SOURCES AND LEVELS OF HUMAN EXPOSURE TO AFLATOXINS IN NANDI COUNTY, KENYA

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A thesis submitted to the University of Nairobi in partial fulfilment of the requirements for Masters degree in Veterinary Public Health

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July, 2013

DECLARATION

This thesis is my original work and has not been presented for award of a degree in any other university

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DEDICATION

To

Mum Hawa, Sis Mariam, Bros Abedy & Misa and in Memory of my late dad Joseph Sirma Kigen

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ACKNOWLEDGEMENTS

First and foremost I wish to sincerely thank the Almighty God for His wisdom, faithfulness and guidance through this work.

My deepest appreciation goes to my supervisors: Prof. Erastus Kang'ethe for linking me up with the Safe Food Safe Dairy (SFSD) project and with much care and attention corrected the scripts and directed me in the research process; Prof. Jackson Ombui and Dr. Isaac Mapenay for their constant guidance throughout the project and eventual thesis write up.

I am grateful to the board of postgraduate studies, University of Nairobi (UON) for granting me the scholarship to pursue this postgraduate course; to SFSD for granting me scholarship to carry out this research. I also thank the Department of PHPT, College of Agriculture and Veterinary Sciences, UON for availing laboratory space and equipment and to the entire staff who in one way or another have given me support: Prof. William Ogara, Prof. P. M. Kitala, Dr. Daniel Senerwa, Mr. Nduhiu Gitahi, Mr. Harrison Njoroge, Mr. Joseph Nderitu, Ms. Caroline Maina, Ms. Martha Jackline and Mr. Martin Odongo just to mention a few.

I'm indebted to the sampled households in Nandi County for being cooperative throughout the survey. To my colleagues in the study: Ms. Sara Ahlberg, Ms. Gatwiri Murithi, Dr. Cameline Mwai, Dr. Elizabeth Ouko, Dr. Christine Mosoti, Ms. Beatrice Mutele, Mr. Vincent Oyugi, Ms. Debra Kiaye, Mr. Samwel Kihara, Dr. Waweru Kabaka, Dr. Royford Bundi, and Ms. Esther Mwangi; I show appreciation to them all for the support we have given each other throughout the journey. Others who deserve my commendation for their diverse roles in my research are Prof. Sheila Okoth, school of biological sciences, University of Nairobi; Prof. Peter Shalo, Egerton University; Dr. Leonard Wamae, Kenya Agricultural Research Institute; Prof. Hannu Korhonen,

Ms. Sari Rämö, Mr. Veli Hietniemi and Dr. Vesa Joutsjoki all from Agrifood Research Finland – MTT; Ms. Erja Lindfors, Finnish Food Safety Authority Evira, and Dr. Joseph Mung'atu (statistician), senior lecturer Jomo Kenyatta University of Agriculture and Technology.

Thanks to my family for their constant encouragement: "*Let's meet at the top*". To all my friends thank you for the support you've offered in one way or another.

Last but not least I thank the Ministry of Foreign Affairs Finland for providing funds that enabled this study to be undertaken.

ABSTRACT

Aflatoxins are by-products of fungal metabolism mainly synthesized by fungus of the genus *Aspergillus*. In Kenya, the risk of chronic exposure to aflatoxins from susceptible foods, especially maize, which is a staple food is of major concern owing to the health hazards associated with it. However, focus in the country has been on acute aflatoxicosis due to the outbreaks that have occurred in the past. The aim of this study was to determine the levels of aflatoxins in dietary foods considered main sources of exposure that include maize, sorghum, millet and cow milk. A cross sectional survey covered 261 households constituting 101, 63 and 97 from Laboret, Kilibwoni and Chepkongony sub-locations. Human milk and urine samples were also collected and analysed to determine the exposure levels to children under age of 5 and their anthropometric measures taken to determine their nutritional status. Questionnaires were administered to household heads to assess knowledge, attitudes and practices towards aflatoxins. Aflatoxins determination was done by Enzyme Linked Immunosorbent Assay (ELISA) and High Performance Liquid Chromatography (HPLC).

Mean household size across the sub-locations was six, with their main source of earnings being from sales of livestock/fish/milk. Gender distribution among study participants was at a ratio of 1:1 (male: female). Education levels significantly differed across the sub-locations ($p<0.001$). Kilibwoni had the highest number of persons having attained university education.

Sixty seven point nine percent (72/106), 73.3% (44/60) and 65.7% (67/102) of maize samples collected from Laboret, Kilibwoni and Chepkongony were contaminated with aflatoxins ranging between 0.17 – 5.3 ppb. Ninety two point nine percent (13/14), 100% (9/9) and 87.5% (14/16) of millet samples from Laboret, Kilibwoni and Chepkongony were positive for aflatoxin at a range of 0.14 – 6.4 ppb. Fifty percent (9/18), 36.4% (8/22) and 27.3% (6/22) of sorghum samples from

Laboret, Kilibwoni and Chepkongony, respectively were contaminated with aflatoxins beyond Kenya Bureau of Standards (KEBS) maximum tolerable limits of 10 ppb.

Prevalence of aflatoxin M1 in cow milk was 50.5% (52/103), 49.2% (32/65) and 55.7% (54/97) from Laboret, Kilibwoni and Chepkongony, respectively but none exceeded the allowable limit of 0.05 ppb by Food and Agriculture Organization (FAO) and World Health Organization (WHO) limits.

Average consumption of maize flour was 0.26, 0.33 and 0.30 kg/person/day from Laboret, Kilibwoni and Chepkongony, respectively. This translated to aflatoxin exposure levels from maize flour of 0.3, 0.4 and 0.2 µg/kg/household/day from Laboret, Kilibwoni and Chepkongony, respectively. The average milk consumption was 0.55 litres/person/day from Laboret and 0.48 litres/person/day each from Kilibwoni and Chepkongony sub-locations. The exposure from milk from Laboret, Kilibwoni and Chepkongony was 0.0005, 0.0007 and 0.0005 µg/l/household/day respectively.

Seventy three point nine percent (17/23), 57.1% (8/14) and 43.3% (13/30) of human milk samples from Laboret, Kilibwoni and Chepkongony, respectively were positive for aflatoxin M1. Amongst urine samples, 94.9% (93/98), 88.9% (56/63) and 88.1% (74/84) from study children from Laboret, Kilibwoni and Chepkongony, respectively were positive for aflatoxin M1.

The percentage of children in various nutritional status categories from Laboret {44.7% (17/38)}, Kilibwoni {31.3% (10/32)}and Chepkongony {30.8% (8/26)} were stunted; 0%, 9.4% (3/32) and 3.8% (1/26) respectively were underweight and 2.6% (1/38), 3.1% (1/32) and 11.5% (3/26) respectively were wasted.

