Screening of aflatoxin M1 in cows' milk in Gadarif town, Sudan

Kamal M. A. Abdalmahmoud
University of Gezira
El Tahir S. Shuiep
University of Gadarif
Ibtisam E El Zubeir (Ibtisamelzubeir17@gmail.com)
Faculty of Animal Production, University of Khartoum, Sudan https://orcid.org/0000-0001-8173-7693
Omer H. M. Arabi
University of Gezira

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Abstract

Background

Milk is a perfect complete perishable food that could be contaminated by any substance throughout the chain of its production. Of these aflatoxins M1 was considered as an important health risk associated with the milk. The objectives of this study are to analyse milk samples for the detection of aflatoxin M1.

Method

Eighty milk samples were collected from different sources randomly; 33 samples from farms, 31 samples from sale points and 16 samples from groceries in Gedarif town. Unisensor kits were used as screening method for detection of aflatoxin M1 in milk.

Results

The occurrence of aflatoxin M1 in raw milk samples were found in 22(27.50%) of the samples. The presence of aflatoxin contamination was high in milk samples collected from sales points (15.0%) followed by farms (11.25%) compared to those obtained from groceries (1.25%).

Conclusion

The presences of aflatoxin M1 in the milk samples might indicate that the cow milk was contaminated with aflatoxins through feed. Hence this study recommended that good hygienic practices should be considered in Gadarif town.

Introduction

Aflatoxins are toxic secondary metabolites of moulds; *Asperigillus flavus* and *A. parasiticus* are the main mycotoxin which could be associated with milk (Abbès et al. 2012; Boudra et al. 2007; Ghorbanian et al. 2008; Iha et al. 2013; Ozay et al. 2008; Sartori et al. 2015). Aflatoxins M1 and M2 are major metabolites of AFB1 and AFB2, respectively and are found in the milk of animals that consume feed contaminated with aflatoxins (Asi et al. 2012; Darsanaki et al. 2013; Mohammed et al. 2016; Siddappa et al. 2012; Zinedine 2007). Moreover Ali et al. (2014) found that the concentration of AFM1 in Khartoum State was affected by the source of concentrated feed but not the farm size. This might be because most of important milk producing areas in Sudan has no rigid systems of inspection on the farms and most of the products of these farms are sold through venders and groceries (Ahmed and El Zubeir 2007). Therefore, the incidence of AFM1 contamination in milk from dairy cow must be considered a risk, and raw milk is continually investigated and surveyed with respect to AFM1 contamination worldwide (Ketney et al. 2017).

The incidence of aflatoxin M1 (AFM1) contamination in milk and milk products is a serious health hazard for human worldwide. Thus, the global monitoring of AFM1 in raw milk has been ongoing for decades (Min et al. 2020). Several surveillance and studies showed the occurrence of AFM1 in milk samples from France (Boudra et al. 2007), Portugal (Duarte et al. 2013), Spain (Cano-Sancho et al. 2010), Greece (Malissiova et al. 2013), Italy (Bellio et al. 2016) and Turkey (Kabak and Ozbey 2012; Sahin et al. 2016; Unusan 2006). Also several surveys have been conducted on the occurrence of AFM1in milk and dairyproducts was reported in developing countries in Asia such as Iran (Kamkar et al. 2014), India (Sharma et al. 2020; Shipra et al. 2004), Pakistan (Asghar et al. 2018) and Saudi Arabia (Bokhari et al. 2017) and Africa such as Nigeria (Atanda et al. 2007), Kenya (Kang'ethe and Lang'a 2009; Kuboka et al. 2019), Tanzania (Mohammed et al. 2016) and Sudan (Ali et al. 2014; Elzupir and Elhussien 2010; Fadlalla et al. 2020; Yousof and El Zubeir 2020). Thus good agricultural practices are needed, which include appropriate drying techniques, maintaining proper storage facilities and taking care not to expose grains or oil seeds to moisture durig transport and marketing (Magan and Aldred 2007). Hence the present study was conducted with the obectives of detection of aflatoxin M1 occurrence in milk from different sources in Gadarif town.

