Presence of mycotoxins in animal milk: a review

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Abstract

Mycotoxins can cause toxicity when ingested by humans and animals. Although the rumen is supposed to be a barrier against mycotoxins, some studies demonstrate that carry-over of mycotoxins to milk is possible. Different studies have found mycotoxin levels in animal milk, mainly related to contaminated feed for ruminants. Aflatoxin M1 is the most studied mycotoxin in milk and levels exceeding the EU maximum level for this mycotoxin in this matrix (0.050 μg/kg) have been found. Maximum levels in milk for other mycotoxins have not been established; however ochratoxin A, aflatoxins G1, G2, B1, B2 and M2, fumonisin B1, cyclopiazonic acid, zearalenone and its metabolites and deepoxydeoxynivalenol have also been found in milk samples. Taking into account that multi-exposure to mycotoxins is the most likely scenario and co-occurrence of mycotoxins could affect their toxicological effects in humans and animals, there is a need to determine the co-occurrence of mycotoxins in milk.

Keywords

Mycotoxin, cow milk, milk, ochratoxin A, aflatoxin M1, human exposure

1. Introduction[1]

Mycotoxins are secondary metabolites produced by filamentous fungi that cause a toxic response when ingested by humans and animals. *Fusarium*, *Aspergillus* and *Penicillium* are the most abundant molds that produce mycotoxins and contaminate human foods and animal feeds through fungal growth prior to and during harvest or during improper storage (Binder, 2007).

Human exposure to mycotoxins occurs directly through the intake of contaminated agricultural products (cereals, corn, fruits, etc.) or indirectly through the consumption of products of animal origin (milk, eggs, etc.) prepared or obtained from animals that were fed with contaminated material (Capriotti, Caruso, Cavaliere, Foglia, Samperi & Lagana, 2012).

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[1] Abbreviations Used: 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), aflatoxin M2 (AFM2), cyclopiazonic acid (CPA), deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM-1), diacetoxyscirpenol (DAS), fumonisin B1 (FB1), fusarenon X (FUS-X), neosolaniol (NEO), nivalenol (NIV), ochratoxin A (OTA), ochratoxin B (OTB), patulin (PAT), sterigmatocystin (STC), toxin T-2 (T-2), toxin HT-2 (HT-2), zearalenone (ZEA), zearalanone (ZAN), α-zearalanol (α-ZAL), β -zearalanol (β -ZAL), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), International Agency for Research on Cancer (IARC), Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA), Provisional Maximum Tolerable Daily Intake (PMTDI), Tolerably Daily Intake (TDI), Limit of Detection (LOD), Limit of Quantification (LOQ).

Many human diseases are related with intake of mycotoxins, especially chronic consumption, with the main toxic effects being carcinogenicity, genotoxicity, hepatotoxicity, nephrotoxicity, oestrogenicity, reproductive disorders, immunosuppression and dermal irritation (Anfossi, Baggiani, Giovannoli & Giraudi, 2010). The well-documented presence of some mycotoxins in feed and food has led the European Union to establish maximum limits (MLs) for certain mycotoxins in food for human (European Commission, 2007; 2010a; 2010b) and animal consumption (European Commission, 2003; 2006).

Cow milk consumption is high because it is important in the diet of all age groups. It provides a number of important nutrients that are essential for humans. Children are the largest consumers of milk as it is one of the principal foods during their first years of life. Thus, it is very important that milk is free of toxic compounds that can be harmful for humans, but especially for children which are more susceptible to the action of toxic compounds.

Milk contamination and contamination of mycotoxins in animals are not only a human health concern, but they also cause economic losses to farmers due to mycotoxin adverse effects that cause poor animal productivity (Bryden, 2012).

Mycotoxin presence in material intended for animal feed and some studies showing the presence of mycotoxins in dairy cow plasma (Winkler, Kersten, Meyer, Engelhardt & Dänicke, 2014) raise the possibility that these toxins could be carried over into the cow milk. Rumen flora is supposed to be a defense against mycotoxins due to the fact that some of them: ochratoxin A (OTA), deoxynivalenol (DON), aflatoxin B1 (AFB1) and zearalenone (ZEA) are metabolized to less toxic compounds. However, other mycotoxins, such as patulin (PAT) or fumonisins, pass the rumen barrier unchanged (Fink-Gremmels, 2008). Moreover, the rumen barrier can be altered by animal diseases,

changes in the diet or high mycotoxin contamination in the animal feed (Pattono, Gallo & Civera, 2011).

It is very important to study the presence of xenobiotics in foods, and consequently in milk, in order to provide data regarding human exposure and so as to be able to evaluate the human health risks associated with the ingestion of low doses of these toxic compounds over long periods of time. Moreover, co-occurrence of mycotoxins in feed and food are likely, and the influence of their simultaneous presence in their toxicological effects should be studied.

Regulation of the maximum presence of mycotoxins in milk and milk products is generally limited for aflatoxin M1 (Table 1). With respect to other mycotoxins, only Slovakia has established 5 μg/kg as maximum limit for Aflatoxin B1, Ochratoxin A, Sterigmatocystin and 50 μg/kg for Patulin; Czech Republic has set 5 μg/kg for Sterigmatocystin.

The aim of this work is to review the research work carried out regarding the potential presence of mycotoxins in milk, presenting a summary of the state-of-the-art on this topic.

2. Fumonisins

Fumonisins are synthesized mainly by *Fusarium verticillioides* (syn. *Fusarium moniliforme*) and *Fusarium proliferatum*. However, other fungal species including *Fusarium napiforme, Fusarium dlamini* and *Fusarium nygamai* are also able to produce fumonisins. In animals, some studies have evidenced that FB1 intake led to a decreased milk production and feed intake (Richard et al., 1996; Whitlow, Diaz, Hopkins & Hagler, 2006; Fink-Gremmels, 2008). Also, fumonisins provoke pulmonary edema, hepatic fibrosis and leukoencephalomalacia or liver disease.

Fumonisin B1 (FB1) has been classified as a 2B carcinogen (possibly carcinogenic to humans) by the International Agency for Research on Cancer (IARC). The European commission has recommended a provisional maximum tolerable daily intake (PMTDI) for fumonisins B1, B2 and B3, alone or in combination, of 2 μg/kg b.w. per day.

In Vitro studies have concluded that FB1 is poorly metabolized in the rumen (Caloni, Spotti, Auerbach, Op den Camp, Gremmels & Pompa, 2000), and therefore, it could reach the milk. At least 2 studies have found natural contamination of FB1 in milk samples for human consumption (Table 2). Maragos & Richard (1994) have reported FB1 in 1 out of 155 analyzed samples, at a concentration of 1 290 ng/L; whereas in a more recent study (Gazzotti, Lugoboni, Zironi, Barbarossa, Serraino & Pagliuca, 2009) it has been reported FB1 in 8 out of 10 tested milk samples, with a maximum level of 430 ng/kg and an average in accordance with the value predicted by the Monte Carlo exposure assessment model developed by Coffey, Cummins & Ward (2009) (predicted mean level = 360 ng/kg). Even when considering the bigger FB1 level encountered in milk (1 290 ng/L), it appears to be unlikely that the proposed PMTDI is surpassed, but more research work regarding FB1 contamination in milk, analyzing a large number of samples, would be recommended.

3. Cyclopiazonic acid

The cyclopiazonic acid (CPA) is a potent neurotoxin produced by *Penicillium* and *Aspergillus*. Surveys regarding the presence of CPA in different foods, including milk, are scarce. However, molds producing this toxin are widespread in different food commodities, thus further studies on its occurrence are needed. Two studies, carried out in Italy (Losito, Monaci, Aresta & Zambonin, 2002) and Brazil (Oliveira, Rosmaninho & Rosim, 2006a) have found milk samples contaminated with CPA in levels up to 9 700 ng/L (Table 2) when only 48 samples were analyzed.

4. Zearalenone

Zearalenone (ZEA) is a non-steroidal mycotoxin produced by *Fusarium cerealis, Fusarium culmorum, Fusarium equiseti, Fusarium graminearum and Fusarium pallidoroseum*. Zearalenone and its metabolites zearalanone (ZAN), α-zearalanol (α-ZAL), β -zearalanol (β -ZAL), α-zearalenol (α-ZEL) and β-zearalenol (β-ZEL) are capable of binding the estrogen receptor and therefore, they could lead to problems in the mammalian reproductive system (Le Guevel & Pakdel, 2001). Moreover, α-ZAL had been widely used for increasing the rate of body weight gain and improving feed conversion in ruminants.

