Available online at **www.ijmrhs.com**

Special Issue: Nursing and Healthcare: Current Scenario and Future Development

ISSN No: 2319-5886

International Journal of Medical Research & Health Sciences, 2016, 5, 7S:68-72

On the occurrence of Aflatoxin M_1 in dairy cattle milk in Varamin Region, Tehran

[1]Mahshid Bolourchian, [2]Shahabeddin Safi and [3]Seyyed Hamed Shirazi Beheshtiha

[1]Veterinary Student, Young Researchers Club, Karaj Branch, Islamic Azad University, Karaj, Iran
[2]Department of Pathobiology, Faculty of Specialized Veterinary Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
[3]Department of Clinical Sciences, Faculty of Veterinary Medicine, Islamic Azad University, Karaj, Iran
Corresponding author: Mahshid_sun90@yahoo.com

ABSTRACT

Mycotoxins are fungal metabolites which have been reported to have toxic, carcinogenic, mutagenic and teratogenic effects. Adverse effects of aflatoxin are recognized on human and animal health, many countries imposed standards for mycotoxin in food and feedstuff. The maximum allowable limit imposed by the European Union is 50 mg/L. 221 raw milk sampleswere collected from four dairy farms in Varamin region, Tehran province during fall 2011. The samples were placed in a refrigerator for 2 hours and then were centrifuged at 2000 g for 5 min. The fat layer was removed and M1 levels were measured using ELISA kits from Tecna Co. Italy. The mean± SD concentration of AFM1 in the studied samples was 21.4 ± 4.7 ng/L ranging from 0 to 405 ng/l. The results of the present study showed that the 26.7% of the studied samples were contaminated with AFM1 which is in accordance with the previous studies affirming the necessity of preventing measures to reduce mycotoxin contamination of feedstuff.

Key words: Aflatoxin, M1, Milk, ELISA

INTRODUCTION

As the role of milk in human nutrition; on specific infants nutrition, has been regarded of high importance; Aflatoxin contamination is considered, a danger to human health. Aflatoxins are a significant type of mycotoxin produced as result of Aspergillus species of fungi, such as A. flavus and A. parasiticus growth in agricultural products. High toxic Mycotoxins; Aflatoxins, are found in agricultural products such as corn, rice, coconut and soybean. Consumption of contaminated milk and dairy products can lead to aflatoxin exposure in human; and cause carcinogenesis effects, immune system suppression and stunted growth. Aflatoxin B1, B2, B3, G1, G2, M1 and M2 are metabolites found in milk and are called tetrahydrofuran (13). Aflatoxins are immune system suppressor, mutagenesis and carcinogenesis (21); and also cause of a variety of other effects. Liver is the target organ of aflatoxin for carcinogenesis. Aflatoxin B_1 is considered the most toxic and is the most abundant aflatoxin in fungal contaminated cattle feed. Mammal's exposure to aflatoxin b1 leads to formation of M1 metabolite. Pasteurization and sterilization of milk have poor effect on Aflatoxin M1 thus as waste product, aflatoxin remains in milk (20, 16).IARK; international agency for research on cancer, has rated aflatoxin B1 as first and aflatoxin M1 as second cause of cancer (17) it is worth mentioning that aflatoxin M1 is resistant to Pasteurization, autoclave and other food processing techniques (18).aiming determination of aflatoxin several methods can be applied; such as TLS, LC, HPLC and ELISA (thin layer chromatography, liquid chromatography, High Performance Liquid Chromatography andenzyme-linked immunosorbent assay).Recognized adverse effects of aflatoxin on human and animal health, many countries imposed standards for mycotoxin in food and feedstuff. The maximum allowable limit imposed by the European Union is 50ng/L; same is imposed by Institute of Standards and Industrial Research of Iran (ISIRI)

highlighting the importance of attention to cattle feed. The objective of this study was the occurrence of Aflatoxin **M_1**in dairy cattle milk in Varamin region, Tehran.

MATERIALS AND METHODS

Sampling

In this study, milk samples from four dairy farm in Varamin region were collected (fall 2012).samples were obtained ; in 50 milliliter sterile tubes, and signed and sent ,in proximity of ice, to the Santral lab (located in Tehran). Samples were kept in refrigerator for 1-2 hours. Spearman collection was conducted pursuant to Institute of Standards and Industrial Research of Iran (ISIRI) protocols.