Materials And Methods

Study area

The study was conducted in Gadarif State (Baladia Locality) situated between latitude 12-17° North and longitude 34-36° East, geographically it has a wide variety as well as heavy rains, which range between 600- 900 mm during the year. The livestock is the most important renewable resource in the state and some of the state's population depend on it, and comes secondly after agriculture. The state is very rich in livestock, which is estimated to 7% of the total census of livestock in Sudan. The number of livestock in Gadarif State is approximately five million heads of different species, this increase to seven million heads in the rainy season as the result of movement of animals from the traditional pastoral system in the prevailing system. According to the Ministry of Animal Resources and Fishery of Gadarif State (MARF 2011), the total number of animals is estimated to be 3.896.134 head. Sheep herds comprise about 48% of the total animal number followed by goats (24%), cattle (24%) and camels are about 4%.

Source of milk samples

Eighty milk samples were collected randomly from Gadarif locality from different sources; 33 samples of raw milk were collected from dairy farms; 31 samples of raw milk was collected directly from sale points and 16 samples of raw milk were collected from groceries.

Collection of milk samples

Raw bulk milk samples were collected in the afternoon during May to June 2018. After collection, the milk samples were kept in ice box till the next morning, and then they were taken to the laboratory of the Faculty of Agricultural and Environmental Sciences for the detection of aflatoxin.

Laboratory examination of milk samples

The raw milk samples were subjected to analysis and detection of aflatoxin.

Test for aflatoxin M1 detection

The Aflasensor kit 041 and its associated accessories (Heatsensor, negative and positive standards, deionized water) that were used in the present study were produced by the Unisensor Company (Liege, Belgium). It is a rapid (10 minutes at 40°C) test that use for the quantification of aflatoxin M1 (AFM1) in raw milk samples. The limit of quantification (LOQ) of the aflsensor is 20 ppt with a range of quantification up to 150 ppt. Aflsensor test requires the use of microwells that containing a predetermined amount of antibody lined to gold particles and a dipstick made up of a set of membranes with specific capture.

The lines for a valid test include the upper red (control line) that should be visible after second incubation time (7 minutes). The test was done by suspending 200 µ1 of milk sample with the reagent from microwell. A specific antibodie will bind the analyses; if present; during the first incubation time (3 minutes). When the dipstick is dipped into the sample, the liquid starts running vertically on the dipstick and passes through capture zones.

The development of a color at the test line indicates that the sample is free of aflatoxin M1. On the opposite, the presence of aflatoxin M1 in the sample will not cause the appearance of the colored signal at the test capture line. The concentration of aflatoxin M1 present in the milk sample will be based on the intensity of the line color that should be started from the bottom line of aflatoxin M1. The results were directly interpreted by visual observation. When the test line was darker in color like the control line, the result was negative, which means that at the given sensitivity of the test, the milk samples contain no aflatoxin M1 or aflatoxin M1 at a lower level than the value stated in the enclosed aflatoxin M1 limit of detection. When the line was as the same intensity or lighter in color than the control line, the result is considered positive (+) and the sample should contain higher concentration than 100 ppt. When there was no test line at all, the milk sample should contain higher concentrations of aflatoxin M1 residues and considered as full positive (++) as was described by UNISENSOR (2013). Figure 1 showed some valid results.

Statistical analysis

The analysis has carried out after obtaining results using a percentage basis for the presentation of the results.

Results

Aflatoxin M1 detection

The results of positive detection of aflatoxin in milk samples collected from farms, sale points and groceries in Gadarif town showed that about 27.50% of the milk samples were contaminated with aflatoxin M1 (Table 1). The highest occurrence of aflatoxin was found in the sale points (15.0%) followed by those obtained from the farms (11.25%) compared to those obtained from groceries (1.25%) as shown in Table 1.

Table 1
Occurrence of aflatoxin M1 in raw milk samples collected from farms, sale points and groceries in Gadarif town

Source of samples	Positive (20-150 PPt)		Negative (>20 PPt)		Total	
	Number	Percent	Number	Percent	Number	Percent
Farms	9	11.25	24	30	33	41.25
Sale points	12	15.00	19	23.75	31	38.75
Groceries	1	1.25	15	18.75	16	20
Total	22	27.50	58	72.50	80	100

The milk samples showing strong positive (level 1) contamination of aflatoxins were 6.25% and they belong to the samples obtained from farms. However the positive milk samples contaminated with aflatoxins from level 2 were 21.25%, of which 5.0% from farms, 15.0% from sale points and 1.25% from groceries (Table 2).

