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The Presence of Mycotoxins in Kenya's Kalenjin Traditional Fermented Milk "Mursik"

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Authors' contributions

This work was carried out in collaboration between all authors. Author KKT designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors CCB, ZWN and TG proof read the protocol and streamlined the manuscript. Author KBL analyses the study data and author MCK managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

The study aimed at carrying out quantification of mycotoxins contaminating *Mursik*. *Mursik* is traditionally fermented milk prepared from freshly milked cow milk. Fermentation does not take place in controlled systems or sterilized conditions; as a result contamination with yeasts, moulds and some pathogenic bacteria would normally occur. These include species from the genus,

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Aspergillus, Penicillium, Fusarium and *Candida*. *Aspergillus, Penicillium* and *Fusarium* has been known as the producers of mycotoxins, which are secondary metabolites of fungi responsible mycoticoses in animals and humans. These mycotoxins include: aflatoxins, fumonisins and deoxynivalenol. The study was laboratory based carried out on *mursik* samples collected from households. All *mursik* samples were processed at mycology laboratory, Center for Microbiology Research (CMR), KEMRI. The research protocol was reviewed and approved by KEMRI.The study was carried in Soliat Location, Kericho County, Kenya. It was conducted between February and August, 2013, period of seven months. 194 samples were collected from farmers and 4 samples commercially sold (packet fermented milk) for controls were bought from local shops. Mycotoxins extraction was done using Envirologix procedure and was subsequently quantified by using a QuickTox kit. 99.5% of the samples were contaminated with Aflatoxins. Fumonisin toxin on quantification, 3 (1.5%) of the samples had detectable quantities, and Deoxynivalenol toxins was detected on 1 (0.5%) sample only. Aflatoxin is the major contaminant of mursik. It is clear that mycotoxins will be of increasing importance for all those involved in milk and milk products production, and food production. There is need to adopt effective strategies for mycotoxin control and mycotoxin detoxification.

Keywords: Mursik'; fermentation; fungi and mycotoxins.

1. INTRODUCTION

'Mursik' is traditionally fermented milk prepared from freshly milked cow milk. Traditional fermentation is a way of food processing, where microbes, lactic acid bacteria for example, *Lactobacillus, Lactococcus, Streptococcus* and *Leuconostoc* species are used. Fermentation is a method of food preservation, used from old age. Many traditional fermented milk products were made in Asia, Africa, the Middle East, and Northern and Eastern Europe [1]. For more than 330 years, it became part of the cultural and traditional norm among the indigenous communities mostly in the third world nations, majorly in Africa [2,3]. In lactic acid (non-alcoholic) fermentation, lactic acid is the main by-product of the fermentation process, the pH of the ferment is always lower than 5. Foods processed using fermentation process include: beverages, dairy products, cereals and even meat products [3]. The calabashes used in fermentation are first treated with charcoal like material obtained from burning a special tree known scientifically as *Senna didymobotrya*. The milk is treated with the same and left to ferment naturally for three to four days [3,4].

Fermentation does not take place in controlled systems or sterilized conditions; as a result contamination with yeasts, moulds and some pathogenic bacteria would normally occur. Milk form a favourable medium for growth and multiplication of microorganisms because of its high nutrient content [5]. It contains proteins, carbohydrates, minerals and vitamins which support the growth of many forms of fungi and yeasts. These include species from the genus, *Aspergillus, Penicillium, Fusarium* and *Candida*. *Aspergillus, Penicillium* and *Fusarium* has been known as the producers of mycotoxins, which are secondary metabolites of fungi responsible for disorders in animals and humans [6]. Mycotoxicosis is the toxic effect of mycotoxins on animal and human health where the severity depends on the toxicity of the mycotoxin, the extent of exposure, age and nutritional status of the individual and possible synergistic effects of other chemicals an individual is exposed [7].