Samples were centrifuged for ten minutes to 2000 g at 4 celsius. Superficial fat layer was removed by Pasteur pipette and skimmed milk was stored at freezer (-70 c). ELISA kit (Tacna co. Italy) was applied to determine the amount of M1 aflatoxin; Competitive immunoassay is the method of the kit.200 microliter of samples and standards were filled in anti aflatoxin M1 antibodies covered sockets, then kept for 30 minutes at room temperature. After being washed 3 time, 200 microliter of Enzyme conjugates was employed and then kept for 15 minute in room temperature, Again washed for 3 times. Next step, 200 microliter of enzyme substrate and chromogen were added and then kept in dark at room temperature. Then 50 microliter of stop solution added and light absorbance at 450 nm was read. By drawing calibration curve, aflatoxin M1 was calculated.

Statistic approach

After determining the concentration, using SPSS software version20, the data of samples were analyzed (ANOVA).

RESULTS

In this study, a total of 221 samples of raw milk from four farms located in Varamin were taken in the fall of 2012. The results showed that of 221 specimens belonging to 4 farms, Mean ± SD of Mycotoxins in all of the samples was 21/4±74 ng. The mean ± standard aflatoxin M1 in the study conducted in four farms is listed in Table 1. The statistical study (one-way ANOVA) showed a significant difference in the amount of AFM1 in studied dairy farms ($p<0.05$).

Table 1: The frequency distribution of AFM1 in pasteurized and sterilized milk

Samples collected from four farms	min ng/l	Standard deviation	mean ng/l	amount	max ng/l
Farm 1	0	0/7	5/2	48	6/8
Farm 2	0	13/7	54/6	59	405
Farm 3	¾	2/5	11/3	56	79
Farm 4	0	1/3	6/6	58	32/4
Total	0	4/7	21/4	221	405

The results of this study suggest that7/26% of the samples collected are contaminated by Aflatoxins above the imposed limit of European Union in milk (50 ng/l).

DISCUSSION

Since contamination with mycotoxins, particularly aflatoxins considered an important issue in many developed countries, so its regular monitoring in milk samples is very important to public health care. Although the toxicity of aflatoxin M1 is less than B1, but its presence in milk and other dairy products is potentially dangerous. The results obtained in Iran, incidence, and different levels of Aflatoxin M1 contamination shows that Iran is facing a serious problem in public health, especially considering children who are the main consumer of milk. So for milk production with acceptable quality, prevention of cattle feed contamination by aflatoxin B1 should be taken into consideration, which is possible by a good Management of food safety and agricultural health in the production and storage of cattle feed. Moreover, adding antioxidants and vitamins, especially vitamin E in the diet of livestock can help the body fighting aflatoxin (4). Based on the studies, linear relationship between dietary AFB1 and AFM1 in animal'smilk is notable. (3). In table No.2 surveys conducted in past two decades in different cities of Iran have been gathered.

Table No.2 Prevalence of milk contaminated with aflatoxin M1 in different cities of Iran

place	count	time	Samples higher than 150/ng
Tehran	73	1998	82/2
Sarab	111	2001	40/ 0
Shiraz	624	2003	17/8
Tehran	128	2005	78/0
Mashad	110	2006	5/4.

Adapted from Karimi and Colleagues article 2007 (12)

The researchers have applied a variety of methods for measuring aflatoxin M1 in milk. Studies show that Elisa technique is a very sensitive and appropriate way to measure aflatoxins even in low concentrations (14). Hence, the ELISA method has been used in this study.

Several reports indicate the prevalence of aflatoxin M1 in milk. Studies in Iran have shown significant percentages of contamination. For example in a study, Karimi. et al., reported 82/2 percent of samples in Tehran were contaminated, indicating that all the milk samples taken from five different ecosystems were contaminated with aflatoxin (11). In the study of Kamkar, 111 samples of raw milk in Sarab city, 85 cases (76/6%) at a concentration of between 0/015 mg and 0/128 mg were contaminated. Amount of aflatoxin in 40% of the positive samples was higher than the Europe Union imposed limit(10). Alborzi and colleagues showed that in Shiraz city in spring and summer in 624 samples of pasteurized milk, 100% contamination was present. 17/8% of the samples exceeded the Europe Union limit (50ng / dl) (1). Gholampour Azizi and colleagues studied amount of aflatoxin M1 contamination in pasteurized and sterilized milk in the city Babol (7). Their results showed that 100% of the samples contamination with aflatoxin M1 exceeded the European imposed limit EU and Codex Alimentarius Committees(ng / dl50). Amount of aflatoxin M1 in pasteurized and sterilized milk in Babol in winter was more than four times above the standard level. Maktabi and colleagues applying ELISA method, also found that levels of aflatoxin M1 contamination was present in 100 percent of samples in Ahvas region (15). Kamkar in Ardebil studied the amount of aflatoxin contamination in raw milk and reported that 75/14% of samples exceed the standard limits (9).

