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Impact of the implementation of tailored management strategies to reduce the occurrence of aflatoxin M1 in milk-supply chain in Italy

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ABSTRACT

In Europe the legal limit for aflatoxin M1 in raw milk, heat-treated milk and milk for the manufacture of milk-based products is set to 50 ng kg^{-1}. In Italy, an 'attention limit' of 40 ng kg^{-1} has been defined in 2013 for aflatoxin M1, while a more stringent attention limit of 30 ng kg^{-1} was set voluntarily by different regions in the following years. In this study we examined the data on aflatoxin M1 contamination in 67,944 milk samples in the framework of the self-control plans of six milk industries in the periods 2004–2008 and 2013–2019. The proportion of positive samples showed a decreasing trend from 2004 to 2019 in relation to the compliance to the EU and both national limits. In addition, no seasonal aflatoxin M1 variation was evidenced after 2013. The data demonstrate how early and rapid detection of aflatoxin M1 applying a stringent self-control strategy resulting in the application of mitigation measures can significantly reduce the aflatoxin M1 concentration in milk. An update on the Estimated Daily Intake, the Hazard Index, and the fraction of hepatocarcinoma cases due to aflatoxin M1 exposure in different population groups, confirmed that infant and toddlers were more exposed than older consumers. Nevertheless, the application of an attention limit of 30 ng kg^{-1} further reduced the risk for young consumers. Taken all together our results demonstrate the efficacy of the tailored management strategies to limit the presence of aflatoxin M1 in milk implemented after the aflatoxin crisis in 2003 and 2013.

1. Introduction

Aflatoxins are bisfuranocoumarin compounds produced primarily by toxigenic strains of the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, but also from *A. minisclerotigenes*, *A. korhogoensis*, *A. aflatoxiformans*, *A. texensis*, *A.novoparasiticus* and *A. arachidicola.* Their presence has been mainly reported in tropical and subtropical regions but is nowadays becoming an unavoidable problem due to climate change and the growing occurrence of hot and drought seasons in several regions of Europe. An increase is also evidenced in case the of bad agricultural practices (Kebede et al., 2012) and in areas with a hot and humid climate (Giorni et al., 2007). In addition, as an impact of climate change, the infected areas may further increase (Miraglia et al., 2009). Toxigenic strains of *Aspergillus* spp. are mainly responsible for the production of aflatoxins in many feed materials, causing the contamination of milk of lactating animals that are fed with the involved feedstuff (Bertocchi et al., 2012; Canever et al., 2004; Giorni et al., 2007; Prandini et al., 2009). In particular, among the different aflatoxins (B1, B2, G1, G2), the M1 hydroxylated metabolite (AFM1) of aflatoxin B1 (AFB1) is the most commonly occurring in milk, appearing after 2 or 3 days from the ingestion and clearing after 5–7 days depending on the amount and duration of the consumption (Masoero et al., 2007). Procedures such as pasteurization or sterilization cannot eliminate or even vary the concentration of the AFM1 once the milk is contaminated, leading to withdrawal of consignments once the legal limit is exceeded.

Only safe food should be placed on the market (Regulation EC 178/2002) and, therefore, food safety could be considered one of the major risks for agribusiness firms, which have the social responsibility of ensuring food safety by following the necessary procedures established by the Food Safety Authorities as well as should incorporate food safety

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measures beyond baseline requirements demanded by regulation or governmental policy (Nganje et al., 2021). Aflatoxins are genotoxic and carcinogenic compounds, specifically AFM1 is classified into Group 1 of carcinogenic substances for humans (IARC 2012), with suggested exposure levels be kept as low as reasonably achievable. The exposure to AFM1 compromises both the health of animals and humans (Kunter et al., 2017) imposing health risk for the consumers. Major concern is for children who are more susceptible to the toxic effects of aflatoxins, due to their underdeveloped metabolic and immune system.

In a previous study, the risk from exposure to AFM1 found in milk from April 2013 to December 2018 in the framework of a self-control plan of six milk processing plants in Italy as well as the risk characterization were calculated in terms of Estimated Daily Intake (EDI), the Hazard Index (HI), and the fraction of hepatocarcinoma cases (HCC) in different population groups.

Since the contamination of milk by mycotoxins poses issues not only regarding food safety and public health policies, but also for the economic sphere, encompassing agriculture and international trading, the most heavily regulated among natural toxins are mycotoxins throughout the globe (Meneely et al., 2022).

