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DETECTION OF AFM_1 IN MILK SAMPLES COLLECTED FROM VISAKHAPATNAM URBAN, INDIA

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ABSTRACT

Aflatoxins are carcinogenic, cancer causing substances which are produced by certain molds. These molds are present everywhere in the environment in soil, water, decay parts of vegetation, hay, grass, grains etc. The toxins can easily enter into the food which can affect the feed stock. In the present study 20 milk samples were collected from Visakhapatnam Urban and Sub-urban areas [6 from different diary farms, 4 from local milking shed vendors, 10 from sub-urban area milk sheds] during September 2017 to August 2018. The collected samples were tested for aflatoxin M_1 [AFM_1] through ELISA plate reader. The results of the samples ranged from 0.0205 to 335.22 ng/L.

KEY WORDS: Aflatoxins, Milk samples, Visakhapatnam

INTRODUCTION

Aflatoxins are carcinogens which are produce by species of certain fungi genera *Aspergilli*: *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins are of great important as it can show its impact on the economic and health, as they have the ability to contaminate the agricultural and dairy products. Most products which get contaminated due to aflatoxins are cereals, oilseeds, chilies, and milk resulting in contamination of human food and animal feeds (Nile, 2016; Peraica, 2002; Yiannikouris, 2002). The presence and distribution of aflatoxins is greatly influenced by different environmental factors, so the extent of contamination depends on geographical conditions, agricultural practices, pre and post harvest of commodities and their storage and processing (Benkerroum, 2016, Fung, 2004). The route of aflatoxins in milk can be either consuming aflatoxin contaminated feed by cattle or subsequent contamination of milk and milk products with fungi producing aûatoxin (Nile, 2016; Sarimehmetoglu, 2003). Exposing to the aflatoxin is accepted as injurious to health (Srivastava, 2001). Aflatoxin B_1, B_2, G_1, G_2, M_1, M_2 and Q_1 are mostly produced from agricultural products and feeds (Rastogi, 2004). In all these toxins Aflatoxin B_1 (AFB_1) considered to be very harmful compound which transforms to Aflatoxin M_1 due to cattle consuming contaminated feed (Jaiswal, 2018). Many authors listed AFM_1 as class 2B carcinogen and possible route to cancer (Rastogi, 2004; Castegnaro, 1998). Worldwide permissible limits were set for the consideration of health. These limits vary from country to country. Milk is consumed by every human being and it is the main staple food for infants and children. As children have more risk to expose to AFM_1 because milk is consumed directly by them. The present study was aimed to detect AFM_1 present in milk samples collected from the Urban and sub-urban of Visakhapatnam by using ELISA plate reader technique.

MATERIALS AND METHODS

Collection of samples

Milk samples of one liter were collected from different farm sheds, local vendors and dairy farms.

A total of 20 samples were collected from these places as the population of Visakhapatnam urban is dependent on it. The selection of sample collection depends on the type of fodder supplement, grazing conditions, milking of cattle, transfer into tins and the hygiene of the surroundings. The collected milk samples were stored in 15-20 ºC ice boxes and sent to laboratory for further analysis, during analysis milk samples were brought to room temperature.

Determination of AFM_1 through ELISA plate reader technique

For the analysis of AFM_1 in milk samples quantitative ELISA technique was used. In ELISA plate reader 100 µL of distilled water was added into each well. Then 100 µL of AFM_1 standard solutions and test samples in duplicate were added to the well. The contents of the plate were mixed gently by manually and the plate was incubated for 20 minutes in room temperature in the dark. The liquid from the wells are removed and washed twice with the distilled water and filled with 250 µL washing buffer and poured the liquid out again. 100 µL of enzyme conjugate was added to each well and mixed the plate manually and incubated for 15 minutes at room temperature in the dark. Next, 100 µL of chromogen was added to each well, mixed thoroughly manually and incubated for 10 minutes at room temperature in the dark. 50 µL of stop solution was added to each well which led to colour change to yellow. The content is mixed manually and the absorbance of each well was measured at 450nm.

RESULTS AND DISCUSSION

In the present study the AFM_1 ranged from 0.0205 to 335.2272 ng/L. All collected milk samples have shown the presence of AFM_1. The milk samples collected from Dairy farms ranged from 0.0205-24.692 ng/L, from local vendors (12.16-19.0102) ng/L, sub-urban milk sheds ranged from 4.12 to 335.227 ng/L indicting threat to human consumption. There might be many factors such as feed, seasonal changes, breed and age of the cattle which might affect the composition of milk. Many studies have been carried out in the past for the determination of AFM_1 contamination in different milk produced by cattle (Nile, 2016, Galvano, 2001, Garg, 2004). Here in this study we have presented AFM_1 contamination in different milk samples collected from different sources represented in Fig. 1. Table 1, 2 and 3 shows the concentration of AFM_1 present in different milk samples. In the present study none of the samples were above the maximum tolerance level 50 (ng/L) which was accepted by EU and Codex Alimentations Commission (50 ng L^{-1}) by (EC) (European Commission, 2006) expect two (Sample No-15 and 19). The less concentration of AFM1 in different samples of the study area might be for providing good feed or fodder, grazing with good quality grains (Nile, 2016; Creppy, 2002). In our study it was observed that in winter season the concentration AFM1 was higher than compared to other two seasons, which was very much correlated to 15. The current study on AFM_1 showed similarity with the reports of other Asian countries (Tekinsen, 2008; Barikbin, 2015) and significantly very high with the reports of European countries (Barbiroli, 2009; Galvano, 1998).