Karimi and colleagues in Mashhad studying the concentration of aflatoxin M1 in milk samples found that all samples were contaminated with AFM1, of which 5/4% more than the maximum permissible contaminant level (12). Studies have been conducted in other countries which we consider to mention a few. Rastogi and colleagues in India studied amount of AFM1 in milk and in children milk products applying competitive ELISA method and reported that out of 87 samples, 87/3% were infected. Almost 99% infected samples exceeded the EU and Codex Alimentarius Committees imposed limit(50ng / dl) and 9% of the contaminated samples exceeded the US standard (500ng / dl) (18). In Greece, Markaki and Melissari used ELISA and HPLC to measure aflatoxin M1 in commercial pasteurized milk of shops. Of 81 milk samples, 32 samples between 2-2/5 , 9 more than 5 and 31 samples 0/5-1 ng/l were contaminated by AFM1 aflatoxin, and 9 were not (14). In a study by Gurbay, applying HPLC, from 27 samples of milk 59/3% were contaminated with AFM1 that only one infected sample exceeded the Europe Union (8).

Table NO.3: Comparison of aflatoxin M1 contamination in milk samples from various studies

year	Contamination percent 50 ng/l >	Researcher	country
2012	26/7	Bolourchian and colleagues	Iran
1998	82/2	Karim and colleagues	Iran
2005	76/6	Kamkar and colleagues	Iran
2006	100	Alborziand colleagues	Iran
1989	100	Gholampour and colleagues	Iran
2011	100	Makatbi and colleagues	Iran
2005	14/75	Kamkar and colleagues	Iran
2003	17/8	Alborzi and colleagues	Iran
2001	40	Kamkar and colleagues	Iran
2004	99	Rastogi et al	India
2004	0	Markaki and Melissari	Greek
2006	3/7	Gürbay et al	Turkey
2001	78	Galvano et al	Italy
2009	28	Dashti et al	Kuwait
2000	76	Kim et al	Korea

AFM1 contamination is also a problem in other countries near Iran, including Turkey. In a similar study conducted in Turket (2005 and 2006) , the contamination of pasteurized and serialized milk were 64 and 47 percent above the limit respectively .that are much higher than the results of this study (17). Galvano et al reported that 78% of milk samples in Italy were infected with aflatoxin M1 (6). In a study in Kuwait, 54 samples of dairy products for

Aflatoxin M1 contamination were investigated, 28% of them were infected (4) .Kim and colleagues in Korea, examined the incidence of AFM1 in pasteurized milk , of which 76% were contaminated (13).

Compared with previous studies, this study shows that contamination levels of aflatoxin M1 in milk, differs so much in various reports. Several reasons for this difference can be explained as differences in sampling time (in cold seasons, stored feed is used for cattle, thus the risk of fungal growth and aflatoxin contamination is higher), the sampling site (milk collection centers, or traditional industrial farms, factories, milk, etc.), different geographical areas with different management systems and different measurement methods (ELISA, HPLC, TLC, etc.). It has been reported that ELISA shows a higher amount of contamination compared to HPLC method (19).

CONCLUSION

Due to the fact that milk contaminated with aflatoxin causes potentially dangerous diseases and also due to some chronic diseases, its importance is well understood. The risk of aflatoxin to human's health, especially liver cancer, has been proven by many researchers. The high cost of treatment is another point that cannot be overlooked. statistical study (ANOVA) showed that aflatoxin M1 levels in various farms has a significant difference. Institute of Standards and industrial Research of Iran (ISIRI) imposed the maximum amount of AFM1, 50 nanograms per liter. and the results of this study, in line with previous studies reports the need to prevent aflatoxin contamination of cattle feed.

To enhance the quality of the milk it is crucial to prevent cattle feed from aflatoxin B1contamination. Though prevention of aflatoxin formation is difficult due to high humidity or temperature, but with proper storage of these products, a great reduction in the amount of alfatoxin can be achieved. It is also recommended to prevent the distribution of contaminated products in the country by supervision on factories producing dairy products.

