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Presence of aflatoxin M_1 in raw, reconstituted, and powdered milk samples collected in Algeria

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Abstract Aflatoxins are potent toxic metabolites produced by *Aspergillus* spp. Aflatoxin M_1 (AFM1) is a metabolite of aflatoxin B_1 that can be present in milk, and it is a public health concern. There is scarce information on the incidence of aflatoxin M_1 contamination in milk consumed in Algeria. The presence of AFM1 was investigated in raw milk samples collected between February and October 2011 from 11 dairy farms representative of Algerian production conditions and that were located around Constantine city. Reconstituted and powdered milk samples were purchased from local supermarkets. The analysis was performed by liquid chromatography-fluorescence detection after immunoaffinity purification. AFM1 was detected in 5 out of 47 samples (11 %) at levels ranging from 9 to 103 ng/L, with one sample exceeding the limit of 50 ng/L set by European regulations. Traces of AFM1 (less than 8 ng/L) were also found in 11 other samples. The incidence of AFM1 contamination was higher in imported powdered milk (29 %) than in raw milk (5 %). Although the concentration of AFM1 in contaminated samples was low, the relatively considerable prevalence found in this exploratory study justifies more detailed and continuous monitoring to reduce consumers' exposure to AFM1.

Keywords Aflatoxin M_1 · Algerian traditional dairies · Milk products · Milk safety

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Introduction

Aflatoxins are secondary toxic metabolites, mainly produced by *Aspergillus* of Flavi section. Aflatoxins are predominantly produced in tropical and warm regions. Cereals and silage are the major source of mycotoxin contamination (Scudamore et al. 1998), particularly when produced and consumed on the farm and not correctly conserved. When ingested, aflatoxin B_1 (AFB1) is absorbed in the gastrointestinal tract and mainly metabolized in the liver into aflatoxin M_1 (AFM1) that is then excreted in urine but also found in the milk of dairy animals. The amount of AFM1 found in milk normally represents 1 to 2 % of the AFB1 ingested. AFM1 is considered to be a potential carcinogen for animals and humans (Cavaliere et al. 2006). The presence of AFM1 in milk and dairy products may have negative health implications for consumers, particularly for infants and children, a part of the population that have a proportionally higher consumption of milk. There is a link between growth impairment in children

and exposition to mycotoxins shown in several epidemiological studies (Khlangwiset et al. 2011) Due to the recognized harmful effect of AFM1, its presence in milk and milk products is regulated and closely monitored in many countries. The European Union fixed the limit of AFM1 in milk at 50 ng/L (EFSA 2004). However, there is no regulation in Algeria. The presence of AFM1 in milk is regularly investigated in EU countries but, to our knowledge, this information is lacking in Algeria. Milk consumption in Algeria was ~140 kg per capita per year in 2013 (faostat3.fao.org), a high consumption compared with other countries of the region. The present study examines the presence of AFM1 in raw, reconstituted, and powdered milk, collected from traditional dairies and local supermarkets over several months in 1 year.

Materials and methods

Sample collection

A total of 47 milk samples (22 raw, 11 reconstituted, and 14 powdered) were collected from February to October 2011 in and around the city of Constantine located at 36° 21′ N 6° 36′ E, Algeria. Raw bulk milk samples were collected from two state-owned pilot farms that had more than 25 cows of the Holstein and Tarentaise breeds (Baaraouia n=28 cows, Kadri n=52 cows) and from nine privately owned farms that had between 6 and 15 dairy cows. Collected milk samples (100 mL) were preserved by adding 1.5 μg/mL of sodium azide, kept on ice during transport from the farm to the laboratory, and stored at −20 °C until analysis. Analyses were done within 6 months as we previously tested that AFM1 was stable for up to 6 months. The powdered milk samples originated from Argentina, New Zealand, and three European countries (France, Switzerland, and Denmark), while reconstituted and pasteurized milk samples were prepared exclusively from imported powdered milk. These powdered and reconstituted milk samples were purchased from different local supermarkets around the city.