From the questionnaire survey, 68.3% (69/101), 60.3% (38/63) and 90.7% (88/97) of respondents from Laboret, Kilibwoni and Chepkongony, respectively identified colour change as the number one criteria for detecting spoilt maize. Twelve point nine six percent (13/101), 22.2% (14/63) and 5.2% (5/97) of respondents from Laboret, Kilibwoni and Chepkongony respectively identified aflatoxicosis as a risk of consuming spoilt grains. In the same order of sub-locations 6.9% (7/101), 0% and 1% (1/97) identified cancer as a health risk of exposure to aflatoxins. On perception of consumption of milk from cows fed on mouldy feed; 51.5% (52/101), 76.2% (48/63) and 72.2% (70/97) of respondents from Laboret, Kilibwoni and Chepkongony felt it was safe.

This study concludes that there is low knowledge on health risks of aflatoxins in Nandi County and that their common dietary foods (cereals and milk) are contaminated with aflatoxins posing a risk of chronic exposure to the residents. There is need for awareness creation on aflatoxins in the study sites and in the country especially in areas where acute aflatoxicosis has never occurred to sensitize people on health hazards associated with aflatoxin contamination. In addition, there is need to promote proper practices of grain production and storage to prevent contamination with aflatoxins and reduce exposure.

CHAPTER 1

INTRODUCTION

Mycotoxins are secondary fungal metabolites that are produced in cereals and forage. Mycotoxins of major concern in the world include aflatoxins, fumonisins, tricothecenes, ochratoxins and zearalenone. Aflatoxins are produced mainly by the moulds *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* (Bennett and Klich, 2003) in nuts and cereals under favourable conditions of temperature, relative humidity/moisture and poor storage (IARC, 2002). There are 4 main aflatoxins commonly encountered in foods/feeds namely: Aflatoxin B1 (AFB1), Aflatoxin B2 (AFB2), Aflatoxin G1 (AFG1) and Aflatoxin G2(AFG2). Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2) are metabolites of AFB1 and AFB2 respectively that can occur in milk and milk products from animals/humans having consumed feeds/foods contaminated with the B aflatoxins (Applebaum *et al*., 1982).

Aflatoxins when ingested cause toxic effects in humans and animals termed as aflatoxicosis. Ingestion of contaminated foods/feeds is primarily the main source of exposure to humans and animals (Wagacha and Muthomi, 2008) but airborne (Mehan *et al*., 1991) and percutaneous exposure (Wagacha and Muthomi, 2008) have also been reported. Several outbreaks of aflatoxicosis have occurred in the world in countries like Uganda, Kenya, USA and India. Kenya has had one major outbreak in 2004 which caused deaths of 125 persons and 317 reported cases in the Eastern province. During that outbreak, market maize was found to be contaminated with aflatoxins at levels ranging 1-46,400 ppb (Lewis *et al*., 2005). The maximum allowable limit in food by the Kenya Bureau of Standards is 10 ppb (KEBS, 2012).

Other than being highly toxic, aflatoxins, are mutagenic, teratogenic and carcinogenic (Massey *et al*, 1995). Aflatoxin B1 is classified as a class 1 human carcinogen by the

International Agency for Research in Cancer (IARC, 1993). Chronic exposure has been linked to the development of liver cancer in humans, which is ranked as the leading cause of cancer death worldwide according to World Health Organization (WHO, 2003). The risk of acquiring liver cancer is higher in people with hepatitis B (Ajayi *et al*., 2007). In children aflatoxin has been found to be associated with stunting (Gong *et al*., 2002; Okoth and Ohingo, 2004). This is worrying for Kenya as children are weaned on cereal based foods at home and also at school, where maize-based rations are provided courtesy of free primary education, a commodity highly susceptible to aflatoxin contamination. In 2011, Proctor and Allan® recalled 28 tonnes of aflatoxin contaminated relief food product branded "unimix" that was to be sent to drought stricken areas in the country; the product was supplied to institutions such as schools and health centres (Business Daily Africa, 2011a).

Economically, aflatoxins cause loss of productivity in animals, animal and human mortalities, loss of trade opportunities and loss to farmers due to disposal of contaminated commodities. During the 2004 acute aflatoxicosis outbreak in Eastern province, the amount of safe food required to replace contaminated food was 166,000 tonnes for 1.8 million people over 6 months (FAO/WHO, 2005). Apart from maize, other commodities have been found to be contaminated with aflatoxins: Milk and feed samples tested for aflatoxins from urban centres in Kenya (2006 – 2007) indicated that 85% (703/830) of feed samples were contaminated with AFB1 among which 63% (442/703) had levels exceeding the Food and Agriculture Organization (FAO) limit of 5ppb; Milk samples contained AFM1 in 474 (77%) of 613 samples (Kang'ethe and Lang'at, 2009). Peanuts from Busia and Homabay districts were found to be contaminated with aflatoxins at levels ranging from 0-7525ppb (Mutegi *et al*., 2009). It has been estimated that more than 5 billion people in developing countries worldwide are at a risk of chronic exposure to aflatoxins through contaminated foods (Strosnider *et al*., 2006).

Owing to the health and economic impacts of aflatoxins, it is necessary to conduct research to mitigate risk and reduce the economic losses. This study was done to assess the exposure level of humans to aflatoxins in Nandi County. Feedback will be given to the communities on the level of contamination of their foods with aflatoxins and the risk factors associated with the contamination. They will then be advised on the best control methods available.

1.1 Objectives of the study

General Objective

To assess level of human exposure to aflatoxins through dietary foods and factors associated with the levels in Nandi County

Specific Objectives

This study was carried out to meet the following specific objectives:

1. To determine the occurrence and level of aflatoxins in maize, sorghum, millet, urine, cow milk and human milk in Nandi County.
2. To determine factors associated with levels of aflatoxins in maize, sorghum, millet, urine, cow milk and human milk.
3. To assess knowledge, attitude and practices related to aflatoxins
4. To estimate the level of human exposure to aflatoxins

1.2 Hypothesis

Null Hypothesis (Ho): Humans in Nandi County are not exposed to high levels of aflatoxins (>10ppb).

Alternate Hypothesis (Ha): Humans in Nandi County are exposed to high levels of aflatoxins (>10ppb).

CHAPTER 2

LITERATURE REVIEW

2.1 Mycotoxins

Mycotoxins are secondary fungal metabolites which are capable of eliciting a toxic response in human or animal host. These metabolites are insignificant for fungal growth and development. The toxic response they elicit is termed mycotoxicosis. Moulds produce mycotoxins in agricultural crops under favourable conditions of temperature, moisture and relative humidity. Twenty five percent of the world's agricultural crops are estimated by FAO to be contaminated by mycotoxins (Smith *et al*., 1995). Human and animals get exposed to mycotoxins through several means, such as dietary, through consumption of contaminated food and feed products, or through dermal and inhalation means. Up to date more than 400 mycotoxins have been discovered and are mainly categorized into groups based on structural similarities (Bennett and Klich, 2003) . Mycotoxins of world importance and their distribution in the world are shown on Table 1, their importance arise from their known or suspected effects on human and animal health.

