Surveillance of aflatoxin M1 in milk from Navsari, Gujarat area

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Abstract: Aflatoxin M1 (AFM1) is produced during metabolism of Aflatoxin B1 by animal. Aflatoxin is a mutagen and carcinogen and hence possess severe health hazard problem. AFM1 can pass from animal to human via milk. Thus, AFM1 contaminated milk pose a serious threat to public health. Hence, present study deals with detection of AFM1 from cow and buffalo milk. 36 raw samples of cow and buffalo and 9 pasteurized milk samples were collected in each three season i.e., winter, summer and monsoon. Samples were extracted for AFM1 from milk. Dried samples were analyzed for AFM1 using strip ELISA test. Total of 23.81 % and 41.67 % samples from cow and buffalo respectively showed positive for AFM1. Higher numbers of samples were positive in winter followed by monsoon. None of the sample was positive for AFM1 in summer. Thus, present study revealed that there is seasonal effect on presence of AFM1 in milk sample.

Keywords: Aflatoxin M1, ELISA, Milk, Mycotoxin, Navsari

Aflatoxins, a one type of mycotoxins produced mainly by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Creppy, 2002). Various factors like type of substrate, temperature, storage time, storage conditions etc are important and play a role in production of aflatoxin (Stack & Carlson, 2003). The major classes of aflatoxin are aflatoxin B1/B2 and aflatoxin G1/G2. If animal feed is contaminated with Aflatoxin B1, animal will metabolize Aflatoxin B1 to AFM1 (Zinedine et al. 2007) and is able to pass the blood-milk barrier. AFM1 is mutagenic and carcinogenic and can pass to animal milk. Aflatoxin M1 is the only mycotoxin of concern to food safety of milk and dairy

products. Moreover, AFM1 is not inactivated by pasteurization or sterilization (Galvano et al. 1996; Jackson and Groopman, 1999). Hence, monitoring of presence of AFM1 is important to prevent health hazard due to AFM1.

Samples were collected from different taluka of Navsari district in sterile bottles and kept into ice box. Analysis was performed within four hours of sample collection. Pasteurized samples were collected from market. AFM1 was extracted as per instruction given in ELISA kit (Abraxis Inc., USA). Aflatoxin M1 was detected using ELISA strip using ELISA test kit. Kit comprised microtiter well which contained colloidal gold labelled antibodies. Sample was added first in microtiter well (after above mention extraction), mixed using dropper provided in kit and incubated as per instruction given in kit. After incubation sample was taken from microtiter well and loaded on ELISA strip which contain control and test line. Colloidal gold labelled antibodies and aflatoxin M1 moves on strip by capillary action. In absence of Aflatoxin M1 in the milk sample, colloidal gold labelled antibody occupied test area and produce a visible line of antibody-antigen reaction. Thus formation of two visible lines indicated a negative result. If Aflatoxin M1 is present in the milk sample, it competes with colloidal gold labelled antibody for binding to Aflatoxin M1 conjugate on test line. If a sufficient amount of Aflatoxin M1 is present in the milk sample or extract, it will fill all of the available binding sites, thus preventing attachment of the gold labelled antibody to the immobilized Aflatoxin M1 conjugate and, therefore no line will develop. The control line is not influenced by the presence or absence of Aflatoxin M1 in the milk sample or extract, and therefore, present in all reactions.

For surveillance of aflatoxin M1, 6 unpasteurized milk samples of cow and buffalo each were tested in winter, summer and monsoon season. Three pasteurized samples were also tested in each season. In winter, three cow milk samples out six samples were positive for AFM1, whereas pasteurized sample was negative for AFM1 (Table 1). Thus, 50 % unpasteurized samples were positive during winter season. In buffalo milk sample, out of six samples, five were positive for AFM1 where as two pasteurized samples also showed positive for AFM1 (Table 1). Thus 83.3 % of unpasteurized and 100 % pasteurized samples showed positive of AFM1 (Fig 1). Thus in winter out of total 7 cow milk samples,

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Fig. 1 Seasonal effect on presence of Aflatoxin M1 in cow and buffalo milk samples

3 showed positive for AFM1, whereas, 7 out of 8 buffalo milk samples were positive for AFM1.

During the summer season, none of the pasteurized and unpasteurized cow milk showed positive for AFM1 (Table 1). Similar pattern was also found in buffalo milk samples. None of the sample showed positive for AFM1 in summer (Table 1). In summer, none of showed positive for AFM1 out of total 6 and 8 samples of cow and buffalo milk respectively.

In monsoon season, out of six cow milk samples, two were positive, whereas none of the pasteurized milk showed positive for AFM1 (Table 1). Thus, 33.3 % of unpasteurized cow milk samples were positive for AFM1. In buffalo unpasteurized milk samples, two out of six were positive of AFM1 (Table 1). Whereas, in pasteurized buffalo milk samples one was positive for AFM1 (Table 1). Thus, 33.3 % of unpasteurized buffalo milk samples and 50 % pasteurized buffalo milk samples showed positive for AFM1 during monsoon season (Fig 1).

In each season, 6 unpasteurized cow milk sample and 1 pasteurized cow milk was analysed for detection of AFM1. Thus total 21 cow milk samples were analyzed samples in three different seasons. Highest AFM1 positive in raw milk samples were detected in winter season (three) followed by monsoon (two). In summer none of the sample was positive for AFM1.

