A survey on Aflatoxin M_1 content in sheep and goat milk produced in Sardinia Region, Italy (2005-2013)

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Abstract

In the present work the results of a survey conducted in Sardinia Region on Aflatoxin M_1 (AFM_1) contamination in milk of small ruminants from 2005 to 2013 are reported. A total of 517 sheep and 88 goat milk samples from bulk tank, tank trucks and silo tank milk were collected. Analyses were performed by the Regional Farmers Association laboratory using high-performance liquid chromatography following the ISO 14501:1998 standard. None of the sheep milk samples analysed during 2005-2012 showed AFM_1 contamination. In sheep milk samples collected in 2013, 8 out of 172 (4.6%) were contaminated by AFM_1 with a concentration (mean±SD) of 12.59±14.05 ng/L. In one bulk tank milk sample 58.82 ng/L AFM_1 was detected, exceeding the EU limit. In none of goat milk samples analysed from 2010 to 2012 AFM_1 was detected. In 2013, 9 out of 66 goat milk samples (13.6%) showed an AFM_1 concentration of 47.21±19.58 ng/L. Two of these samples exceeded the EU limit, with concentrations of 62.09 and 138.6 ng/L. Higher contamination frequency and concentration rates were detected in bulk tank milk samples collected at farm than in bulk milk truck or silo samples, showing a dilution effect on AFM_1 milk content along small ruminants supply chain. The rate and levels of AFM_1 contamination in sheep and goat milk samples were lower than other countries. However, the small number of milk samples analysed for AFM_1 in Sardinia Region in 2005-2013 give evidence that food business operators check programmes should be improved to ensure an adequate monitoring of AFM_1 contamination in small ruminant dairy chain.

Introduction

An increased frequency of Aflatoxins (AFs) contamination in corn has been reported in the last few years, where feed imported from third countries or crop raised in different European countries, mainly in West and South Europe were implicated (Streit *et al.*, 2012; EU-RASFF, 2014) (Figure 1). Corn and related products are widely used as feedstuff in dairy animals as an important source of fermentable carbohydrates. Recent works also showed an increased AFs contamination rate in corn produced in Italy (Causin, 2013). From 2003 to 2012, regions of Northern Italy – which are the main national corn producers – were affected by particular climatic conditions. An increase in temperature and drought stress caused a high rate of corn crops contaminated with Aflatoxin B_1 (AFB_1). In 2012, during a wide survey conducted on corn produced in Northern Italy, 31.326 samples taken at storage plants were analysed. The results showed that AFB_1 contamination above the EU limit of 20 µg/kg, was detected in samples representative of about 784.000 corn tons, corresponding to 45.2% of the total production (Causin, 2013). A correspondence between AF contamination in corn and the presence of AFs metabolites in Italian cow milk and dairy products was observed (Bolzoni *et al.*, 2013). As a consequence of the last AFs contamination crisis, the Italian Ministry of Health enforced measures to minimise the risk of contamination of milk and dairy products by Aflatoxin M_1 (AFM_1). Preventive measures along bovine dairy production chain and more stringent requirements for food business operators (FBOs) own-check monitoring programmes were established (Italian Ministry of Health, 2013).

Aflatoxin M_1 contamination levels in goat and sheep milk is generally lower as compared to cow's milk (Virdis *et al.*, 2008). Sheep and goat mainly graze on pasture and their lower intake in concentrate reduces the exposure to AFs. The use of concentrate and feedstuff in the formulation of small ruminants feeding is limited due to economic reasons and to effectiveness on milk production (Molle *et al.*, 2008).

The ability to convert the AFB_1 ingested with feedstuff to AFM_1 excreted with milk, referred to as carry-over, is also variable between large and small ruminants. Previous works reported carry-over values ranging between 0.35 and 3% in cows (Veldman *et al.*, 1992; Frobisch *et al.*, 1986) and between 0.018 and 3.1% in goats (Goto and Hsieh, 1985; Nageswara Rao and Chopra, 2001; Ronchi *et al.*, 2005). Lower carry-over rates were found in dairy sheep, ranging between 0.08 and 0.33% (Battacone *et al.*, 2005).

The own-check programmes developed in Sardinia Region by FBOs in the last years included monitoring for AFM_1 in sheep and goat milk only in few cases. Only few cheese-making factories conducted AFM_1 analysis by means of rapid detection methods on internal laboratories. Most of the analyses for AFM_1 detection were carried out at the Regional Farmers Association laboratory using high-performance liquid chromatography (HPLC).

