JOURNAL OF PURE AND APPLIED MICROBIOLOGY, September 2013. Vol. 7(3), p. 1769-1773

Natural Occurrence of Aflatoxin M_1 in Egyptian Milk and Cheese

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(Received: 04 November 2012; accepted: 22 December 2012)

A survey was carried out to detect the natural occurrence of aflatoxin M_1 in four hundred and seventy seven milk (168) and cheese (309) samples collected totally from smallholder dairy farming households belong to four governorates (Egypt). The total contamination percent of the milk samples was 37.48% with contamination average ranged between 8-480 ng/l, minimal average of M_1 was detected in camel milk. The homemade cheese (Aresh cheese) was the most contaminated cheese in all governorates except Giza with maximum contamination average (21-87 ng/Kg). All contaminated samples were higher than the maximum admissible levels of EU and US.

Key words: Aflatoxin M_1, Milk, Dairy products, Egypt.

Aflatoxins are secondary toxic metabolites produced naturally mainly by *Aspergillus flavus* and *Aspergillus paraciticus*[1]. Aflatoxin M_1 (AM_1) is the hydroxylated metabolite of Aflatoxin B_1 (AB_1) produced from mammalian that ingest AB_1-contaminated diets, can be found in, milk and dairy products[2]. The natural contamination of milk and dairy products with AM_1 is serious problem for public health due to its hepatotoxic and carcinogenic potential[3]. AM_1 has been reported in milk and dairy products in many countries including Thailand[4], Malaysia[2], Turkey[5], Iran[6], Kuwait[7] and Egypt[8].

The maximum allowable concentration of AM_1 in the European countries is 50 ng/L[9], however, in the developed countries such as Egypt have been reported up to more than 250 ng/L due to improper handling procedures of animal fodder, feed and their main components such as maize and soybean which allowed to their contamination with high level of AB_1[10]. There is increasing interest among researchers in determining contamination levels of milk and dairy products due to its widespread occurrence, increasing health risk concerns and development of new technologies to detoxification. Since AM_1 is potential carcinogen, its quantity in milk and dairy products is closely monitored and regulated in most countries.

In Egypt, the number of cancer, kidney failure and hepatic failure increase day by day, due to many cases of contamination of milk and dairy products with high and not acceptable levels of AM_1[11]. Therefore, this study aimed to determine incidence levels of AM_1 in Egyptian milk and dairy products with special reference to traditionally prepared.

MATERIALS AND METHODS

Samples collection

Milk (168) and cheese (309) samples were collected from smallholder dairy farming households in Sharkia, Ismailia, Dakahlia and Giza governorates, Egypt during the summer of 2012. The samples were collected in sterile 250 ml tubes and transferred to the lab under cooling.

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Extraction and estimation of AM_1

The cheese as well as butter samples were homogenated with dichloromethane, filtered and the filtrates evaporated. The residue was dissolved in aqueous methanol (3:1, v/v) and shaked well with equal volume of Hexane in separating funnel. The aqueous layer separated and AM_1 extracted with chloroform by shaking. The milk samples were skimmed by centrifugation (3.500 g for 10 min) and AM_1 extracted with chloroform. The clean up procedures of AM_1 extracts were carried out using immuno affinity column[12], methanol used for elution. The quantitative determination of AM_1 was carried out by High Performance Liquid Chromatography [HPLC][13]. Standard AM_1 (Sigma) used as reference (0.1-1.0 ng/ml).

Statistical analysis

The data were statistically analyzed and means were compared using the protected least significant difference (LSD) values.

RESULTS

The present regional report summarizes the natural contamination of milk with AM_1 in smallholder dairy farming households belong to four governorates in Egypt namely Sharkia, Ismailia, Dakahlia and Giza. The highest contamination percent of milk was recorded in Dakahlia (52.25% of samples were contaminated) followed by Ismailia (48.11%); Sharkia (40.45) and lastly Giza (37.47). The total contamination percent of the milk samples (168) was 37.48% with contamination average ranged between 8-480 ng/l. (Table 1). The buffalo milk was the most contaminated milk in all governorates except Giza, in which cow milk was the most contaminated one. The camel milk was free of contamination with AM_1 except in Giza, 18.87% of milk samples were contaminated with minimal average ranged (8-43).

