Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Annals of Biological Research, 2011, 2 (3) :292-296
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0976-1233
CODEN (USA): ABRNBW

Aflatoxin M1 contamination in the marketed cow's raw milk of Tabriz, Iran

Mohammad Hosein Movassagh

Department Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran

---

ABSTRACT

Milk is a good source of many nutrients. However it could be a source of toxic substances like aflatoxin M1 (AFM1). The purpose of this study was to investigate of AFM1 contamination level in the marketed cow's raw milk of Tabriz, Iran. A total of 10 marketed cow's raw milk samples were collected randomly from Tabriz dairy stores between January to July 2009. Determination of AFM1 was based on ELISA (Enzyme Linked Immunosorbent Assay). Results show that in 5 samples (50%) the AFM1 concentrations were over the maximum tolerance limit (50ng/l) accepted by European Union and in 2 samples (20%) were over the maximum tolerance limit (100ng/l) accepted by the Iranian food standard. It can be concluded that AFM1 levels in the samples purchased in Tabriz city, appear to be a serious public health problem at the moment.

Keywords: Aflatoxin M1, Cow, milk, ELISA, Tabriz.

INTRODUCTION

Aflatoxins, a group of several toxic secondary fungal metabolites produced by some *Aspergillus* spp., are found in a wide variety of foods and feeds around the world [1].

Contaminated milk with AFM1 could be a threat for children are consumed milk and dairy products. Aflatoxin may be produced by three species of *Aspergilus-A.flavus*, *A.parasiticus*, and rare *A.nomius* – that contaminate plants and its products. *A.flavus* produces only B aflatoxins, while the others produce both B and G aflatoxins aflatoxins M1 and M2 are the hydroxilated metabolites of aflatoxin B1 and B2 and may be found in milk products obtained from live stock that have ingested contaminated feed [2].

AflatoxinM1 contamination of milk results primarily from the conversion of aflatoxin B1 that is metabolized by enzymes found primarily in the liver. After aflatoxin M1 is formed, it is excreted

in the urine and milk of the cow. The action level for aflatoxinB1 is 20 ppb for feed fed to lactating dairy cows. As both aflatoxins B1and M1 may cause cancer in humans, the action level of 0.5 ppb of aflatoxin M1in milk is strictly enforced by the United States Food and Drug Administration (FDA) [3].

AFM1 is resistant to thermal inactivation; pasteurization, autoclaving and other varieties of food processing procedures are not effective in the reduction of this toxin [4, 5].

To protect consumers each countries have established legislation to regulate the levels of AFB1 in feeds and AFM1 in milk. The Food and Drug Administration (FDA) of US has established an action level of 500 ng/l in whole milk [6], whereas the EU has set a maximum level of 50 ng/l in raw milk [7] and in Iran, the Iranian food standard, the maximum level of AFM1 is 100 ng/l [8].

Monitoring surveys are conducted in several parts of Iran to determine the level of AFM1 in milk and dairy products.

This is the first report, as far as we are aware, of AFM1 contamination of marketed cow's raw milk in Tabriz, Iran.

MATERIALS AND METHODS

A total of 10 marketed cow's raw milk samples were collected randomly from Tabriz dairy stores between January to July 2009. The milk samples were centrifuged in 10 ° C for 10 min with 3500 × g. After centrifugation, upper cream layers were completely discarded and the lower phases were freezed for the quantitative test. The quantity of AFM1 was determined by I' screen aflatoxin M1 test (Tecna, Italy) which is a competitive enzyme immunoassay based on antigen–antibody reaction. Sample solutions of 100 µl were added to the wells to occupy the binding sites proportionately then mixed gently and incubated for 45 min at room temperature (20-25 ° C). The liquid was poured out of the wells and the wells filled with 250 µl washing buffer and poured out the liquid again. This washing step repeated four times. In the next stage 100 µl of enzyme conjugate were added to occupy the remaining free binding sites and incubated for 15 min at room temperature and repeated washing step. Then 100 µl of developing solution was added to each well and incubated for 15 min at room temperature. By using a multichannel pipette, 50 µl of stop solution was added to each well. The measurement of AFM1 was done photometrically at 450 nm against air blank within 60 min in ELISA reader (Sunrise, USA). [2].