In the current work the results of the AFM_1 monitoring programme conducted in Sardinia Region on sheep and goat milk during eight years period, from 2005 to 2013, are presented.

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Materials and Methods

During the period from 2005 to 2013 a total of 517 sheep milk and 88 goat milk samples were collected for the detection of AFM_1. Samples were represented by: 75 bulk tank milk samples, of which 56 and 19 were obtained from sheep and goat farms, respectively; 443 milk tank trucks samples, 401 from sheep and 42 from goat farms and 87 milk samples from silo tanks (60 from sheep milk and 27 from goat milk processing plants). All the analysis were performed by the Regional Farmers Association laboratory using the HPLC 1100 series (Agilent Technologies Inc., Santa Clara, CA, USA) with automatic sampler LAS G1313A and a fluorescence detector (FLD) G1321, following the ISO 14501:1998 standard.

After AFM_1 extraction, samples were processed using HPLC-FLD method. Briefly, 50 mL of each milk sample were centrifuged at 4000 r/min for 15 min to separate the fat fraction and the skimmed sample injected into an immune-affinity columns (VICAM) with a flow of 2 mL/min. Each column was washed with 10 mL of ultrapure water (MillQ; Millipore, Billerica, MA, USA) with a flow of 2 mL/min and the AFM_1 eluted from the column using 4 mL of acetonitrile. Then, the eluate was dried at 45-50°C with a nitrogen flow and the dried residue resuspended with 500 μL of water-methanol (50:50 w/v). Finally, 10 μL of the solution were loaded into a Zorbax SB C18 column 150×4.6 mm with a 5 μm diameter (Agilent Technologies Inc.). The mobile phase (water-methanol-acetonitrile, 63:26:11 w/v) was injected with a flow of 1 mL/min in isocratic condition. All the standard for the AFM_1 detection were dissolved in methanol-water solution (10 μg/mL) and stored at 4°C until use. The calibration curve was determined by loading 5 AFM_1 standard solution at the concentration of 0.012, 0.025, 0.050, 0.100, 0.200 and 0.300 μg/L.

Results

A total of 517 sheep (Table 1) and 88 goat (Table 2) bulk tank milk samples collected at farm level, from trucks and from silo tanks were analysed for the detection of AFM_1. In all milk samples collected from 2005 to 2012, 345 (66.7%) and 22 (25%) from sheep and goats, respectively, the presence of AFM_1 was never detected. For both species the presence of AFM_1 was observed only in samples collected in 2013, when were analysed 172 (33.3%) milk samples from sheep and 66 (75%) from goat. Of sheep milk samples collected in 2013, 8 (4.6%) showed an AFM_1 contamination greater than 8 ng/L, with a concentration (mean±SD) of 12.59±14.05 ng/L, range between 8.72 and 58.82 ng/L. In two bulk tank milk samples (7.4%) collected from sheep farms AFM_1 was detected (34.19±34.83 ng/L), and in one of these a concentration of 58.82 ng/L, exceeding the EU limit. Aflatoxin M_1 was also detected in 4 samples (4.5%) from tank trucks (13.54±6.80 ng/L, range between 9.48 and 23.71 ng/L) and in 2 samples (3.6%) from silo (13.67±6.99

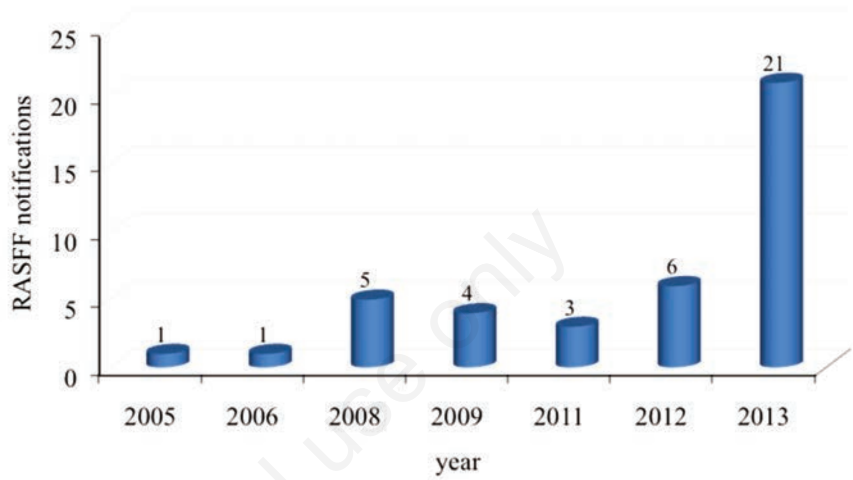

Figure 1. Rapid Alert System for Food and Feed notification on Aflatoxins contamination in corn used for feedstuffs production from 2005 to 2013.