Initially, in Italy the milk controls were only sporadic and only in the autumn of 2003, following an alarming amount of positivity in the self-control plan, special monitoring plans were coordinated for milk and feed (Decastelli et al., 2007). Probably the abnormal AFB1 contamination that occurred in maize grown in Italy, was the consequence of particularly unusual climatic conditions (high temperatures and drought lasting more than four months) that characterised the summer of 2003. Back in those days the Directive 2003/100/EC of 31 October 2003 on undesirable substances in animal feed set limits for AFB1 in terms of mg/kg of feed with a humidity rate of 12%. In milk, a limit of 0.05 μg/kg for AFM1 was earlier set by European Legislation 466/2001/EC. The same maximum level is nowadays applied in Europe by Commission Regulation (EC) No 1881/2006 for raw milk, heat-treated milk and milk for the manufacture of milk-based products, while for infant food it is limited to 0.025 μg/kg. Criteria for sampling and analysis of aflatoxins are specified in Commission Regulation (EC) No 401/2006. In addition, specific import conditions have been put in place for certain feed and food commodities from selected third countries related to the presence of aflatoxins (i.e. Commission Regulation (EC) No 669/2009 and Commission Implementing Regulation (EU) No 884/2014). In parallel to official controls, industries have been applying risk management strategies in order to detect unacceptable levels of contamination in the framework of self-control plans. However, as stated by Trevisani et al. (2014), this regulation, similarly with other provisions worldwide, does not indicate the frequency of sampling or give an indication for seasonal or regional stratification. Therefore, the frequency of sampling must be evaluated on the basis of acquired previous knowledge on the risk for specific aspects. On the national territory, the Italian Ministry of Health issued a note in 2013 (Ministry ofHealth. Note prot. n. 855 del, 16/1/2013) defining an 'attention limit' (AL) of 40 ng kg^{-1}, to be applied every time that extreme weather conditions are registered. In addition, these guidelines, provide operators in the feed and food sectors with specific operational indications in order to allow the reduction of aflatoxin levels with an holistic approach for the dairy chain, focused not only on the food product but also on feed by means of cleaning techniques or other physical treatment. This because any milk sample with aflatoxin above this limit has to be regarded as suspect and preventive checks and measures at farm level must consequently be performed. Following the note of 2013, Regions are demanded to apply more stringent controls by means of regional plans when needed. In particular, in Calabria region, one dairy plant has applied the most stringent level of 30 ng kg^{-1}. In 2016 another note has been issued by the Italian Ministry of Health (Ministry of Helath. Note prot. n.11850 del, 29/03/2016) in order to declare the need to intensify aflatoxin official control and to underline the obligation of FBO (Food Business Operators). In 2017 also Emilia Romagna Region applied the more stringent AL of 30 ng kg^{-1}.

Based on these not ordinary events, the dairy industry performed several risk reduction strategies based on the specific scenarios observed in the different industries and well-programmed interventions have been defined from every dairy plant in its self-control plan, each year. Ten years have passed from the note of 2013, and what was previously an out of order strategy is now a routinely applied procedure. Thus, further data mining and analysis are needed to both define and update the actual real scenario in Italy for the hazard aflatoxin in milk as well as define appropriate sampling plans for milk and milk products.

This paper presents data on the concentration of AFM1 in milk sampled in 6 Italian dairy plants between 2004 and 2008 and between 2013 and 2019. In addition, a retrospective evaluation was performed to evaluate the effectiveness of the risk reduction strategy performed by these dairy industries and the evolution of AFM1 presence in milk during a long period of time. These data were used to update the information produced by a previous study (Serraino et al., 2019) regarding human exposure and potential risk of consumers in different age categories. Results allow both to identify potential different exposure and risk scenarios based on different AFM1 contamination data in milk in Italy, and to evaluate the effect of different AFM1 milk monitoring as a result of the implementation of more stringent AL in EDI, HI, and HCC cases reduction.

2. Materials and methods

2.1. Aflatoxin M1 concentration data collection

The data on AFM1contamination in milk from the self-control plan records of six milk processing plants located in Northern, Central, and Southern Italy, were gathered in the years from 2004 to 2008 and from 2013 to 2019. The dairy plants involved in the study collected altogether almost 465 million liters of milk per year, comprising high quality milk, normal quality milk and organic milk, that were analysed within a self-control plan, following the same protocol. Data comprised a total of 67,944 samples that were tested for AFM1 concentration at arrival to the plant, using the ELISA kit Immunoscreen (Tecna srl, Trieste, Italy), in order to avoid the contamination of more milk at a later stage tank. The ELISA test was validated within the range of 5–100 ng kg^{-1} (Rosi et al., 2007). Specifically, prior to unloading, milk samples were taken from the compartments of each truck, transporting milk provided from different farms. The procedures performed in the self-control plans and successive revisions by the six dairy plants to control the AFM1 in milk before and after 2013 and also into the several years considered in the study are different but a defined and rigorous framework is the same for all the dairy plants. Briefly, before milk is discharged on every milk truck entering the milk processing plant a bulk milk sample is analysed with a commercial immunochromatographic rapid test (Charm), detecting AFM1 at or above 25 ng kg^{-1} in milk and suitable to indicate the compliance with EC limit of 50 ng kg^{-1}. In case the analytical record within the truck exceeds the AL, an ELISA test is further performed to better quantify the concentration of the mycotoxin. The milk can be processed only if the legal limit is not overcome, whereas in case levels result higher than 50 ng kg^{-1}, the milk truck awaits the AFM1 concentration measurement performed by HPLC method for the unloading. Liquid chromatography mass spectrometry and liquid chromatography-fluorescence detection methods are also used for the determination AFM1 and the reported LOQs are typically between 0.0007 and 0.014 lg/kg. Whenever the analytical results show that AFM1 exceeds the EC limit the milk is discarded as Category 1 material as stated in Article 8 (d) of Regulation EC 1069/2009. In parallel, the FBO must proceed with testing samples collected at charge of milk of every farm in order to identify the dairy farm or farms exceeding the limit, and the Veterinary Competent Authority is informed of the analytical record at the milk processing plant as well as at farms level in accordance with the Italian law (Ministry ofHealth. Note prot. n. 855