Table 2
The levels of aflatoxin M1 in raw milk samples collected form farms, sale points and groceries in Gadarif town

Source of samples	Strong positive (++)		Positive (+)		Total	
	N	Percent	N	Percent	N	Percent
Farms	5	6.25	4	5.00	9	11.25
Sale points	0	0.0	12	15.00	12	15.00
Groceries	0	0.0	1	1.25	1	1.25
Total	5	6.25	17	21.25	22	27.50
Strong positive (100-150 PPt)						
Positive (20-100 PPt)						
Negative (>20 PPt)						

Discussion

The raw milk samples tested during this study (Table 1) showed that 22 (27.5%) of the samples were found positive for aflatoxin M1 with the highest occurrence in the samples obtained from sale points and farms (15% and 11.5%, respectively). Moreover all strong positive (level 1; 0.05 to 0.1) contaminated samples were obtained from the farms (22.72%). Aflatoxin M_1 (AFM1) is a hydroxylated metabolite of aflatoxin B1 (Zinedine et al. 2007). Hence the high level of contamination in raw milk samples from the farms might be due to the contamination of dairy cow rations with aflatoxins B1 (Ali et al. 2014; Elteib et al. 2012). Higher occurrence were also reported in India, the range of contamination with AFM1 was 28-164 μg/l and that 99% of the contaminated milk samples exceeded the European Communities recommended limit (Shipra et al. 2004). Also Kang'ethe and Lang'a (2009) detected 99% of milk samples were contaminated with aflatoxin in Kenya. Bokhari et al. (2017) in KSA, tested 160 milk samples and found that 74.47% of milk samples were contaminated with aflatoxin. Ali et al. (2014) concluded that the levels of AFM1 in the raw milk samples indicated that the feeds offered to the cows were contaminated with aflatoxin B_1 in such a level that might cause a serious public problem. Aflatoxins are absorbed in the gastrointestinal tract but not been biotransformed in the liver can also be excreted (Scaglioni et al. 2014). Thus aflatoxin can accumulate through the food chain posing a serious health concern to both humans and animals (Gavrilova et al. 2014; Otim et al. 2005; Patel et al. 2015). However according to the survey conducted throughout North western Italy between 2012 and 2014, the overall AFM contamination rate was 2.2% (36 samples out of 1668 samples) and less than 1% of milk samples of were non-compliant with EU limits (Bellio et al. 2016). However the positive samples with level 2 (0.1 to 0.15) were detected in the samples collected from sale points (15.0%) compared to those collected from farms (5.0%) and groceries (1.25%) as shown in Table 2. This might indicate that the higher concentration of the contaminated milk from the farms was dilated to a lower level in the sale points, because in the sale points and groceries they bulked the milk from different farms. Similarly Sharma et al. (2020) was able to detect AFM1contamination in the milk sold by local traders (14/50) and vendors (16/50) in India. This study suggested the presence of aflatoxin in animal feed, which supported that of Omer et al. (2004); Elteib et al. (2013) who showed the presence of aflatoxin content in groundnut seeds and cakes, respectively. Aflatoxins are generally classified into B1, B2, G1 and G2, which metabolized to aflatoxins M1 and M2 (Boudra et al. 2007). Aflatoxins B1 is a potent mutagenic and carcinogenic agent found in numerous agricultural and dairy products consumed by humans (Madrigal-Santillan et al. 2007). Moreover Aflatoxins are highly carcinogenic and mutagenic in nature (Ehrlich et al. 2003; Ozay et al. 2008; Williams et al. 2004). Aflatoxins contaminated corn and cotton seed meal in dairy rations has resulted in aflatoxins M1 contaminated milk and milk products (Van Eijkeren et al. 2006; Zinedine et al. 2007). Hence regular monitoring of AFM1 is necessary for evaluating their contamination and improvement status. Simultaneously, more precautions could be implemented on hygiene controls in order to limit AFM1 contamination in dairy products (Min et al. 2020).