Table 1. Concentration of Aflatoxin M_1 detected in sheep milk samples collected from 2005 to 2013 using the high-performance liquid chromatography-fluorescence detector method.

Year	Samples (n)	AFM_1 concentration (ng/L)			
		<8	≥8-20	>20-50	>50
2005	12	12	-	-	-
2006	58	58	-	-	-
2007	51	51	-	-	-
2008	46	46	-	-	-
2009	52	52	-	-	-
2010	40	40	-	-	-
2011	41	41	-	-	-
2012	45	45	-	-	-
2013	172	164	6	1	1
Total	517	509	6	1	1

AFM_1, Aflatoxin M_1.

Table 2. Concentration of Aflatoxin M_1 detected in goat milk samples collected from 2010 to 2013 using the high-performance liquid chromatography-fluorescence detector method.

Year	Samples (n)	AFM_1 concentration (ng/L)			
		<8	≥8-20	>20-50	>50
2010	4	4	-	-	-
2011	5	5	-	-	-
2012	13	13	-	-	-
2013	66	57	2	5	2
Total	88	79	2	5	2

AFM_1, Aflatoxin M_1.

ng/L). A summary of AFM_1 contamination levels in sheep milk samples collected in 2013 is reported in Table 3.

In 9 (13.6%) out of 66 goat milk samples collected in 2013, AFM_1 was detected at a concentration of 47.21±19.58 ng/L (Table 4), range between 10.45 and 138.16 ng/L. Contamination by AFM_1 was observed also in 2 samples (22.2%) from bulk tank milk (80.00±82.25 ng/L), in 6 samples from milk tank trucks (34.38±19.92 ng/L, range between 10.45 and 62.09 ng/L) and in 1 sample (3.8%) from silo tank (30.40 ng/L). AFM_1 was detected at a concentration exceeding the EU limit in one bulk tank milk sample (138.6 ng/L) and in 1 tank truck milk sample (62.09 ng/L).

Discussion

In the past years, the monitoring of AFM_1 contamination in milk of small ruminants in Sardinia Region has been carried out only on a small number of samples. However, in 2013 AFM_1 contamination in cow milk and in several cases also in sheep and goat milk was reported. With respect to the crisis occurred in Sardinia Region in 2003, the last one was better managed with a faster response of the public veterinary authorities. As in other areas of Italy this was mainly due to the experience gained in previous emergencies (Bolzoni *et al*., 2013). Therefore, from 2013 the Competent Authority increased the official control on AFs contamination throughout the small ruminants dairy chain (Sardinia Region, 2013). In the same year, the number of milk samples analysed in the own-check monitoring programme showed

Table 3. Detection of Aflatoxin M_1 in sheep milk samples collected from bulk tank, milk tank truck and silo tank using the high-performance liquid chromatography-fluorescence detector method.

Tank	Milk samples (n)	AFM_1 concentration (ng/L)			
		<8	≥8-20	>20-50	>50
Bulk	27	25 (92.6)	1 (3.7)	-	1 (3.7)
Milk truck	89	85 (95.5)	3 (3.4)	1 (1.1)	-
Silo	56	54 (96.4)	2 (3.6)	-	-
Total	172	164 (95.3)	6 (3.5)	1 (0.6)	1 (0.6)

AFM_1, Aflatoxin M_1.

Table 4. Detection of Aflatoxin M_1 in goat milk samples collected from bulk tank, milk tank truck and silo tank using the high-performance liquid chromatography-fluorescence detector method.

Tank	Milk samples (n)	AFM_1 concentration (ng/L)			
		<8	≥8-20	>20-50	>50
Bulk	9	7 (77.8)	-	1 (11.1)	1 (11.1)
Milk truck	31	25 (80.6)	2 (6.5)	3 (9.7)	1 (3.2)
Silo	26	25 (96.2)	-	1 (3.8)	-
Total	66	57 (86.4)	2 (3.0)	5 (7.6)	2 (3.0)

AFM_1, Aflatoxin M_1. Values in brackets are expressed as percentage.