Table 1 Distribution of mycotoxins in the world

Distribution (Continent)	Mycotoxin
Africa and Asian Sub continent	Aflatoxin, Fumonisin
Australia	Aflatoxin and Fumonisin
North America	Aflatoxin, Ochratoxin, Zearalenone (ZEN) and Deoxynivalenol (DON)
South America	Aflatoxin, Fumonisins, Ochratoxin ZEN, DON,
Eastern Europe	ZEN and DON
Western Europe	Ochratoxin, ZEN and DON

(Bhat *et al*., 2010)

2.1.1 Aflatoxins

Aflatoxins are a group of highly toxic compounds synthesized mainly by the moulds *A. flavus, A. parasiticus* and *A. nomius* (Klich and Pitt, 1988). The name AFLATOXIN acronym formed from the following combinations: first letter "A" for the genus Aspergillus, the next 3 letters "FLA" for species flavus and the noun toxin meaning poison. They were first discovered in the early 1960's as the probable cause for turkey "X" disease in Great Britain, which caused a loss of 100,000 turkey poults due to liver damage (Sargeant *et al*., 1961).

There are several known types of aflatoxins but type B1, B2, G1 and G2 are the major ones encountered in most organic crops of cereals, oil seeds, spices and tree nuts (Table 2). They are classified on the basis of the colour of the fluorescence exhibited on thin layer chromatography; B for Blue and G for Green fluorescence (Bennett and Klich, 2003). Aflatoxins B2 and G2 were established as the dihydroxy derivatives of B1 and G1, respectively. Metabolites M_1 and M_2 of aflatoxins were first isolated from milk of lactating animals fed aflatoxin preparations; hence, the M designation (Applebaum *et al*., 1982).

Aflatoxin B1 (AFB1) is the most potent of the aflatoxin types (Williams *et al*., 2004). This was the type isolated from maize samples that caused the death of 125 people in Eastern province, Kenya during an acute aflatoxicosis outbreak in 2004 (Azziz-Baumgartner *et al*., 2005). *A. nomius* and *A. parasiticus* produce both B and G toxins (Vaamonde *et al*., 2003). *A. flavus* produces the B toxins only (Klich and Pitt, 1988). *A. flavus* can be further subdivided into S and L strains (Cotty, 1997); S strain isolates produce more aflatoxin than the L strains (Cotty and Cardwell, 1999).

The chemical structures of aflatoxins are shown on Fig. 1

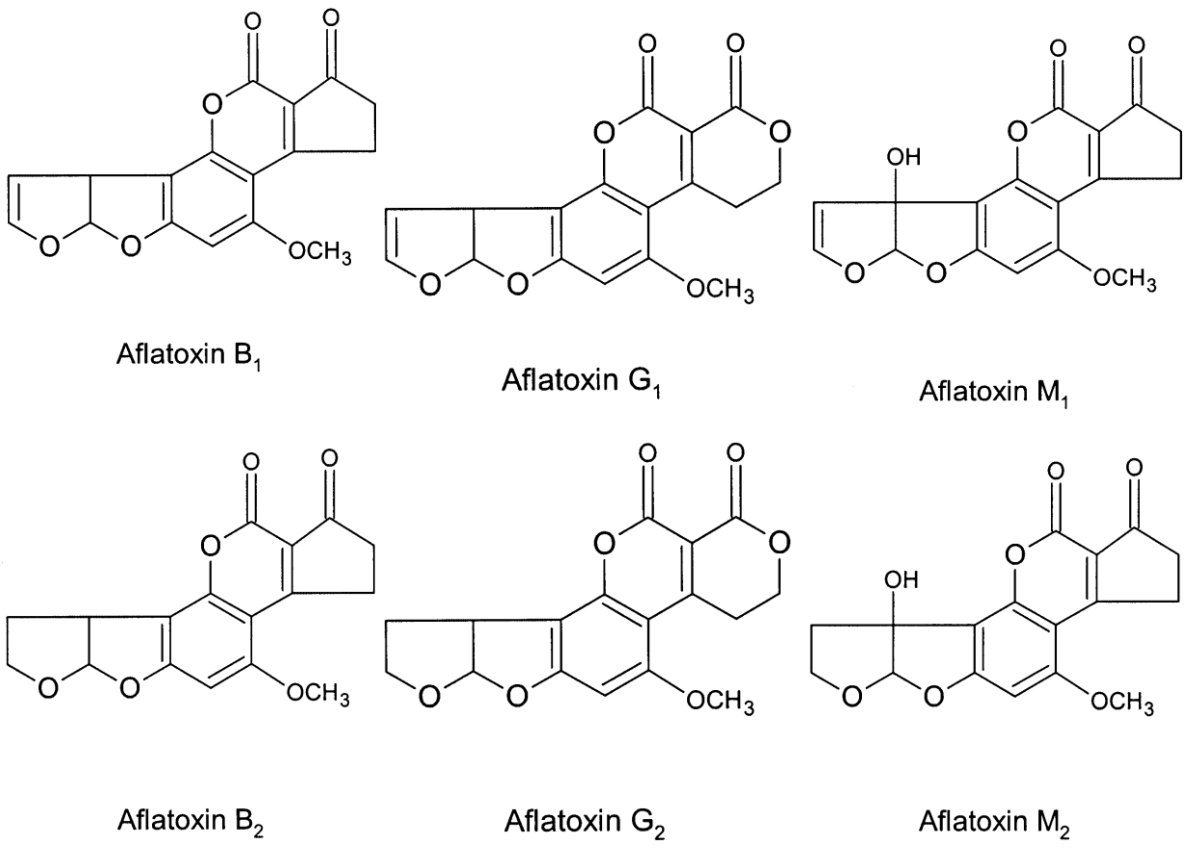

Figure 1. Structure of various aflatoxins; (IARC, 1993)

Table 2 Properties of aflatoxins

Properties	**Aflatoxin**					
	B1	**B2**	**G1**	**G2**	**M1**	**M2**
Chemical Formula	$C_{17}H_{12}O_6$	$C_{17}H_{14}O_6$	$C_{17}H_{12}O_7$	$C_{17}H_{14}O_7$	$C_{17}H_{12}O_7$	$C_{17}H_{14}O_7$
Molecular weight	312	314	328	330	328	330
Melting point	268-269(D)[1]	287-289 (D)	244-249 (D)	230	299 (D)	293
Flourescence	425 nm	425 nm	450 nm	425 nm	425 nm	425nm

[1] D = Decomposition, Source: (IARC, 1993).

2.2 Conditions favoring aflatoxins production

Aflatoxins are produced in cereals and forages under ideal conditions of moisture, temperature and humidity. Common substrates for fungal colonization and aflatoxin production include maize, groundnuts, wheat, rice and sorghum (Lisker *et al*., 1993). Aflatoxins have also been detected in cassava, liver, milk and milk products (Kaaya and Eboku, 2010; Kang'ethe and Lang'at, 2009; Murugavel *et al*., 2007).

Colonization of susceptible substrates by aflatoxin producing fungi and subsequent aflatoxin production can occur during pre-harvest, storage or processing periods. It is therefore influenced by several factors. Relative humidity of more than 85% and a temperature range of 20 - 35^{o}C are optimal for *Aspergillus g*rowth and aflatoxin production (Diener *et al*., 1987). Kenya National and Cereals Produce Board recommend a moisture content of 12 – 15% in maize to prevent fungal growth and subsequently aflatoxin production. Insect damage to intact seeds or nuts creates wounds and a microclimate that encourages fungal colonization, and the insect themselves serve as vectors of fungal spores (Munkvold *et al*., 1999; Waliyar *et al*., 2003). Hell *et al*. (2000), in their survey found that maize free of insect damage had no aflatoxin contamination, but maize with 70% of the cobs damaged by insects had 30.3% of the cobs contaminated with aflatoxin.

