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A Survey of Aflatoxin M_1 Contamination in Bulk Milk Samples from Dairy Bovine, Ovine, and Caprine Herds in Iran

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Abstract A total of 150 bovine (60), ovine (42), and caprine (48) bulk milk samples were analyzed using a commercially available competitive ELISA kit. Overall, AFM1 was found in 46.7 % of the analyzed samples by an average concentration of 40.3 ± 22.2 ng/L. The incidence rates of AFM1 contamination in bovine, ovine, and caprine bulk milk samples were 66.7, 31.0, and 35.4 %, respectively. The concentration of AFM1 in 37.5 % of AFM1-positive bovine milk samples and 5.9 % of AFM1-positive caprine milk samples were higher than 50 ng/L.

Keywords Aflatoxin M_1 · Milk · Iran

Aflatoxins (AFs) produced mostly by *Aspergillus flavus* and *Aspergillus parasiticus*, are a group of hepatocarcenogenic metabolites (AFB_1, AFB_2, AFG_1, and AFG_2) which may contaminate plant and plant products. Mammals that ingest AFB_1-contaminated diets excrete the principal 4-hydroxylated metabolite known as aflatoxin M_1 (AFM_1) into milk (Van Egmond 1989; Prandini et al. 2009). AFM_1 has been categorized by International Agency for Research on Cancer (IARC 1993) as a class 2B toxin, a possible human carcinogen. AFM_1 is resistant to thermal inactivation and not destroyed completely by pasteurization, autoclaving, or other food processing procedures (Motawee et al. 2009). Therefore, the presence of AFM_1 in milk and milk products is considered undesirable (Galvano et al. 1996; Van Egmond 1989; IARC 1993). Due to serious health concerns, many countries have set maximum limits for aflatoxins, which vary from country to country. The European Community and Codex Alimentarius have limited the level of AFM_1 in milk and milk products to 50 ng/L (Codex Alimentarius Commission 2001; European Commission Regulation 2001), whereas according to US Food and Drug Administration the level of AFM_1 in milk should not be higher than 500 ng/L (US FDA 1996). The Institute of Standards and Industrial Research of Iran (ISIRI) has accepted 500 ng/L as the action level for AFM_1 in milk (ISIRI 2005). Enzyme linked immunoassay (ELISA) is the most widely used immunoassay for determination of AFM_1 concentration in milk and dairy products because it is a simple and rapid method with high diagnostic sensitivity and specificity. The purpose of this survey was to detect the level of AFM_1 in bovine, ovine, and caprine bulk milk samples from dairy farms in Chaharmahal Va Bakhtiari province, Iran using a commercially available ELISA kit.

Materials and Methods

Bovine, ovine, and caprine herds were randomly selected from 260 herds in Chaharmahal va Bakhtiari province, Iran. This province is located in the central and southern part of Iran with about 850,000 inhabitants. Dairy farming is an important industry in this province and a large portion of milk and dairy products are exported to other provinces and other countries. From December 2008 to December 2009 a total of 60 bovine bulk milk samples were collected from 10 commercial dairy herds (Holstein cows). From September to November 2008 and March to May 2009 a

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total of 42 ovine bulk milk samples were collected from 12 sheep breeding farms (Lori-Bakhtiari breed) and a total of 48 caprine bulk milk samples were collected from 15 goat breeding farms (Lori-Bakhtiari breed) (Table 1). The samples were immediately transported to the laboratory in a cooler with ice packs and stored at −20°C until analysis.

The milk samples were first centrifuged at 3,500 *g* for 10 min at 10°C and then the upper cream layer was removed completely. To measure AFM_1 in milk samples, a competitive ELISA was employed using RIDASCREEN® aflatoxin M_1 kit (R-Bipharm AG, Germany). The assay was performed according to the manufacturer's recommendation and as described elsewhere (Lopez et al. 2003). The mean lower detection limit of the assay was 5 ng/L. All experiments were performed in triplicate. All statistical analyses were performed using SPSS software, version 16 (SPSS Chicago, IL, USA) and the data were expressed as mean ± standard deviation (SD). A repeated measures analysis of variance (ANOVA) and Tukey's test were used to compare the mean of AFM_1 concentration in bovine, ovine, and caprine milk samples in different seasons and the differences were considered significant at values of $p < 0.05$.

