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Short communication

Seasonal variation of aflatoxin M_1 in raw milk from the Yangtze River Delta region of China

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ABSTRACT

The objective of this study was to evaluate the occurrence of aflatoxin M_1 (AFM_1) in raw milk samples from 18 dairy farms in the Yangtze River Delta region during four different seasons. A total of 72 tank milk samples was collected with 18 samples for each season. Milk AFM_1 was detected using LC-MS/MS. The AFM_1 was detected in 43 milk samples (59.7%) ranging in concentration from 10 to 420 ng/L. The concentration of AFM_1 in raw milk was significantly higher during the winter (123 ng/L) than during other seasons ($P < 0.05$). There was no significant difference between the spring (29.1 ng/L), summer (31.9 ng/L), and autumn (31.6 ng/L) (P 0.05) seasons. This indicates that raw milk collected during the winter is at high risk for AFM_1 and that seasonal factors should be considered for the management of aflatoxins in both the feed and milk.

1. I

Aflatoxins are secondary hepatotoxic metabolites that are produced primarily by *Aspergillus flavus, A. parasiticus, A. bombycis, A. ochraceoroseus, A. nomius, and A. pseudotamari* that contaminate plants and plant products (Bennet & Klich, 2003; Cheraghali et al., 2007; Iqbal, Paterson, Paterson, & Asi, 2010). The presence of aflatoxins in food and feed are of great concern worldwide because of their harmful mutagenic, teratogenic, carcinogenic and immunosuppressive effects (Kamkar, 2005; Oveisi, Jannat, Sadeghi, Hajimahmoodi, & Nikzad, 2006; Zinedine & Manes, 2009). Aflatoxins occur naturally in agricultural commodities and food. There are four main types of aflatoxin: B_1, B_2, G_1 and G_2. Aflatoxin M_1 (AFM_1), the monohydroxylated derivate of aflatoxin B_1 (AFB_1), is excreted into the milk of dairy cows that ingest AFB_1 contaminated feed and subsequently contaminates other dairy products (Allcroft, Roberts, & Butler, 1967). Because of the hepatotoxic and carcinogenic damage caused by aflatoxins, AFB_1 and AFM_1 are classified as class 1 human carcinogens (IARC, 1993; IARC, 2002).

Because of serious health concerns, the maximum residue level (MRL) for AFM_1 in milk and dairy products has been established in many countries to protect consumers. The international regulations for the MRL vary from 0 to 1.0 μg/kg depending on the country (Dashti et al., 2009; Stoloff, Van Egmond, & Parks, 1991). The European Commission (EC) has set an MRL of 50 ng/kg for AFM_1 in milk (EC, 2001). However, the MRL for both the USA (US FDA, 1996, p. 219) and China (MoH, 2011) is 500 ng/kg, which is 10-fold higher than the EC (2001).

The Yangtze River Delta (YRD) is a subtropical zone where temperature and moisture favor the growth of toxigenic *Aspergillus* (Mostrom & Jacobsen, 2011; Pan, Wang, Zeng, Xie, & Miao, 2011; Schindler, Palmer, & Eisenberg, 1967). The YRD is also an important raw milk production area where a total of 1.228 million tons of raw milk were produced in 2010 (*The fifth dairy industry, 2012*), with a major milk consumption district by 94.79 million of population in 2009 (Xu, 2012). However, little information is available regarding the seasonal occurrence of AFM_1 contamination of raw milk in the YRD, although some researchers have reported seasonal differences in AFM_1 contamination in milk in other areas of the world (Asi, Iqbal, Ariño, & Hussain, 2012; Assem, Mohamad, & Oula, 2011; Nemati, Mehran, Hamed, & Masoud, 2010).

In this study, the occurrence of AFM_1 was determined in raw milk samples from dairy farms in the YRD region during four different seasons.

2. Ma a a

2.1. Sample collection

This study occurred from November 2011 to September 2012. A total of 72 raw milk samples was collected from 18 dairy farms in

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four major cities, Shanghai ($n = 6$), Hangzhou ($n = 4$), Nanjing ($n = 4$) and Jinhua ($n = 4$), including the main farms of raw milk production in the YRD. These dairy farms with the capacity of 15–40 tons of raw milk production per day were using three types of collection system: pipeline milking, parallel milking and rotary milking. All the raw milk was pumped into milk-holding tanks with refrigeration system. Approximately 600 mL of all-day milk was collected from the bulk-tanks of each dairy farm, and bronopol (Broad Spectrum Microtabs II, D and F Control Systems Inc., Dublin, CA) was added to the samples as a preservative. The milk samples were stored at −20 °C until analysis using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

2.2. Chemicals and supplies

HPLC grade methanol and acetonitrile were purchased from Alfa Aesar (Ward Hill, MA). The AFM_1 standard was obtained from Sigma–Aldrich (A6428, Sigma Chemical Company, St Louis, MO). A stock solution of AFM_1 was made from the AFM_1 standard dissolved in trichloromethane, which was then diluted with a blank matrix solution, a standard milk that is negative for AFM_1, for the preparation of different concentrations of working standard solutions. All stock and working standard solutions were stored in brown vials milk

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dominant precursor ion was obtained from the electrospray ionization source at a cone voltage of *m/z* 329, and the product ions for quantification and confirmation were obtained at *m/z* 273 and *m/z* 259. The limit of quantification was based on the minimum amount required to produce a signal-to-noise ratio of 10. The limit of quantitation was set at 10 ng/L AFM_1.

Prior to analysis of the samples, the analytical procedure was validated on the basis of recoveries and the relative standard de-

et al., 2011). A large amount of fresh animal feed, such as pasture and green fodder, are available during the other three seasons compared to the winter, resulting in a low contamination of milk by AFB_1 (Asi et al., 2012).

The seasonal differences in the AFM_1 levels in raw milk are summarized in Table 3. Consistent with the results of the current study, the milk produced during the hot seasons has less AFM_1 contamination than cold seasons. This may be because of the similarities in climate changes and feed management practices in the districts studied. However, Marnissi, Belkhou, Morgavi, Bennani, and Boudra (2012) found that the AFM_1 concentration in raw milk during autumn was higher than other seasons, which is different from our findings. This difference may be explained by the wide variations in geography, different study design, analytic methods and dairy farm management practices. Additionally, the AFM_1 levels detected in the raw milk in the current study are lower than a previous study by Pei, Zhang, Eremin, and Lee (2009). One possible reason is that the Chinese government, dairy farms and dairy plants have recently focused attention on AFM_1 levels in milk and milk products. Supervisory organizations regularly inspect for AFM_1 concentrations in milk and milk products, and dairy farms have improved the feed quality and hygiene by many quality control measures, such as importing high quality feed and improving storage conditions. In addition, Zheng et al. (2013) reported that AFM_1 was detected in 54.9% of UHT milk with levels of 6–160 ng/L, indicating that the safety of Chinese milk has improved. Moreover, the majority of dairy farms in the current study are modern, large and have enough funds to organize skilled technicians and advanced instruments for feed and milk quality analysis.

The present study is only an initial survey of AFM_1 contamination in raw milk in the YRD using LC-MS/MS. A large-scale investigation is necessary to complete the risk assessment.

4. C

The incidence and concentration of AFM_1 in raw milk from the YRD during the winter was higher than in other seasons and seasonal factors should be considered for the management of aflatoxins in both the feed and the milk. It was recommended that raw milk from the YRD during the winter season should be strictly supervised to produce safer milk and dairy products.

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