Due to their usage, considerable attention has been paid to the potential human health risk. Thus, in 1996, the Council Directive 96/23/EC included these resorcylic acid lactones in Group A (substances having anabolic effect and unauthorized substances), and they were banned within the EU in order to protect consumer health.

Due to their toxic effects, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has recommended a PMTDI of 0.5 μg/kg b.w. for ZEA and metabolites.

Levels of ZEA, ZAN and α-ZAL of up to 12.5 μg/kg, have been detected after analysis of approximately 400 milk samples from Hungary, Egypt, UK and China. For details, see Table 2. In the worst case scenario (12.5 μg/kg of ZEA) (El-Hoshy, 1999), an adult (50-70 kg) should drink 2 - 2.8 L of milk daily to reach the PMTDI. Therefore, human exposure to ZEA from milk is not considered to be a health risk. However, toxicity from ZEA metabolites should be taken into consideration; for instance, α-ZEL is 3 times more estrogenic than ZEA (Mirocha, Pathre & Robison, 1981). This compound has been found in milk samples in China in levels up to 73.5 ng/kg (Huang et al., 2014).

5. Sterigmatocystin

Sterigmatocystin (STC) is produced by many *Aspergillus* species, with *Aspergillus versicolor* being the main STC producer. It was classified as a 2B carcinogen by the IARC. STC is of interest because of its structural relationship to aflatoxin B1, being a precursor in aflatoxin biosynthesis.

Although STC has frequently been detected in different foodstuffs, there is no summarized information about its occurrence and analysis in food. There is no European Union legislation for STC and therefore, no official control and monitoring programs. There are some papers (Versilovskis, Van Peteghem & De Saeger, 2009; Versilovskis & De Saeger, 2010) that report the presence of STC in cheese. Some of them point out indirect contamination which results from lactating animals ingesting contaminated feed. Regarding to this assumption, some studies have found STC in essential components of the milking cow diet like hay (Buckle, 1983) and corn (Tian & Liu, 2004).

6. Trichothecenes

Produced particularly by molds belonging to the genus *Fusarium,* trichothecene mycotoxins are the largest group of toxins, and they are ubiquitous in moderate climate areas. Approximately 170 trichothecene mycotoxins have been discovered so far. They are divided into different groups according to their characteristic functional groups: Type A, including diacetoxyscirpenol (DAS), neosolaniol (NEO) and the highly toxic HT-2 and T-2 toxins, and Type B, including deoxynivalenol (DON, vomitoxin), 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), nivalenol (NIV) and fusarenon X (FUS-X). With regard to their toxic effects, trichothecenes type A causes vomiting, diarrhea, leukopenia, necrotic lesions and hemorrhage while

trichothecenes type B causes food refusal and vomiting, kidney problems and immunosuppression (Capriotti et al., 2012). The European Commission has recommended a PMTDI of 60 ng/kg b.w. for T-2 and HT-2 toxins, alone or in combination and 1 μg/kg b.w. for DON.

In milk, DON has been studied along with its metabolite DOM-1. Sørensen and Elbæk (2005) have found up to 0.3 ng DOM-1/mL in 5 out of 20 milk samples collected in Denmark (Table 2).

7. Patulin

Patulin is a mycotoxin produced by certain species of the genera *Aspergillus* and *Penicillium,* including *Aspergillus clavatus, Penicillium expansum, Penicillium patulum, Penicillium aspergillus and Penicillium byssochlamys.* Exposure to this mycotoxin is associated with neurological, immunological, carcinogenic, teratogenic, genotoxic, and gastrointestinal outcomes (Capriotti et al., 2012; Puel, Galtier & Oswald, 2010). However, the mechanisms through which patulin causes toxicity are still not well-understood.

At the time of writing this manuscript, no studies reporting the occurrence of patulin in milk could be found. However, some studies reported the presence of fungi producers of this mycotoxin in cheese (Taniwaki, Silva, Banhe & Lamanaka, 2001). Cheese could be contaminated indirectly, through contaminated milk, or directly, when fungal contamination appears. Regarding to this assumption, at least one study has found PAT in an essential component of the milking cow diet like grass silage (Buckle, 1983).

8. Ochratoxins

Among them, the most important ochratoxin, due to its prevalence in foods and toxicity is ochratoxin A. It is a stable compound that is not destroyed by common food

preparation procedures. Temperatures above 250ºC for several minutes are required in order to reduce the toxin concentration (Boudra, Le Bars & Le Bars, 1995). Ochratoxin B (OTB) is a dechloro analog of OTA. Ochratoxins A and B are produced by several fungal species in the *Penicillium* and *Aspergillus* genera, primarily *Penicillium verrucosum*, *Aspergillus ochraceus*, *Aspergillus melleus,* and *Aspergillus petrakii*, among others. Exposure to OTA has been associated with distinct endemic renal diseases in the Balkans, referred to as Balkan Endemic Nephropathy (BEN) and Urinary Tract Tumors (UTT). The IARC classified it as being possibly carcinogenic to humans (2B group). Thus, the European Commission has recommended a Provisional Tolerable Weekly Intake (PTWI) of 120 ng/kg b.w. for OTA.

Although OTB may have co-existed in some of the naturally contaminated materials tested for toxicity in animal studies, the concentrations are generally low and this mycotoxin appears to be much less toxic than OTA (Mally et al., 2005).

OTA concentrations found in the 505 analyzed samples of bovine milk were low. Studies carried out in Italy, Sweden, Norway, France and China found OTA at levels in the range of 5-84.1 ng/L, low enough to be considered that the PTWI could not be reached in an adult. However, some exceptions may occur if cows are ingesting large amounts of OTA (González-Osnaya, Soriano, Moltó & Mañes, 2008). In addition, sudden changes in feed composition or the inclusion of a high percentage of protein-rich concentrates in the diet may alter the capability of rumen microorganisms to degrade OTA (Skaug, 1999; Fink-Gremmels, 2008). In Sudan, a sample contaminated at 2 730 ng/L has been found (Table 2).

Even though only low concentrations of OTA may be present in milk, these small amounts may be important to consumers of large quantities of this product, particularly children. Skaug (1999) has found that small children who consume large quantities of

milk may have a total daily intake of OTA which is greater than the Tolerably Daily Intake (TDI) of 5 ng/kg b.w./day. Moreover, other ochratoxin sources could be in the diet of children. However, the European Commission has not established regulations for OTA contamination of milk and dairy products.

9. Aflatoxins

Aflatoxins (AFs) are toxic metabolites produced by the molds *Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius* which grow especially in areas with hot, humid climates and they can be found in several types of foods destined for both human and animal consumption. From a chemical standpoint, aflatoxins belong to the difuranocoumarin group.

Among AFs (B1, B2, G1 and G2), AFB1 is the most commonly found in food. It is highly toxic, in terms of both acute and chronic toxicity, and it is classified by the IARC as a human carcinogen (group 1).

Exposure to aflatoxins is typically through the ingestion of contaminated foodstuff, while dermal exposure results in slow and insignificant absorption. Aflatoxins are most commonly known for causing acute or chronic liver disease, but they are also considered immunosuppressive, hepatotoxic, mutagenic, teratogenic, and carcinogenic. In animals, exposure to aflatoxins results in impairment of liver function and reduced food intake, which might also explain the reduced milk production in dairy cattle exposed to aflatoxins (Fink-Gremmels, 2008).

A small number of studies on AFs in milk -apart from aflatoxin M1 (AFM1)- has been published and they report 112 analyzed milk samples. Herzallah (2009) has reported levels of AFB1, AFB2, AFG1, AFG2 and aflatoxin M2 (AFM2) in Jordan and Nakajima et al. (2004) have reported levels of AFM2 in Japan (See details in Table 2).

Most of the studies regarding mycotoxin presence in milk are focused on AFM1 analysis. It is the main hydroxylated aflatoxin metabolite in milk from dairy cows that have consumed foodstuffs containing AFB1. Stoloff (1997) and Battacone et al. (2003) have reported that AFM1 can be found in animal milk within 12-24h after the first ingestion of AFB1 and can last up to 3 days after the last ingestion of the mycotoxin. In humans, it is also present in milk from nursing mothers who consumed a diet contaminated with AFB1 (Navas, Sabino & Rodriguez-Amaya, 2005).

