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Occurrence of aflatoxin M1 in pasteurized and UHT milks in China in 2014–2015

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ABSTRACT

This survey was performed to assess the safety of milk in China, specifically by assessing the presence of aflatoxin M1 (AFM1) residues in pasteurized and ultra high temperature (UHT) milk. In 2014–2015, 193 samples of UHT milk were collected from different cities in China. In 2015, 38 samples of pasteurized milk were collected from different cities in China. AFM1 was detected using an enzyme-linked immunosorbent assay (ELISA). AFM1 positivity was defined as a concentration exceeding the detection limit of the assay (0.005 μg/kg). Other cut-offs that were used were the legal AFM1 limits in the European Union (EU) and China (0.05 and 0.5 μg/kg, respectively). In 2014 and 2015, 88.6% and 59.6% of UHT milk samples were AFM1-positive, respectively. The pasteurized milk samples were less frequently AFM1-positive (47.4%). In 11.9% of the 2014–2015 UHT milk samples, the AFM1 levels exceeded the EU limit. This is lower than the frequency we recorded in 2010 (20.3%). None of the pasteurized milk samples exceeded the EU limit in 2015. The UHT milk samples from the north of China were less likely to be contaminated than the samples from the south in both 2014 and 2015. None of the samples exceeded the Chinese legal limit.

1. Introduction

Aflatoxins are poisonous and carcinogenic metabolites of *Aspergillus* species, especially *Aspergillus flavus* and *Aspergillus parasiticus*. Aflatoxins have several forms. Of the approximately 18 aflatoxins that have been identified to date, aflatoxin B1 (AFB1) is considered to be the most toxic. AFB1 is frequently found in dairy cow feed (Decastelli et al., 2007): Han et al. (2013) found that 42% of dairy cow feed samples in China contained AFB1 in the range of 0.05–3.53 μg/kg. Aflatoxin M1 (AFM1) is a metabolite of AFB1. When dairy cows are given AFB1-contaminated feed, their milk will be contaminated with AFM1 (Diaz & Espitia, 2006). This is problematic because AFM1 is heat stable, only being degraded at temperatures of at least 250 °C (Ellis, Smith, Simpson, Oldham, & Scott, 1991). Campagnollo et al. (2016) discussed the presence of AFM1 in relation the milk processing. As raw milk is contaminated by AFM1, this mycotoxin will likely be found in the final product. Thus, AFM1 contamination can be a substantial public health concern (Sassahara, Netto, & Yanaka, 2005; Tajkarimi et al., 2008). Due to the toxic and carcinogenic effects of AFM1, International Agency for Research on Cancer (IARC) reconsidered its carcinogenic categorization and changed it from Group 2B to Group 1, as a proved carcinogen (IARC, 2012).

To address this problem, most countries have established maximum legal AFM1 residue levels in milk. These range from 0.05 μg/kg in the European Union (EU) to 0.5 μg/kg in the United States of America (USA) (Rama, Latifi, Bajraktari, & Ramadani, 2015). Other countries have similar legal limits. For example, in Iran, Brazil, and Serbia, they are 0.5, 0.5, and 0.05 μg/kg, respectively (Fallah, 2010; Kos, Lević, Đuragić, Kokić, & Miladinović, 2014; Shundo, Navas, Lamardo, Ruvieri, & Sabino, 2009). In China, the

Abbreviations: AFB1, aflatoxin B1; AFM1, aflatoxin M1; ELISA, enzyme-linked immunosorbent assay; EU, European Union; SD, standard deviation; UHT, ultra high temperature; USA, United States of America.

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legal limit is 0.5 μg/kg, which is the same as the limit in the USA and higher than the limit in the EU.

When Pei, Zhang, Eremin, and Lee (2009) tested 12 raw milk and 104 liquid milk samples from the northeast of China for AFM1 contamination in 2008, they found that 67% of the raw milk samples and 40% of the liquid milk samples had AFM1 levels of 0.32–0.50 μg/kg. Although all samples were below the Chinese legal limit, this finding indicated that further research on the degree of AFM1 contamination in milk in China was warranted. Therefore, in 2010, we assessed 153 ultra high temperature (UHT) milk samples and 26 pasteurized milk samples from the 25 most populous cities in China for AFM1 contamination (Zheng et al., 2013). AFM1 was detected in 54.9% of the UHT milk samples and 96.2% of the pasteurized milk samples. Although the AFM1 levels in these milk samples were all far below China's legal limit, 20.3% of the UHT milk samples and 65.4% of the pasteurized milk samples exceeded the EU legal limit of 0.05 μg/kg. The high frequency of AFM1 contamination in the milk samples also suggested that further precautions should be taken to reduce the possibility of AFM1 contamination in milk in China and that additional studies screening for AFM1 in milk products are needed to assure milk safety.

In the present study, we measured the AFM1 levels in 38 pasteurized and 193 UHT milk samples collected in China in recent years. The samples were collected in the supermarkets of various cities and provinces in China to better depict the general situation of milk products in China.

