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***S. E. SULIMAN and M. A. ABDALLA*[1]**

PRESENCE OF AFLATOXIN M1 IN DAIRY CATTLE MILK IN KHARTOUM STATE-SUDAN

SUMMARY

This study was carried out to detect aflatoxin M1 in dairy cattle milk in Khartoum State - Sudan. A total of 143 fresh milk samples were randomly sampled during 2011 in Khartoum. The enzyme-linked immunosorbent assay (ELISA) was used to detect aflatoxin M1 in the milk samples. AFM1 was found in 100% of the examined milk samples; 141 (98.6%) of the samples had AFM1 greater than the maximum tolerance limit (50ng/L). AFM1 levels in the samples in Khartoum state appear to be a serious public health problem.AFM1 contamination in the samples of dairy cattle milk in Khartoum state appears to be prevalent and may pose a public health problem at the moment. Awareness must be conveyed to producers, handlers and specialists.

Keywords: Aflatoxin M1, dairy cattle milk, Khartoum State, Sudan.

INTRODUCTION

Livestock is vital to lives and livelihood of people and to the economics of many countries especially developing ones. In the Sudan, animals is the main source of food particularly protein for human diets. Moreover, consumption of livestock and livestock products in the Sudan is increasing, which increase issues of public health impact of food of animal origin. Aflatoxins are mutagenic, teratogenic and hepatocarcinogenic compounds produced as secondary metabolic products (Whitlow and Hagler, 2002; Santacroce et al, 2008). Aflatoxin (AFM1) is a hydroxylated metabolite of aflatoxin B1 that may be found in milk and milk products obtained from livestock that have ingested contaminated feed (Creppy, 2002). The occurrence of AFM1 in milk is a public health concern so that AFM1 was classified by the international agency of research on cancer as, possible human carcinogens (Boudra et al, 2007).Animal exposure to Aflatoxins reveals a variety of symptoms depending on the animal species. However, in all animals, Aflatoxins can cause liver damage, decreased reproductive performance, reduced milk or egg production, embryonic death, teratogenicity, tumors and suppressed immune system function, even when low levels are consumed (Akande et al, 2006). Aflatoxins are toxic by-products produced by Aspergillus flavus and Aspergillus parasiticus (Harper, 2003). When animals eat food stuff containing AFB1, it will be metabolized and excreted as AFM1 in the urine and also in milk

[1] S. E. Suliman, (corresponding author: siham_suliman@yahoo.co.uk), M. A. Abdalla, College of Veterinary Medicine, Sudan University of Science and Technology P.O. Box 204, Khartoum North, Sudan.

(Gurbay et al, 2006). Apart from this, exposure to Aflatoxin can be through ingestion of contaminated milk containing AFM1. The parameters affecting levels of AFM1contamination in milk are the source of animal feeds, ecologic and economic factors on the farm, and also farm management. It seems that the kind of the animal feed and the harvesting time and temperature could be effective parameters in this regard (Kuiper, 1999). According to the European Union and Codex Alimentarius the maximum level of AFM1 in liquid milk and dried or processed milk products should not exceed 50 ng/ kg (CAC, 2001). But based on the US regulations it should not be higher than 500 ng/kg (Stoloff et al, 1991; Tajkarimi et al, 2007). The percentage of AfM1 contamination that has been reported in Sudan was 95.45% with contamination level ranging between 0.22 and 6.90 μg L^{-1} and average concentration of 2.07 μg L^{-1} (Elzupir and Elhussein 2010). The residues of AFM1 remain soluble when milk is processed by heat or is fermented (Yousef and Marth, 1988; Blanco et al, 1988). The aim of this study was to investigate the occurrence of AFM1 in dairy cattle milk.

MATERIAL AND METHODS

Sampling - A total of 143 fresh milk samples were randomly sampled during 2011 in Khartoum. The samples were transported to the laboratory in an insulated container at about 4°C. The samples were kept at −20 °C deep-freeze till tested. At the time of analysis samples were brought up to room temperature (Richard et al, 1993).

ELISA test procedure - The quantity of aflatoxin M1 was determined according to Enzyme-Linked Immuno Sorbent Assay (ELISA) by using the Ridascreen® aflatoxin M1 (R-biopharm, Darmstadt, Germany) test kit which is a competitive enzyme immunoassay based on antigen-antibody reaction (Anonymous, 1999). The milk samples were centrifuged for 10 minutes with 3500 rpm in 10°C. The upper creamy layer was completely removed by aspirating through a Pasteur pipette. The skimmed milk (100 μL) was used directly in each well for quantitative test. Addition of the enzyme conjugate (100 μL) and substrate (50 μL) to all wells. The washing of the wells was used after each step by buffer solution. Finally, the measurement of AFM1 was done photometrically at the wavelength of 450 nm against the air blank in ELISA reader. Absorbance percentages were taken to the calibration curve performed with standards at different concentrations.