REFERENCES

[1] Alborzi S, Pourabbas B, Rashidi M, Astaneh B. Aflatoxin M1contamination in pasteurized milk in Shiraz (south of Iran). Food Control. 2006;17, 582-4.
[2] Creppy E.E. Update of survey, regulation and toxic effects of mycotoxins in Europe.Toxicol Lett. 2002;127, 19-28.
[3] D'Mello J.P.F, MacDonald A.M.C. Mycotoxins.Anim Feed Sci Tech. 1997;69, 155-166.
[4] Dashti B, Al-Hamli S, Alomirah H, Al-Zenki S, Bu Abbas A, Sawaya W. Levels of aflatoxin M1 in milk, cheese consumed in Kuwait and occurrence of totalaflatoxin in local and imported animal feed. Food Control 2009;686-690.
[5] Deshpande S.S. Fungal toxins. In: Deshpande SS, Editor, Handbookof food toxicology. New York: Marcel Decker. 2002;387-456.
[6] Galvano F, Galofaro V, Ritieni A, Bognanno M, DeAngelis A, Galvano G. Survey of the occurrence of aflatoxinM1 in dairy products marketed in Italy: Second year of observation. Food Addit Contam. 2001;18 (7): 644-6.
[7] Gholampour Azizi I, S.H. Khoshnevis, S.J. Hashemi. Study on the occurrence of aflatoxin M1 in pasteurized and sterilized milk consumption in Babol city. Tehran University Medical J. 2007; 65(1): 20-24
[8] Gürbay A, Aydın S, Girgin G, Engin AB, Şahin G. Assessment of aflatoxin M1 levels in milk in Ankara, Turkey. Food Control.2006; 17, 1-4
[9] Kamkar A. A study on the occurrence of aflatoxin M1 in Iranian Feta cheese.Food Control. 2005;16, 257-61.
[10] Kamkar A. A study on the occurrence of aflatoxin M1 in raw milk produced in Sarab city of Iran. Food Control. 2005;16, 593-599.
[11] Karim G, Bekaeei S, Khorasani A. Contamination of raw milk with aflatoxin M1 in Tehran using ELISA. Pajoohesh J. 1998; 40(3):163-5. [Persian]
[12] Karimi G, Hassanzadeh M, Teimuri M, Nazari F, Nili A. Aflatoxin M1 Contamination in pasteurized milk in Mashhad, Iran. Iranian Journal of Pharmaceutical Sciences.2007; 3(3):153-156
[13] Kim D.H, Shon D, Ryu J.W, Park H.J, Hwang and Y.B Kim. Occurrence of aflatoxin M1 in Korean dairy products determined by ELISA and HPLC. Food Addit Contam. 2000;17, 59-64.
[14] Markaki P, Melissari E. Occurrence of aflatoxin M1 in commercial Pasteurized milk determined with ELISA and HPLC. Food Addit Contam. 2004; 21, 592-597.
[15] Moktabi S, M.R. Haji Hagikolaie, M Ghorbanpour, M Pourmehdi. Determination of Aflatoxin M1 in UTM, pasteurized and GSM milks in Ahvaz (South-West of Iran) using ELISA. Global Veterinaria. 2011;7(1):31-34
[16] Nemati M, MesgariAbbasi M, ParsaKhankandi H, Ansarin M. A survey on the occurrence of aflatoxin M1 in milksamples in Ardabil, Iran. Food Control. 2010; 21(7): 1022-1024.
[17] Nurhan U. Occurrence of aflatoxin M1in UHT milk in Turkey. J Food ChemToxicol. 2006; 44, 1897-1900

[18] Van Egmond H. P. Mycotoxins: regulations, quality assurance and reference materials. Food Addit Contam.1995;12 (3):321-30
[19] Rastogi S, Dwivedi D.P, Khanna K.S, Das M. Detection of aflatoxin M1 contamination in milk and infant milk products from Indian markets by ELISA. Food Control .2004; 15, 287-290.
[20] Riazipour M, Tavakkoli H.R, Razzaghi. Abyane M, Rafati H, Sadr Momtaz S.M. Measuring the amount of M1 Aflatoxin in pasteurized milks. Kowsar Medical Journal. 2010;15(2): 89-93
[21] Tajkarimi M, ShojaeeAliabadi F, Salah Nejad M, Pursoltani H, Motallebi AA, Mahdavi H. Seasonal study of aflatoxin M1 contamination in milk in five regions in Iran. Int J Food Microbiol. 2007;116, 346-349.