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Presence of Aflatoxin M1 in Raw Milk for Human Consumption in Palestine

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Abstract

The absences or insufficient food control program result in the occurrence of mycotoxin in milk and milk products, which poses a serious risk for humans and can be a public health concern. This study was conducted to highlight the occurrence of aflatoxin M1 in Palestine raw milk collected at farms from Tulkarm, Nablus and Jenin. Aflatoxin M1 was determined by direct competitive ELISA technique. 85 % (34 of 40) of the total examined raw milk samples tested were positive. The aflatoxin M1 contamination levels were between 3 - 80 ppt with a mean of 29.57 ppt. There was a high incidence rate with 92 % (11 of 12) and the highest means of contaminated with aflatoxin M1 in the samples tested in Tulkarm city ($P \leq 0.05$). 20 % of the analyzed samples (8 of 40) exceeded the maximum permissible limit (50 ppt) in European Codex, with a range of 2 - 80 ppt.

Keywords: Palestine, aflatoxin M1, raw milk, ELISA

Introduction

Aflatoxins are mycotoxins produced by fungi especially *Aspergillus flavus* and *A. parasiticus* [1]. These mycotoxins are related to aflatoxin B1 which occurs in animal feed, and their production is enhanced by several factors including temperature and humidity. After ingestion of aflatoxin B1 contaminated feed, it is metabolized in the liver to give aflatoxin M1 and is excreted in milk [2]. Aflatoxin M1 is responsible for many serious diseases in human and animals. Persons living in developing countries are chronically exposed to largely uncontrolled amounts of the toxin; children are most susceptible as they are the major milk consumers. Clinically, aflatoxin M1 is responsible for hepatotoxicity, cancer, nutritional interference, immunosuppressive and teratogenic in humans [3]. In farm and laboratory animals, chronic exposure to aflatoxins compromises immunity and interferes with protein metabolism and anti-nutritional effect, which result in decreased milk and egg production, and recurrent infection. While the young of a species are most susceptible, all ages are affected but in different degrees for different species [4]. Aflatoxin M1 has been found in raw and processed milk products, and it is relatively stable and unaffected by pasteurization and ultra-high-temperature (UHT) treatment or processing. The best method to limit exposure to aflatoxins is to ensure that foods consumed have the lowest practical aflatoxin concentration, and this done by imposing regulatory limits on commodities intended for use as food and feed [5,6].

Many countries have carried out surveillance studies about the occurrence of AFM1 in milk, [7-9]. Unfortunately, in Palestine, in spite of the fact that the dairy products are consumed daily in large quantities, there are very few data (if not) about aflatoxin M1 presence in milk and milk products. For that, the present investigation will be concerned with aflatoxin M1 presence and levels in raw milk available in Palestine for human

consumption, and to compare these levels with maximum aflatoxin M1 limits adopted by European regulations and to highlight the potential risk posed to human health by the consumption of these products. This is the first report about the occurrence of aflatoxin M1 in dairy products in Palestine. We aim to establish a research and regulatory starting point for the safety and quality control of milk and milk products with reference to mycotoxins residues, and bring attention to the presence and level of it in Palestinian marketed dairy products.

Materials and methods

Samples

All milk samples were collected at farms in Nablus, Tulkarm and Jenin in Palestine. Normal samples were chilled and degreased by centrifugation for 10 min at 3,500 g, then the upper cream layer completely removed by aspirating through a pasteur pipette. 100 µl of the defatted milk was applied directly in the enzyme-linked immunosorbent assay (ELISA) kit for aflatoxin M1 determination.