Altogether, 309 cheese samples were examined for the possibility of AM_1 contamination (Table 2). The samples were collected from Sharkia (54), Ismailia (71), Dakahlia (100), and Giza (84). The most contaminated samples were recorded in Sharkia (17.56%), followed by Ismailia (13.73%), Giza (7.94%) and Dakahlia (7.4%). Aresh cheese (home made cheese) was the most contaminated cheese in all governorates except Giza with maximum contamination average (21-87 ng/Kg). On the other hand, Feta cheese was the least contaminated cheese also except in Giza. The White

Table 1. Natural occurrence of AM_1 (ng/l) in milk samples collected from different governorates, Egypt

Governorate	Milk source	Number of collected samples	Number of contaminated samples	Percent of contaminated samples	Range of aflatoxin contamination (ng/l)
Sharkia	Buffalo	12	7.38	61.5	27-210
	Cow	4	1.52	38.0	8-75
	Camel	6	ND	ND	ND
Total	22	8.9	40.45		
Ismailia	Buffalo	18	12.42	69.00	18-287
	Cow	22	8.75	39.77	32-115
	Camel	4	ND	ND	ND
Total	44	21.17	48.11		
Dakahlia	Buffalo	24	15.37	64.04	215-425
	Cow	13	7.62	58.61	67-110
	Camel	7	ND	ND	10-31
Total	44	22.99	52.25		
Giza	Buffalo	32	3.47	10.84	110-480
	Cow	18	4.92	27.33	57-147
	Camel	8	1.51	18.87	8-43
Total	58	9.9	17.07		
Total samples	168	62.96	37.47		

ND= Not detected under the experimental conditions.

Table 2. Natural occurrence of AM1 (ng/kg) in cheese samples collected from different governorates, Egypt

Governorate	Cheese source	Number of collected samples	Number of contaminated samples	Percent of contaminated samples	Range of aflatoxin contamination (ng/l)
Sharkia	Aresh	17	10.04	59.06	27-58
	White	22	6.52	29.64	8-15
	Feta	15	1	6.67	22
Total	54	17.56	32.52		
Ismailia	Aresh	12	8.24	68.67	32-87
	White	24	2.34	9.75	14-21
	Feta	35	3.15	9.00	10-18
Total	71	13.73	19.34		
Dakahlia	Aresh	27	4.75	17.59	21-65
	White	41	ND	ND	ND
	Feta	32	2.65	8.28	14-97
Total	100	7.4	7.4		
Giza	Aresh	41	1.62	3.95	28-37
	White	15	3.78	25.20	9-27
	Feta	28	2.54	9.07	15-37
Total	84	7.94	9.45		
Total samples	309	46.63	15.09		

ND= Not detected under the experimental conditions.

cheese recorded moderate AM_1 contamination between the other cheeses (Aresh and Feta). Generally, the contamination average in all cheese samples ranged between 8-87 ng/Kg. It is necessarily to mention here that the contamination average in cheese samples (8-87 ng/Kg) was lower than those of milk (8-480 ng/Kg).

DISCUSSION

In the present study, AM_1 was detected in all buffalo and cow milk samples collected from all governorates in high level, however camel milk samples were very less contamination. Also, the results of our survey indicated that Aresh cheese was the most contaminated cheese with higher levels of AM_1. In Egypt, similar studies reported high levels of AM_1 contamination in buffalo as well as cow milk and cheeses however the camel milk samples were below the EU maximum admissible levels of AM_1 contamination[8,11]. The contamination with AM_1 occasionally reach 40 to 50 % and even much higher in some developing countries such as Egypt[10] due to the use of improper handling procedures and lack of methods to prevent decay and deterioration of animal feed and fodder[14]. Worldwide natural contamination of milk and dairy products with AM_1 mainly depends on the contamination of feed with AB_1 and has been reported in most countries. It has been reported that up to 6% of the ingested AB_1 is secreted into the milk as AM_1[15]. In other developed countries such as Sudan, high level of AM_1 has been reported in animal[16] and breast[17] milks in very high levels. Similar results were reported in other African countries as Kenya[14], Libya[18] and South Africa[19].

The milk and dairy products are consumed daily with primary importance in the diet of adult and children, consequently most countries have set up maximum admissible levels of AB_1 in feed[20] and of AM_1 in milk[21] ranged between 50 to 500 ng/kg in EU and US, respectively,[22]. In parallel with US and EU, many Asian countries recorded similar low levels of AM_1 in their milk and dairy products.

It was reported that the natural contamination of AM_1 in Turkish dairy products ranges between 50 to 205 ng/kg[23]. Kamkar *et al*.,[24] reported less than 15% of the raw milk samples were contaminated with AM_1 with average range of 14 ng/L in Iran. In Saudi Arabia, the natural

contamination of dairy products with AM_1 was minimum[25] however, in developed Saudi provinces such as Najran, very high level of contamination was reported[26].

ACKNOWLEDGMENTS

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University for its funding of this research through the Research Group Project no RGP- VPP-271.

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