del, 16/1/2013). Immediately, FBO must adopt for the food product the procedures laid down in Regulation EC 178/2002, article 9, whereas, at farm level, a supplementary in-depth analysis of the AFM1 level of contamination of milk is performed with programmed checks at fixed frequencies depending on the estimated AFM1 concentration until the full resolution of the non-conformity. Whenever the EC limit is exceeded at farm level, the milk consignment is not performed. The same procedure is applied when the milk exceeds the AL set by the Region.

In addition, following the visit of the veterinarian performed on the same day of the notification, the FBO has also to adopt corrective actions in all the implicated dairy farms. As mitigation measure, the feed provided to the animals is replaced in order to reduce the animal exposure to aflatoxins favoring the use of maize reserves from previous production seasons or changes in the components of the ration, with for example sorghum or other cereals such as barley and wheat. Finally, both cooperative and dairy plants perform further additional analysis by Charm and/or ELISA, with a minimum of twice a month, to test AFM1 concentration of milk of the different dairy farms.

2.2. Statistical analysis

Descriptive statistical parameters (mean, standard deviation, median, percentile) were calculated for all the years included in the study. Moreover, the percentages of sample above the EC compliance limit of 50 ng kg^{-1}, the AL levels of 40 ng kg^{-1} and 30 ng kg^{-1} were computed. Additionally a comparison between the AFM1 values of plants with different Als, for the 2013–2019 period, was made to investigate eventual differences. Data are illustrated in Tables 1 and 2.

The data were tested for normality using Kolmogorov-Simrnov test and for equality of variance using Levene's test, resulting not normally distributed and with non-equally distributed variances, hence were analysed using Chi-squared test, Mann–Whitney *U* test, Kruskal-Wallis test and Dunn's multiple comparison test considering significant a $p \leq 0.01$. All statistical analysis was made using *R Studio* (2022.2.3.492), *ggstatplot* (Patil, 2021) and *ggplot2* (Wickham, 2016) packages.

2.3. Dietary exposure and risk characterization

For the risk assessment, the food consumption data used as well as the information for exposure assessment and hazard assessment were obtained as previously described (Serraino et al., 2019). Briefly, food consumption data were obtained from the Comprehensive Food Consumption Database of EFSA (https://www.efsa.europa.eu/en/data-report/food-consumption-data), containing derived from the Italian National Food Consumption Survey (INRAN-SCAI) conducted in 2005–2006 (Leclercq et al., 2009). The exposure assessment is based on the mean "Cattle milk" consumption data of "consumers only" of six population groups: infants (0–0.9 years), toddlers (1–2.9 years), other children (3–9.9 years), adolescents (10–17.9 years), adults (18–64.9 years), elderly (65–74.9), and very elderly (>75).

The estimated daily intakes (EDI: ng kg^{-1} bw day^{-1}) of the population groups were calculated as:

$$EDI = \frac{\Sigma\left[WM_{AFM1} concentration\left(\frac{ng}{kg}\right) \times AC\left(\frac{kg}{day}\right)\right]}{[mean\ body\ weight\ (kg)]}$$

EDI values were calculated from the weighted mean (WM) AFM1 concentrations unloaded from the tankers in the given period and the average (AC) portion size (consumption data (kg/day), the calculations were carried on separately for plants using the more restrictive AL to assess whether this had an impact on the exposure to AFM1.

To calculate HI, the average EDIs were divided by 0.2 (Kuiper-Goodman, 1990), in line with the approach of Serraino et al. (2019). $BMDL_{10}$ of AFB1 (870 ng kg^{-1} bw day^{-1}) was used as a conservative value since no value for AFM1 is available. Margin of exposure (MoE) was calculated as reported by Serraino et al. (2019) as well as risk potency (calculated assuming 2% prevalence of carriers of hepatitis B). The calculation was carried out for the same groups as for the EDI and HI.

Table 2
Descriptive statistics for the level of aflatoxin M1 (ng kg^{-1}) during 2004–2008/2013-2019.

	All data 2004–2018/2013–2019	2004–2008	2013–2019
Number of samples	67944	24177	43767
Mean	12.90	16.45	10.94
SD	10.6	14.05	7.38
Median	10	13	9
P 0.95	32	39	26
P 0.99	48	64	38
Max	280	280	58
CM1 $\geq$ **50 ng kg** $^{-1}$ ***(%)***	0.92	2.52	0.03
CM1 $\geq$ **40 ng kg** $^{-1}$ ***(%)***	2.31	4.93	0.86
CM1 $\geq$ **30 ng kg** $^{-1}$ ***(%)***	6.66	13.27	3

Note: P, percentile; CM1 $\geq$ 50-40-30 ng kg $^{-1}$(%) is the proportion of consignments above the limit in relation to number of samples.