The presence of AFM1 in animal milk has been reported in several studies (Tables 3 - 6) worldwide. From 22 189 milk samples analyzed for AFM1 contamination that were taken into account in this review, at least 9.8% of them (2 190 samples) from all around the world exceeded the ML established by EU (0.05 µg/kg). This percentage of samples could be higher because many studies report only the number of samples exceeding the US regulation for AFM1 (0.5 µg/kg) (FDA, 2005), which is higher than in European regulations.

In regard to the geographic distribution of the total number of samples, the reference literature showed that the largest number of samples tested came from the continent of Europe (61.1%) (Table 3), and the smallest number of samples assayed came from Africa (Table 4), contributing 4.4% of the total number of samples. In this survey, Turkey has been considered part of Asia.

In regard to the number of samples by continent exceeding the ML set by the EU, at least 1 709 were from Asia (7.7% of total samples and 26.8% of Asian samples) (Table 5), 253 from Africa (1.1% of total samples and 25.8% of African samples) (Table 4), 119 from Europe (0.5% of total samples and 0.9% of European samples) (Table 3), and 109 from America (0.5% of total samples and 8.6%% of American samples) (Table 6). It can be observed that Asia accounts for the highest percentage of milk samples exceeding

MLs, despite the fact that the total number of Asian samples accounts for only 28.7% of the total number of samples tested worldwide.

In regard to all the countries from where occurrence of AFM1 has been detected, milk samples from Egypt, Kenya, Libya, Morocco, Nigeria, Sudan, Argentina, Brazil, Colombia, Mexico, Trinidad, China, India, Iran, Jordan, Korea, Kuwait, Pakistan, Syria, Thailand, Turkey, Albania, Croatia, Italy, Greece and Portugal have reported levels exceeding the maximum allowed level in Europe. Milk samples from Austria, Cyprus, Finland, France, Germany, Ireland, Japan, Lebanon, Netherlands, Spain, Sweden and UK have presented AFM1 levels, but they do not reach the maximum established by EU.

The highest levels of AFM1 found in milk from each of the continents has been reported as follows: India (Asia) at levels of 48 000 ng/L (Thirumala-Devi et al., 2002); Egypt (Africa), up to 8 000 ng/L (El-Sayed, Neamat-Allah & Soher, 2000); Brazil (America), up to 1 000 ng/L (Oliveira, Germano, Bird & Pinto, 1997); and in Europe, Albania recorded the highest levels, reaching 850 ng/kg (Panariti, 2001).

In fact, over the past 5 years, at least Sudan (Elzupir & Elhussein, 2010), Iran (Fallah, 2010a), Pakistan (Iqbal, Asi & Jinap, 2013), China (Zhang et al., 2012) and Brazil (Iha, Barbosa, Okada & Trucksess, 2013) have found high AFM1 levels (510 – 6900 ng/L) that exceed not only EU legislation but also U.S. maximum limits.

With respect to the type and origin of milk samples, the presence of AFM1 has been studied in milk samples from cows and other species such as sheep, goats and buffalos. Concentrations of AFM1 were found to be above the European Limit in at least one sample of each type of milk (Tables 3 - 6).

9.1. Influence of type of Feeding

At least 8 studies coincide in that milk from animals fed by grazing present lower levels of AFM1 in comparison with milk from animals fed with compound feed and/or stored foodstuff. Panariti (2001) found relatively higher levels of AFM1 when cows have a diet composed mainly of stored feedstuffs rather than when the cows were at pasture (Table 3). Another study (Diaz & Espitia, 2006) reports that batches of contaminated milk were produced at farms using feed supplements such as corn by-products or cottonseed meal, as opposed to farms where the cows were only grazing and did not received supplemental feeds (Table 6). Thirumala-Devi et al. (2002) analyzed milk from rural and peri-urban areas in India and found that most of the milk samples that contained high AFM1 concentrations were obtained from peri-urban areas where cows were fed with cotton cake, groundnut cake, rice bran and straw. A study published for Bognano et al. (2006) revealed that the contamination of samples obtained from stabulated ewes fedwith compound feed was higher than that from grazing ewes. Four studies (Ghanem & Orfi, 2009; Hassan & Kassaify, 2014; Rahimi, Bonyadian, Rafei & Kazemeini, 2010; Srivastava, Bu-Abbas, Alaa-Basuny, Al-Johar, Al-Mufti & Siddiqui, 2001) found a high level of AFM1 in cow milk compared with that from other animals (e.g. water buffalos, camels, sheep and goats). All these authors postulate that these low levels could be related to the fact that these species are mainly fed by grazing.

9.2. Seasonal changes in AFM1 concentrations in milk

It is known that hot and humid climates are favorable to the growing of aflatoxin-producing fungus but nevertheless, not all studies are in agreement regarding seasonal influence. Blanco et al. (1988) and El Marnissi et al. (2012) found high contamination in milk samples collected in autumn; Diaz & Espitia (2006) found the highest AFM1 concentration predominantly in the rainy season; Markaki & Melissari (1997) and Rodriguez, Calonge & Ordonez (2003) reported no seasonal influence on the aflatoxin

content in the milk samples they analyzed. Panariti (2001), Tajkarimi et al. (2007; 2008), Hussain & Anwar (2008), Ozsunar, Gumus, Arici & Demirci (2010), Nemati, Mehran, Hamed & Masoud (2010), Heshmati & Milani (2010), Ruangwises & Ruangwises (2010) and Iqbal, Asi & Jinap (2013) have found higher AFM1 concentrations in winter compared with other seasons. Most of the authors presumed that the marked higher contamination of AFB1 in foodstuffs during winter could be a result of the feed, consisting mainly of stored foodstuff, sometimes keep under humid conditions and thereby facilitating the growth of fungi and accumulation of toxins. Future studies must record weather conditions (temperature, humidity, rainfall, etc.) and stored conditions that could help clarify the apparent discrepancies of the current studies.

9.3. Effects of processing

The presence of AFM1 has been studied in raw, pasteurized, powder, organic, concentrated and ultra-high temperature treated (UHT) milk samples. At least one study in each type of milk found concentrations of AFM1 above the European Limit (Tables 3 - 6). Studies regarding the effects of heat processing on the amount of AFM1 in dairy products are ambiguous, but most of them indicate that treatments such as pasteurization and sterilization do not cause an appreciable change in the concentration of AFM1 in the product. With respect to other processes applied to milk, with or without heating, such as evaporation, concentration or drying, large losses of AFM1 were reported in some studies, whereas in other studies, they did not affect the AFM1 content (Prandini, Tansini, Sigolo, Filippi, Laporta & Piva, 2009).

10. Mycotoxin carry-over into the milk

Although the rumen is supposed to be a barrier against mycotoxin contamination, the analysis of milk samples demonstrates that the carry-over of mycotoxins into the milk is possible in some cases.

Few works have studied the possibility of the carry-over of fumonisins from feed into milk, and the results obtained were contradictory. Scott, Delgado, Prelusky, Trenholm & Miller (1994), Richard et al. (1996) and Prelusky et al. (1996) did not detect fumonisins in milk after oral administration of up to 5 mg FB1/kg b.w. (even when a 3 mg FB1/kg b.w. dose was administered daily for 14 days).

Spotti, Caloni, Fracciolla, Pompa, Vigo & Maffeo (2001) demonstrated that fumonisins are able to pass through the mammary barrier into the milk when FB1 is perfused into the blood of the udder. Scott et al. (1994) and Prelusky et al. (1996) did not detect fumonisins in milk after intravenous administration of up to 0.2 mg FB1/kg b.w. On the other hand, Hammer, Blüthgen & Walte (1996) detected FB1 after intravenous administration of approximately 0.046 - 0.067 mg FB1/kg b.w. According to European Food Safety Authority (EFSA) (2005), the carry-over of fumonisins into milk is limited and does not significantly contribute to total human exposure. Dorner, Cole, Erlington, Suksupath, McDowell & Bryden (1994) administered CPA to 3 lactating ewes at a rate of 5 mg/kg b.w. per day and found levels of CPA in milk which were 236 ng/g (the day after the first dose) and 568 ng/g (the day following the second dose). After 2 days, dosing of CPA was discontinued because of marked and rapid toxic effects in the ewes. Milk production at 48 h was only 20% of the expected and feed intake dropped substantially.