Table 5. Aflatoxin M_1 occurrence in bulk tank sheep milk samples in different countries.

Year	Samples (n)	Country	Positive samples (%)	Mean±SD (ng/L)	Detection method	References
2005-2006	23	Syria	13 (57)	67±18.4	ELISA	Ghanem and Orfi, 2009
2007	24	Pakistan	4 (16.7)	2.0±4.0°	HPLC	Hussain *et al*., 2010
2007-2008	51	Iran	19 (37.3)	28.1±13.7	ELISA	Rahimi *et al*., 2010
2007-2008	814	Spain	387 (47.5)	-	ELISA	Rubio *et al*., 2011
2008-2009	42	Iran	13 (31.0)	25.8±15.1	ELISA	Rahimi and Ameri, 2012
2009	118	Italy	1 (0.8)	5.2	ELISA	Cossu *et al*., 2011
2013	19	Croatia	0 (0.0)	3.7±0.91°	ELISA	Bilandžić *et al*., 2014

SD, standard deviation; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography. °Concentration mean was determined on all the samples.

Table 6. Aflatoxin M_1 occurrence in bulk tank raw goat milk samples in different countries.

Year	Samples (n)	Country	Positive samples (%)	Mean±SD (ng/L)	Detection method	References
2003-2004	208	Italy	36 (17.3)	14.5±8.4	ELISA	Virdis *et al*., 2008
2005-2006	11	Syria	7 (64)	19±13.8	ELISA	Ghanem and Orfi, 2009
2007	30	Pakistan	6 (20.0)	2.0±5.0°	HPLC	Hussain *et al*., 2010
2007-2008	60	Iran	19 (31.7)	30.1±18.3	ELISA	Rahimi et al., 2010
2008-2009	48	Iran	17 (35.4)	31.8±13.7	ELISA	Rahimiand Ameri, 2012
2013	32	Croatia	2 (6.2)	7.6±8.94°	ELISA	Bilandžić *et al*., 2014

SD, standard deviation; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography. °Concentration mean was determined on all the samples.

an increasing trend (Tables 1 and 2). However, the number of small ruminants milk samples investigated for AFM_1 detection is still limited and it should be increased (Tables 5 and 6). Until 2012, AFM_1 contamination in all analysed samples was not detectable. On the other hand, in 2013, several sheep (7.4%) and goat (22.2%) milk samples were found contaminated with AFM_1. Previous works conducted in the same production areas using the ELISA detection method, showed a prevalence of positive samples of 0.8 and 17.3% for sheep and goat milk, respectively (Virdis *et al*., 2008; Cossu *et al*., 2011). However, the present study demonstrated that in Sardinia Region, the prevalence of sheep milk samples contaminated with AFM_1 was lower than reported in other countries (Table 5).

Conclusions

The current own-check monitoring programme on AFM_1 contamination in milk of small ruminants produced in Sardinia Region require a revaluation of framework arrangement and resources. A larger amount of samples should be analysed to cover a large number of milk producers and cheese-making factories. The wider use of rapid screening test should be promoted, limiting HPLC as a confirmatory method. Developing own-check monitoring and controlling programmes in small ruminant dairy chains is more complex as compared to the dairy cow sector. This is mainly related to the high number of sheep and goat farms that should be submitted to a monitoring plan, while a limited amount of milk per farm is delivered to the cheese making plants. For this reason, in order to evaluate the AFM_1 contamination levels, the FBOs have increased the number of tank truck and silo tank milk samples (Tables 3 and 4). In small ruminant dairy chains, tank truck and silo gather bulk milk from a larger number of farms than in cows, resulting in potential stronger dilution effect of contaminants in the milk delivered. In the present work a reduction of AFM_1 concentration and rate of positive samples was observed in relation to the origin of samples, decreasing from samples taken at farm level to truck tank and silo. In Italy, an attention level of 40 ng/kg for AFM_1 in bulk tank cow milk was established. In small ruminants dairy chains, the own-check programmes for monitoring AFM_1 consider the milk tank truck as a main target and so attention level should be set at a lower level than thsoe provided for cows, due to the observed dilution effect of the contamination.

References

Battacone G, Nudda A, Palomba M, Pascale M, Nicolussi P, Pulina G, 2005. Transfer of Aflatoxin B_1 from feed to milk and from milk to curd and whey in dairy sheep fed artificially contaminated concentrates. J Dairy Sci 88:3063-9.