Harvesting practices such as late harvest, mechanical damage during harvest, improper drying and poor transportation promote conditions conducive to contamination by aflatoxins as well (Kaaya *et al*., 2005).

2.3 Toxicity mechanism of aflatoxins

Aflatoxins exert their toxicity by binding to DNA. Metabolism of aflatoxins in the body has only been described for AFB1. AFB1 once in the circulatory system is activated by cytochrome P450 to AFB1-8,9-*exo*-epoxide and AFB1-8,9-*endo*-epoxide. The former binds to DNA to form 8, 9-dihydro-8-(N7-guanyl)-9-hydroxy-AFB1 (AFB1-N7-Gua) adduct (Iyer

et al., 1994- see figure 2). These adducts are usually removed by nucleotide excision repair pathway; however, if unrepaired they may cause point mutations, in which a purine is substituted by a pyrimidine, or vice versa, and subsequent cellular changes that may lead to cellular transformation (Bennett and Klich, 2003). Other naturally occurring aflatoxins (B2, G1 and G2) are poorer substrates for epoxidation and, consequently are less mutagenic, carcinogenic and toxic compared to AFB1.

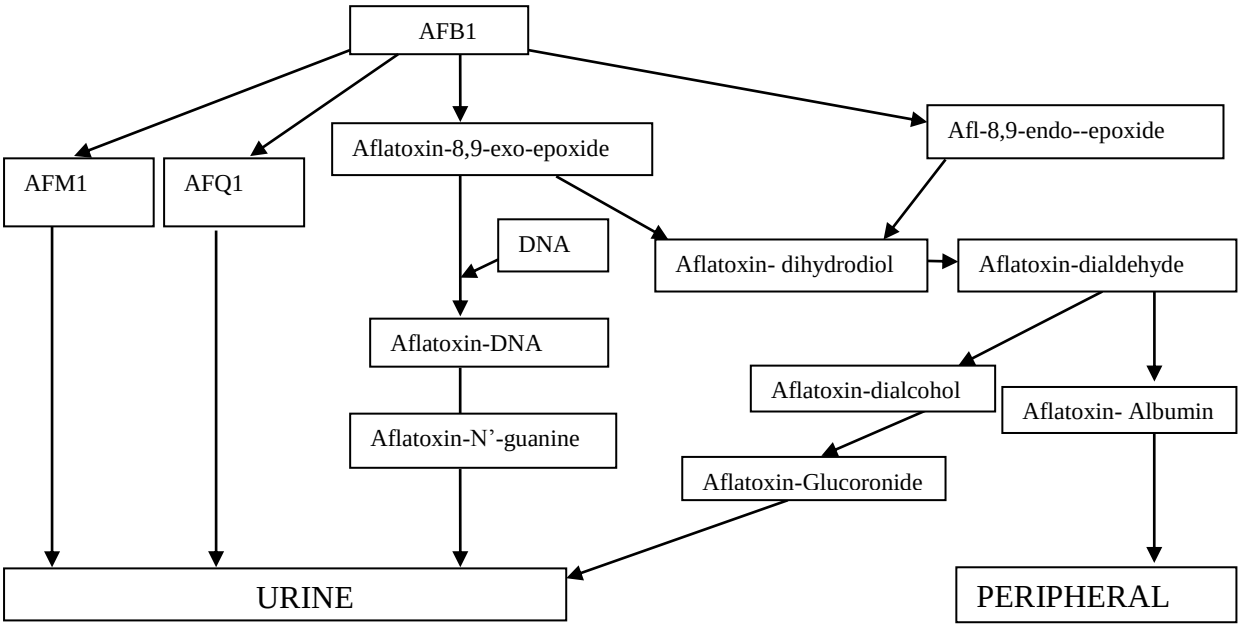

Figure 2. Metabolism of aflatoxin B1; Source: Wild and Turner, (2002)

2.4 Regulatory limits for aflatoxins

Regulation of aflatoxins is done in parts per billion (ppb) or parts per trillion (ppt) ranges with the maximum permitted levels in various commodities depending on the country setting it and how the commodity will be used. Worldwide, 99 countries have set legislation for mycotoxins with the limit of aflatoxin in food ranging between 1 – 20 ppb (FAO/WHO, 2003). Kenya has adopted the Food and Agriculture Organization (FAO) and World Health Organization (WHO) limit of 10 ppb total aflatoxins and 5 ppb AFB1 in food. Limit for AFM1 in milk is set at 50 ppt by the FAO/WHO. Despite Kenya having established regulations, this is mainly implemented for foods that pass through formal market, and not for the local markets, from where majority of Kenyans get their food, thus placing them at a high risk of exposure.

2.5 Occurrence of aflatoxins in food/feed commodities in Kenya

Food/feed aflatoxin contamination data available in Kenya generally show that a number of commodities are contaminated and that a significant proportion of them are contaminated far above the allowed levels. In samples from Makueni district, an area which suffered acute aflatoxicosis during the 2004 outbreak, 35.5% (37/104) of the locally grown maize was found to have levels of aflatoxins exceeding FAO/WHO recommended maximum limit of 10ppb, of those 20.2% (21/104) had levels of aflatoxin above 100ppb, with 10.6% having levels above 1,000ppb (Mwihia *et al*., 2008). Peanuts from Busia and Homabay districts were found to be contaminated with aflatoxins with levels ranging from 0 to 2687.6 ppb and 0 to 7525 ppb respectively (Mutegi *et al*., 2009).

A survey by Kang'ethe and Lang'at, (2009) on aflatoxin contamination of milk and feed samples from urban centres in Kenya showed 77% and 85% contamination of milk and feed samples by AFM1 and AFB1 respectively. Among the contaminated feed samples 63% (442/703) had AFB1 levels exceeding the FAO/WHO limit of 5 ppb. In another study to

determine maize meal contamination in Nairobi, AFB1 and AFB2 were found at levels ranging 0.4 – 20 ppb (Muriuki and Siboe, 1995). Okoth and Kola, (2012) also found contamination of maize from retail shops in Nairobi sampled between the years 2006-2009; more than 83% of the samples were contaminated with levels above FAO/WHO regulatory limit of 10ppb with maximum determined level being 4593.93 ppb.