3. Results

3.1. AFM1 results in milk

A total of 67,944 milk samples were considered in this study. All the statistics describing the distribution of AFM1 sorted by year and by season, are showed in Tables 1–4 AFM1 mean values ranged between 25.9 and 7.9 ng kg^{-1} and the median between 24 and 7 ng kg^{-1} indicating a positive skewed distribution which implied a non-normal distribution of the data, as confirmed by the Kolmogorov-Simrnov test. We evidenced significantly ($p \leq 0.01$) different year-to-year variation in AFM1 prevalence and average contamination levels in the analysed milk samples. This result applies to the whole studied period, except for 2005 vs 2008, 2015 vs 2016 and 2018 vs 2019 which were not significantly different. The proportion of samples above the EC compliance limit (i.e., 50 ng kg^{-1}) varied between 6.7 and 0%, with a decreasing trend from year 2004 to year 2019. The same tendency was observed for the

Table 1
Descriptive statistics for the level of aflatoxin M1 (ng kg^{-1}) sorted by year.

	2004	2005	2006	2007	2008	2013	2014	2015	2016	2017	2018	2019
Number of samples	5079	4930	5040	4619	4509	3826	9180	6114	6600	7062	6251	4734
Mean	25.94	15.18	13.98	11.41	15.08	17.63	11.21	11.72	12.86	8.84	8.31	7.95
SD	15.10	13.16	12.70	10.95	13.09	9.04	7.46	8.25	8.08	5.02	4.74	4.02
Median	24	12	11	9	13	16	9	9	11	8	7	7
P 0.95	54	35	36	28	32	35	26	29	28	17	17	16
P 0.99	75	62	53	45.82	60.92	46	37.21	40	40	26.39	25	22
Max ng kg $^{-1}$	175	181	228	197	280	50	50	48	50	47	58	48
CM1 $\geq$ **50 ng kg** $^{-1}$ ***(%)***	6.73	1.74	1.27	0.78	1.82	0.18	0.01	0.00	0.05	0.00	0.03	0.00
CM1 $\geq$ **40 ng kg** $^{-1}$ ***(%)***	13.15	3.20	2.88	1.56	3.30	2.59	0.84	1.82	1.08	0.14	0.10	0.04
CM1 $\geq$ **30 ng kg** $^{-1}$ ***(%)***	35.26	9.80	9.50	3.49	6.54	9.88	3.10	4.81	4.17	0.55	0.46	0.27

Note: P, percentile; CM1 $\geq$ 50-40-30 ng kg $^{-1}$(%) is the proportion of consignments above the limit in relation to number of samples.

proportion of samples above the AL of 40 ng kg^{-1}, ranging from 13.1 to 0.04%, and 30 ng kg^{-1}, ranging from 35.3 to 0.3% (Table 1). Overall, in the studied period, 0.92% of the samples were above the 50 ng kg^{-1} EC limit, and 2.31% above the 40 ng kg^{-1}, while 6.66% above the 30 ng kg^{-1} ALs (Table 2). A total of 36.4% of the samples above the EC compliance limit were detected during the critical season (September to November), with the highest levels of AFM1 detected in September 2006 and September 2008, respectively with a concentration of 228 and 280 ng kg^{-1}. Regarding seasonal variability, the observed AFM1 prevalence has shown an interesting periodic fluctuation over the surveyed period, as shown in Table 3. Although significantly higher ($p \leq 0.01$) values were observed during the fall season (36.6%), the 23.8 and 21% of EC non-compliant samples ($\geq$50 ng kg^{-1}) were observed in spring (March to May) and winter (December to February) respectively (Fig. 1). Almost all non-compliant samples (97.9%) were received between 2004 and 2008. Among them, more than 50% of the samples were referred to 2004 (Fig. 2) while 2.1% to the period 2013–2019. During the latter, only 13 of the 43,767 samples (i.e., 0.03%) were contaminated with levels above the 50 ng kg^{-1} limit and 376 (0.85%) above the AL of 40 ng kg^{-1}. For the 2013–2019 period an overall reduction of AFM1 level was observed (Table 4). In particular, a statistically significant ($p \leq 0.01$) reduction in the proportion of samples above the 30 and 40 ng kg^{-1} AL limits was observed in plants with a 30 ng kg^{-1} AL compared to plants with 40 ng kg^{-1} AL.

3.2. *Exposure assessment*

Average EDI, HI, and liver cancer incidence (LCI) values were calculated for the 2013–2019 period using AFM1 values from all the plants but dichotomized in two groups, one with lower AL (namely considering 30 ng kg^{-1} AL from 2013 in Calabria Region and from 2017 in Emilia Romagna Region) and the other with AL laid down by regulation in force (namely 40 ng kg^{-1} AL in the 5 remaining plants from 2013 to 2017 and all the remaining 4 plants since 2017). The result of EDI calculation, based on the mean “cattle milk” consumption data of “consumers only”, sorted by different population age, and for both AL values are reported in Fig. 3. Among the different population groups, EDI values varied between 0.02 and 0.24 ng kg^{-1} bw day^{-1} for the 30 ng kg^{-1} AL and between 0.03 and 0.30 ng kg^{-1} bw day^{-1} for the 40 ng kg^{-1} ALs, within both groups infants and toddlers had the highest mean EDI values while adults the lowest (Fig. 3).