Bilandžić N, Božić Đ, Đokić M, Sedak M, Kolanović BS, Varenina I, Cvetnić Ž, 2014. Assessment of aflatoxin M_1 contamination in the milk of four dairy species in Croatia. Food Control 43:18-21.

Bolzoni G, Zanardi G, Bertocchi L, Delle Donne G, 2013. Risultati delle analisi dell'autocontrollo per la contaminazione da Aflatossina M_1 nel latte in Lombardia. Large Anim Rev 19:251-5.

Causin R, 2013. Mycotoxins contamination in Italy and management experiences. Available from: www.micotossine.it/public/pag_2029.pdf

Cossu F, Scarano C, Moniello G, Spanu C, Pittau D, Virdis S, De Santis EPL, 2011. Detection of aflatoxin M_1 in bulk-tank milk and sheep cheese. Ital J Food Safety 1:93-7.

EU-RASFF. European Commission-Rapid Alert System for Food and Feed portal. Available from: https://webgate.ec.europa.eu/rasff-window/portal/

Frobisch RA, Bradley DD, Wagner DD, Long-Bradley PE, Hairston H, 1986. Aflatoxin residues in the milk of dairy cows after ingestion of naturally contaminated grain. J Food Protect 49:781-5.

Ghanem I, Orfi M, 2009. Aflatoxin M_1 in raw, pasteurized and powdered milk available in the Syrian market. Food Control 20:603-5.

Goto T, Hsieh DP, 1985. Fractionation of radioactivity in the milk of goats administered 14^C-Aflatoxin B_1. J Assoc Off Ana Chem 68:456-8.

Hussain I, Anwar J, Asi MR, Munawar MA, Kashif M, 2010. Aflatoxin M_1 contamination in milk from five dairy species in Pakistan. Food Control 21:122-4.

Italian Ministry of Health, 2013. Contaminazioni da aflatossine nel mais e nella catena alimentare. In: Official Journal, L 855-P, 16/01/2013.

Molle G, Decandia M, Cabiddu A, Landau SY, Cannas A, 2008. An update on the nutrition of dairy sheep grazing Mediterranean pastures. Small Ruminant Res 77:93-112.

Nageswara Rao SB, Chopra RC, 2001. Influence of sodium bentonite and activated charcoal on Aflatoxin M_1 excretion in milk of goats. Small Ruminant Res 41:203-13.

Rahimi E, Ameri M, 2012. A survey of Aflatoxin M_1 contamination in bulk milk samples from dairy bovine, ovine, and caprine herds in Iran. Bull Environ Contam Toxicol 89:158-60.

Rahimi E, Bonyadian M, Rafei M Kazemain HR, 2010. Occurrence of aflatoxin M_1 in raw milk of five dairy species in Ahvaz, Iran. Food Chem Toxicol 48:129-31.

Ronchi B, Danieli PP, Vitali A, Sabatini A, Bernabucci U, Nardone A, 2005. Evaluation of AFB_1/AFM_1 carry-over in lactating goats exposed to different levels of afb_1 contamination. In Proc 56th Ann Meet EAAP, Uppsala, Sweden, pp 1-10.

Rubio R, Licon CC, Berruga M, 2011. Occurrence of aflatoxin M_1 in the Manchego cheese supply chain. J Dairy Sci 94:2775-8.

Sardinia Region, 2013. Piano di controllo per la prevenzione e gestione del rischio aflatossine nel mais, destinato all'alimentazione umana e animale, e nella filiera lattiero casearia. Available from: www.regione.sardegna. it/documenti/1_38_20130422 175030.zip

Streit E, Schatzmayr G, Tassis P, Tzika E, Marin D, Taranu I, Tabuc C, Nicolau A, Aprodu I, Puel O, Oswald IP, 2012. Current situation of mycotoxin contamination and co-occurrence in animal feed-focus on Europe. Toxins 4:788-809.

Veldman A, Meijst JAC, Borgrevve GJ, Heeres-van der Tol JJ, 1992. Carry-over of Aflatoxin from cows' food to milk. Anim Prod 55:163-8.

Virdis S, Corgiolu G, Scarano C, Pilo AL, De Santis EPL, 2008. Occurrence of aflatoxin M_1 in tank bulk goat milk and ripened goat cheese. Food Control 19:44-9.