According to Lanyasunya et al. [8], *Aspergillus* species has been known to be among the contaminants of most agricultural commodities, milk included. *Aspergillus flavus* and *A. parasiticus*, are the major producers of Aflatoxins, secondary metabolic products. Aflatoxins are a family of extremely toxic, mutagenic, and carcinogenic compounds produced. Two structural types of aflatoxins are known, B and G types, of which aflatoxin B_1 is considered the most potent and mostly found in Kenya during outbreaks [9,10]. Toxigenic *A. flavus* species produce aflatoxins B1 and B2, and toxigenic *A. parasiticus* species produce aflatoxins B 1, B2, G1, and G2. Aflatoxin B1 is a carcinogen and is excreted in milk in the form of aflatoxin M1 [11]. Aflatoxin B1 has been known to be carcinogenic in humans leading to hepatocarcinoma [12]. Aflatoxin M1 cannot be denatured through pasteurization process or in yoghurt and cheese processing [3].

Fumonisin is a secondary metabolite produced by the fungus of the genus *Fusarium.* The most

common forms of fumonisins are B1, B2 and B3 [13,14]. Fumonisin in humans has been related with neural tube defects in people that rely on maize as a staple food [15], but most prominently it has been indicated as a potential cause of oesophageal cancer in humans [14]. *Fusarium* species has also been known to produce Deoxynivalenol (DON) toxins, which has been associated with polyribosomal breakdown in mammalian cell lines.

In Kenya, the ability for organized food monitoring and managing of mycotoxin contamination at subsistence and consumer level is not in place. During the frequent aflatoxicosis episodes in Eastern province, only aflatoxins were investigated. However, it is likely that the population is all the time exposed to other toxic fungal metabolites such as fumonisins and deoxynivalenol or vomitoxin which have also been linked with serious health consequences [16]. In the rural areas, populations' are at high risk and simple measures of control of fungal infestations, inspections, and awareness campaigns can interrupt mycotoxin exposure and provide long term solutions to the problem [11].

These mycotoxins are produced by fungi in warm humid conditions. Kenya's climate is relatively warm (temperatures of 18°C-37°C) thus forming favorable conditions for growth of mycotoxin producing moulds. In Kenya, a larger number of people consume fresh and fermented milk in the rural areas. No report has been documented on the mycotoxin contamination of the milk in these areas which poses a health risk. Additionally, fermented milk undergoes a form of a process which is traditionally practiced, providing favorable conditions for milk contamination with mycotoxin producing fungi. Thus the main objective of the study was to quantify the mycotoxins in the traditionally fermented milk consumed majorly by cattle raring communities, especially the Kalenjins.

2. MATERIALS AND METHODS

Fermented milk (*mursik*) samples were collected from Soliat location, Kericho County, Kenya. 194 *mursik* samples were collected from different households and packet fermented milk from dairy industries were purchased from local different shops in the area to be the control samples and transported to Mycology Laboratory at Kenya Medical Research Institute. The mycotoxins assayed and quantified were Aflatoxins, Fumonisins and Deoxyvalenol with their tolerable levels being 0.05 ppb, 2.0 ppb and 1.0 ppb respectively. Mycotoxins were extracted using Envirologix procedure (Envirologix Inc.). Briefly, 25 ml of 'mursik' was measured into a disposable sample cup with lid and two volumes of 50% ethanol (100 ml); the sample cup was closed tightly and shaken using a shaker at a high speed for 1 minute. The extract immediately separated into two layers where the extracted mycotoxin will float as the top layer; a yellowish layer for each mycotoxin. The top yellowish layer was used for testing for the presence of mycotoxins. Using a calibrated pipette with a new tip, 100 µl of DB4 Buffer provided in the kit was put into a reaction vial. Exactly, 100 µl of the top yellowish layer of the sample was then added into the reaction vial containing the buffer. The buffer and sample extract was then mixed thoroughly by drawing the liquids up and down in the pipette tip.

Mycotoxins were quantified by using a QuickTox kit. QuickTox Strips was removed from the canister and placed into the reaction vial containing the Buffer and sample extract. The arrow tape on the end of the strip pointed into the reaction vial. The sample extract was allowed to travel up the strip; reaction vials standing on their own, the strip was allowed to develop for 5 minutes. The bottom section of the strip covered by the arrow tape was immediately cut off and discarded. Strip was inserted into the QuickScan reader for quantitation. The readings were saved in a computer and copied to files (Envirologix Inc.).