2.6 Impact of aflatoxins

2.6.1 Health Impact

Health effects of aflatoxins are varied and range from a minor irritation to death. The toxicity in both humans and animals is dependent on a number of factors including species, ingestion levels, susceptibility (Hussein and Brasel, 2001), age (Meissonnier *et al*., 2005), aflatoxin concentration (Meissonnier *et al*., 2005), gender, and duration of exposure. Acute effects are linked to exposure to large doses of aflatoxin and may cause acute toxicity manifested by symptoms such as hepatitis, jaundice and gastrointestinal injuries with high morbidity and mortality (Jolly *et al*., 2007; Nyikal *et al*., 2004)

Exposure to low aflatoxin doses for prolonged periods may lead to carcinogenic and immunosuppressive effects and stunted growth in children (Barrett, 2005; Gong *et al*., 2002; Okoth and Ohingo, 2004), liver cirrhosis and reproductive problems (Cousin *et al*., 2005), micronutrient deficiencies in animals (Williams *et al*., 2004) and kwashiorkor in children (Hendrickse *et al*., 1982). Hepatitis B and C carriers have a high risk of developing liver cancer on exposure to aflatoxins (Williams *et al*., 2004). A recent study by Gong *et al*., (2012) has raised the possibility of an association between aflatoxin exposure and childhood hepatomegaly.

In poultry, main symptoms include liver damage, decreased egg production, reduced egg shell quality, poor carcass quality and increased susceptibility to disease (Wyatt, 1991).

Carcinogenicity in livestock is not recognized because the animals do not receive contaminated diets for a sufficient time prior to marketing for slaughter (Robens and Richard, 1992).

2.6.2 Economic impact

Aflatoxin contamination of food/feeds and exposure to humans and animals results in significant economic effects. These effects include human and animal deaths, veterinary and physician costs, reduced productivity of animals, loss of livelihoods, cost of control, loss of trade, loss to farmers through disposal of contaminated foods/feeds and investment in aflatoxin research to come up with mitigation strategies. Recently the Government of Kenya received Ksh40 million to fight aflatoxins from United States Agency for International Development (Business Daily Africa, 2011b).

In the country farmers have incurred losses due to contamination of their maize with aflatoxins. In 2010, it was approximated that farmers would lose 2.8 billion from banning of sale of contaminated maize in the Coast and Eastern provinces by the government; on the other hand the government spent Ksh1.4 billion to buy the contaminated maize (www.businessdailyafrica.com; accessed 17/09/2012). It is estimated that African food exporters of cereals and dried fruit incur an annual loss of 670 million USD by trying to meet European Union aflatoxin standards (Otsuki *et al*., 2001).

2.7 Exposure assessment of aflatoxins

Environmental and biological monitoring is used to determine human exposure to aflatoxins. Environmental exposure is determined by measuring levels of aflatoxins in food, air or other samples whereas biological exposure is by measuring directly residues, adducts and metabolites present in tissues, fluids and excreta (Bennett and Klich, 2003). Exposure assessments done in the country have relied on measuring levels in foods/feeds. Assessing the level of aflatoxin adducts in urine gives a reliable indication of recent (24-48hours) exposure

to aflatoxins. Measuring levels of aflatoxin-albumin adduct in peripheral blood indicates a person's sub-acute exposure as it has a half-life of 30-60 days in the body (Williams *et al*., 2004).

2.8 Methodologies for aflatoxin determination

Currently available methodologies for aflatoxin determination have been summarized by Pascale and Visconti (2008), and include: Thin Layer Chromatography (TLC), Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC), Liquid Chromatography/Mass Spectrometry (LC/MS), Enzyme-Linked Imunosorbent Assay (ELISA), and rapid tests. These methods have been verified by Association of Analytical Chemists International (AOAC) (IARC, 1993; AOAC, 2000) and by various international committees (ISO, 1998; ISO 2001).

The test to be used is dependent on various factors such as cost effectiveness, precision, and number of samples being analyzed. Most preferred method for analysis of aflatoxin is ELISA owing to its simplicity, speed, cost effectiveness, adaptability and sensitivity (ICRISAT, 2007). It allows for analysis of multiple samples which is ideal for screening purposes. HPLC is ideal for validation and quantification as it is highly sensitive and has good selectivity and is easily automated. However, HPLC's disadvantage is the high cost, making it unsuitable for routine procedures.

Emerging technologies for mycotoxins analysis include lateral flow devices (LFDs), Fluorescence Polarization Immunoassay (FPIA), Infrared Spectroscopy, capillary electrophoresis, fibre-optic immunosensors and molecularly imprinted polymers (Pascale and Visconti, 2008). Whichever method that is used should enable detection of tolerance levels, to facilitate monitoring programs and ensure international trade safety (Pascale and Visconti, 2008).

2.9 Methodologies for Detoxification, Control and Prevention of aflatoxins

Methodologies for control and prevention for aflatoxins can be classified as physical, chemical or microbiological:

2.9.1 Physical methods

Sorting, sieving, steeping, density segregation of grains and nuts significantly reduces the aflatoxin content of grains. Sorting can be done either manually (Awuah *et al*., 2009; Dorner, 2008) or commercially by use of electronic sorting machines (Dorner, 2008).

Park *et al*. (2002) have shown a reduction of aflatoxin content by 40-80% following physical cleaning and separation procedures of contaminated and physically damaged kernels. Promotion of rapid and effective drying methods of grains after harvesting has also proved effective (Bruns, 2003). Grains should be dried to a moisture content of 12-15%.

2.9.2 Chemical methods

Ammoniation: This method is effective for detoxification as it converts the parent aflatoxin compound to numerous products that exhibit greatly decreased toxicity (Ahmed *et al*, 1996).

Nixtamalization: This is a process of treating maize with Calcium Hydroxide and heating it.

Enterosorption: Use of certain chemicals and clays bind aflatoxins in the gut preventing their absorption into the body and thus minimize health and nutrition effects on humans. Aluminosilicates are the most preferred adsorbents (Huwig *et al*, 2001).

2.9.3 Microbiological methods

The use of bio-controls is proposed as a better control tool as use of fungicides or chemicals can add to production costs of commodities such as maize, millet and sorghum. Bio-control agents have been shown to reduce field aflatoxin contamination by 77-98% (Horn and Dorner, 2009). An endophytic bacterium from the subgroup of *Bacillus subtilis* and atoxigenic strains of *A. flavus* and *A. parasiticus* have been tested as pre- and post-harvest bio-controls of aflatoxin accumulation, respectively. These operate under the principle of

competitive exclusion, reducing aflatoxin concentration in plants by reducing the highly toxic strains (Horn and Dorner, 2009).

CHAPTER 3

MATERIALS AND METHODS

3.1 Study selection and sites

This study was carried out in Nandi County in Nandi north, central and south districts. The study districts were purposively selected because of their high maize growing activity and dairy keeping. Within the three districts, study divisions, locations and sub-locations were as well purposively selected based on high maize growing activity and population of dairy cattle. The selection was done with the assistance of a district and divisional team. District team comprised of District Veterinary Officer (DVO); District Agricultural Officer (DAO); District Medical officer of Health (DMoH); District Public Health Officer (DPHO) and District Livestock Production Officer (DLPO). Divisional team comprised of staff from Veterinary, Animal production and Agriculture. Ethical approval for the study was acquired from the Kenya National Council for Science and Technology

3.1.1 Nandi North District

Nandi North district borders Kakamega north district to the North-West, Eldoret West district to the North-East and Nandi central district to the South East. The district occupies an area of 736km^2. Its altitude ranges from 1300m to 2500m above sea level in the highlands. It has 3 administrative divisions, 29 locations and 67 sub-locations. In this district, the study was conducted in Kipkaren division, Laboret location and sub-location.