According to the EFSA scientific opinion in 2004, ZEA has a limited tissue deposition and a low transmission rate into milk (EFSA, 2004a). Some transmission experiments in which cows were administered low levels of ZEA (0.5-165 mg daily, 0.77-340 µg/kg b.w.) detected no ZEA or metabolites in milk (Shreeve, Patterson & Roberts, 1979; Prelusky, Scott, Trenholm & Lawrence, 1990; Goll, Valenta & Oldenburg, 1995). In the study of Prelusky and others, a dose of 544.5 mg ZEA (approx. 940 –1 130 µg ZEA/kg b.w.) during 21 days had to be applied in order to observe transmission of ZEA and metabolites into milk (2.5 ng ZEA/mL, 3 ng α-ZEL/mL). In another study, the maximum detected ZEA levels in the milk of cows fed daily with 25-100 mg ZEA (55.6 - 222.2 µg/kg b.w.), during 6 days, were 0.39 and 1.16 ng/mL (Usleber, Renz, Martlbauer & Terplan, 1992). These authors concluded that the ZEA level of contamination is low, even after high oral ZEA doses. However, Mirocha and others found a high level of ZEA and metabolites of 1 359 ng/mL seven days after starting a daily dose of 200 mg ZEA.

Although the study of Hagler, Danko, Horvath, Palyusik & Mirocha (1980) showed low levels of ZEA and metabolites transmitted into the ewe milk, they were enough to cause strogenic effects in its offspring. The ewe used in the experiment had a 1-week-old female lamb which received all the milk not used for analysis. Within 10 days after administration of ZEA to the ewe, the lamb showed signs of hyperestrogenic syndrome. Transmission studies regarding trichothecenes have been carried out only with DON, NIV, FUS-X and T-2.

Studies performed on cows conclude that DON is very rapidly biotransformed to deepoxy-deoxynivalenol (DOM-1) in the rumen (Côté, Dahlem, Yoshizawa, Swanson & Buck, 1986; Seeling et al., 2006; Keese et al., 2008) and unmetabolized DON is excreted at an extremely low rate into the milk (0.0001% of the dose, 1 - 3 ng DON/mL) (Prelusky, Trenholm, Lawrence & Scott, 1984). Levels of up to 26 ng DOM-1/mL have

been detected in cow milk by Côté et al. (1986) when 2 933 - 5 867 µg DON/kg b.w. was administered daily. One study carried out on ewes, and with higher doses of DON administered (16 500 – 18 860 µg DON/kg b.w), reported levels of up to 17 ng DON/mL and 205 ng DOM-1/mL when DON was orally administered, but with a lower and intravenously single dose of DON (4000 µg DON/kg b.w.), the levels in milk reached up to 61 ng of DON/mL and 1 220 ng of DOM-1/mL (Prelusky, Veira, Trenholm & Foster, 1987).

Poapolathep, Sugita, Phitsanu, Doi & Kumagai (2004) have performed transmission studies of NIV and FUS-X in mice and also their distribution in whey, fat and casein acid into mouse milk. They concluded that NIV is transmitted to milk without metabolization, and FUS-X is metabolized and transmitted mainly as NIV.

A study determined that 0.2% of the T-2 dose given to one Jersey cow was transmitted into the milk in the form of T-2, HT-2, NEO, 4-deacetylneosolaniol along with 4 more unknown metabolites, 3 of which accounted for 60 - 70% of the total T-2 and metabolites excreted (Yoshizawa, Mirocha, Behrens & Swanson, 1981).

Although bio-transfer of OTA into milk has been demonstrated in other animal species (Breitholtz-Emanuelsson, Palminger-Hallén, Wohlin, Oskarsson, Hult & Olsen, 1993; Ferrufino-Guardia, Tangni, Larondelle & Ponchaut, 2000), in ruminants, the resident microflora of the rumen decreases bioavailability through hydrolysis of OTA to its metabolite ochratoxin α (OTα) (Skaug, 1999). Only 2 transmission studies on cows have been carried out. Shreeve and others (1979) could not detect OTA or OTα in milk after feeding 2 milking cows with levels of up to 25 µg OTA/kg b.w. daily for 11 weeks.A higher dose (13 300 µg OTA/kg b.w) administered by Ribelin, Fukushima & Still (1978) produced 650 mg of OTA and 4 500 mg of OTα in the milk measured the day after administration.

There are five studies where levels of up to 200 µg of AFB1 or AFB1 and OTA or ZEA were administered daily to cows (Shreeve et al., 1979; Veldman, Meijs, Borggreve & Heeres-van, 1992; Masoero, Gallo, Moschini, Piva & Diaz, 2007; Pietri, Bertuzzi, Piva, Binder, Schatzmayr & Rodrigues, 2009; Britzi et al., 2013) and they showed that AFM1 appears in milk after AFB1 administration. Inasmuch as AFB1 carry-over into the milk in the form of AFM1 is very well known, most studies have been focused on different factors that can influence AFM1 transmission. The carry-over of AFB1 (excreted as AFM1) into cow milk appears to be influenced by the lactation period of the cow. Cows in early lactation stage allow higher transmission of AFM1 and this gradually decreases through mid- and late-lactation stages. Lafont, Lafont, Mousset & Frayssinet (1980) and Veldman, Meijs, Borggreve & Heeres-van (1992) coincide in that the carry-over of AFM1 in cows in the early lactation period is 3.3 – 3.5 times higher than in cows in the late lactation period. Thus, it is important that transmission studies consider the lactation period when milk samples are collected. More studies which analyze dose-dependent transmission are needed.

11. Multi-exposure and co-occurrence

Co-occurrence of mycotoxins in feed and food is likely to appear. One type of fungi can produce more than one type of mycotoxins and more than one type of fungi could be present in the same substrate. In addition, mycotoxin metabolites could have similar or more toxic effects than the parent mycotoxin (Mirocha et al., 1981; Valimaa, Kivisto, Leskinen & Karp, 2010). The co-existence of different mycotoxins within one food product could affect their toxicity, showing additive or even synergistic effects. For instance, the co-occurrence of fumonisins and aflatoxins, even at low concentrations, has synergistic toxicological and carcinogenic effects (Gelderblom, Marasas, Lebepe-Mazur, Swanevelder, Vessey & Hall Pde, 2002).

One study suggests that OTA may influence the accumulation of AFM1 in kidney. Shreeve, Patterson & Roberts (1979) administered OTA and AFB1 daily to 2 Jersey cows and ZEA and AFB1 to another 2 cows for several weeks. Although the AFM1 levels in milk were similar, the kidneys from cows fed with OTA-contaminated diet presented AFM1 levels at least twice as high as those from cows that received ZEA.

Fungi producing CPA are widespread in nature and are able to simultaneously produce aflatoxins (*Aspergillus*) and ochratoxins (*Penicillium*). An association between CPA and aflatoxins toxic effects when both are present at the same time was first revealed by a retrospective study on the “Turkey X disease” (Cole, 1986); subsequently, a significant synergism between these two mycotoxins was reported by Pier, Belden, Ellis, Nelson, & Maki (1989).

Two studies carried out in 2011 and 2012 have analyzed aflatoxins, fumonisins, OTA, ZEA and DON in more than 4000 food samples from all around the world (BIOMIN, 2014). They have shown that only 26% (2011) and 18% (2012) of the samples were free of these 5 mycotoxins that were tested. Moreover, the study confirmed the presence of 2 or more types of mycotoxins in at least 41% of the samples tested in 2011; for the samples tested in 2012, this percentage increased to 50%.

Most recently, Huang et al. (2014) have detected up to 4 mycotoxins in single milk samples in China. The method they used was able to simultaneously analyze AFM1, OTA, ZEA and α-ZEL by UHPLC-MS/MS. Although no sample exceeded the Chinese and EU maximum residue levels for AFM1, of the 50 milk samples tested, 15% were contaminated with 2 mycotoxins, 45% for 3 mycotoxins and 22% for 4 mycotoxins. This finding coincides with a previous Chinese study mentioned by the author (Chen, 2011).

The MLs in food and feed have been established considering the toxicity of each mycotoxin, but the interaction of different mycotoxins may be not only additive, but also, synergistic (Alassane-Kpembi et al., 2013; Bouaziz, Bouslimi, Kadri, Zaied, Bacha & Abid-Essefi, 2013; Pier et al., 1989; Tatay, Meca, Font & Ruiz, 2014). Even at nontoxic concentrations of individual mycotoxins, when together, toxic effects are observed (Wan, Turner & El-Nezami, 2013). Therefore, since the co-occurrence of them is highly probable in one food, the mere presence of multiple mycotoxins should be considered to be a risky factor even when the values corresponding to each one is below the maximum permitted limit.