3. QUESTIONNAIRE

The questionnaire was administered to the farmers to determine on the form they ferment milk. For example, batch fermentation or continuous fermentation and also to determine the feeds used on their cows like use of commercial feeds, grass naturally obtained from the field or by use of contaminated maize grains.

4. DATA ANALYSIS

The data collected was entered using Ms. Excel, a computer package, and uploaded to statistical computer software, SPSS version 17 and analyzed for descriptive statistics.

5. RESULTS AND DISCUSSION

Mycotoxins are secondary metabolites of fungi that are toxic. The word Mycotoxin plainly means

poison from fungi. Amid the thousands of species of fungi, only about 100 are known to produce mycotoxins. There are three major genera of fungi that produce mycotoxins: *Aspergilius*, *Fusarium* and *Penicilium*. Although between 300 and 400 mycotoxins are known, those mycotoxins of most concern, based on their toxicity and occurrence, are aflatoxin, deoxynivalenol (DON) or vomitoxin and fumonisin [8].

Mycotoxins are not only accumulated in muscles of all animal species, but through metabolism it is excreted in urine and faeces. It is also found in eggs of poultry and animal milk. The mean rate of presence in milk varies according to the mycotoxins' minimum levels which range from 0.3-2.2% for AFB1 to 0.05% for FB1 and T2-toxin. Ochratoxin A and Vomitoxin residues can only be found in cow's milk when high quantities of toxins have been administered to animals. Occurrence of AFM1 in milk is a matter of concern in relation to the transfer of mycotoxins in the dairy food chain [17].

In the current study, 99.5% of the samples were contaminated with Aflatoxin B1 (Fig. 1). The Aflatoxin levels range from 0 to 12 ppb, giving the mean of 4.67 ppb and a mode of 4.8 ppb (Table 3). Of these samples, the average level of contamination was 4.67 ppb levels exceeding 0.05ppb as per EU, FAO/WHO and Food and Drug Adminstration (FDA) permissible levels for milk. The levels of contamination of fermented milk reported in this study are similar to those reported in Kenya by Kang'ethe and Lang'a, [11]. Reports of contamination of milk in varius parts of the world have been reported by De Sylos et al. [18], in Brazil, Rousi et al. [19] in Greece and Diaz et al. [20] in Colombia. However, in this study a higher proportion of samples exceeding the FAO/WHO limit of 0.05 ppb are reported. Higher proportions have been reported in India where 99% of the contaminated raw milk, milk based cereal weaning formula and infant formula exceeded the 0.05 ppb [21].

Aflatoxins, are secondary metabolites produced by species of *Aspergilus*, specifically *Aspergilus flavus* and *parasiticus* fungi, which are naturally occurring contaminants of food and elaborate the toxins under favorable conditions of temperature, relative humidity and poor storage conditions. There is high risk of farmers feeding AFB1contaminated animal feeds to their animals. Aflatoxin M1 (AFM1) is the principal hydroxylated AFB1 metabolite present in milk of cows fed with a diet contaminated with AFB1 and excreted within 12 hours of administration of contaminated feeds [11].

Aflatoxins belong to two structural types of aflatoxins are known, B and G types, of which aflatoxin B_1 is considered the most potent and mostly found in Kenya during outbreaks [9,10]. Toxigenic *A. flavus* species produce aflatoxins B 1 and B2, and toxigenic *A. parasiticus* species produce aflatoxins B 1, B2, GI, and G2. Aflatoxin B1 is a carcinogen and is excreted in milk in the form of aflatoxin M1 [11].

Exposure to aflatoxins is a result of ingestion of contaminated foods. Ingestion of aflatoxin poisoned food causes hepatic and gastrointestinal injury, immunosuppression, teratogenic, and oncogenic effects [22,23]. Constant exposure to low-level of aflatoxin enhances the danger of hepatocellular carcinoma [24]. Acute liver injury, morbidity and mortality have been associated with high exposure to aflatoxins [25]. Intake of $2x10^3$-$6x10^3$ ppb/day of aflatoxin for a month can result in acute hepatitis leading to death [25]. Aflatoxin in swine leads to reduced weight gain, immunesupresion, hepatitis and death [26]. The Food and Drug Administration (FDA) has established action levels of 20 parts per billion (ppb) for grain and feed products, and 0.05 ppb for milk. Grain, feed, or milk having aflatoxin at or above these levels cannot be allowed for consumer consumption [27]. Recommended limits in feed are: 20 ppb for dairy animals, 100 ppb for breeding cattle, breeding swine, and mature poultry [28].