In all the three season 6 unpasteurized and 2 pasteurized buffalo milk samples were analyzed for AFM1. Thus, total 24 samples were tested for AFM1. Higher AFM1 positive samples were reported in winter followed by monsoon. None of the sample was positive in summer season. Thus, in both cow and buffalo milk samples higher numbers of positive samples were detected in winter followed by monsoon. Data revealed that out of total 45 samples analyzed 27.27 % were positive for AFM1. This ratio for cow and buffalo milk was 23.81 % and 41.67 % for AFM1

positive respectively. Moreover, unpasteurized samples showed higher number of positive compared to pasteurized samples and AFM1 positive samples were more in buffalo milk sample compared cow's milk.

Quality surveillance study was conducted in Navsari area suggested that number of *E. coli* were higher in winter followed by monsoon and summer (Vyas et al. 2016). Thus, in winter and monsoon season perishable items like milk is more prone to pathogens and toxin.

In one study conducted for mycotoxin detection revealed that 59.3% (n = 64) of milk and cheese samples were detected AFM1, but no sample exceeded the EU legal levels (Batinou et al. 2020). The levels of AFM1 were found significantly lower in ultra-high temperature pasteurised milk (long-life milk) than in pasteurized milk. In another surveillance of AFM1 in Hisar city of Haryana, India revealed that out of 150 milk samples, 40 samples contained AFM1 below the limit of detection (LOD), 46 raw milk samples contained above LOD and 64 samples showed above limit of quantitation (LOQ) (Sharma et al. 2019). 31 samples showed the AFM1 concentration above 0.5 µg/kg prescribed by FSSAI regulation.

The fungi grow in animal feed and produce aflatoxin B1. Hence, there is great impact of season, storage condition and storage time on mycotoxin production by fungi. There is seasonal effect on AFM1 concentrations in milk samples. Puga-Torres and coworker (2020) reported that 100 % samples were positive for mycotoxin when analyzed by lateral flow immunochromatographic assays. They have reported that all the tested samples were positive for mycotoxin and 59.3 % exceeded the European Union regulatory limit of AFM1. Moreover, they have reported that there is significant difference between season and higher AFM1 positive were in dry season.

Table 1 Presence of aflatoxin M1 in various season in cow and buffalo milk samples

Type of Milk	Pasteurized/Unpasteurized	Total	Winter	Summer	Monsoon
Cow	Total Sample	21	7	7	7
	Total Unpasteurized +Ve	5	3	0	2
	Total Pasteurized +Ve	0	0	0	0
	Total AFM1 +Ve (%)	23.81	-	-	-
	Total Unpasteurized +Ve (%)	-	50	0	33.30
	Total Pasteurized +Ve (%)	-	0	0	0
Buffalo	Total Sample	24	8	8	8
	Total Unpasteurized +Ve	7	5	0	2
	Total Pasteurized +Ve	3	2	0	1
	Total AFM1 +Ve (%)	41.67	-	-	-
	Total Unpasteurized +Ve (%)	-	83.30	0	33.30
	Total Pasteurized +Ve (%)	-	100	0	50

Generally, AFM1 is not inactivated by heat. Thus, pasteurization will not affect the presence of AFM1. However in one research where 85 pasteurised milk samples collected from Ankara, Turkey, were analysed for AFM1 by ELISA method (Celik et al. 2005). They have reported that 88.23 % (75 samples) were contaminated with AFM1. Moreover, 64 % samples were exceeded the legal level as per Turkish Food Codex and Codex Alimentarius limit. Batrinou et al. (2020) reported that AFM1 levels were found significantly lower in ultra-high temperature pasteurised milk (long-life milk) than in pasteurized milk.

Visconti et al. (1985) conducted a surveillance of AFM1 in southern Italy. They have collected raw milk (31 samples), heat-treated milk (66 samples) and dried milk (9 samples). Out of 106 samples tested 76 (72%) samples were positive for AFM1. The AFM1 concentration was in the ranged between 4 to 480 ng/Kg. Higher incidence of AFM1 contamination was reported in commercial milk (91%) than farm milk (26 %). However, the highest AFM1 was reported in dried milk (100 %). One of the reason for high incidence in processed milk was probably the processed feeds used for cattle destined the commercial milk production. One study conducted in Tamilnadu where 45 samples of UHT milk and 52 raw milk samples analyzed were also showed positive of AFM1 (Siddappa et al. 2012). 38% of UTH milk samples contained more than 0.5 µg/kg prescribed limit of Codex Alimentarius Commission and FSSAI Regulations, 2011. Also 61.6% of 52 samples tested showed positive of AFM1 from Karnataka and Tamilnadu area.

In one surveillance of AFM1 conducted by Anand Agricultural University, Anand reported that 32 out of 38 buffalo milk samples and 30 out of 34 cow milk samples collected around Anand were positive for AFM1 (Choudhary et. al, 1997). AFM1 concentration was 0.076 µg/l and 0.143 µg/l from buffalo and cow milk samples respectively.

Conclusions

Present study revealed that there is seasonal effect on presence of AFM1 in milk samples. Moreover, there was higher incidence of AFM1 in buffalo milk than in cow's milk samples. However, detail study with higher number of samples will provide more insight on seasonal effect and type of milk i.e., cow or buffalo.

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