12. Conclusions

Mycotoxins in milk could be a risk to human and animal health. High contamination in feed may result in a significant mycotoxin level in milk when animals are in a state of physiological imbalance or when they are mainly fed with highly contaminated foodstuff. The increasing efficiency of milk production and the ongoing striving for higher milk yields may facilitate such situations. Several studies performed on different animal species have shown that the transmission of mycotoxins to the milk is possible. Moreover, mycotoxin intake may reduce milk production or alter milk composition.

The most studied presence of a mycotoxin in milk has been that of AFM1. It has been studied in samples worldwide, and AFM1 appears as a natural contaminant in the milk of different animals, before or after milk processing. Approximately 10% of the milk samples analyzed presented AFM1 levels above the maximum level legislated in the EU for this mycotoxin in this human food. The highest occurrence and levels found in the papers which were reviewed are from Asian samples. Few studies have been carried out for monitoring mycotoxins other than AFM1 in milk. Nonetheless, the occurrence of FB1, FB2, OTA, ZEA, ZAN, α-ZAL, α-ZEL, DOM-1, CPA, AFG1, AFG2,

AFB1, AFB2 and AFM2 have been detected in natural milk samples, although at low levels. This review shows the importance of continuous monitoring of mycotoxins as natural contaminants in milk regarding the safety aspects of milk and milk products as human food. Moreover, the presence or absence of mycotoxins in milk could be used for indirect analysis of animal health.

Very few studies have been performed for the purpose of analyzing the co-occurrence of more than one type of mycotoxins in milk and, in cases where they co-ocurred, their synergistic or antagonistic effects are unknown. The European Commission recommendations state that “Member States should ensure that samples are simultaneously analyzed for the presence of deoxynivalenol, zearalenone, ochratoxin A, fumonisin B1 + B2 and T-2 and HT-2 toxin so that the extent of co-occurrence can be assessed”. Analysis of simultaneous mycotoxins in human food and animal feed is an analytical challenge because of the very different physico-chemical characteristics, but it is essential in order to protect human and animal health. Analytical methods that can simultaneously detect and quantify a broad number of mycotoxins, belonging to different families, in milk with low limits of detection and quantification are needed in order to reduce analytical costs and allow more frequent monitoring of mycotoxins in milk. In addition, with these tools, synergistic effects could be studied and better risk assessment could be made.

Furthermore, and due to the fact that dairy animals are mainly contaminated through animal feed, this matrix should be included in studies for monitoring mycotoxin contamination.

Abbreviations Used

3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), aflatoxin B1 (AFB1), aflatoxin M1 (AFM1), aflatoxin M2 (AFM2), cyclopiazonic acid (CPA),

deoxynivalenol (DON), deepoxy-deoxynivalenol (DOM-1), diacetoxyscirpenol (DAS), fumonisin B1 (FB1), fusarenon X (FUS-X), neosolaniol (NEO), nivalenol (NIV), ochratoxin A (OTA), ochratoxin B (OTB), patulin (PAT), sterigmatocystin (STC), toxin T-2 (T-2), toxin HT-2 (HT-2), zearalenone (ZEA), zearalanone (ZAN), α-zearalanol (α-ZAL), β -zearalanol (β -ZAL), α-zearalenol (α-ZEL), β-zearalenol (β-ZEL), International Agency for Research on Cancer (IARC), Joint FAO/WHO Expert Committee on Food Additives (JECFA), European Food Safety Authority (EFSA), Provisional Maximum Tolerable Daily Intake (PMTDI), Tolerably Daily Intake (TDI), Limit of Detection (LOD), Limit of Quantification (LOQ).

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Table 1. Legislation for AFM1 levels in milk worldwide.

Country	Foodstuffs	Aflatoxin M1 maximun level
Austria, Belgium, Bosnia and Herzegovina, Cyprus, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Liechtenstein, Lithuania, Luxembourg, Netherlands, Norway, Poland, Portugal, Slovenia, Spain, Sweden, Turkey, United Kingdom, EU (European Commission, 2010a)	Raw milk, heat-treated milk and milk for the manufacturer of milk-based products	0.050 µg/kg
Saudi Arabia, United Arab Emirates, Kuwait, Bahrain, Oman, Qatar, GCC (GSO, 2013)		
Belarus, Bulgaria, Chile, Czech Republic, Estonia, Honduras, Hungary, Iran (Islamic Republic of), Israel, Malta, Morocco, Switzerland, Turkey(FAO, 2004)	Milk	0.05 µg/kg
Argentina, Brazil, Paraguay, Uruguay, MERCOSUR(2002)	Fluid milk	0.5 µg/L
	Powder milk	5 µg/kg
Codex Alimentarius (FAO/WHO, 1995)	Milk	0.5 µg/kg
USA (FDA, 2005)		
India (Indian MoH, 2011)		
South Africa (South African MoH, 2004)		
Kenya (Kubo, 2012)		
Armenia, Barbados, Croatia, South Korea, Latvia, Republic of Moldova, Montenegro, Peru, Romania, Russian Federation, Serbia, Singapore, Slovakia, Taiwan, Ukraine, Venezuela, Vietnam (FAO, 2004)		
China (Chinese MoH, 2011)	Milk and milk products (for milk powder, calculated on a fresh milk basis)	0.5 µg/kg
Mexico (Secretaría Mexicana de Salud, 2002)	Pasteurised, ultrapasteurised, sterilised and dehydrated milk, milk formula and combined milk products	0.5 µg/L
Syria (FAO, 2004)	Liquid milk	0.2 µg/kg
	Dried milk (not used in baby food)	0.05 µg/kg
Indonesia (FAO, 2004)	Milk	5 µg/kg

EU: European Union, GCC: Cooperation Council for the Arab States of the Gulf. MoH:Ministry of Health.

Table 2. Occurrence of mycotoxins in animal milk samples worldwide.

Mycotoxin	Milk origin	Collection year	Milk type	Total samples	Number of positive samples	% positive sample	Mycotoxin concentration	Method of analysis	Reference
FB1	USA	1993	R	155	1	0.6	1 290 ng/L	GC/MS	(Maragos & Richard, 1994)
	Italy	n.r.	R,P,O	10	8	80	260[a] 430[b] ng/kg	LC-MS/MS-QQQ	(Gazzotti et al., 2009)
CPA	Italy	n.r.	P	20	3	15	6 333[a] 8 300[b] ng/L	LC-MS/MS-ion trap	(Losito et al., 2002)
	Brazil	2004	P,U	48	2	4.2	8 050[a] 9 700[b] ng/L	LC-UV-VIS	(Oliveira, Rosmaninho & Rosim, 2006b)
ZEA	Hungary	1980	R	n.r	16	n.r	n.r.	TLC-UV and GC	(Sándor, 1984)
	Egypt	n.r.	R	20	4	20	6 900[a] 10 100[b] ng/kg	LC-FLD	(El-Hoshy, 1999)
			P	20	3	15	5 000[a] 7 200[b] ng/kg		
			D	20	6	30	6 400[a] 12 500[b] ng/kg		
			C	20	5	25	4 400[a] 9 300[b] ng/kg		
	UK	n.r.	n.r.	100	3	3	500[a] 5 500[b] ng/kg	n.r.	(SCOOP, 2003)
	China	2012	R	30	n.r	n.r	14.9[a] 45.8[b] ng/kg	UHPLC-MS/MS	(Huang et al., 2014)
			D	8	n.r	n.r	11.6[a] 12.4[b] ng/kg		
			L	12	n.r	n.r	20.5[a] 28.3[b] ng/kg		
ZAN	China	n.r.	n.r	103	1	0.97	370 ng/L	LC-MS/MS-QQQ	(Xia et al., 2009)
α-ZAL			n.r	103	1	0.97	3 030 ng/L		
α-ZEL	China	2012	R	30	n.r	n.r	24.3[a] 73.5[b] ng/kg	UHPLC-MS/MS	(Huang et al., 2014)
			D	8	n.r	n.r	43.1[a] 64.3[b] ng/kg		
			L	12	n.r	n.r	36.7[a] 45.1[b] ng/kg		
DOM-1	Denmark	n.r.	R	20	5	25	180[a] ng/L 300[b] ng/L	LC-MS/MS	(Sørensen & Elbæk, 2005)

[a] mean value, [b] maximum value, C = condensed cow milk, D = powder cow milk , P = pasteurized cow milk, R = raw cow milk, L= liquid cow milk, O = organic, U = UHT-treated, n.r. = not reported. GC = gas chromatography, LC = liquid chromatography, UHPLC = ultra high pressure liquid chromatography, TLC = thin layer chromatography, FLD = fluorescence detector, UV-VIS = ultraviolet and visible detector, MS/MS = tandem mass spectrometry, QQQ = triple quadrupole, IPC = ion-pair

chromatography.