Findings from the current study show a remarkably low contamination of samples (1.5%) with Fumonisin B1 (Table 1). However, the mean concentration of contamination was 0.008 ppb (Table 3) which was below recommended levels of 2.0ppb; same results were also reported by Maragos and Richard, [29]. Fumonisin is a secondary metabolite produced by the fungus of the genus *Fusarium*. The most common forms of fumonisins are B1, B2 and B3 [13,14]. Ingestion of these secondary metabolites may cause a range of adverse effects in different animal species. Variety of malignancies in many consumers has been associated with the consumption of fumonisin contaminated maize [30].

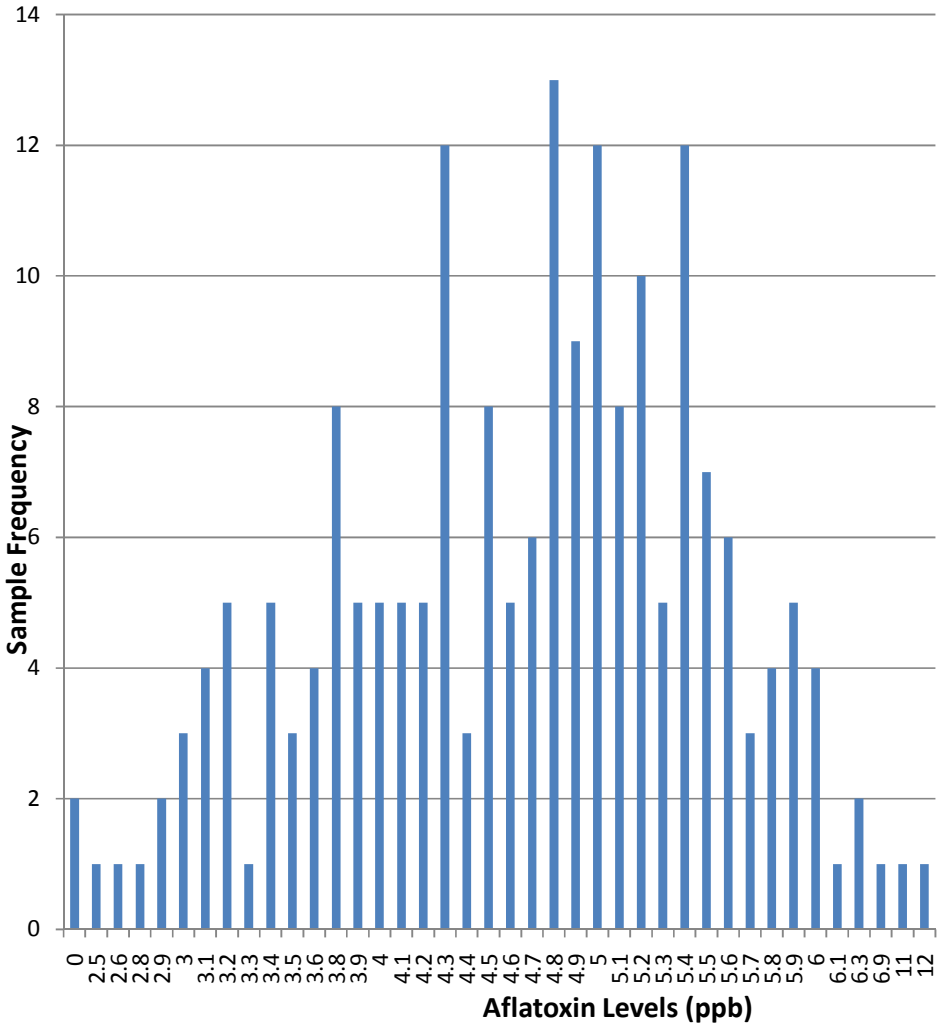

Fig. 1. Chart showing Aflatoxin levels and sample frequency

Table 1. Fumonisin quantified levels

Toxin level	Frequency	% of total
0	195	98.5
0.33	1	0.5
0.39	1	0.5
0.77	1	0.5
	198	**100**