Assay

The method used in this study was the direct competitive ELISA, for quantitative analysis of aflatoxin M1 in milk. Commercial ELISA kits were purchased from Biopharm: RIDASCREEN® aflatoxin M1 30/15 (Art. No.: R1111) (RIDASCREEN aflatoxin M1, R-Biopharm, Germany). 100 µl of standard solutions and prepared samples were added into microtitre wells and incubated for 30 min at room temperature (20 - 25 °C) in the dark. The liquid was then poured out and the wells were washed with washing buffer (250 µl) twice in ELISA plate washer (DAS Inc., Italy). After that, 100 µl of the diluted enzyme conjugate was added to the wells, mixed gently by shaking the plate manually and incubated for 15 min at room temperature in the dark. Again, the wells were washed twice with washing buffer. Afterwards, 100 µl of substrate/chromogen was added, mixed gently and incubated in the dark at room temperature for 15 min. Finally, 100 µl of the stop reagent (1 N H_2SO_4) was added into the wells and the absorbance was measured at 450 nm against air blank in ELISA plate reader (DAS Inc., Italy) within 15 min. The detection limit for milk samples were 5 ppt (ng/l), with recovery rates (10 - 80 ppt) of 95 %, and the cross reactivity is 100 % and 30 % for aflatoxin M1 and aflatoxin M2 respectively.

Statistical analysis

The optical density (O.D.) values of the standards and the samples were divided by the mean O.D. value of the zero standards, the percentage of the absorbance values obtained for the standards and the samples were plotted on semilogarithmic graph paper against the aflatoxin M1 standards concentration in ppt (**Figure 1**). The virtually linear calibration curve used to obtain of aflatoxin M1 concentration (ppt) in samples. The aflatoxin M1 concentration corresponding to the extinction of each sample was obtained directly from the calibration curve. The statistical software package SPSS version 10 was employed to analyze the results.

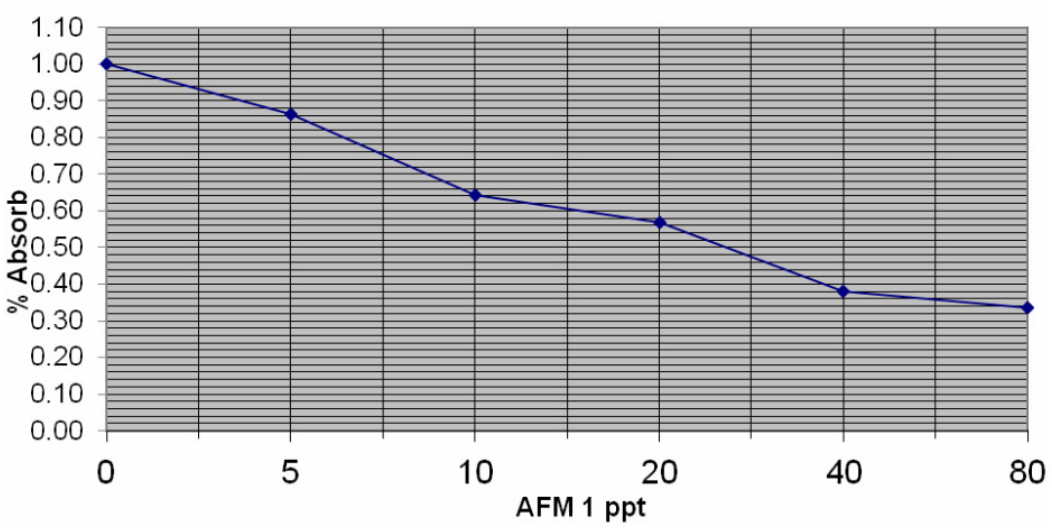

Figure 1 Calibration curve of aflatoxin M1 standards 0 ppt (zero standard), 5 ppt, 10 ppt, 20 ppt, 40 ppt, and 80 ppt. The optical density (O.D.) of each standard based on the zero standard.

Result

In this study, a total of 40 raw milk samples were analyzed with the competitive ELISA (RIDASCREEN Aflatoxin M1, R-Biopharm, Germany) kit. The occurrence and levels of aflatoxin M1 in raw milk samples in Nablus, Tulkarm, and Jenin (North Palestine) during October 2010 are shown in **Tables 1** and **2**.

Aflatoxin M1 contamination was detected in 85 % of the total examined raw milk samples. The aflatoxin M1 contamination levels were between 3 - 80 ppt with a mean of 29.57 ± 5.07 ppt. There was a high incidence rate of 92 % (11 of 12) and the highest means of contaminated with aflatoxin M1 in the samples tested were in Tulkarm. There are significant differences in the occurrence of aflatoxin M1 in Tulkarm with regard Jenin and Nablus ($P \leq 0.05$), and no significant differences in occurrence of aflatoxin M1 between Jenin and Nablus ($P \leq 0.05$), **Table 2**.