Results and Discussion

The results of our survey on the occurrence of AFM1 in bovine, ovine, and caprine bulk milk samples in Chaharmahal and Bakhtiari province is presented in Table 1. In the present study, the concentration of AMF1 in 15 of 40 (37.5 %) AMF1-positive bovine bulk milk samples were higher than the EU action level of 50 ng/L of AFM1. In contrast, all 13 AMF1-positive ovine milk samples contained lower than the EU action level for AFM1 and only 1 of 17 (5.9 %) AMF1-positive caprine bulk milk samples exceeded the EU action level for AFM1. In this study, the level of AMF1 in all of the bovine, ovine, and caprine positive-milk samples were lower than ISIRI accepted action level of 500 ng/L. Overall, the percentage of bovine AMF1 positive-milk samples was significantly ($p < 0.05$) higher than ovine and caprine milk samples. The low incidence of AFM1-positive ovine and caprine milk samples in this study could be at least partially due to the fact that sheep and goat herds are fed on stored grains for only 3–4 months and are raised on range lands with free grazing range plants for major part of the year. It has been suggested that out-pasturing of dairy animals is an effective way to reduce the level of AFM1 in milk (Kamkar 2005; Lopez et al. 2003). Moreover, cotton-seed cake, corn, and concentrate feed which are the major source of aflatoxin contamination are not used for feeding sheep and goat in this area.

In the present study, the overall contamination level of AFM1 in bovine bulk milk samples was lower than those reported by Kamkar (2005), Hussain and Anvar (2008), and Rahimi et al. (2010). Our results, however, was higher than those reported by Galvano et al. (2001), Motawee et al. (2009), Hussain et al. (2010), and Bilandžić et al. (2010). In this study, the overall contamination level of AFM1 in ovine and caprine bulk milk samples was lower than those reported by Motawee et al. (2009), and Rahimi et al. (2010); however, it was higher than those reported by Roussi et al. (2002), and Hussain et al. (2010). The differences could be due to several factors including different analytical techniques employed, samples size, season of the year, livestock management, and dairy processing systems. In addition, the AFM1 level in the milk has been found to be significantly affected by the geographical region (Galvano et al. 1996).

Although no significant differences were noted in the percentages of AFM1-positive bovine milk samples in different season, the results of this study showed that the mean concentration of AFM1 in bovine milk samples taken in winter was significantly ($p < 0.05$) higher than other seasons (Table 2). A marked seasonal variation in the level of AFM1 concentration in milk samples has been previously reported (Kamkar 2005; Ruangwises and Ruangwises 2010). This variation is possibly a result of toxin accumulation during storage under hot and humid condition. It has been suggested that surveillance for AFM1 contamination in milk must be continuous and widespread, since AFB1 recurs in feeds over long periods of time based on the overall climate and it may or may not be present in a

Table 1 Occurrence of aflatoxin M_1 in bulk milk samples from dairy bovine, ovine, and caprine herds in Chaharmahal Va Bakhtiari province, Iran

Species	No. of herds studied	No. of milk samples	No. (%) of positive milk samples	Mean ± SD (ng/L)	Range (ng/L)
Cow	10	60	40 (66.7)	48.7 ± 23.5	11–115
Sheep	12	42	13 (31.0)	25.8 ± 15.1	8–50
Goat	15	48	17 (35.4)	31.8 ± 13.7	15–61
Total	37	150	70 (46.7)	40.3 ± 22.2	8–115

Table 2 Seasonal distribution of aflatoxin M_1-positive bulk milk samples from dairy bovine, ovine, and caprine herds in Chaharmahal Va Bakhtiari province, Iran

Species	Month	No. of milk samples	No. (%) of positive milk samples	Mean ± SD (ng/L)	Range (ng/L)
Cow	Winter	18	12 (66.7)	60 ± 26	29–115
	Spring	14	9 (64.3)	44 ± 18	19–67
	Summer	13	8 (61.5)	39 ± 12	26–60
	Fall	15	11 (73.3)	47 ± 28	11–93
Sheep	Spring	19	7 (36.8)	24 ± 17	8–50
	Fall	23	6 (26.1)	28 ± 15	13–46
Goat	Spring	20	7 (35.0)	33 ± 12	19–47
	Fall	28	10 (35.7)	31 ± 15	15–61

particular year depending on the weather for that period (Galvano et al. 2001).

In conclusion, the results of the present study showed the importance of periodically monitoring the level of AFM1 in bovine, ovine, and caprine milk in Chaharmahal and Bakhtiari province. In order to reduce AFM1 contamination of milk and milk products, we suggest that more emphasis should be given to the routine AFB1 inspection in feedstuffs of dairy animals by governmental agencies and both farmers and dairy companies should be informed about the consequences of AFM1 presence in their products.

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