Table 2 (continued). Occurrence of mycotoxins in animal milk samples worldwide.

Mycotoxin	Milk origin	Collection year	Milk type	Total samples	Number of positive samples	% positive sample	Mycotoxin concentration	Method of analysis	Reference
OTA	Italy	n.r.	O	63	3	4.8	70 - 110 ng/L	LC-FLD	(Pattono et al., 2011)
	Sweden	1991	R	36	5	14	10 - 40 ng/L	IPC-FLD	(Breitholtz-Emanuelsson, Olsen, Oskarsson, Palminger & Hult, 1993)
	Norway	1997-98	O	47	5	11	15 - 28 ng/L	LC-FLD	(Skaug, 1999)
		1995-96	R	40	6	15	11 - 58 ng/L		
	France	2003	R	264	3	1	5 - 6.6 ng/L	LC-FLD	(Boudra, Barnouin, Dragacci & Morgavi, 2007)
	Sudan	2009	R	5	1	20	2 730 ng/L	LC-UV	(Elzupir, Makawi & Elhussein, 2009)
	China	2012	R	30	n.r	n.r	56.7 [a] 84.1 [b] ng/kg	UHPLC-MS/MS	(Huang et al., 2014)
			D	8	n.r	n.r	27.0 [a] 49.4 [b] ng/kg		
			L	12	n.r	n.r	26.8 [a] 57.9 [b] ng/kg		
AFB1	Jordan	2007	R,RS, RG, P, PS, PG	60	5	8.3	2 270 [a] ng/kg	LC-FLD LC-UV	(Herzallah, 2009)
AFB2					5	8.3	210 [a] ng/kg		
AFG1					5	8.3	80 [a] ng/kg		
AFG2					5	8.3	90 [a] ng/kg		
AFM2					5	8.3	< 50 [a] ng/kg		
AFM2	Japan	2001-02	U	52	25	48	0.9 [a] 3.4 [b] ng/kg	LC-FLD	(Nakajima et al., 2004)

[a] mean value, [b] maximum value, C = condensed cow milk, D = powder cow milk , P = pasteurized cow milk, R = raw cow milk, L=liquid cow milk, O = organic U = UHT-treated, n.r. = not reported. GC = gas chromatography, LC = liquid chromatography, UHPLC = ultra high pressure liquid chromatography, TLC = thin layer chromatography, FLD = fluorescence detector, UV-VIS = ultraviolet and visible detector, MS/MS = tandem mass spectrometry, QQQ = triple quadrupole, IPC = ion-pair chromatography.

Table 3. Occurrence of Aflatoxin M1 in animal milk samples in Europe

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
Albania	n.r.	R	120	n.r.	n.r.	< 50 - 850 ng/kg	70	58.3	TLC	(Panariti, 2001)
Austria	1999	n.r.	20	0	0	< 10 ng/kg	0	0	n.r.	(EFSA, 2004b)
Croatia	2009	R	61	n.r.	n.r.	0.6 – 58.6 ng/L	1	1.64	ELISA	(Bilandžić, Varenina & Solomun, 2010)
Cyprus	1992	n.r.	270	26	9.6	< 10 - 50 ng/kg	0	0	n.r.	(EFSA, 2004b)
Finland	1999	n.r.	296	1	0.3	< 10 - 50 ng/kg	0	0	n.r.	
France	1999	n.r.	234	0	0	< 30 ng/kg	0	0	n.r.	
Germany	1999	n.r.	6537	211	3.2	< 10 - 50 ng/kg	0	0	n.r.	
	2000	n.r.	3618	4	0.1	< 10 - 50 ng/kg	0	0	n.r.	
Greece	1995 - 1996	P	81	72	88.9	0.5 - 177 ng/L	3	3.7	ELISA/LC-FLD	(Markaki & Melissari, 1997)
	1999 - 2000	C	15	14	93.3	5 - > 50 ng/L	2	13.3	LC-FLD	(Roussi, Govaris, Varagouli & Botsoglou, 2002)
	1999 - 2000	P	82	70	85.4	5 – 50 ng/L	0	0		
	1999 - 2000	R	30	22	73.3	5 - > 50 ng/L	1	3.3		
	1999 - 2000	RG	10	4	40	5 – 50 ng/L	0	0		
	1999 - 2000	RS	12	8	66.7	5 – 50 ng/L	0	0		
	1999 - 2000	U	17	14	82.3	5 – 50 ng/L	0	0		
	2000 - 2001	P	54	43	79.6	5 – 50 ng/L	0	0		
	2000 - 2001	R	51	36	70.6	5 - > 50 ng/L	1	2		
	2000 - 2001	RG	12	8	66.7	5 - 50 ng/L	0	0		
	2000 - 2001	RS	15	11	73.3	5 - > 50 ng/L	1	6.7		
	2010	R,O,U	196	91	46.5	10 ng/L [a]	2	1	ELISA	(Tsakiris, Tzatzarakis, Alegakis, Vlachou, Renieri & Tsatsakis, 2013)
Ireland	1999		62	0	0	< 20 ng/kg	0	0	n.r.	(EFSA, 2004b)

[a] mean value, C = condensed cow milk, O = organic cow milk, P = pasteurized cow milk, R = raw cow milk, RG= raw goat milk, RS = raw sheep milk, U = UHT-treated cow milk, n.r. = not reported. ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, MS = mass spectrometry detector

Table 3 (continued). Occurrence of Aflatoxin M1 in animal milk samples in Europe

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
Italy	1993	R	43	43	100	1 - 100 ng/kg	14	32.5	LC-FLD	(Pietri, Bertuzzi, Moschini & Piva, 2003)
	1994	R	55	55	100	1 – 406 ng/kg	9	16.3		
	1995	R	63	51	81	1 – 50 ng/kg	0	0		
	1996	R	45	42	93.3	1 – 100 ng/kg	1	2.2		
	1996	U	161	125	78	< 1 – 23.5 ng/L	0	0	LC-FLD	(Galvano, Galofaro, Ritieni, Bognanno, De Angelis & Galvano, 2001)
	1998	R	66	66	100	1 – 397 ng/kg	4	6	LC-FLD	(Pietri et al., 2003)
	1999	R	60	60	100	1 – 50 ng/kg	0	0	LC-FLD	
	2000	RS	240	195	81	2 – 108 ng/L	3	1.3	LC-MS	(Bognanno et al., 2006)
	2003 - 2005	P, R	316	n.r.	n.r.	2 – 90 ng/L	2	0.6	LC-FLD	(Nachtmann, Gallina, Rastelli, Ferro & Decastelli, 2007)
	2004 - 2005	R	341	5	1.5	until 50 ng/L	3	1	ELISA/LC	(Decastelli et al., 2007)
	2012	R	12	6	50	<3 - 10 ng/L	0	0	LC-FLD	(Santini et al., 2013)
		RG	3	1	33.3	<3 - 5 ng/L	0	0		
		RS	34	20	58.8	<3 - 20 ng/L	0	0		
Netherlands	1999	n.r.	30	5	16.7	10 - 50 ng/kg	0	0	n.r.	(EFSA, 2004b)
Portugal	1999	R	31	25	80.6	5 - 50 ng/kg	0	0	n.r.	(Martins & Martins, 2000)
	1999	U	70	60	84.2	5 - 61 ng/kg	2	2.9	n.r.	
Spain	2000	R	92	5	5.4	14 – 24.9 ng/L	0	0	ELISA	(Rodriguez Velasco et al., 2003)
Sweden	1999	n.r.	11	0	0	< 10 ng/kg	0	0	n.r.	(EFSA, 2004b)
UK	2001	n.r.	100	3	3	10 - 50 ng/kg	0	0	n.r.	(EFSA, 2004b)
TOTAL			13 566				119			

[a] mean value, C = condensed cow milk, O = organic cow milk, P = pasteurized cow milk, R = raw cow milk, RG= raw goat milk, RS = raw sheep milk, U = UHT-treated cow milk, n.r. = not reported. ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, MS = mass spectrometry detector.