Furthermore in Khartoum State, higher prevalence was found compared to the present study and the percentage of AFM1 contamination has been found in 42/44 (95.45%) samples with contamination level ranging between 0.22 and 6.90 lg L_1 and average concentration of 2.07 lg L_1 (Elzupir and Elhussien 2010). Also Ali et al. (2014) showed that the average concentration for AFM1 in raw milk samples ranged between 0.1 and 2.52 ppb with 100% exceeding the limits of European countries. The presence of AFM1 was detected in a concentration that ranged between 20150 ppt and that 88.7% of the processed milk samples were found to be contaminated with aflatoxin M_1 compared to 92% of the raw milk samples (Fadlalla et al. 2020). The average concentration for AFM1 in Nigeria revealed 2.04 μg/k (Atanda et al. 2007), although Nigeria set a limit of 1.0 μg kg-1 (Iqbal et al. 2015). However the European communities and Codex Alimentarius recommend limits of 0.05μg/kg and 0.5μg/kg, respectively. (Kemboi et al. 2020) stated that despite their regulation being stricter, the EU is a major destination of trade for most African countries, and hence the EU regulatory and guidance values are used for comparison since they may negatively impact trade and in addition they cover a wide variety of feeds for different species. In China, the situation of AFM1 contamination in milk proved the improvement of surveillance (Xiong et al. 2018; Zheng et al. 2017).

The variations in AFM1 levels among milk samples from farms and distributors (Table 1 and 2) could be attributed to forage and feed quality, cow's diet, genetic variation in dairy cows, and geographical and seasonal variations (Mohammed et al. 2016; Sahin et al. 2016). On the other hand, the mean concentration of AFM1 in milk samples collected in summer ((96.3%) was significantly ($P<0.05$) higher than that obtained in winter ((89.0%) in Karachi, Pakistan (Asghar et al. 2018). They added that seasonal variations tend to increase the growth rate of fungi and AFs contamination, ultimately resulting in higher AFM1 contamination in summer when compared to winter. Based on 171 different milk samples, the results showed that all age's categories, especially children were exposed with high risk related to presence of AFM1 in milk in Serbia (Kos 2014). However Ahmad et al. (2019) found that the dietary exposure data of AFM1 among six different groups was indicated that the male children population was the most vulnerable group to AFM1, up to 6.68 ng L−1 per day and the least affected one was the female group above 20 years of age with 1.13 ng L−1 per day. The economic impact of aflatoxins leads directly to crop and livestock losses as well as indirectly to costs of regulatory programmers designed to reduce risks to animal and human health (Martins et al. 2007).

Conclusions

The presence of aflatoxin in raw milk samples need further monitoring and control, although the present frequencies are low compared with the previous studies. The presences of aflatoxin in milk samples might indicate that the cow milk was contaminated with aflatoxin through feed because all samples showing strong positive were detected in the milk samples collected from the farms. The present study recommended that good practices should be adopted for dairy cows' feeding, and the sale point of milk should be improved. Also education and awareness should be conducted especially among farmers and livestock producers on the health hazards of aflatoxins. Moreover strict lows and legislations should be implemented for the milk producers in order to minimize occurrence of aflatoxins and to ensure the quality of milk and dairy products in country.

Abbreviations

AF: Aflatoxins

MARF: Ministry of Animal Resources and Fishery

LOQ: limit of quantification

ppb: Part per billion

µ1: Mictoliter

SSMO: Sudanese Standard and Metrology Organization

KSA: Kingdom of Saudi Arabia

EU: European Union

Declarations

Availability of data and material:

All the generated data obtained during this study are included with the submission of the paper.

Competing interests

The authors declare that they have no any competing financial interests or personal relationships that could have influencing the work done in this paper.

Funding:

Not applicable.

Authorship contribution

KMAA, IEME and OHMA conceptualized the research proposal and study design; **KMAA, ESS and IEME** were involved in data acquisition including the collection of the milk samples; **KMAA and ESS** were involved in laboratory analysis, statistical analysis and interpretation of the results; **KMAA, ESS, IEME and OHMA involved in the preparation of the first** original draft.