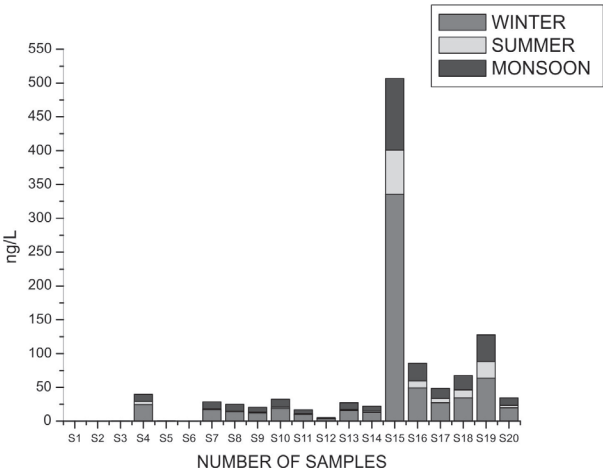

Fig. 1. Graphical representation of AFM_1 milk samples in different areas

The feeding practices for cattle in urban and semi-urban is different compared to rural areas, so the concentration of AFM_1 might be higher than rural areas. In rural areas, green fodder is the primary source of feed for cattle but in urban and semi-urban regions due to lack of green fodder, they depend on concentration feeds such as paddy straw, wheat straw, corn cobs (Hussain, 2008). Many people use milk and its products in their daily intake, especially for children and infants so AFM_1 levels in milk and its products are important (Sarimehmetoglu, 2003). As AFM_1 has been identified as the possible human carcinogen and cancer risk causing (Sugiyama, 2008). Some of the studies in India (Nile, 2016) and the present study specify the importance of AFM_1 in milk samples

Table 1. Collection of milk samples from Dairy farms

S. No.	Sample No.	Sample identity	AFM AFM1 1 (ng/L)	Winter AFM1 (ng/L)	Summer (ng/L) Monsoon
1	Sample 1	Dairy-1 (good milk)	0.0205	0.0000	0.0015
2	Sample 2	Dairy-1 (toned milk)	0.0300	0.0100	0.0100
3	Sample 3	Dairy-1 (Pasteurised milk)	0.0500	0.0000	0.0600
4	Sample 4	Dairy-2 (Full cream milk)	24.6924	4.5132	10.5914
5	Sample 5	Dairy-2 (Long life)	0.1030	0.0030	0.0930
6	Sample 6	Dairy-2 (toned milk)	0.0305	0.0015	0.0105

Table 2. Collection of milk samples from local vendors

S. No.	Sample No.	Sample identity	AFM1 Winter (ng/L)	AFM1 (ng/L) Monsoon	AFM1 (ng/L) Summer
1.	Sample 7	Local vendor-1	17.2050	1.2050	10.3165
2.	Sample 8	Local vendor-2	14.1860	0.9750	9.7431
3.	Sample 9	Local vendor-3	12.1670	1.2780	7.2526
4.	Sample 10	Local vendor-4	19.0102	1.9134	11.9862

Table 3. Collection of milk samples from sub-urban farm sheds

S. No.	Sample No.	Sample identity	AFM1 (ng/L) Winter	AFM1 (ng/L) Summer	AFM1 (ng/L) Monsoon
1.	Sample 11	Sub-urban milk shed-1	10.0000	0.9650	6.1490
2.	Sample 12	Sub-urban milk shed-2	4.1200	0.1369	1.2150
3.	Sample 13	Sub-urban milk shed-3	16.0340	1.3352	9.9502
4.	Sample 14	Sub-urban milk shed-4	13.0000	2.1050	7.2470
5.	Sample 15	Sub-urban milk shed-5	335.2272	65.3461	106.3184
6.	Sample 16	Sub-urban milk shed-6	49.2670	10.3510	26.2790
7.	Sample 17	Sub-urban milk shed-7	27.3900	6.1651	15.3091
8.	Sample 18	Sub-urban milk shed-8	34.6900	11.5321	21.5321
9.	Sample 19	Sub-urban milk shed-9	63.5400	24.6715	39.5641
10.	Sample 20	Sub-urban milk shed-10	20.1000	3.1520	11.4530

which implies that more emphasis should be given to the routine inspection of AFM_1 in milk samples. Moreover, government agencies should educate farmers and dairy farms about the importance of AFM_1 and AFB_1 and their impacts on milk and their products.

CONCLUSION

In this study, it was identified the serious risk for public health of all age group of people who consume milk through AFM_1. It is very important to note that AFM_1 should be maintained at very low levels in the feeds of dairy cattle. There are many agencies and commissions like European Community which regulated the maximum permissible limit as 0.05 µg/kg AFM_1 for raw milk, heat-treated milk and milk for the manufacture of milk-based products, similarly other nations like USA and Turkey have made maximum allowable limit up to 0.5 µg/kg. Although the regulation or allowable limit for AFM_1 of milk and its products is lacking, the study might give good information which could be useful for identification of presence of AFM_1 in milk samples which further make regulations for consumer health.

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