Nandi north district has a cool and moderately wet climate. On average the district receives between 1,490 mm and 2,179 mm of rainfall per annum. The long rains start in early March and continue to the end of June, while the short rains usually fall from mid-September to end of November. Most parts of the district experience mean temperatures of between 18^{o}C and 22^{o}C during the rainy season while higher temperatures averaging 23^{o}C are recorded during the drier months of December and January. The coolest temperatures, as low as 12^{o}C, are

experienced during the cold spell of July and August. Main topographic features of the district include rivers, steep slopes, hills and swamps. The area has 7 main agro-ecological zones: lower humid highland (LH1) covering 17% and dominated by dairy and tea; lower sub humid highland (LH2) occupying 26%, with wheat and maize being the major crops; lower semi humid highland (LH3) covering 14% and is a zone suited for wheat, barley and pyrethrum; upper humid highland (Um1) occupying 3.6%, suited for coffee production; upper sub-humid midland (UM2) and upper midland (UM3) (that covers 13% and is appropriate for coffee production.

The district has a human population of 160,605 (CBS, 2009). Half of the population is estimated to fall below poverty line. This is attributed to factors such as high cost of farm inputs, under-utilization and inequitable distribution of resources, low standard and inadequate education, unemployment, poor infrastructure and inaccessibility to credit (Nandi North district development plan, 2009).

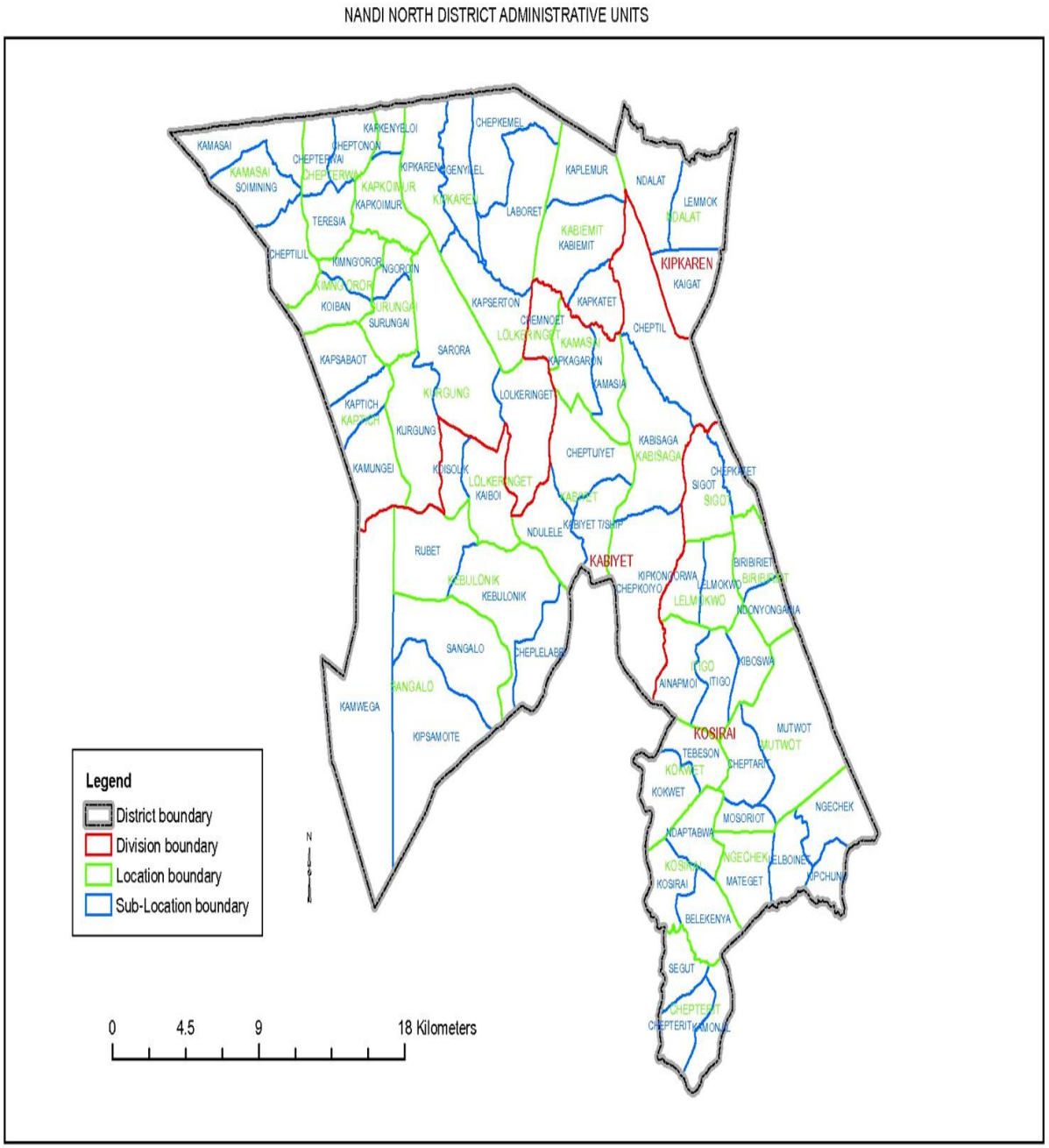

Figure 3. Nandi North District administrative units; Source: District commissioner's office, Nandi north district

3.1.2 Nandi Central district

Nandi Central district is situated in the Western part of the Rift Valley Province, it borders Kakamega district to the North West, Nandi North district to the North East, Nandi East district to the East and Nandi South district to the South East. It has an area of 961.8km^2 (Nandi Central district development plan, 2009). On administrative units, the district has 2 divisions, 22 locations and 66 sublocations. In this district the study was carried out in Kilibwoni division, Kilibwoni location and Kilibwoni sub-location.

Physiographically, Nandi Central district has two distinct features: rolling hills to the west, the Kapsabet plateau and the Kingwal swamp in the Baraton/Chepterit area. The altitude of the area ranges from 1,300m to 2,500m above sea level. Major rivers in the area are Kimondi, Kingwal and Yala. The district has a cool and moderately wet climate and receives mean rainfall of between 1200 – 2000 mm per year. The rainfall is bimodal with dry spells experienced between December and March. The district has 7 main agroecological zones: lower humid highland (LH1) covering 17%; lower sub humid highland (LH2) occupying 26%,; lower semi humid highland (LH3) covering 14%; upper humid highland (UM1) occupying 3.6; upper sub humid midland (UM2) and upper midland (UM3) that covers 13% (GOK, 2005).

Nandi Central district is populated with a total of 142,419 persons; male-70,344; female-72,075 (CBS, 2009). Fifty four percent of the population is estimated as poor (Nandi Central district development plan, 2009). The causes vary and include: low standards of education, high cost of farm inputs, under-utilization and inequitable distribution of resources and inaccessibility to credit (Nandi Central district development plan, 2009).