2. Materials and methods

2.1. Sample collection

UHT milk samples were collected in both 2014 and 2015. In 2014, 79 UHT milk samples were collected from 20 supermarkets located in two municipalities (Beijing and Tianjin cities) and 18 provincial capitals (An'hui, Fujian, Gansu, Guangdong, Guangxi, Hebei, Heilongjiang, Hubei, Inner Mongolia, Jiangsu, Liaoning, Ningxia, Shaanxi, Shandong, Sichuan, Xinjiang, Yunan, and Zhejiang provinces). Thus, 59 of these samples were collected from the north of China (Beijing and Tianjin cities, Gansu, Hebei, Heilongjiang, Inner Mongolia, Liaoning, Ningxia, Shaanxi, Shandong, and Xinjiang provinces) while the remaining 20 were from the south (An'hui, Fujian, Hubei, Jiangsu, Guangdong, Guangxi, Sichuan, Yunan, and Zhejiang provinces).

In 2015, 114 UHT milk samples were collected from 23 supermarkets located in three municipalities (Beijing, Chongqing, and Tianjin cities) and 20 provincial capitals (An'hui, Fujian, Gansu, Guangdong, Hebei, Heilongjiang, Henan, Hubei, Inner Mongolia, Jiangsu, Jiangxi, Jinlin, Liaoning, Ningxia, Shaanxi, Shandong, Shanxi, Xinjiang, Yunan, and Zhejiang provinces). Thus, 94 of these samples were collected from the north of China (Beijing and Tianjin cities, Gansu, Hebei, Heilongjiang, Henan, Inner Mongolia, Jinlin, Liaoning, Ningxia, Shaanxi, Shandong, Shanxi, and Xinjiang provinces) while the remaining 20 samples were from the south (Chongqing city, An'hui, Fujian, Guangdong, Hubei, Jiangsu, Jiangxi, Yunan, and Zhejiang provinces).

The 38 pasteurized milk samples were collected in 2015 from 16 supermarkets located in three municipalities (Beijing, Shanghai, and Tianjin cities) and 13 provincial capitals (Fujian, Guangdong, Hubei, Hunan, Jiangsu, Jiangxi, Liaoning, Ningxia, Shaanxi, Shandong, Shanxi, Yunnan, and Zhejiang provinces).

The pasteurized and UHT samples were chosen by a convenience sampling as previous study (Felicio et al., 2013), in order to obtain the representative samples of the pasteurized and UHT milks commercially available in China. Subsequently, the UHT samples were stored at room temperature. All pasteurized milk samples were stored at 4 °C before analysis. All analyses were completed before the expiration date of the samples.

2.2. Measurement of AFM1 levels in the milk samples

An enzyme-linked immunosorbent assay with a minimum detection limit of 0.005 μg/kg (RIDASCREEN Aflatoxin M1 test kit, R-Biopharm AG, Germany) was used to measure the AFM1 levels in the samples. AFM1 standards (including 0, 0.005, 0.01, 0.02, 0.04, and 0.08 μg/kg) are contained in the test kit. The test was used according to the manufacturer's instructions. The validation parameters including limit of detection (LOD), limit of quantification (LOQ), recovery, and relative standard deviation calculated under repeatability conditions (RSDr) were showed as followed: LOD = 0.005 μg/kg, LOQ = 0.0085 μg/kg, recovery = 80–120%, RSDr<10% in this study. A sample was considered to be positive for AFM1 if the levels exceeded the minimum detection limit of the assay. Other cut-offs that were used were the EU and Chinese legal limits of AFM1 contamination (0.05 and 0.5 μg/kg, respectively).

3. Results

Tables 1 and 2 indicate the frequency and concentrations of AFM1 in the UHT and pasteurized milk samples obtained in 2014–2015.

3.1. AFM1 positivity in UHT milk samples collected in 2014

In total, 70 of the 79 samples (88.6%) collected in 2014 were positive for AFM1 (>0.005 μg/kg). The mean ± standard deviation (range) AFM1 concentration in the 79 samples was 0.041 ± 0.0424 (0.005–0.263) μg/kg. All positive UHT milk samples had AFM1 levels below the national legal limit of China (0.5 μg/kg). However, in 22 samples (27.8%), the AFM1 concentration exceeded the EU limit (>0.05 μg/kg).

3.2. AFM1 positivity in UHT milk samples collected in 2015

Analysis of 114 UHT milk samples the next year showed that 68 (59.6%) were AFM1-positive. Thus, the incidence of positivity had dropped compared with the previous year. The mean ± standard deviation (range) AFM1 level in the 114 samples was 0.012 ± 0.0094 (0.005–0.065) μg/kg. Of the 68 positive samples, only one had exceeding the EU limit of AFM1 levels (0.065 μg/kg).

3.3. AFM1 positivity in pasteurized milk samples collected in 2015

Of the 38 pasteurized milk samples, 18 (47.4%) were positive for AFM1 contamination. The mean ± standard deviation (range) AFM1 level in these 38 samples was 0.017 ± 0.0099 (0.007–0.040) μg/kg. All positive samples were below the EU limit.

3.4. AFM1 positivity in UHT milk samples from the north and south of China

Analysis of the UHT milk samples showed that AFM1 contamination was less common in the samples collected from the north of China than in the samples from the south of China: this was particularly true for 2014 (86.9% in the north *vs*. 94.6% in the south) but was still observed to some degree in 2015 (58.9% in the north *vs*. 52.6% in the south). Moreover, in both years, the samples from the north were less likely to have AFM1 levels exceeding the EU limit (0.05 μg/kg) than the samples from the south (in 2014, 24.6% *vs*. 38.9%; in 2015, 0% *vs*. 5.3%). Notably, in 2015, all the AFM1-