Table 1 Aflatoxin M1 number, percentage of positive, mean ±SEM, maximum, and minimum, and values exceeding limits established by the European Commission Regulation of raw milk samples in Nablus, Tulkarm, and Jenin in Palestine (ppt).

City	N	Positive N (%)	Mean of positive ± SEM	Minimum	Maximum	Exceeding EC regulations N (%)	Range
Nablus	14	10 (71 %)	11.33 ± 3.58	3	35	0 (0 %)	5 - 35
Tulkarm	12	11 (92 %)	36.21 ± 10.92	7	80	7 (58 %)	7 - 80
Jenin	14	13 (93 %)	9.12 ± 2.53	2	75	1 (7 %)	2 - 75
Total	40	34 (85 %)	29.57 ± 5.07	2	80	8 (20 %)	2 - 80

Table 2 Aflatoxin M1 mean differences with least significant differences of (LSD) raw milk in Nablus, Tulkarm, and Jenin in Palestine (ppt).

1.00		Mean Difference (I-J)	Std. Error	Sig.	95 % Confidence Interval	
(I) CITY	(J) CITY				**Lower Bound**	**Upper Bound**
Nablus	**Tulkarm**	–37.77*	9.79	0.000	–57.62	–17.93
	Jenin	–1.04	9.41	0.913	–20.10	18.03
Tulkarm	**Nablus**	37.77*	9.79	0.000	17.93	57.62
	Jenin	36.74	9.79	0.001	16.90	56.58
Jenin	**Nablus**	1.04	9.41	0.913	–18.03	20.10
	Tulkarm	–36.74*	9.79	0.001	–56.58	–16.90

* The mean difference is significant at the 0.05 level.

Discussion and conclusions

According to results obtained in this study, aflatoxin M1 was found in 85 % of raw milk samples with 20 % of the samples were higher than the permissible level of 50 ppt prescribed by the European Community for maximum limit of aflatoxin M1 in liquid milk and dried or processed milk products [10]. The trace occurrence of aflatoxin is a critical topic, because of the vital daily consumption of milk in an agricultural community like in Palestine, especially by infants and children. Since aflatoxin M1 is a metabolite of aflatoxin B1 excreted in milk, detecting high concentrations of aflatoxin M1 in raw milk samples implies the presence of very high aflatoxin B1 levels in feed, particularly in hay. Many factors may affect the level of aflatoxin B1 in animal feeds. Geographic and climate changes can affect the farm management practices and feed quality. These effects can lead to the wide variations in aflatoxin B1 levels in milk [11]. Tulkarm city has high incidence of 92 % (11 of 12) of the samples tested were positive, and 58 % (7 of 12) were higher than the maximum limit of aflatoxin M1 in liquid milk prescribed by the European Commission Regulation [10]. As a coastal city, with very wet winter seasons, the dairy cattle farmers in Tulkarm harvest hay in the summer, store it until the next season, and feed it to the cattle during the year. This enhances the growth and aflatoxin B1 production from fungi present in haystacks, stimulated by the high humidity, high temperature, and inappropriate storage conditions. As feed aflatoxin B1 levels increase, it will be metabolized in the liver and lead to elevated levels of aflatoxin M1 excreted in milk, Therefore, it is important to reduce the occurrence of aflatoxin B1 toxins in feedstuff and take prophylactic measures to prevent factors enhancing toxin production. Management practices in harvest and storage regarding the aforementioned factors could decrease aflatoxin B1 occurrence in feed.

It is important to prevent toxin production in feed, as well as creating effective detoxification processes. Implementing a food supervision control system in the dairy products industries, and application of strict regulations by government and other food control agencies, also frequent analytical surveillance are required to control the incidence of aflatoxin in dairy products in Palestine. All this will limit mycotoxin contamination in the Palestinian's food products.

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