IEME finalized the reviewing and editing of the manuscript. All the authors read and approved the final version for submission.

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Figures

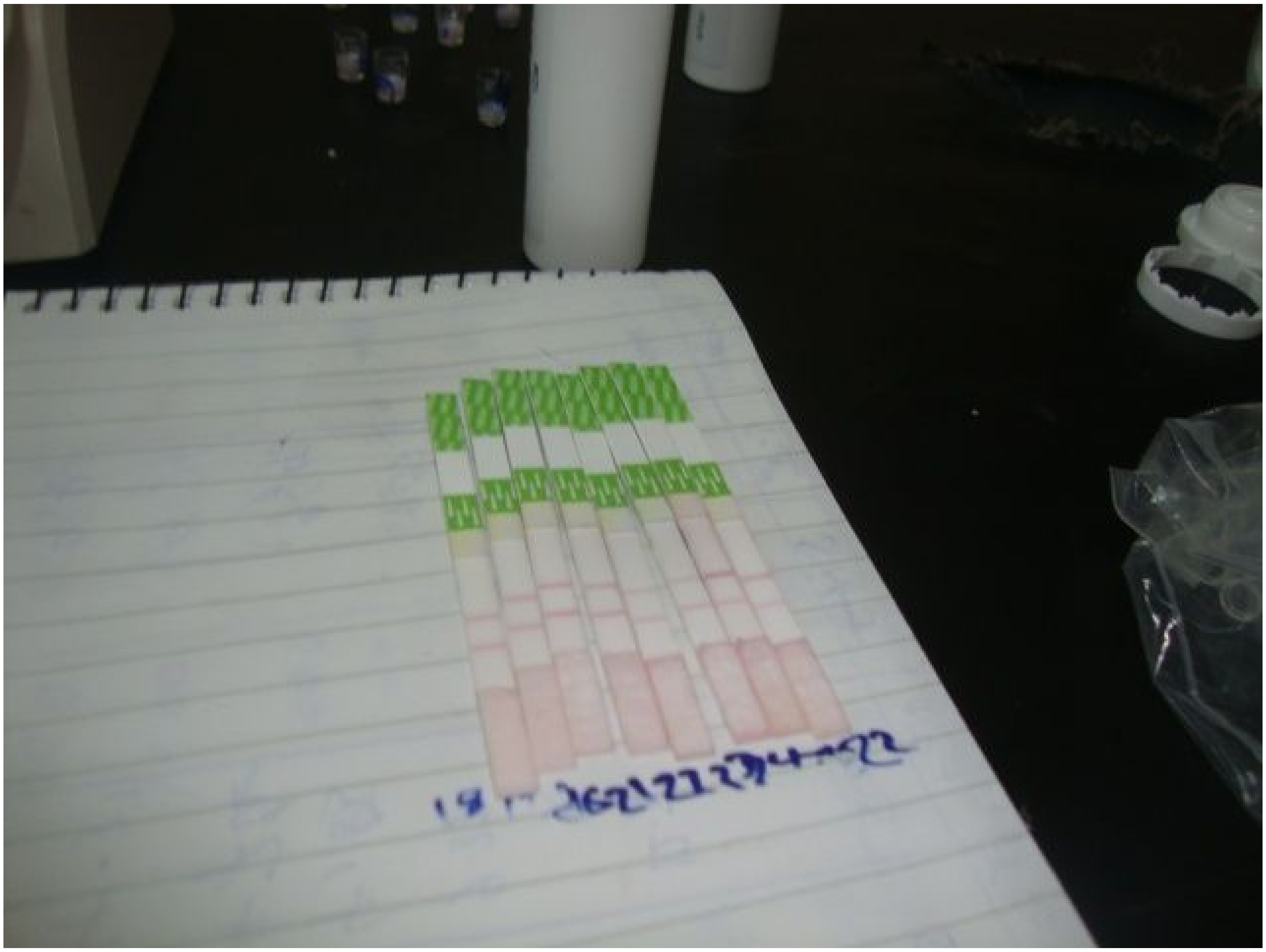

Figure 1

A valid test for aflasensor

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- aflatoxinldata.xlsx