Aflatoxin M_1 analysis

Before analysis, powdered milk samples were reconstituted by mixing 10 g with 100 mL of deionized water in a horizontal shaker for 5 min. AFM1 was analyzed using the official French method NF EN ISO 14501 (Dragacci and Grosso 2001). The method had

Table 1 AFM1 concentrations in Algerian raw, reconstituted, and powdered milk samples

Milk samples	Collection season	Number of samples	Number of positive samples (AFM1, ng/L)
Raw milk			
Dairy 1	Autumn-winter	2	0
	Spring-summer	1	0
Dairy 2	Autumn-winter	2	1 (11)
	Spring-summer	1	0
Small farms (n=9)	Autumn-winter	7	0 (T, n=2)
	Spring-summer	9	0
All		22	1
Reconstituted milk		11	0 (T, n=4)
Powdered milk			
Argentina		4	3 (T, 19, 20, and 103)
Denmark		1	0
France		1	0
New Zealand		2	0 (T)
Switzerland		1	0
Other[a]		5	1 (T, n=3 and 9)
All		14	4
Total		47	5

Samples collected in and around Constantine between February and October 2011. Dairy 1 (28 cows) and 2 (52 cows) are state-owned farms; the rest are private farms with less than 15 cows

T AFM1 trace concentrations that ranged from 5 ng/L to less than 8 ng/L (limit of quantification)

[a]Country of origin not stated in the label

been validated in a previous study (El Marnissi et al. 2012) in terms of linearity, precision, accuracy, recovery, as well as stability after 6 months of storage at −20 °C. Variability of the method expressed in terms of precision and accuracy was less than 16 %. The limit of quantification of AFM1 was 8 ng/L, and it was calculated by using a signal-to-noise ratio of 3:1.

Results

The occurrence of AFM1 in raw, reconstituted, and powdered milk is shown in Table 1. Five out of 47 samples (11 %) were contaminated with AFM1 at low levels, ranging from 9 to 103 ng/L, while 11 samples (23 %) contained traces (less than 8 ng/L). The greatest incidence and level of AFM1 contamination was observed in imported powdered milk 29 % (4/14) with one sample exceeding the European limit of 50 ng/L, while only one positive raw milk sample was found albeit at low concentration. Traces of AFM1 (≤8 ng/L) were found in 11 samples without any particular predominance for raw, reconstituted, or powered milk.

Discussion

In this study, AFM1 was detected in 11 % of milk consumed in the city of Constantine, notably in powdered milk samples. No contaminated samples were found in reconstituted milk although it is produced from imported powdered milk. Aflatoxin M_1 is not affected by the reconstitution process, including pasteurization, and the absence of positive samples may be simple explained by the use of non-contaminated powdered milk batches. Local raw milk had only one contaminated sample at low level and two more samples at trace levels. These samples were all collected in autumn-winter when diets are more complemented with barley, bran wheat, and dry bread than during spring-summer season. The sampling area has a characteristic Mediterranean climate, and both types of farms, state-owned or private, are representative of the Algerian milk production system. To our knowledge, this study is the first report on AFM1 contamination in Algeria.

The incidence of AFM1 contamination in the present study is lower than that reported in neighboring regions with a similar climate. In Morocco, authors observed a higher incidence of AFM1 contamination in reconstituted milk (89 %) (Zinedine et al. 2007) than in raw milk (27 %) (El Marnissi et al. 2012). Similarly, the incidence of AFM1 contamination in Libya was 71 % (Elgerbi et al. 2004) and in Egypt was 98 and 60 % for raw and powdered milk, respectively (Ghareeb et al. 2013). High incidence of contamination of processed milk has also been observed in other countries where control is absent or inefficient. In Lebanon, 5 out of 13 imported powdered milk samples were contaminated with AFM1 in the range of 9.18 to 16.5 ng/L (Assem et al. 2011). Studies undertaken in various regions of Iran showed a high incidence of AFM1 in raw milk (57 %) (Mahmoudi and Norian 2014) and pasteurized milk (72 to 100 %) (Tajkarimi et al. 2007; Fallah 2010). In contrast, in surveys conducted in Europe where the control is stricter, the incidence of AFM1 contamination is much lower at ~1 % (Boudra et al. 2007; Velasco et al. 2003).

Conclusion

This study demonstrated a moderate incidence of AFM1 contamination of imported milk consumed in the north-east of Algeria. Although the level of contamination was generally below the EU regulation limit, the results demonstrate the insidious presence of AFM1 in consumed milk and highlight the need for continuous monitoring to decrease risk for consumers.

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Conflict of interest The authors declare that they have no competing interests.

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