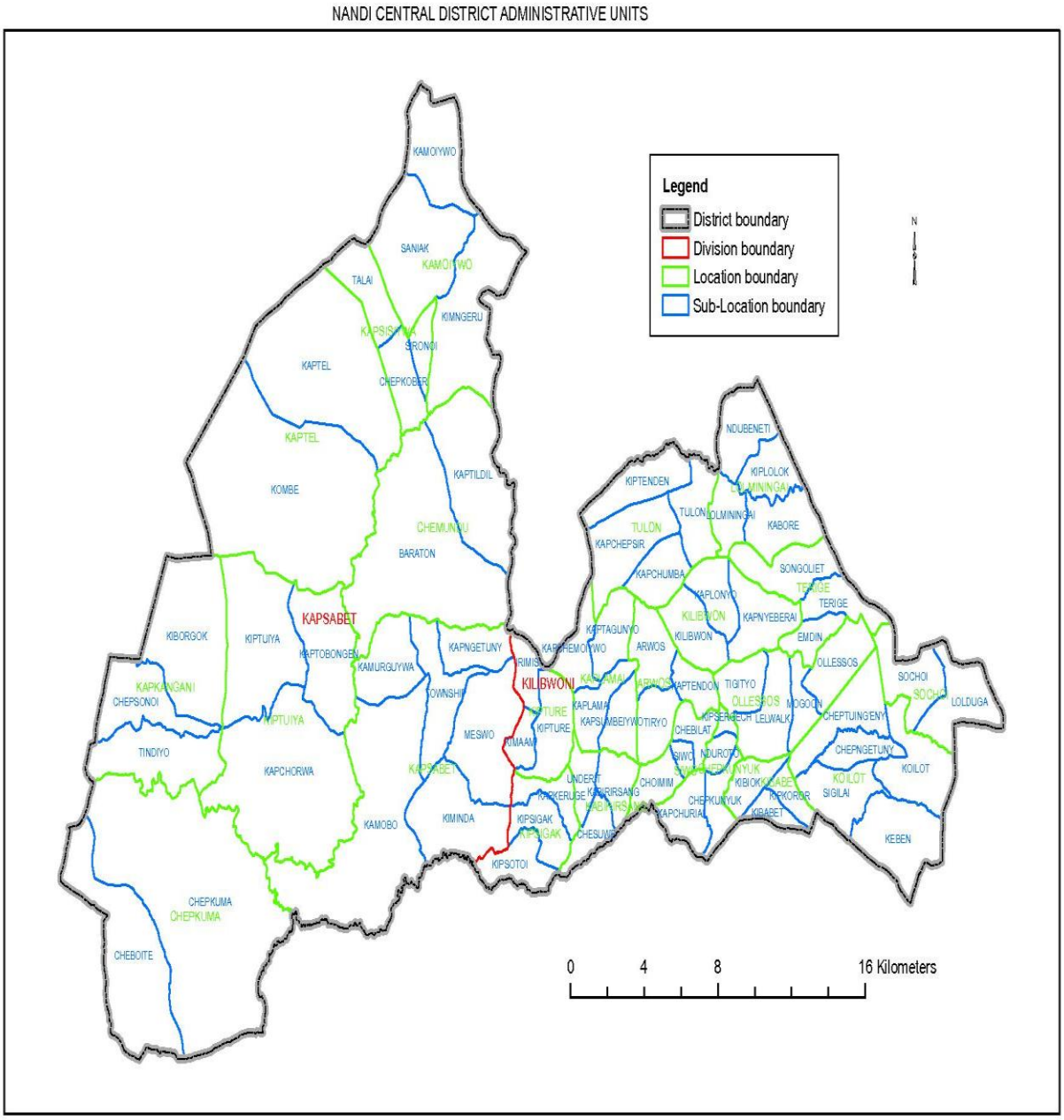

Figure 4. Nandi Central District administrative units. Source: District commissioner's office Nandi Central district

3.1.3 Nandi South District

Nandi South district occupies an area of 1,437.7 sq. km and is bordered by Kakamega district to the west, Nandi central district to the north, Kericho to the south east and Kisumu district to the south. The altitude ranges from 1,400m to 2,400m above sea level in the highlands. The district is divided into 5 administrative divisions: Nandi hills, Tinderet, Lessos, Kaptumo and Aldai. In this district the study was carried out in Kaptumo division, Kaptumo location and Chepkongony sub-location.

Nandi South district has moderately cool and wet climate. The precipitation varies from 1,200mm to 2000mm, annually. The month of March marks the onsets of long rains which continue to the end of June. The short rains regularly start from mid-September to end of November. Dry spell is usually experienced from end of December to March. The area experiences average temperatures of 18-25^{o}C. Nandi South has three main agro-ecological zones: upper highland (UH) that covers about 5%, lower highlands (LH1-2) covering about 24% and upper midlands (UM1-2) occupying 56%.

Nandi South has a total population of 157, 967 persons, having human concentration ranges from 285 persons per square kilometre in Aldai division to 162 individuals per km^2 in Tinderet; averaging density of 226 inhabitants per square kilometre (CBS, 2009). Nearly half of the district's residents are categorized as absolutely poor but has agriculture as the major sector of the districts economy, sustaining over 90% of the economically active individuals and accounts for nearly 52% of households earnings generated in the region (Nandi South district development plan, 2009).