The results of mean HI for the different population groups are reported in Fig. 4, as can be seen only infants and toddlers had values greater than 1 while all the other groups were well below the concern limit. Between the two AL groups there were no significant differences even though the group with lower limit had lower mean HI values, with toddlers, for example, being slightly above (1.03) the concern limit.

The fraction of incidence of HCC or liver cancer incidence (LCI) attributable to the intake of AFM1 was evaluated based on MoE considering the estimated mean exposure. The average LCI values calculated per 100,000 people for the studied period showed, in alignment with EDI and HI results, the highest values in infant and toddlers (Fig. 5). Among the two age groups, values for the 30 ng kg^{-1} ranged from 0.0003 to 0.0038, while for the 40 ng kg^{-1} from 0.0004 to 0.0048 per 100,000 people.

4. Discussion

The strict control of AFM1 in the commercialized milk is extremely relevant for protecting public health because this aflatoxin is a carcinogenic compound classified in the Group 1 (IARC 2012). To this aim the implementation of a risk assessment approach can help to identify risk management strategies reducing the consumer exposure to AFM1.

The current EC Regulation sets the maximum levels of AFM1 in milk at 5 ng kg^{-1} but does not indicate the frequency of sampling nor give an indication for seasonal or regional sampling stratification. Therefore, the frequency of sampling must be evaluated on the basis of acquired previous knowledge on the distribution of the hazard. Fig. 2 clearly shows three time-frames relevant in our dataset, during which the AFM1 concentrations in milk were significantly different: the year 2004 and the period 2005–2008 before the introduction of the AL and the period 2013–2019 after the introduction of the AL. The median of the AFM1 concentration from 2004 to 2019 was 10 ng kg^{-1} but is it important to highlight that after the introduction of the AL it decreased ($p \leq 0.01$) from 13 ng kg^{-1} to 9 ng kg^{-1}.

Our results are in line (a bit lower considering values after 2013) with the ones reported by other authors in European countries such as France 14.3 ng kg^{-1} in raw milk (Boudra et al., 2007) and Spain 9.69 ng kg^{-1} in UHT milk (Cano-Sancho et al., 2010). Besides, our values are lower than Portugal 23.4 ng kg^{-1} in pasteurized milk (Duarte et al., 2013), Croatia 46.6 ng kg^{-1} (Bilandžić et al., 2022) and Serbia 71 ng kg^{-1} in raw milk (Milićević et al., 2017) as well as other extra-EU countries such as Brazil 66.9 ng kg^{-1} (Picinin et al., 2013) and China 51.9 ng kg^{-1} (Li et al., 2017). However, it is important to consider that a higher maximal residual limit may be in force, for instance China and Brazil have a 500 ng kg^{-1} compliance limit.

The percentage of cow’s raw milk sample noncompliant with the EC 50 ng kg^{-1} limit reported for the whole studied period was 0.92%, with a reduction from 2.52 to 0.03% before and after the introduction of the AL. Nations such as Greece (3.6%) (Roussi et al., 2002), Croatia (9.36%) (Bilandžić et al., 2022) extra-EU Serbia (30%) (Milićević et al., 2017), as well as China (1.1%) (Li et al., 2017) and Brazil (14%) (Picinin et al., 2013) report higher values, while Spain (0%) and France (0%) (Boudra et al., 2007; Cano-Sancho et al., 2010) lower ones. The wide variations in mycotoxin levels among studies could be related to the sample size, but also to the analysed geographic, temporal and climatic differences, as well as to the identification methods. Nevertheless, it is to notice that if we consider the studies conducted in Italy after 2013, our results (0.03%) are lower than those reported by Serraino (0.20%) and Roila (0.89%) (Roila et al., 2021).

Our data, in accordance with (Kerekes et al., 2016) demonstrate how the application of a stringent self-control strategy, where the application

Table 3
Descriptive statistics for the level of aflatoxin M1 (ng kg^{-1}) sorted by month of consignment in the 2013–2019 period.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Number of samples	4764	4517	5415	5363	5829	5693	6197	6114	5948	6328	6233	5543
Mean	12.66	14.01	12.98	13.84	13.42	13.10	11.89	12.19	14.91	13.46	11.39	11.33
SD	9.57	11.56	10.31	10.47	10.10	9.87	8.64	9.93	14.63	11.63	9.70	8.66
Median	10	11	10	11	10	10	9	9	11	10	9	9
P 0.95	30	38	34	34	33	32	29	31	39	33	28	28
P 0.99	45.37	58	49.86	48.38	47	47	42	46.87	68	48	43	42
Max	185	95	91	105	111	95	105	112	280	247	175	125
CM1 ≥ 50 ng kg^{-1} (%)	0.63	1.73	1.02	0.91	0.75	0.81	0.36	0.80	2.30	0.92	0.51	0.41
CM1 ≥ 40 ng kg^{-1} (%)	1.64	3.74	2.53	2.18	1.99	1.88	1.37	2.11	4.69	2.78	1.67	1.28
CM1 ≥ 30 ng kg^{-1} (%)	5.44	9.14	7.46	8.26	7.27	6.39	4.60	5.92	10.68	7.08	4.36	3.84

Note: P, percentile; CM1 ≥ 50-40-30 ng kg^{-1}(%) is the proportion of consignments above the limit in relation to number of samples.

