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Aflatoxin M_1 in Ultra High Temperature Milk Consumed in Sharjah, United Arab Emirates

M. Mohamadin [1*], A. Rama [1], R. Seboussi [2]

1. *Department of Veterinary Science, Faculty of Health Sciences, Higher Colleges of Technology, P.O. Box 7946, Sharjah, United Arab Emirates*
2. *Department of Veterinary Science, Faculty of Health Sciences, Higher Colleges of Technology, P.O. Box 17155, Al Ain, United Arab Emirates*

HIGHLIGHTS

- Aflatoxin M_1 (AFM_1) was detected in 4 (9.5%) of Ultra High Temperature (UHT) milk samples.
- The concentration range of AFM_1 was 2.8-7.4 ng/L in the UHT milk samples.
- None of the positive samples had AFM_1 levels exceeding the maximum permissible limit (50 ng/L).
- AFM_1 seems to be no serious public health problem in Sharjah, United Arab Emirates.

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Acronyms and abbreviations
AFM_1=Aflatoxin M_1
ELISA=Enzyme Linked Immunosorbent Assay
UHT=Ultra High Temperature

ABSTRACT

Background: Aflatoxin M_1 (AFM_1) is a mycotoxin found in milk that has a carcinogenic effect and poses significant public health risks. Since the human population's consumption of milk and milk products are quite high, thereby increasing the risk of exposure to AFM_1 is of great threat. To assess public health hazards associated with the occurrence of AFM1 in Ultra High Temperature (UHT) milk, a survey was carried out in Sharjah, United Arab Emirates (UAE).

Methods: A total of 42 UHT milk samples from different commercial brands were collected from January to April 2020. The occurrence and concentration range of AFM_1 in the samples were investigated by applying the competitive Enzyme Linked Immunosorbent Assay (ELISA) method.

Results: AFM_1 was detected in four positive samples (9.5%) with a concentration range of 2.8-7.4 ng/L and a mean concentration of 5.2±1.9 ng/L. However, none of the positive samples had AFM_1 levels exceeding the maximum permissible limit (50 ng/L) as set by the European Commission.

Conclusion: AFM_1 incidence in the samples selected from UHT consumed milk in Sharjah-UAE is very low and seems to be no serious public health problem at the moment. Frequent analytical surveillance by food control agencies is highly recommended to keep controlling of the incidence of mycotoxin contamination in dairy products consumed in the UAE.

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Introduction

Milk is considered to be one of the most valuable natural foods for all ages posing high nutrition and health benefits to humans (Li et al., 2018). However, milk and dairy products may be contaminated with Aflatoxin M_1

* Corresponding author (M. Mohamadin)
✉ E-mail: mmohamadin@hct.ac.ae
ORCID ID: https://orcid.org/0000-0003-1886-0028

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(AFM_1) which has a potential risk to public health (Škrbić et al., 2014). AFM_1 is a member of a group of mycotoxins called aflatoxins which are secondary metabolites produced mainly by fungi of the genus *Aspergillus flavus* and *A. parasiticus* (Ahmad et al., 2014). There are more than 18 different types of aflatoxins that have been isolated, but the most important ones from the toxicological point of view are aflatoxins B_1, B_2, G_1, G_2, and M_1 (Benkerroum, 2020). AFM_1 is the metabolite of aflatoxin B_1 (AFB_1) that is excreted in the milk of ruminants that have consumed contaminated feeding stuff with AFB_1 (De Freitas et al., 2018).

AFM_1 had been classified by the International Agency for Research on Cancer (IARC) as a group 2 human carcinogen (IARC, 1993). Recently, the demonstrated toxic and carcinogenic effects of AFM_1 led to a change in its carcinogenicity classification from group 2 to group 1 (IARC, 2002a,b). Milk is the major source of introducing AFM_1 into the human diet. Since the human population's consumption of milk and milk products are quite high, thereby increasing the risk of exposure to AFM_1 is of great threat (Rastogi et al., 2004).

Due to serious health concerns, many countries have regulated the maximum permissible limits of AFM_1 in milk and dairy products. The European Commission (EC) has set a limit of 50 ng/L for AFM_1 in raw milk, heat-treated milk, and milk-based products (EC, 2006). The United Arab Emirates has considered the same limit of 50 ng/L for AFM1 in milk and milk products.

