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Short communication

A survey of aflatoxin M1 in milk and sweets of Punjab, Pakistan

Asma Sadia [a,b,*], Makhdoom Abdul Jabbar [c], Youjun Deng [b], Erum Akbar Hussain [a], Sualeha Riffat [d], Saima Naveed [c], Muhammad Arif [c]

[a] Department of Chemistry, Lahore College for Women University, Jail Road, Lahore, Pakistan
[b] Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474, USA
[c] Department of Food and Nutrition, University of Veterinary and Animal Sciences, Syed Abdul Qadir Jillani Road, Lahore, Pakistan
[d] Department of Pharmacology and Toxicology, University of Veterinary and Animal Sciences, Syed Abdul Qadir Jillani Road, Lahore, Pakistan

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ABSTRACT

Aflatoxin M1 (AFM1) occurs in milk and dairy products, resulting from metabolism of ingested aflatoxin B1 (AFB1) in contaminated feedstuffs. The toxic effects of AFM1 include carcinogenesis, reduced protein synthesis, and immunosuppressant effects. The aim of this study was to determine the levels of aflatoxin M1 in milk and milk products of Punjab, the major milk producing province in Pakistan. Two hundred thirty two milk samples from local shops, household farms, and large scale farms; and 138 sweets (Mithae) from local shops were collected from the 36 districts of Punjab province. These samples were analyzed for aflatoxin M1 by ELISA technique. The AFM1 incidence rates in milk and sweets were 76.3% and 97%, respectively. Average AFM1 concentration in milk was 0.252 μg/L and in sweets was 0.48 μg/kg. Results were compared with the European Union permissible levels of aflatoxin M1 in milk (0.05 μg/L) and milk products (0.05 μg/kg). Contamination level in 75 milk and 108 sweets sample was higher than these levels. No positive correlation was observed between the level of AFM1 in milk and the level of AFM1 in sweets samples, which was likely caused by the large variations in milking and sweets processing. The results indicated that the community is constantly exposed to these toxins. Regulatory mechanism should be implemented to control the toxins in milk and milk products. As feed is the main source of AFM1 in milk the level of aflatoxins in livestock feeds should also be kept to the minimum.

1. Introduction

Aflatoxins are a group of hepatotoxic, carcinogenic and immunosuppressive fungal metabolites (Williams et al., 2004). Aflatoxins are mainly produced by *Aspergillus flavus and A. parasiticus* grown on different crops and feed stuffs (Prandini et al., 2009), such as corn, wheat, corn gluten, and kitchen waste. International Agency for Research on Cancer has classified aflatoxins B1, B2, G1, G2 and M1 as group 1 carcinogen (IARC, 2002, pp. 173–245). Aflatoxin B1 is the most toxic compound and it is one of the biosynthetic precursors of the other aflatoxins (Pei, Zhang, Eremin, & Lee, 2009).

When lactating mammals such as cows, sheep and goat are fed with AFB1 contaminated feed, the ingested AFB1 is converted to AFM1 by hydroxylation at the tertiary carbon of the difuran ring system, metabolized by cytochrome P450 enzyme system in the liver (Fallah, Rahnama, Jafari, & Saei-Dehkordi, 2011). About 0.3–6.2% of AFB1 in animal feed is transformed to AFM1 in milk (Pei et al., 2009). The transformation rate may vary from animal to animal. The AFM1 starts appearing in milk approximately after 12–24 h of the first AFB1 ingestion (Rahimi, Bonyadian, Rafei, & Kazemeini, 2010).

Aflatoxins are a worldwide threat to public health and economy and therefore, extensive surveillance on the levels of aflatoxins B1, B2, G1, G2 and M1 has been conducted throughout the world including developing countries like India and Pakistan. The presence of aflatoxin M1 in milk and dairy products makes it a particular risk for humans because these products are largely consumed by children including infants who are considered more susceptible to the adverse effects of AFM1. More than 60 countries have set maximum permissible level for AFM1 in milk (Iaha, Barbosa, Okada, & Trucksess, 2011). The European regulatory maximum levels for AFM1 in liquid and powder milk is 0.05 μg/kg (European Commission, 2001).

Pakistan is the fifth largest milk producer in the world with an approximately 29 million tons annual production (Zia, 2007). Milk in Pakistan is mainly produced by small farmers at small scales.

* Corresponding author.
E-mail address: asma.sadia1@gmail.com (A. Sadia).

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Feed storage conditions in small scale farms are often not satisfactory due to economic backwardness of the producers and would not reduce the occurrence of the mycotoxins in the feed or the milk.

Most milk (70%) in Pakistan is produced by buffalo (Garcia, Mahmood, & Hemme, 2003). About 98% of the milk is sold in raw form with only 2% going to processing. A significant portion of milk is converted into dairy products. Among the dairy based products, mithae is a popular traditional dessert in Pakistan. Different types of mithae such as burfi, ras gulla, laddu, chamcham are the essential element of many celebrations in Pakistan's culture.