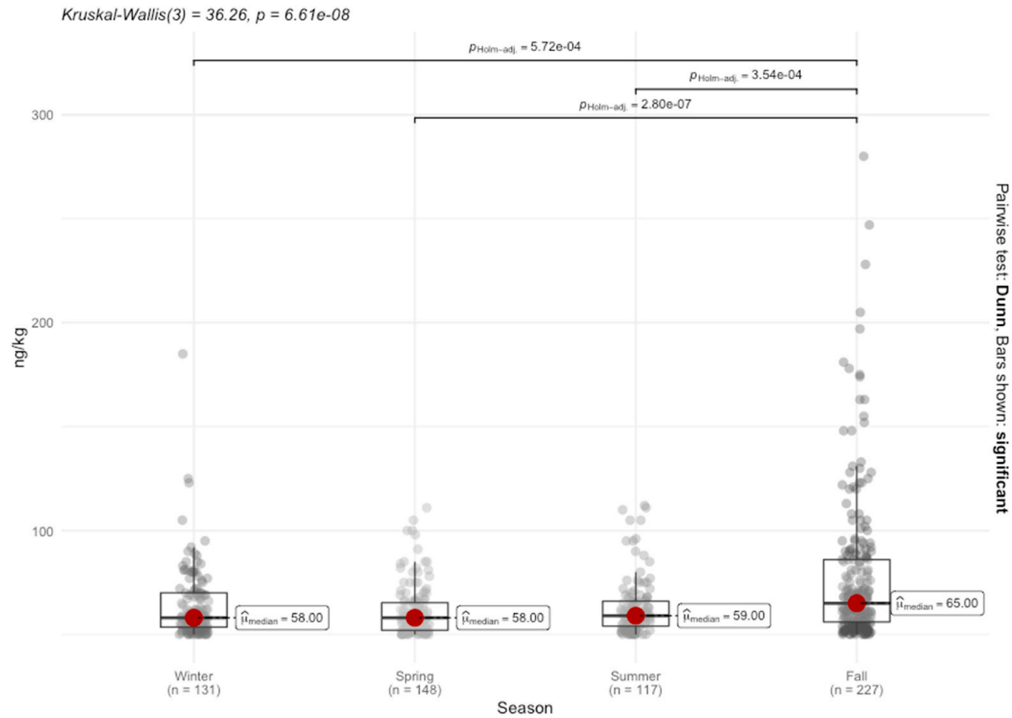

Fig. 1. Information about statistics and distribution of samples above the EC 50 ng kg^{-1} limit, grouped by season. In the graph are reported median ($\widehat{\mu}$ $_{median}$), **number of samples (n), and statistically significant differences between groups ($p_{Holm-adj.}$).**

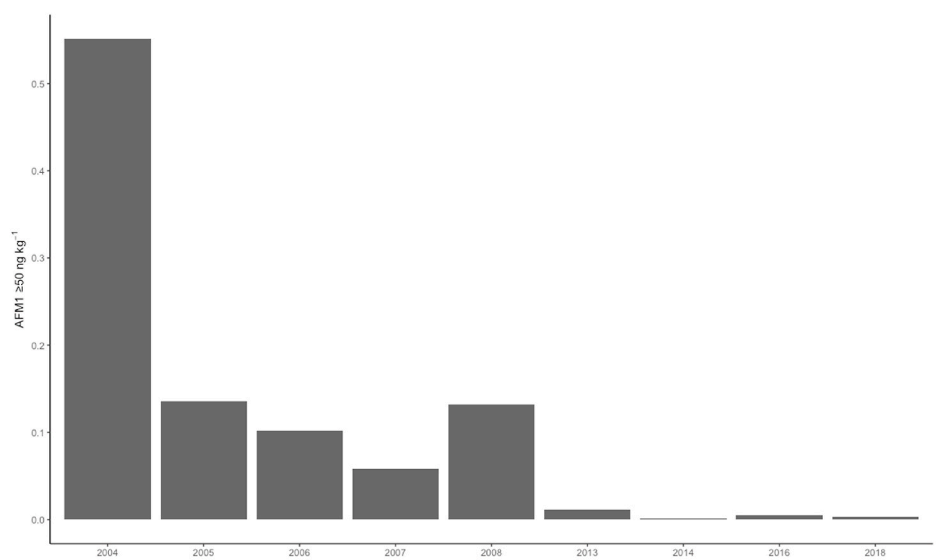

Fig. 2. Frequency of samples above the EC 50 ng kg^{-1} limit, grouped by year.

of an AL and the subsequent accomplishment of corrective measures is performed, can significantly diminish the risk for public health due to AFM1in milk. These actions synergically intercept possible ascending trends enabling the application of early countermeasures, preventing health problems. This impact is clearly showed in Table 2 displaying that the AFM1 concentration in milk quantified after the application of the AL was significantly ($p \leq 0.01$) lower in comparison to the previous period. Moreover, the proportion of samples exceeding the EC limit in 2008 was 10 times higher in comparison to 2013 (Table 1) and a decreasing trend was kept up to 2019.