This study was done to evaluate the occurrence of AFM_1 in Ultra High Temperature (UHT) milk consumed in Sharjah, the United Arab Emirates (UAE) to evaluate the potential of AFM_1 contamination of UHT milk.

Materials and methods

Study areas and samples collection

In this study, the AFM_1 content was examined in 42 UHT milk samples of different commercial brands, 13 local milk samples (manufactured in industrial dairy units in the UAE), and 29 imported milk samples from foreign producers. Samples were collected from the retail stores in Sharjah, UAE from January to April 2020 by a simple random sampling method. The samples were transported to the Veterinary Laboratory at the Higher Colleges of Technology, Sharjah in an insulated container and stored at 4 ˚C for next analysis.

Sample preparation

AFM_1 concentration in UHT milk was measured using an AFM_1 competitive Enzyme Linked Immunosorbent Assay (ELISA) Kit (MyBioSourse, San Diego, United States). Milk samples were prepared according to the manufacturer's instructions. Samples were centrifuged at 3,000 g for 10 min. The upper creamy layer was removed by Pasteur pipette and 50 µl from the lower phase was used for the analysis.

AFM_1 analyses by ELISA

AFM_1 analyses were performed according to the test kit's instructions. The procedure was based on the competitive inhibition enzyme immunoassay technique. Briefly, 50 µl of the AFM_1 standard solutions (50 µl/well) and test samples (50 µl/well) were added in duplicate to the wells of the microtiter plate. Then, 50 µl of HRP conjugate and 50 µl of AFM_1 antibody were added to each well and incubated for 30 min at 25 °C. The washing step was repeated four times, then 100 µl of the substrate was added to each well and mixed thoroughly and incubated for 15 min in the dark. Following the addition of 50 µl of the stop solution to each well, the absorbance was measured at 450 nm in ELISA reader (ELX-800, Bio-Tek Instruments, USA). The color development is inversely proportional to the AFM_1 concentration in the sample. The concentration of AFM_1 was calculated from the calibration curve which was obtained using standards with the following concentrations: 0, 2, 6, 18, 54, and 162 ng/L. According to the MyBioSourse kit guidelines, the detection range is 2-162 ng/L and the lower detection limit is 2 ng/L for milk. The absorbance values were obtained for the standards and the samples, and then data were analyzed using the GEN5 software system.

Results

The ELISA method was validated to ensure data quality. Validation of ELISA was carried out by determination of recoveries and the mean variation coefficient for UHT milk spiked with different concentrations of AFM_1 (2, 6, 18, 54, and 162 ng/L). The results are expressed in Table 1. The standards of AFM_1 concentrations from 2 to 162 ng/L were used to generate the calibration/standard curve. The results showed the linearity of the standard curve over the range studied. Figure 1 gives the calibration curve of standard solutions of AFM_1 with concentrations of 2, 6, 18, 54, and 162 ng/L by ELISA analysis.

Analytical results showed that the presence of AFM_1 in the tested UHT milk samples was very low. AFM_1 were detected in four milk (9.5%) samples ranged between 2.8 and 7.4 ng/L and a mean concentration of 5.2±1.9 ng/L. The distribution of AFM_1 levels in the UHT milk samples by origin is presented in Table 2. Interestingly, none of the positive samples had AFM_1 in concentrations exceeding the EC Permissible limit (50 ng/L).

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Table 1: Validation data of the competitive Enzyme Linked Immunosorbent Assay (ELISA) for Aflatoxin M_1 (AFM_1)

AFM_1 spiked (ng/L) (*n*=5)	AFM_1 (ng/L)	Recovery (%)	Variation coefficient (%)
2	2.1	105.00	5
6	6.5	108.33	8.3
18	17.2	95.56	4.4
54	54.9	101.67	1.6
162	161.1	99.44	0.5