Punjab is the major milk producing province (67% milk share of the total production) of Pakistan (Garcia et al., 2003). Limited research in Pakistan (Ahmad, Khan, Shamsuddin, & Khan, 1996; Hussain & Anwar, 2008; Hussain, Anwar, Asi, Munawar, & Kashif, 2008; Hussain, Anwar, Asi, Munawar, & Kashif, 2010; Maqbool, Anwar-ul-Haq., & Ahmed, 2009; Raza, 2006) has been carried out to estimate aflatoxin M1 in milk from a few selected areas of Pakistan. There was no study on the incidence of AFM1 in milk and milk products of all areas of Punjab.

The objectives of this study were (1) to investigate the aflatoxin M1 contamination status in milk and sweets (Mithae) samples taken in the 36 districts of Punjab Province, Pakistan, and (2) to find if the contamination in milk and sweets are correlated.

2. Materials and methods

2.1. Milk and mithae samples

Forty householder milk, 175 shop milk, 17 large scale dairy farm milk, and 138 burfi (a type of sweets or mithae) samples were collected from 36 districts of Punjab Province, Pakistan (Fig. 1). The dairy farm samples were analysed to check if the larger scale milk production in Pakistan had better control on aflatoxins. Milk and sweets samples were transported to laboratory in 250-mL bottles kept in an icebox. All samples (232 milk and 138 burfi samples) were kept in freezer till use. Samples were brought to room temperature before analysis.

2.2. AFM1 quantification kit and chemicals

The AFM1 quantification was performed by solid phase competitive enzyme immunoassay using kits supplied by Helica

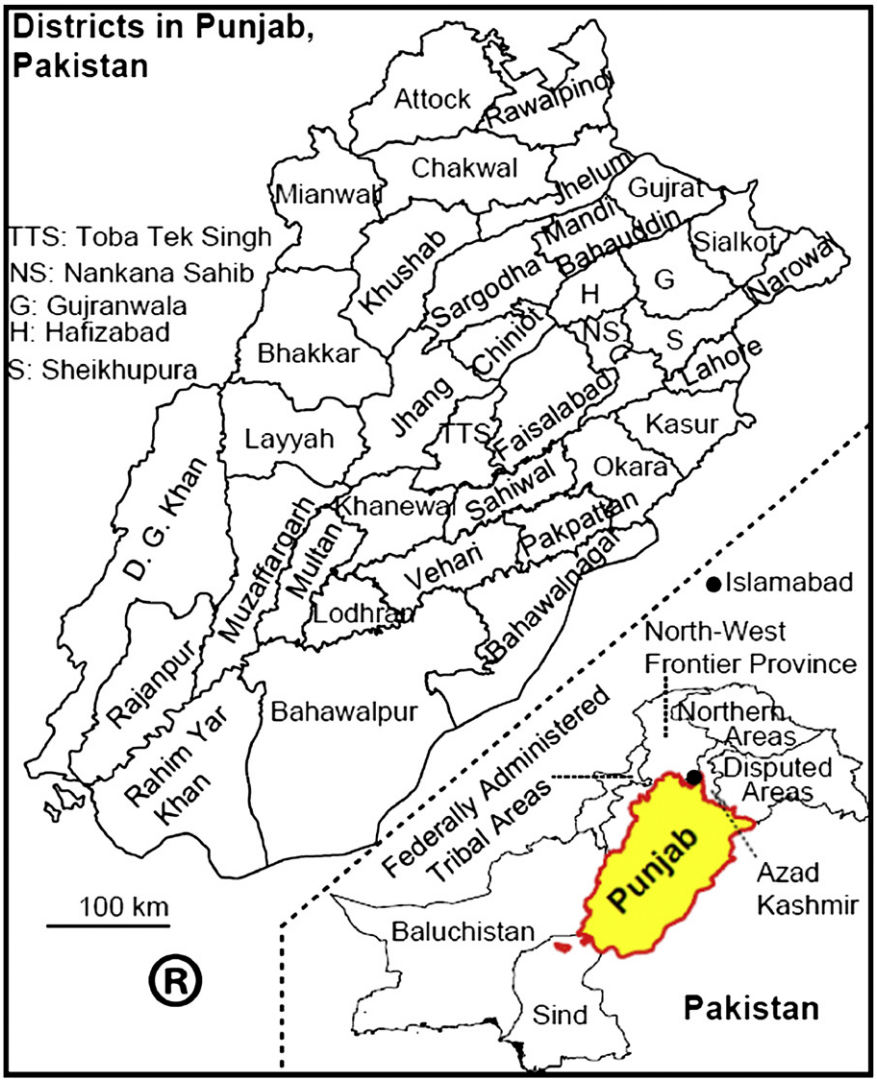

Fig. 1. Geographical locations of milk and sweets samples taken in the 36 districts of Punjab province, Pakistan. GIS data source: http://pakgis.blogspot.com/2009/03/district-boundaries-of pakistan.html