RESULTS

The standard curve for AFM1 detection by competitive ELISA is given in fig 1. As can be seen from the figure, the calibration curve was found virtually linear in the 5-250 ng/l range. The detection limit was found to be 5 ng/l. AFM1 was found in 30% of the analyzed samples. Results show that in 3 samples (30%) the AFM1 concentrations were less than 5 ng/l.

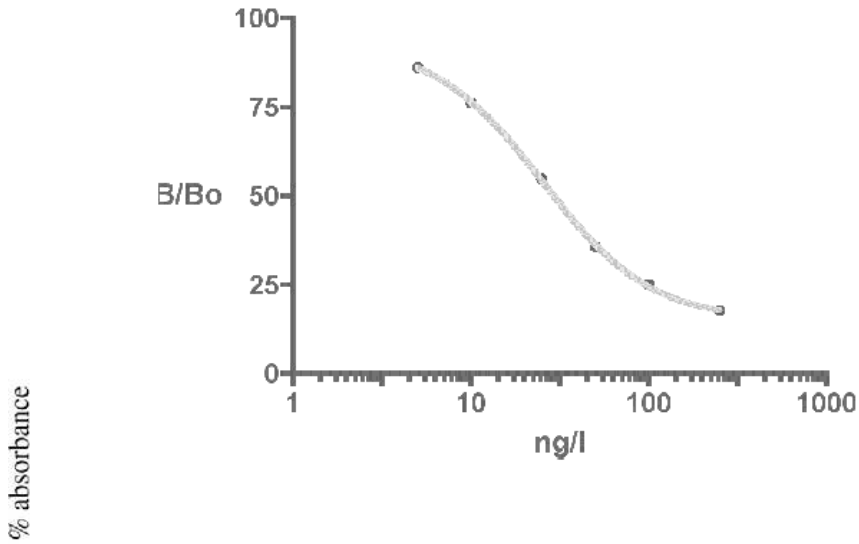

Fig. 1 Calibration curve of AFM1

DISCUSSION

In the Iranian food standard, AFM1 levels in raw milk were limited to 100 ng/l.. Results show that in 5 samples (50%) the AFM1 concentrations were over the maximum tolerance limit (50ng/l) accepted by European Union and in 2 samples (20%) were over the maximum tolerance limit (100ng/l) accepted by the Iranian food standard.

Table-1 The prevalence of cow's milk contamination in other studies

Location	Milk samples (number)	Number of contaminated milk samples > 50 ng/l	Percent	Reference
Babol (Iran)	78	78	100	12
Japan	208	207	99.5	13
Tehran (Iran)	73	60	82.2	14
Libya	49	35	71.4	2
Korea	180	143	76.6	15
Tabriz (Iran)	50	31	62	2
Turkey	90	35	44.3	2
Sarab (Iran)	111	44	40	14
Brazil	139	29	20.9	2
Shiraz (Iran)	624	101	17.8	16
Mashhad (Iran)	110	6	5.4	14
Pakistan	168	1	0.6	17
Germany	379	2	0.5	2
Argentina	77	0	0	2
Italy	161	0	0	2

According to the results obtained by Bognanno et al in Italy, aflatoxin M1 was found in 81% of examined ewe milk samples and 1.25% of all samples were over the legal limits (50 ng\l) [9].