Table 4. Occurrence of Aflatoxin M1 in animal milk samples in Africa

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
Egypt	1999 - 2000	n.r.	15	3	20	5 000 – 8 000 ng/L	3 [a]	20 [a]	LC-FLD	(El-Sayed et al., 2000)
		D	10	n.r.	n.r.	5 000 ng/L	1	10		
	2010	D	125	54	43.2	0.3 – 21.8 ng/L	0	0	ELISA	(El-Tras, El-Kady & Tayel, 2011)
Libya	2002	R	49	35	71.4	30 – 2 680 ng/L	34	69.4	LC-FLD	(Elgerbi, Aidoo, Candlish & Tester, 2004)
Morocco	2006	P	54	50	88.8	1 – 117 ng/L	4	7.4	LC-UV	(Zinedine, González-Osnaya, Soriano, Moltó, Idrissi & Mañes, 2007)
	2009 - 2010	R	48	13	27	10 – 100 ng/L	4	8	LC-FLD	(El Marnissi et al., 2012)
Nigeria	2006	R	22	3 [b]	13.6 [b]	2 040 – 4 000 ng/L	3 [b]	13.6 [b]	TLC-2D	(Atanda, Oguntubo, Adejumo, Ikeorah & Akpan, 2007)
Kenya	2006	P, U	613	473	77.2	5 – 780 ng/kg	162	26.4	ELISA	(Kang'ethe & Lang'a, 2009)
Sudan	2009	R	44	42	95.5	220 – 6 900 ng/L	42	95.5	LC-FLD	(Elzupir & Elhussein, 2010)
TOTAL			980				253			

[a] > 500 ng/L, [b] detection limit = 2000 ng/L, D = powder cow milk, P = pasteurized cow milk, R = raw cow milk, U = UHT-treated cow milk, ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, UV = ultraviolet detector, TLC-2D = Two-dimensional thin layer chromatography.

Table 5. Occurrence of Aflatoxin M1 in animal milk samples in Asia

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
China	2012	R,L,D	50	n.r.	n.r.	42.9[b] -237.4 ng/kg	n.r.	n.r.	UHPLC-MS/MS	(Huang et al., 2014)
	n.r.	n.r.	50	3	6	10 - 250 ng/kg	1 a	n.r.	LC-MS/MS	(H. Wang, Zhou, Liu, Yang & Guo, 2011)
	n.r.	P,U	50	15	30	6 – 250 ng/mL	1 a	n.r.	LC-MS/MS	(H. Wang, Zhou, Liu, Yang & Guo, 2010)
	n.r.	n.r.	15	13	86.7	12.1 – 301.2 ng/L	8	53.33	LC-FLD	(Guan, Li, Zhang, Zhang, Zhang & Jiang, 2011)
		D	9	7	77.7	19.1 – 253.4 ng/L	3	33.33		
	n.r.	DG	3	1	33.3	118 ng/kg	1	33.3	LC-FLD	(Y. Wang et al., 2012)
		R,P,U,D	23	7	30.4	38 - 212 ng/kg	6	26.1		
	n.r.	n.r.	27	21	77.78	13.3 – 1 061.3 ng/L	14	51.85	ELISA	(Zhang et al., 2012)
	2006 - 2007	U	233	112	48	21.49 [b] - 95.73 ng/kg	48	20.6	ELISA	(Guo, Yuan & Yue, 2013)
India	n.r.	DB	10	n.r.	n.r.	>500 – 15 000 ng/L	5 a	50	ELISA	(Thirumala-Devi et al., 2002)
		PB	44	n.r.	n.r.	>500 – 30 000 ng/L	16 a	36		
		RB	216	n.r.	n.r.	>500 – 48 000 ng/L	110 a	50.9		
	n.r.	P	12	4	32	164 ng/kg	3	33	ELISA	(Rastogi, Dwivedi, Khanna & Das, 2004)

[a] > 500 ng/L, [b] mean value n.r. = not reported, D = powder cow milk, DB = powder buffalo milk, DG=powder goat milk, P = pasteurized cow milk, PB = pasteurized buffalo milk, PG = pasteurized goat milk, PS = pasteurized sheep milk, R = raw cow milk, RB = raw buffalo milk, RC = raw camel milk, RG = raw goat milk, RS = raw sheep milk, U = UHT-treated cow milk. ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, MS/MS = tandem mass spectrometry, TLC = thin layer chromatography, AC = affinity chromatography.

Table 5 (continued). Occurrence of Aflatoxin M1 in animal milk samples in Asia

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
Iran	n.r.	R	73	60	82.2	n.r.	60	82.2	n.r.	(Tajik, Rohani & Moradi, 2007)
	2001	R	111	85	76.6	15 – 280 ng/L	44	40	TLC	(Kamkar, 2005)
	2003	P	624	624	100	< 45 - > 80 ng/L	111	17.8	ELISA	(Alborzi, Pourabbas, Rashidi & Astaneh, 2006)
	2003 - 2004	R	98	98	100	8 - 76 ng/L [b]	37	37.7	LC-FLD	(Tajkarimi et al., 2007)
	2004	R	319	172	54	57 ng/L [b]	73	23		
	2005	P	128	128	100	31 – 113 ng/kg	100	78	ELISA	(Oveisi, Jannat, Sadeghi, Hajimahmoodi & Nikzad, 2007)
	2005 - 2006	P	72	72	100	5.1 – 28.5 ng/L	0	0	ELISA	(Tajik et al., 2007)
		R	72	72	100	4.3 – 91.8 ng/L	9	12.5		
	2006	P	72	72	100	178.8 - 253.5 ng/L	72	100	ELISA	(Sefidgar, Mirzae, Assmar & Naddaf, 2011)
	2006	R	120	120	100	4 – 352.3 ng/L	68	56.7	ELISA	(Sefidgar, Azizi, Khosravi & Roudbar-Mohammadi, 2008)
	2006	R, P	90	90	100	2.9 – 85 ng/kg	30	33.3	ELISA	(Nemati et al., 2010)
	2007	P	40	n.r.	n.r.	23.2 ng/L [b]	n.r.	n.r.	ELISA	(Mohamadi & Alizadeh, 2010)
		U	40	n.r.	n.r.	19.5 ng/L [b]	n.r.	n.r.		
	2007 - 2008	R	75	59	78.7	60.1 ng/L [b]	27	36	ELISA	(Rahimi et al., 2010)
		RB	75	29	38.7	31.9 ng/L [b]	6	8		
		RC	40	5	12.5	19 ng/L [b]	0	0		
		RG	60	19	31.7	30.1 ng/L [b]	4	6.7		
		RS	51	19	37.3	28.1 ng/L [b]	2	3.9		
		U	210	116	55.2	8 - 249 ng/L	70	33.3		
	2008	P	196	196	100	19 - 126 ng/L	158	80.6	ELISA	(Mohamadi Sani, Nikpooyan & Moshiri, 2010)
	2008 - 2009	P	116	83	71.5	5.8 – 528.5 ng/L	31	26.7	ELISA	(Fallah, 2010a)
		U	109	68	62.3	5.6 – 515.9 ng/L	19	17.4		
	2009	P	75	75	100	5 - >50 ng/kg	3	4	ELISA	(Arast, Mohammadian & Behnamipour, 2012)
	2009	P	91	66	72.5	13 – 250 ng/L	n.r.	n.r.	TLC	(Fallah, 2010b)
	2011	P	60	60	100	2 – 64 ng/L	1	1.6	LC-FLD	(Sani & Nikpooyan, 2012)
	2012	P	45	45	100	8.8 - 64 ng/L	13	28.9	ELISA	(Riahi-Zanjani & Balali-Mood, 2013)

[a] > 500 ng/L, [b] mean value n.r. = not reported, D = powder cow milk, DB = powder buffalo milk, DG=powder goat milk, P = pasteurized cow milk, PB = pasteurized buffalo milk, PG = pasteurized goat milk, PS = pasteurized sheep milk, R = raw cow milk, RB = raw buffalo milk, RC = raw camel milk, RG = raw goat milk, RS = raw sheep milk, U = UHT-treated cow milk. ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, MS/MS = tandem mass spectrometry, TLC = thin layer chromatography, AC = affinity chromatography.