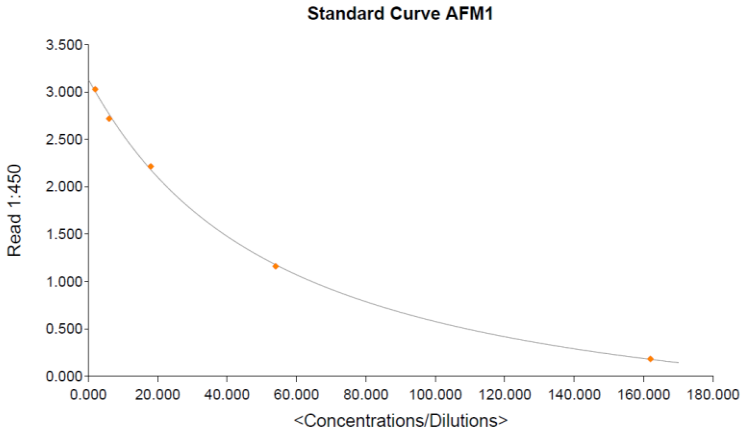


Figure 1: Calibration curve of standard solutions of Aflatoxin M_1 (AFM_1) with concentrations of 2, 6, 18, 54, and 162 ng/L by competitive Enzyme Linked Immunosorbent Assay (ELISA) analysis

Table 2: The distribution of Aflatoxin M_1 (AFM_1) levels by origin in the Ultra High Temperature (UHT) milk samples consumed in Sharjah, United Arab Emirates

Samples origin	No. of samples	Positive samples			Exceed European Commission limit
		No.	Range (ng/L)	Mean±SD (ng/L)	
Local	13	1 (7.7%)	2.8	-	0
Imported	29	3 (10.3%)	3.5-7.4	5.8±1.7	0
Total	42	4 (9.5%)	2.8-7.4	5.2±1.9	0

Discussion

AFM_1 contamination of milk and milk products can cause serious health problems. This toxin may accumulate in the human body and cause mutagenic, teratogenic, and carcinogenic effects (Miliţă et al., 2010). Many countries have established acceptance levels for AFM_1, from 50 ng/L in most European countries (EC, 2006) to 500 ng/L in the United States (FDA, 2005), however, the regulatory limits throughout the world are influenced by economic considerations, degree of development and may vary from one country to another (Van Egmond et al., 1997). The UAE Authority for Standards and Metrology has laid down general standard for contaminants and toxins in food and feed which mentioned that permissible limit for AFM_1 in milk and milk products is 50 ng/L.

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In the current study, we aimed to evaluate the AFM_1 contamination levels in UHT milk consumed in Sharjah, UAE. To the best of our knowledge, only one study by Saad et al. (1989) has been published on milk contamination with AFM_1 in UAE concerning camel milk in Abu Dhabi. In this study, AFM_1 was detected in 6 out of 20 camel milk samples (30%), at levels ranging from 25 to 80 ng/L. Our study showed a very low incidence of AFM_1 in UHT milk consumed in Sharjah, UAE as only four samples were positive out of 42 with AFM_1 levels ranging from 2.8 and 7.4 ng/L and mean concentration of 5.2±1.9 ng/L which means that the occurrence of AFM_1 in the positive samples was far below the European Permissible Limit of AFM_1 (50 ng/L).

The results of the current study are corresponding with some studies reported by recent surveys carried out in some countries. In Najran, Saudi Arabia, a total of 96 samples of cow's UHT milk were investigated for AFM_1 contamination with a minimum concentration of 10 ng/L and a maximum concentration of 190 ng/L and the mean value was 58±5.3 ng/L (Abdallah et al., 2012). In Morocco, 54 samples of pasteurized milk were surveyed for the presence of AFM_1 and 7.4% were above the maximum level of 50 ng/L set by the Moroccan and European regulations for AFM_1 in liquid milk (Zinedine et al., 2007). In Iran, the level of AFM_1 in raw and pasteurized milk produced in Alborz province was investigated in a study by Sarvar Taherabadi et al. (2016) as AFM_1 was detected in 20 samples of pasteurized milk with various concentration levels ranging from 2.4 to 231 ng/L, and 5% of the contaminated milk samples had higher levels of AFM_1 than the maximum recommended limit (50 ng/L).

Contamination of milk may be mitigated either directly by decreasing the AFM_1 content in contaminated milk or indirectly by decreasing AFB_1 contamination in the feed of dairy animals (Giovati et al., 2015). The low incidence of AFM_1 in the UHT milk in the UAE is probably due to a variety of strategies to mitigate the aflatoxin contamination in the dairy industry. The UAE government applied the Hazard Analysis and Critical Control Point (HACCP) system in food control agencies. The HACCP-based food control system is a preventive approach that addresses the biological, chemical, and physical hazards through anticipation and prevention, rather than through end-product inspection and testing. This system identifies specific hazards and measures for their control to ensure the safety of food consumed in the UAE (Al-Kandari and Jukes, 2011). Moreover, the process of approving imported animal feed in the UAE requires a lot of procedures to ensure that the product is free from any harmful substances and the product is safe for animals and the environment. These procedures include a certificate from the manufacturer regarding adherence to the maximum limits of mycotoxins in the product. In addition, to ensure the health and safety of the consumers, the milk and dairy products in UAE must undergo a conformity assessment test conducted by the Emirates Authority for Standardization and Metrology (ESMA).

