Global Veterinaria 6 (3): 313-315, 2011
ISSN 1992-6197

Detection of Aflatoxin M1 in Cow's Raw Milk in Miandoab City, West Aazerbaijan Province, Iran

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Abstract: The aim of this study was detection of Aflatoxin M1 contamination in cow's raw milk samples in Miandoab city (Iran) by Enzyme Linked Immunosorbent Assay) ELISA). Ten cow's raw milk samples from different milk stores in Miandoab city (a city in West Azerbaijan province, Iran) were collected during 3 months (May to July 2009). AFM1 was found in 50% of the analyzed samples. Results showed that in all positive samples (50%) the AFM1 concentrations were less than 5 ng/l. It can be concluded that AFM1 levels in Miandoab region, appear to be safe at the moment and dairy farmers must be educated by the government authorities on potential health consequences of aflatoxins.

Key words: Aflatoxin M1 · Cow Raw Milk · ELISA · Miandoab

INTRODUCTION

Aflatoxins are mycotoxins of major concern to the dairy industry. Aflatoxin M1 is a hydrolyzed metabolite from Aflatoxin B1 accumulated in animal's livers, which is excreted through urine and milk (Fig. 1) [1]. Contaminated milk as well as dairy products with AFM1 could be a threat for consumingchildren milk. Because AFM can survive pasteurization, there is a concern about exposure to it through milk or dairy products, especially for infants fed with breast milk or milk formula, as they are typically more susceptible to chemicals [1-4].

The International Agency for Research on Cancer (IARC) in the previous classification from 1993 year put AFM1 in second group of carcinogens as a potent carcinogen, but in next classification from 2002 year this toxin was moved to the first group, G2 as a proved carcinogen but is considered to have only 10% of potential carcinogenic and genotoxicity from its precursor Aflatoxin B1 [5].

The level of AFM1 in milk should not exceed 500 ng/l according to US regulations but the level is set at 50 ng/l in most European countries and in the Codex Alimentarius [6].

AFM1 is resistant to thermal inactivation; pasteurization, autoclaving and other varieties of food processing procedures are not effective in the reduction of this toxin [7,8]. This study was carried out to evaluate the prevalence of cow's raw milk contamination with AFM1 in Miandoab city (a city in West Azerbaijan province, Iran). This is the first report, as far as we are aware, of AFM1 contamination of cow's milk in Miandoab, Iran.

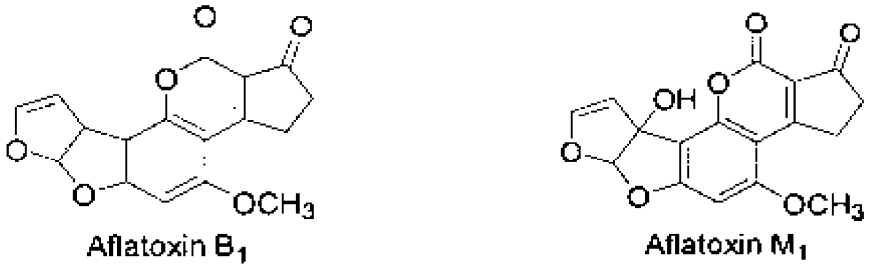

Fig. 1: Structure of Aflatoxin B1 and M1

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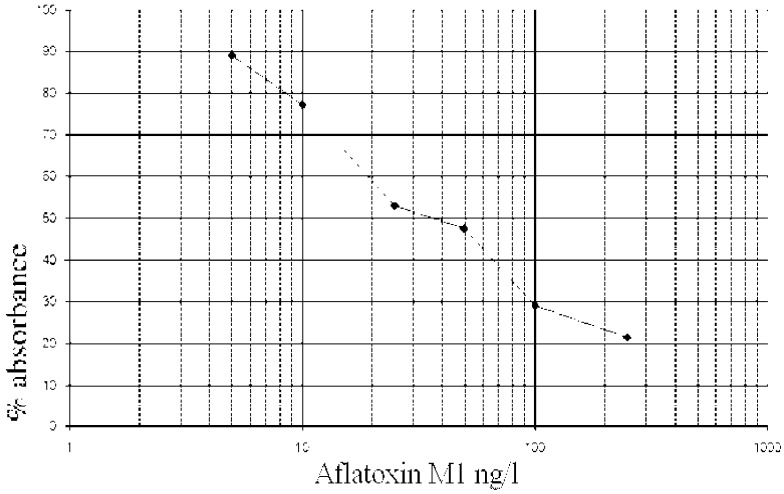

Fig. 2: Calibration curve of AFM1.

MATERIALS ABD METHODS

A total of 10 cow's raw milk samples from different milk stores in Miandoab city (a city in West Azerbaijan province, Iran) was collected randomly during 3 months (May 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) [9].

RESULTS

The standard curve for AFM1 detection by competitive ELISA is given in figure 2. 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 50% of the analyzed samples. In all positive samples AFM1 concentrations were less than 5 ng/l.

DISCUSSION

In the present study, Aflatoxin M1 was found in 50% of examined cow's raw milk samples and in all positive samples AFM1 concentrations were less than 5 ng/l. In the Iranian food standard, AFM1 levels in raw milk were limited to 100 ng/l [10]. In Babol, Iran, Gholampour Azizi *et al.* [11] studied on 78 milk samples and all samples were over the legal limits(50ng/l) accepted by European Union. Karimi *et al.*[12] found that out of all 73 milk samples in Tehran, Iran, 82% were contaminated by AFM1. Movassagh [9,13] reported 71.4% contamination of AFM1 over the legal limits(50ng/l) in pasteurized milk in Tabriz, Iran and there was no contamination of AFM1 in ewe's milk in Tabriz, Iran. In other cities of Iran, like Sarab [9], Shiraz [14], Mashhad [12] and Sanandaj [6], AFM1 contamination were 40, 17.8, 5.4 and 4.4%, respectively. The milk samples in Italy and Argentina had the same contamination as our findings [9]. Bognanno *et al*. [15] in Italy reported that 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) in EU regulation. The wide variations in AFM1 levels among studies could be related to geographic and climatic differences but also to differences in feeding systems and farm management practices. This study showed that contamination with AFM1 in Miandoab is lower than standard levels. It is concluded that consuming of cow's milk is safe for people in Miandoab region.

ACKNOWLEDGMENTS

The author gratefully acknowledges the contribution of Dr.Yaghobei for technical help and Shabestar Branch, Islamic Azad University for partially funding this study.

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