Temperature, humidity, rainfall patterns and the frequency of extreme weather events are already affecting farming practices, crop production and the nutritional quality of food crops, and therefore have an effect on aflatoxin presence. The impact of seasonality on AFB1 contamination in feed has been observed both in Italy (Trevisani et al., 2014) and in other countries (Bilandžić et al., 2022; Li et al., 2017). Before 2013, our data report higher AFM1 levels in September 2006 and 2008. On the contrary, after the introduction of the AL of 40 ng kg^{-1} and the subsequent decrease of non-conformities, not only the seasonal trend was absent, but in addition the highest number of samples (n = 13) exceeding EC limit was detected during spring (n = 6), followed by summer (n = 5), autumn (n = 1) and winter (n = 1) (Data not shown). It is also important to highlight that we observed a lowering trend of AFM1 levels in the years 2015 and 2018 when the highest temperatures in

Table 4
Descriptive statistics for the level of aflatoxin M1 (ng kg^{-1}) sorted by attention limit in the 2013–2019 period.

	30 ng kg^{-1}	40 ng kg^{-1}
Number of samples	10572	34330
Mean	9.21	11.64
SD	5.44	7.90
Median	8	9
P 0.95	19	28
P 0.99	29	40
Max	58	50
CM1 ≥ 50 ng kg $^{-1}$ (%)	0.019	0.032
CM1 ≥ 40 ng kg $^{-1}$ (%)	0.36	1.06
CM1 ≥ 30 ng kg $^{-1}$ (%)	0.99	3.84

Note: P, percentile; CM1 ≥ 50-40-30 ng kg $^{-1}$(%) is the proportion of consignments above the limit in relation to number of samples.

comparison to the previous last 10 years where registered (Locatelli et al., 2022) in the area from which part of samples where tested. The decreasing trend in the presence of AFM1 is also certainly due to a new consolidated way of thinking of the farmers who are now used to manage the aflatoxin hazard as an ordinary and intrinsic problem for milk production and are aware of the impact climate changes on its occurrence.

In Italy, the regulation that introduced the AL dates back to 2013 and was specifically emitted in order to prevent and manage aflatoxin risk contamination "in case of extreme climatic conditions". After the note of Italian Ministry of Health, aflatoxins control was implemented by feed manufacturer, supplier and feed business operators in their respective fields. Moreover, it was implemented by farmers in relation to the drying procedures able to prevent aflatoxins contamination of feed leading to the consequent AFM1 reduction in milk. The effectiveness of the effort is showed by Ferrari et al. (2022), who demonstrated that almost the totality of feed matrices analysed between 2013 and 2021 were compliant with the EC legal limit.

Considering that self-control strategies for limitation of the presence of mycotoxins in milk are expensive, our results provide the basis for redefining a risk-based sampling plan and assure an appropriate level of compliance of milk and milk products with the legal limits. Based on the very low AFM1 concentrations in milk observed from 2013 to 2019, the probability of non-compliant milk could be considered negligible in this specific scenario. Therefore, an AFM1 monitoring plan based on a reduced sampling frequency but incorporating a precise early warning system able to intercept increasing trends in AFM contamination, allows to quicky identify the most critical dairy farms. Regretfully, the impact of rising feed costs as well as the farmers uncertainty in the relative tight feed supply, for which feed quality is not always ensured, might nullify the actually applied AFM1 risk reduction strategy.

Infant and toddlers, due to the relatively large milk intake compared to their body weights, confirmed to be more exposed than older consumers in line with literature (Roila et al., 2021; Tsakiris et al., 2013) and independently from the AL applied. Our results are in line with previously reported mean EDIs of 0.08 ng kg^{-1} bw day^{-1} (n = 40) in Portugal (Duarte et al., 2013), 0.09 ng kg^{-1} bw day^{-1} (n = 16) in France (LeBlanc et al., 2005), and 0.18–0.20 ng kg^{-1} bw day^{-1} (n = 1233) in Serbia (Milićević et al., 2017). The implementation of a more stringent AL, yield a decrease of EDI, HI, and LCI. Specifically the HI was lowered, even if not statistically significant, to a value (1.03) almost below the concern limit for toddlers, while the LCI for both infants and toddlers (the most at risk groups), by 0.001 per 100,000 people, showing its efficacy as strategy to reduce the risk related to AFM1.