Some other studies showed a high rate of AFM_1 contamination in other regions of the world. In a study on 100 UHT milk samples consumed in Turkey, 62% of the examined samples contained AFM_1 ranged from 10 to 630 ng/L and 31% of UHT milk samples exceeded the maximum tolerable limit of the EC (Tekinşen and Eken, 2008). In a study conducted in India, the incidence of contamination of AFM_1 in infant milk, milk-based cereal weaning food, and liquid milk samples was 87% with 99% of contaminated samples exceeding the European Union (EU)/Codex recommended limits (Rastogi et al., 2004). In another study conducted in India by Hattimare et al. (2022), AFM_1 was detected in 52 milk samples (35.6%) with concentration levels up to 2,608 ng/L and 94.2% of the contaminated milk samples had higher levels of AFM1 than the maximum recommended limit (50 ng/L). In Pakistan, the AFM_1 in raw milk samples from 14 districts of the Punjab province was detected in all samples and 99.4% of samples exceeded the EU limit, i.e. 50 ng/L (Hussain and Anwar, 2008). In Bangladesh, a total of 145 samples of raw milk, pasteurized milk, UHT milk, and fermented milk products such as yoghurt were tested for determination of AFM_1 levels through competitive ELISA and 78.6% of milk and milk products samples contaminated in the range of 5.0 to 198.7 ng/L (Sumon et al., 2021). In Ethiopia, a study was conducted on a total of 100 raw milk samples for AFM_1 analysis in the South Gondar Zone from January to February 2020 and AFM_1 was detected in the 99% of raw milk samples with 41% exceeded the limit of the EU (Admasu et al., 2021). Concerning the regional distribution of AFM_1 contamination, the high occurrence of AFM_1 in the higher temperature and relative humidity regions may be associated with the storage of the rations or silage under inadequate conditions which can lead to the opportunity the contamination with the toxigenic *Aspergillus* fungi and aflatoxins formation. Cows may be kept in dairy farms and fed on the contaminated rations with AFB_1 hence they may produce contaminated milk with AFM_1.

The quality and safety of milk in some regions of the world have improved in recent years probably because of the regular testing of AFM_1 and the enhancement of the milk examination techniques. In China, the concentration of AFM_1 in 547 milk samples was at an average of 19.6 ng/L in positive samples and only 5.3% of the positive samples were higher than the maximum residue level of 50 ng/L set by the EU (Xiong et al., 2021). In Brazil, 108 goat milk samples were tested for the occurrence of AFM_1 in 2021 and all positive goat milk samples were below the maximum recommended limit (50 ng/L; De

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Matos et al., 2021). In Albania, a survey on AFM_1 contamination in 119 cow milk samples from retail markets was conducted in 2019-2020 and the mean AFM_1 concentration for the analyzed samples was 22 ng/L (Topi et al., 2022).

Conclusion

The results of this survey indicated that the occurrence of AFM_1 concentrations in UHT milk samples consumed in Sharjah, UAE was very slight and far below the EC limits. This is probably because of the implementation of a food control system, such as the Hazard HACCP system in the UAE food industries.

Since there are not enough studies in the UAE about the AFM_1 content of milk, more studies are required. Frequent analytical surveillance by food control agencies is highly recommended to keep control of the incidence of mycotoxin contamination in dairy products consumed in the UAE. This study emphasized the role of improving manufacturing processes that enhance the quality and safety of milk products through inspection and routine sampling.

Author contributions

M.M., A.R., and R.S. conceived and designed the project and analyzed the data; M.M. and A.R. executed the experiments and wrote the paper; A.R. and R.S. critically reviewed the manuscript for important intellectual contents and approved the final version. All authors read and approved the revised manuscript.

Conflicts of interest

The authors declare no potential conflict of interests.

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