Table 2. Deoxynivalenol (DON) quantification

Toxin level	Frequency	% of total
0	197	99.5
0.25	1	0.5
Total	198	100

Fumonisin in humans has been related with neural tube defects in people that rely on maize as a staple food [15], but most prominently it has been indicated as a potential cause of oesophageal cancer in humans [14]. In certain regions around the world including Bomet County in Kenya, where high daily intake of maize and maize-derived commodities occurs, an associations between either mouldy maize, *F. verticillioides* or fumonisin and the incidence of oesophageal cancer have been reported [14,31]. The toxic concentrations of fumonisin differ much depending on the animal species [13]. Absorption of about 5 – $10x10^3$ ppb/kg fumonisin in feed induces neurotoxic effects in horses [32]. In pigs the ingestion of 4 – $16x10^3$ ppb/kg body weight may result in liver cirrhosis and more than $16x10^3$ ppb/kg body weight result in pulmonary edema [33]. Chickens can withstand higher concentrations of fumonisin in feed, up to $75x10^3$ ppb/kg and cattle seem not to be affected by high fumonisin concentrations [34].

Table 3. Shows the mean and range of the mycotoxins levels

	Aflatoxin	Fumonisin	Deoxynivalenol
N	198	198	198
Mean	4.671	0.008	0.001
Range	12	0.77	0.25
Minimum	0	0	0
Maximum	12	0.77	0.25

This study found that 0.5% of the samples (Table 2) were contaminated with with Deoxynivalenol toxins, with an average contamination of 0.00126 ppb (Table 3) lower than the recommended levels of 1.0ppb of EU, FDA and FAO/WHO. Deoxynivalenol (DON) is a secondary metabolite produced by a *Fusarium* species. It is one of the most mycotoxin commonly detected in feed. DON is also called vomitoxin because it was first associated with vomiting in swine. Studies have shown DON to be a primary mycotoxin associated with swine disorders including feed refusals, diarrhea, reproductive failure, and deaths. Dairy cattle consuming feeds contaminated primarily with DON have led to reduction in milk production. Research has shown that DON causes polyribosomal breakdown in mammalian cell lines.

Human DON, exposure may be within the range of doses shown to be immunotoxic in rodents, human exposures and responses to this toxin are ill defined. Several thousand people were affected by gastrointestinal distress in an incident in the Kashmir Valley of India in 1987 [17].

In the study, all the participants did not use cereals to feed cattle or any kind of commercial feeds. However, the calabashes were opened daily, for further addition of fresh milk, hence increasing the likely hood of contamination with *Aspergillus, Penicillum* and *Fusarium* species, as reported in the study.

6. CONCLUSION

This finding suggests that there is exposure of humans to mycotoxin through contaminated milk. Aflatoxin is the major contaminant of *mursik* with mean quantities of 4.671 ppb. However, there is less contamination of *mursik* with Fumonisin and DON mycotoxins due to their insignificant mean quantities of 0.008 and 0.001 ppb respectively. It is clear that mycotoxins will be of increasing importance for all those involved in milk and milk products production, and food production. There is need to adopt effective strategies for mycotoxin control and mycotoxin detoxification. The formulation and implementation of mycotoxins regulatory limits, regular analysis of animal feed and feed ingredients and employment of proper mycotoxin control and surveillance strategy will help to reduce the economic losses and health consequences of mycotoxin exposure.

Quality of raw materials, prevention of the occurrence of mycotoxins, control and testing systems are all essential to reducing the exposure of humans and animals to mycotoxins. Milking, storage and maintaining milk containers is of great concern since farmers have no proper handling procedures where it will reduce contamination thus calling for education on the risk of microorganisms contaminating their milk and milk products and the adverse effects accompanied by these contaminations.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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