Statistical analysis - All statistical analyses were performed using the software SPSS, version 11.5. Analysis of variance ANOVA was employed after logarithmic conversion when necessary to detect significant differences among means. A probability level of $p<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

In this study, a total of 143 raw milk samples were analyzed with the competitive ELISA technique. Aflatoxin M1 contamination was detected in 141

(98.6%) of the examined raw milk samples. The aflatoxin M1 contamination levels were between 18-86 ng/L with the mean of 69.40±12.32 ng/L. The most positive samples were 134 samples of raw milk greater than the maximum tolerance (50 ng/l or Kg).

The results (2.04ug/L^{-1}) of this study are similar to the results obtained (Atanda et al, 2007), but higher than that estimated by previous studies (Unusan 2006, Nachtmann et al, 2007, Zinedine et al, 2007, Heshmati and Milani 2010). Our findings lend support to those obtained in human breast milk (Coulter et al, 1984), when 13 out of 99 (13.13%) of the samples were contaminated with aflatoxin (0.019 mL^{-1}). The rate of aflatoxin M1 contamination levels in this study is higher than that raw milk samples examined by Tajik et al (2007) and it is lowered than the data obtained by Kamkar (2005). In many countries of Europe the low level of aflatoxin M1 have been found may be due to stringent regulation of aflatoxin B1 in complementary feedstuffs for dairy cattle. But almost 99% of contaminated samples exceeded the European communities and Codex Alimentarius recommended limits (EC, 1992; Shipra et al, 2004). Also the incidence of contamination of aflatoxin M1 was reached 87.3% in liquid milk samples in India. In another research by Hussain et al (2008) revealed that all of the raw milk samples examined from 14 districts of Punjab, Pakistan the contamination with aflatoxin M1 reached 99.4% more than European Union limit (0.05 mg/l). Dashti et al (2009) showed that all fresh milk samples except one from Kuwaiti markets were contaminated with aflatoxin M1 ranging from 4.9 to 68.7 ng/L, but 8 samples exceed maximum tolerant limit. Elzupir et al (2010) analyzed 44 bulk milk samples in Khartoum state. The percentage of AfM1 contamination has been found in these samples were 42/44 (95.45%) with contamination level ranging between 0.22 and 2.07mg/$L^{-1.}$ The present contamination of AfM1 in these samples was not unexpected as it is consistent with the presence of AfB1 in feed (Elzupir et al, 2010). Monitoring of AfM1 levels in animal studies has shown that the rate between the amount AFB1 ingested by cattle and the quantity excreted in milk is usually 0.32- 6.2% (Heshmati and Milani, 2010). Therefore, dairy cattle AfB1 exposure must be reduced by manufacturing and good practices (GMP).

In conclusion, AfM1 contamination in the present investigation of the samples of dairy cattle milk is alarming high and may pose a serious public health problem to human health. It is important to maintain control and to apply an ideal recommended limit to minimize the health hazard from AfM1 contaminants in milk which it can be used by infants and children. Moreover, the prevention of aflatoxin formation in feeds (AfB1) is very important, because the consumption of contaminated feeds by dairy animals cause AfM1 formation in milk. AfM1 contamination in the samples of dairy cattle milk in Khartoum state appears to be prevalent and may pose a public health problem at the moment. Awareness must be conveyed to producers, handlers and specialists.

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S. E. SULIMAN and M. A. ABDALLA

PRISUSTVO ALFATOKSINA M1 U KRAVLJEM MLIJEKU U DRŽAVI KARTUM - SUDAN

SAŽETAK

Ovo istraživanje je obavljeno u cilju utvrđivanja alfatoksina u kravljem mlijeku u državi Kartum – Sudan. Tokom 2011. godine je u Kartumu izvršeno nasumično uzorkovanje ukupno 143 uzoraka svježeg mlijeka. Korišćen je test enzimskog imunoodređivanja (ELISA) da bi se otkrio alfatoksin M1 u uzorcima mlijeka. AFM1 je otkriven u 100% ispitanih uzoraka mlijeka; 141 (98,6%) uzorak je imao AFM1 iznad maksimalne tolerisane granice (50ng/L). Nivo AFM1u uzorcima u državi Kartum predstavlja ozbiljan problem za javno zdravlje. Čini se da je kontaminiranost uzoraka kravljeg mlijeka AFM1-om u Kartumu rasprostranjena i može da u ovom trenutnku predstavlja problem po javno zdravlje. Svijest o tome se mora prenijeti na proizvođače, upravljače i stručnjake.

Ključne riječi: Aflatoksin M1, kravlje mlijeko, država Kartum, Sudan.