Given that milk containing AFM1 ≤ 10 ng kg^{-1} should be used for producing milk and milk-based products specifically for young children because HI is estimated below 1 (Serraino et al., 2019), the AL of 30 ng kg^{-1} would allow a mean and median AFM1 concentration respectively

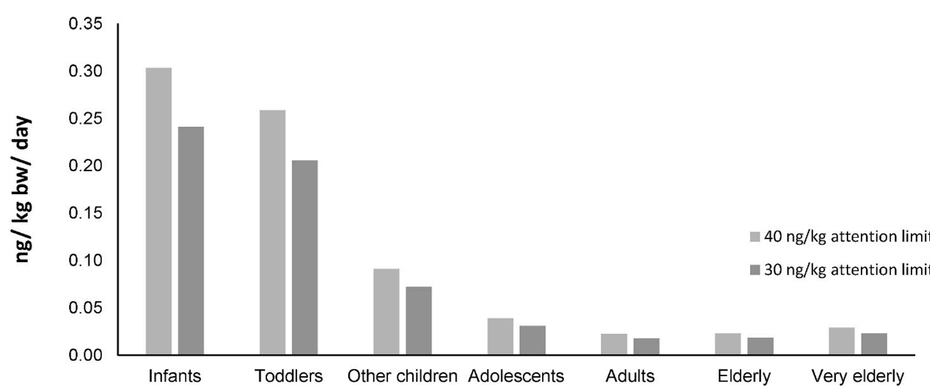

Fig. 3. Estimated Daily Intake (EDI) values for different population groups and for different attention limits in the 2013–2019 period.

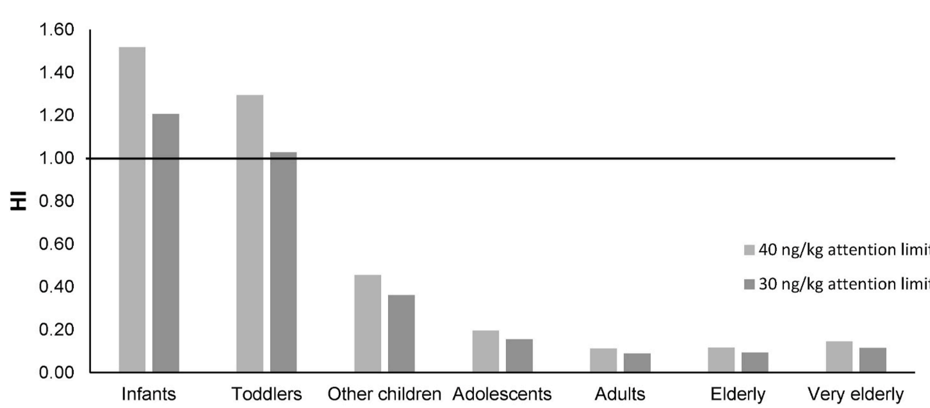

Fig. 4. Mean Hazard Index (HI) values for different population groups and for different attention limits in the 2013–2019 period.

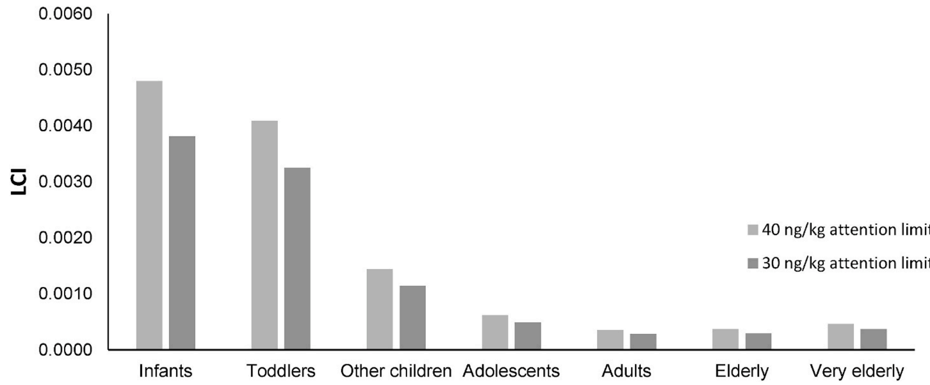

Fig. 5. Estimated average liver cancer incidence (LCI) (cases per 100,000 people) in the Italian population by age groups and by attention limit during 2013–2019.

of 9.21 and 8 ng kg^{-1}, meaning that almost all these commingled milk batches might be used, stored and processed for the youngest population, with a remarkable advantage for milk industry to assure safety also of this population groups.

5. Conclusion

The results obtained in this study demonstrate the efficacy of the management strategies to limit the presence of AFM1 in milk implemented after the aflatoxin crisis in 2003 and 2013. Moreover, they represent baseline data to define risk-based sampling plans to detect AFM1 contamination in milk thus lowering the human exposure to AFM1. The application of tailored sampling strategies when FBO must face either expected situation, as global climate changes, or unexpected crises, as the disruption in the supply chain due for instance to geopolitical reasons, can certainly help to limit the presence of aflatoxins in the food and feed systems.

CRediT authorship contribution statement

Federica Giacometti: conception and design of the study, Writing – original draft. **Federico Tomasello:** Formal analysis, Writing – original draft. **Federica Savini:** Data curation, and, Writing – original draft. **Valentina Indio:** Writing – review & editing. **Andelka Bacak:** Resources. **Alessandra Canever:** Resources. **Paolo Bonilauri:** Methodology. **Alessandra De Cesare:** Writing – review & editing. **Andrea Serraino:** Conceptualization.

Declaration of competing interest

Andelka Bacak and Alessandra Canever were employed by company Granarolo S.p.A., Bologna, Italy.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

The data that has been used is confidential.

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