Table 5 (continued). Occurrence of Aflatoxin M1 in animal milk samples in Asia

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
Japan	2001 - 2002	U	208	207	99.5	1 – 29 ng/kg	0	0	LC-FLD	(Nakajima et al., 2004)
	2004	R	299	n.r.	n.r.	5 -11 ng/L	0	0	LC-FLD	(Sugiyama, Hiraoka & Sugita-Konishi, 2008)
Jordan	2007	R,RS, RG, P, PS, PG	60	5	8.3	140 ng/kg [b]	n.r.	n.r.	LC-FLD, LC-UV	(Herzallah, 2009)
	2011 - 2012	R	50	50	100	7.05 -129.79 ng/kg	21	42	ELISA	(Omar, 2012)
Korea	1997	D	24	18	75	26 – 342 ng/L	n.r.	n.r.	ELISA, LC-FLD	(Kim, Shon, Ryu, Park, Hwang & Kim, 2000)
		P	70	53	75.7	2 – 52 ng/L	n.r.	n.r.		
Kuwait	1998	P	25	13	58	10 – 210 ng/L	6	24	LC-FLD	(Srivastava et al., 2001)
Lebanon	n.r.	P	60	n.r.	n.r.	11.88 - 26.33 ng/L	0	0	ELISA	(Hassan & Kassaify, 2014)
		PG	4	n.r.	n.r.	4.55 ng/L [b]	0	0		
		PS	4	n.r.	n.r.	2.1 ng/L [b]	0	0		
		R	60	n.r.	n.r.	13.89 - 35.60 ng/L	0	0		
		RG	4	n.r.	n.r.	6.85 ng/L [b]	0	0		
		RS	4	n.r.	n.r.	3.33 ng/L [b]	0	0		

[a] > 500 ng/L, [b] mean value n.r. = not reported, D = powder cow milk, DB = powder buffalo milk, DG=powder goat milk, P = pasteurized cow milk, PB = pasteurized buffalo milk, PG = pasteurized goat milk, PS = pasteurized sheep milk, R = raw cow milk, RB = raw buffalo milk, RC = raw camel milk, RG = raw goat milk, RS = raw sheep milk, U = UHT-treated cow milk. ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, MS/MS = tandem mass spectrometry, TLC = thin layer chromatography, AC = affinity chromatography.

Table 5 (continued). Occurrence of Aflatoxin M1 in animal milk samples in Asia

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
Pakistan	2005	R	168	167	99.4	10 – 700 ng/L	167	99.4	AC-FLD	(Hussain & Anwar, 2008)
	2007	R	120	63	52.5	4 – 87 [b] ng/L	n.r.	n.r.	LC-FLD	
	2007	RB	360	153	42.5	4 – 75 [b] ng/L	n.r.	n.r.		
	n.r.	R	21	18	85.7	14 – 40 ng/L	0	0	ELISA	(Maqbool, Anwar-Ul-Haq & Ahmad, 2009)
	2012	R	104	39	37.5	4 - 890 ng/L	26	25	LC-FLD	(Iqbal et al., 2013)
	2012	U	84	35	41.7	4 - 510 ng/L	20	23.8		
Syria	2005 - 2006	D	8	1	1	12 ng/L	0	0	ELISA	(Ghanem & Orfi, 2009)
		P	10	10	100	8 - 765 ng/L	8	80		
		R	74	70	95	20 – 690 ng/L	41	59		
		RG	11	7	64	8 - 54 ng/L	1	14		
		RS	23	13	57	6 - 634 ng/L	3	23		
Thailand	2006 - 2007	R	240	240	100	14 – 197 ng/L	167	70	LC-FLD	(Ruangwises & Ruangwises, 2010)
Turkey	2004	U	50	50	100	5 – 244 ng/L	10	20	ELISA	(Gundinc & Filazi, 2009)
	2005	U	129	75	58.1	10 - 543.64 ng/L	61	47	ELISA	(Unusan, 2006)
	n.r.	U	24	14	58.3	< 10 – 50.5 ng/L	1	3.7	LC-FLD	(Gurbay et al., 2010)
		P	3	2	66.7	< 10 – 50 ng/L	0	0		
	2006 - 2007	U	150	89	59	< 5 - > 100 ng/kg	16	10.7	ELISA	(Aydemir Atasever, Adiguzel, Atasever, Ozlu & Ozturan, 2010)
	2010	R	36	22	61.1	1 - 10 ng/kg	0	0	ELISA	(Aksoy, Yavuz, Guvenc, Das, Terzi & Celik, 2010)
	n.r.	R	135	60	44.4	5 – 68.3 ng/L	n.r.	n.r.	ELISA	(Ozsunar et al., 2010)
TOTAL			6 376				1 709			

[a] > 500 ng/L, [b] mean value n.r. = not reported, D = powder cow milk, DB = powder buffalo milk, DG=powder goat milk, P = pasteurized cow milk, PB = pasteurized buffalo milk, PG = pasteurized goat milk, PS = pasteurized sheep milk, R = raw cow milk, RB = raw buffalo milk, RC = raw camel milk, RG = raw goat milk, RS = raw sheep milk, U = UHT-treated cow milk. ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, MS/MS = tandem mass spectrometry, TLC = thin layer chromatography, AC = affinity chromatography.

Table 6. Occurrence of Aflatoxin M1 in animal milk samples in America

Milk origin	Collection year	Milk type	Total samples	positive samples	% positive samples	C.min –C.max	samples >50ng/L	% >50ng/L	Method of analysis	Reference
Argentina	1999	D	5	4	80	10 – 14 ng/L	0	0	ELISA	(López, Ramos, Ramadán & Bulacio, 2003)
		P	16	8	50	10 – 17 ng/L	0	0		
		R	56	6	10.7	12 - 30 ng/L	0	0		
	2007	R	94	60	64	10 - 70 ng/L	10	11	LC-MS/MS	(Alonso, Monge, Larriestra, Dalcero, Cavaglieri & Chiacchiera, 2010)
Brazil	1992	P	52	4	7.7	70- 370 ng/L	4	7.7	LC-FLD	(De Sylos, Rodriguez-Amaya & Carvalho, 1996)
	n.r.	D	300	33	11	100 – 1 000 ng/L	33	11	ELISA	(Oliveira et al., 1997)
	1999 - 2000	P	79	58	73.4	15 - 500 ng/L	12	15.2	LC-FLD	(Garrido, Iha, Santos Ortolani & Duarte Favaro, 2003)
		U	60	53	88.3	15 - 500 ng/L	17	28.3		
	2004 - 2005	DG	12	8	66.7	< 10 – 100 ng/L	4	33.3	LC-FLD	(Oliveira & Ferraz, 2007)
		PG	12	7	58.3	< 10 – 200 ng/L	3	25		
		UG	12	10	83.3	< 10 – 200 ng/L	6	50		
	n.r.	D	12	12	100	20 - 760 ng/L	1 [a]	n.r.	LC-FLD	(Iha et al., 2013)
		P	47	39	83	9 - 437 ng/L	1 [a]	n.r.		
		U	17	13	76.5	8 – 215 ng/L	1 [a]	n.r.		
Colombia	2004	P	120	83	69.2	10.7 – 213 ng/L	1 [a]	n.r.	LC-FLD	(Diaz & Espitia, 2006)
	2005	P	121	96	79.3	10.6 – 288.9 ng/L	1 [a]	n.r.		
Mexico	n.r.	R	40	32	80	6 - 65 ng/L	1 [a]	n.r.	ELISA	(Reyes Velázquez, Patricio Martínez, Isaías Espinosa, Nathal Vera, De Lucas Palacios & Rojo, 2009)
Trinidad	n.r.	P	37	0 [a]	0 [a]	500 ng/L	n.r. [a]	n.r. [a]	RIA	(Offiah & Adesiyun, 2007)
		R	175	13 [a]	7.4 [a]	500 ng/L	13 [a]	7.4 [a]		
TOTAL			1 267				109			

[a] >500 ng/L, n.r. = not reported, D = powder cow milk, DG= powder goat milk, P = pasteurized cow milk, PG= pasteurized goat milk, R = raw cow milk, U = UHT-treated cow milk, UG = UHT-treated goat milk, ELISA = Enzyme-Linked ImmunoSorbent Assay, LC = liquid chromatography, FLD = fluorescence detector, MS/MS = tandem mass spectrometry, RIA = Radioimmunoassay.