In Spain, Cano-Sancho et al. reported aflatoxin M1 contamination in 94.4% (68/72) of whole UHT milk samples, in 2.8% (2/72) of yoghurt samples and not detected in cheese. The maximum level was detected in one yoghurt sample with 51.58 ng/kg, only this sample being over the legal EU limit of 50 ng/kg. Milk, cheese and yoghurt mean concentrations were 9.2972.61, o12.5 and 13.2274.82 ng/kg, respectively[10].

Kamkar analyzed aflatoxin M1 levels in 52 samples of UHT milk that were produced by different plants in province of Tehran were analyzed by competitive ELIZA. AFM1 was found in 100 percent of 52 of the UHT milk samples that were analyzed in this study. AFM1 contamination in summer and autumn samples ranged from 22.40 to 84.80 and 19.40 to 93.60 ng/kg respectively, while the mean values were 69.22 and 65.50 ng /kg respectively[11].
The result of this study has been compared to other studies on cow's milk, which is shown in Table 1.

It can be concluded that the contamination of aflatoxin M1 in marketed cow's raw milk appear to be a serious public health problem at the moment in Tabriz. However, more samples of the dairy products will have to be taken for analysis during a long period.

Acknowledgments
The author gratefully acknowledges the contribution of Tabriz Blood Transfusion Organization to this work, and Islamic Azad University, Shabestar Branch for partially founding this study.

REFERENCES

[1] L. Lin, F. M. Liu, Y. M. Fu, D. Y. Chih- Shih, *Journal of Food and Drug Analysis*, **2004**, 12, 2, 154-160.
[2]M.H. Movassagh Ghazani, Food and Chemical Toxicology, **2009**, 47, 1624-1625.
[3] J. A. Penningtone, Division of Agriculture, University of Arkansas, http://www.uaex.edu, **2011**.
[4] S.S. Deshpandeh, *Fungal toxins*, **2002**, 387-456.
[5]D.L. Park, *Advances in Experimental Medicine and Biology*, **2002**, 504, 173-179.
[6] I. Kaniou-Grigoriadou, A. Eleftheriadou, T. Mouratidou, P. Katidou, *Food Control*, **2005**, 16, 257-261.
[7] M.T. Montagna, C. Napoli, O. De Giglio, R. Iatta, G. Barbuti, *Int. J. Mol. Sci*., **2008**, 9, 2614-2612.
[8] Anonymous, **2010**, Institute of Standards and Industrial Research of Iran, Amendment No: 1.
[9] M. Bognanno, L. La Fauci, A. Ritieni, A. Tafuri, A. De Lorenzo, P. Micari, L. Di Renzo, S. Ciappellano, V. Sarullo and F. Galvano, *Mol. Nut. Food Res*, **2006**, 50, 300-305.
[10] G.C. Sancho, S. Marin, A.J. Ramos, J.P. Vicente, V. Sanchis, *Rev Iberoam Micol*. **2010**, 27, 3, 130–135.
[11] A. Kamkar, J.Vet. Res., **2008**, 63, 2, 7-12.
[12] I. Gholampour Azizi, S.H. Khoushnevis, and S.J. Hashemi, Tehran University Medical Journal, **2007**, 65, 20-24.

[13] M. Nakajima, S. Tabata, H. Akiyama, Y. Itoh, T.Tanaka, H.Sunagawa, T.Tyonan, T.Yoshizawa, S. Kumagai, *Food Additives and Contaminants*, **2004**, 21, 472-478.
[14] G. Karimi, M. Hassanzadeh. M. Teimuri, F.Nzari, A. Nili, *Iran. J. Pharm. Sci*. ,**2007**, 3(3):153-156.
[15] N. Nuryano, A. Agus, S. Wedhastri, Y.B. Maryudani, F.M.C. Sigit Setyabudi, J. Bohm, *Food Control*, **2009**, 20, 721-724.
[16] S. Alborzi, B. Pourabbas, M. Rashidi, B. Astaneh, *Food Control*, **2006**, 17, 582-584.
[17] I. Hussain, J. Anwar, *Food Control*, **2008**, 19, 393-395.