent with literature data, which frequently report the presence of several of these types of molds in milk. [19,20]. Similarly, these observations are part of the context described by Quigley *et al.* [21] who showed that fungal contamination of raw milk is strongly influenced by hygiene, handling and storage conditions.

From a microbiological point of view, the presence of the genera *Aspergillus* and *Penicillium* is particularly important, as these genera include species capable of producing mycotoxins under favorable conditions, such as high humidity, a temperature between 20°C and 30°C and high water activity, as is the case with raw milk [10]. However, the mere presence of these genera does not allow to conclude that there is an effective production of mycotoxins, which depends on the specific strain and environmental conditions. The genus *Rhizopus* is generally associated with environmental contamination, which can occur during the storage or transport of milk, reflecting inadequate hygiene conditions. Thus, its presence in the samples of analyzed milks indicates non-compliance with hygiene rules by producers and/or sellers.

The qualitative research of aflatoxins B1, B2, G1 and G2 by thin layer chromatography revealed no contamination of the twenty-six samples studied. However, this result should be interpreted with caution due to the limitations of the method used. Indeed, thin-layer chromatography is a qualitative method whose sensitivity is lower than that of reference chromatographic methods such as HPLC or LC-MS/MS [1]. Thus, the presence of low concentrations of mycotoxins cannot be totally excluded. After ingestion of aflatoxin B1 by the animal, it is metabolized at hepatic level to aflatoxin M1, which is then excreted in milk. [25] The absence of aflatoxins B and G therefore does not allow to conclude a total absence of mycotoxin contamination. Leite *et al.* [22] observed an exposure which does not pose a health risk to milk consumers, though it is important to recognize vulnerable age groups. They confirmed that currently, only aflatoxin M1 is regulated in milk products and pointed out also that it is important to recognize the occurrence of multiple toxins and

their co-occurrence in highly consumed food products, since feed materials and complete feed present a multi-mycotoxin composition and are the main mycotoxin source into milk. Other researchers like Chen *et al.* [23] showed significant metabolic changes among the 9 mycotoxins, with zearalenone (ZEN) demonstrating notably higher metabolic conversion rates compared with other mycotoxins (α -zearalanol, HT-2 toxin, T-2 toxin, ochratoxin A, fumonisin B1, deoxynivalenol, aflatoxin M1, and aflatoxin B1) in the transitions from feed to serum, from feed to milk, and from feed to feces. These findings emphasize the importance of strict monitoring and regulation of these toxins in dairy products to protect human health.

All the results suggest a potential health risk associated with the consumption of raw milk marketed in the district of Abidjan. The systematic contamination by yeasts, the high frequency of contamination by molds and the presence of potentially toxigenic types of mold are indeed matters of concern, particularly for vulnerable people. Infants, young children, pregnant women, the elderly and immunocompromised individuals represent the groups most vulnerable to microbiological and mycotoxicological risks associated with the consumption of food products including raw milk [18, 24].

Furthermore, the conditions for marketing raw milk commonly observed in the Abidjan district, notably the absence of refrigeration, exposure to open air, and repeated handling, reported by FAO [5], may promote the multiplication of microorganisms and increase the risk of contamination of this food. Even if aflatoxins B1, B2, G1 and G2 were not detected in the milk samples studied, the results obtained justify the strengthening of health surveillance measures in the dairy sector, notably by improving hygiene practices, the awareness of the actors involved and the establishment of a regular microbiological and mycotoxicological monitoring system.

Furthermore, the conditions for marketing raw milk commonly observed in the Abidjan district, notably the absence of refrigeration, exposure to open air, and repeated handling, reported by the FAO [5], may promote the multiplication of microorganisms and increase the risk of contamination of this food.

Even if aflatoxins B1, B2, G1 and G2 that are the major aflatoxins [10], were not detected in the milk samples studied, the results obtained justify the strengthening of health surveillance measures in the dairy sector because Aflatoxin M1 (the hepatic hydroxylated metabolites of AFB1) in milk and milk products is considered to pose certain hygienic risks for human health. These metabolites are not destroyed during the pasteurization and heating process. That's one more reason to improve hygiene practices, the awareness of the actors involved and the establishment of a regular microbiological and mycotoxicological monitoring system.

6 CONCLUSION

The objective of this study was to characterize the fungal contamination in raw cow's milk produced and marketed in Abidjan. For this purpose, mycological analyses were carried out on raw milk samples collected in different municipalities of the district.

The results obtained showed a significant fungal contamination of the analyzed samples. Yeast and mold loads showed variability between sampling sites, with particularly high levels in certain areas such as Yopougon (Lievre-rouge) and N'Dotre. Yeasts showed a frequency of occurrence of 100% in all samples, while molds exhibited variable frequencies depending on the municipalities studied. These results show an overall insufficient microbiological quality of the analyzed raw milks and probably reflect the influence of milking conditions, handling, transport and marketing.

The morphological identification of mold isolates highlighted the presence of several fungal genera, including *Aspergillus*, *Penicillium*, *Rhizopus* and *Saccharomyces*. The presence of the genera *Aspergillus* and *Penicillium* is of particular health interest, as some species belonging to these genera are known for their toxigenic potential.

Ultimately, the significant fungal contamination observed highlights the need to improve hygiene practices throughout the production and marketing chain of raw milk. Controlling these factors remains essential to ensure the health quality of dairy products and protect consumer health.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

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Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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