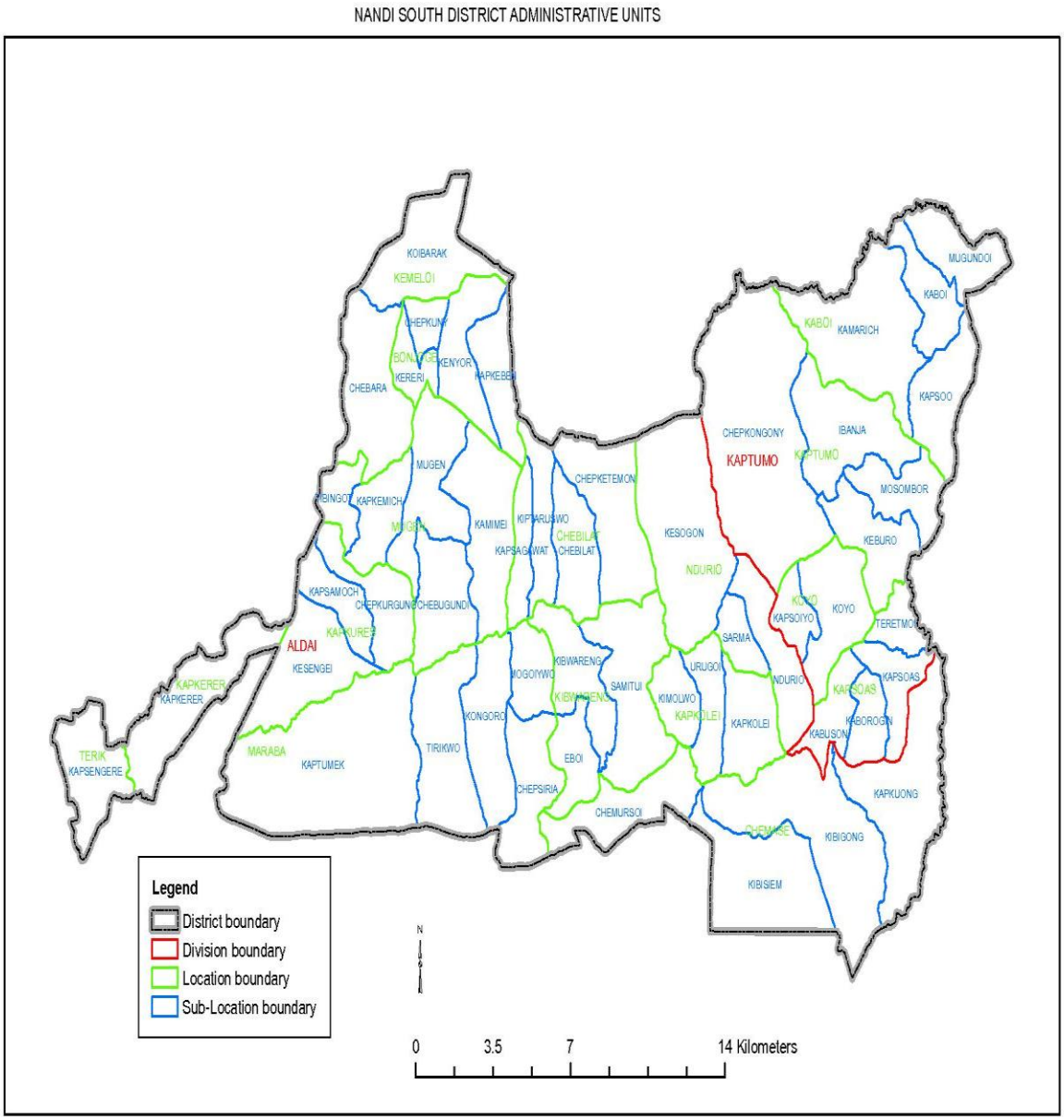

Figure 5. Nandi South district administrative units. Source: district commissioner's office Nandi South

3.2 Household selection

Selection of households (HH) within the selected locations qualifying for the study was done purposively based on the following criteria:

i. Rearing of dairy cattle
ii. Maize growing
iii. Having children below five years

However the following were also looked into as increasing chances of inclusion into the qualifying HH but not mandatory:

iv. Growing of sorghum or millet
v. Breast feeding mother

Households within each of the selected location that fit the criteria were listed (Table 3) and households randomly picked using Rand Between function of Ms Excel® for the study depending on the sample size.

3.3 Sample size determination

The calculation of the sample size was based on Martin *et al*. (1987) formula using a prevalence of 71%, level of confidence 95% and desired level of precision 5%.

$$n = \frac{Z^2 P(1-P)}{L^2}$$

Where n= sample size
Z= Z statistic for a level of confidence
P= proportion with the attribute of interest in the population
L= precision

$$p = \frac{915}{1289} = 0.71$$

$$n = \frac{(1.96)^2 (0.71)(0.29)}{(0.05)^2} = 317$$

Proportionate sampling (Table 3):

Nandi North 351/915 x 317 = 121 households

Nandi Central 221/915 x 317 = 76 households

Nandi South 343/915 x 317= 118 households

Table 3 Total household and population in the three study districts

District	Total HH	Population	Qualifying HH
Nandi Central	412	1927	221
Nandi North	457	4533	351
Nandi South	420	15000	343
Totals	1289	21460	915

3.4 Study Design

This was a cross sectional study where the selected households were visited once to collect samples and data using structured questionnaires. The data was mainly collected by interviewing household heads/spouses on their attitudes, knowledge and practices in relation to aflatoxins. The samples collected from the households included cow and human milk, maize, sorghum, millet, urine and anthropometric measures (weight, height and upper arm middle circumference) from the index child. Index child referred to the youngest child in the household amongst the ones who were under 5 years of age. The samples collected do not match with the number of households interviewed as some households did not provide the samples or in the case of children there were none below five years of age.

3.5 Samples

All collected samples were given identification number corresponding to household identity numbers for ease of tracing the results back to the particular households.

The following samples were collected:

i. Cow's milk – bulked and not boiled (n=265)

Cow's milk was collected so as to measure the levels of aflatoxin M1 in it. Aflatoxin M1 is a metabolite of Aflatoxin B1 which is formed following ingestion of feeds contaminated with AFB1. The milk was sampled into 50ml bottles and then transported in cool boxes to the laboratory where it was kept frozen, until analyzed within three months of collection.

ii. Human milk (n=67)

Human milk was collected to determine levels of aflatoxin M1, which if available will be transmitted to the breast-feeding children. The milk was collected by suction into 15 ml sterile tubes, transported in cool boxes to the laboratory where it was kept frozen, until analysed within 3 months of collection.

iii. Urine from children under 5 years of age (n=245)

Urine was collected to determine levels of aflatoxin M1 from index child. The levels of aflatoxin M1 found were used to infer to children exposure to aflatoxins and the effect on growth. Urine was collected into 20ml bottles and kept frozen until analysed within 3 months of collection

iv. Cereals { maize grains, millet and Sorghum (n=413) }

All cereals were collected in paper bags and kept at room temperature to be analysed within three months of collection.

v. Anthropometric measures of children less than 5 years of age (n=245)

Anthropometric measures (height, weight and upper middle arm circumference) were taken from the index child in the household using tape measure and a weighing scale. Weight for Age Z-score (WAZ), Height for Age Z-score (HAZ) and Weight for Height Z scores (WHZ) were calculated according to the median value of the international reference population recommended by National Centre for Health Statistics (NCHS)/ World Health Organization (WHO, 1986). A child with a Z-score less than minus 2 standard deviations (-2SD) for HAZ is considered short for their age (stunted) and one with less than minus 3 standard deviations (-3SD) is considered severely stunted. Children with less than -2SD for WHZ are considered thin (wasted) and those with less than -3SD are considered severely wasted. WAZ is a composite index for height-for-age and weight-for-height; children whose WAZ is less than -2SD are classified as underweight and those with less than -3SD classified as extremely underweight.

3.6 Questionnaire Survey

Questionnaires (Appendix 1) were administered to household heads/spouses on their attitudes, knowledge and practices in relation to aflatoxins. Data captured comprised of the following:

3.6.1 Household identification and characteristics

Households (hh) in each location were given identification numbers that were computer generated. To capture hh characteristics, hh heads were asked to name their members stating their position in the home, their sex, age and formal education. Age was categorized into: < 1 year, 2-5, 6-18, 19-35, 36-60 and 61 plus. Formal education was categorized into: primary, secondary, tertiary (post-secondary), university and any other where the members were required to specify.

3.6.2 Evaluation of household income

To assess household income, household heads were interviewed on their sources of income. Sources of income were categorized as: livestock/fish/milk sales, remittances, paid employment and self-employment. Any other source of income was specified. The income assessment was also based on the households' possessions which were calculated to produce a continuous variable and the mean asset values determined for each district.

3.6.3 Food consumption

To capture data on food consumption, household respondent (the spouses) were asked to state their source of the foods (maize, sorghum, millet and milk), the preparation in which they are taken, the amount and frequency of intake per day. To estimate ugali consumption in kilograms, respondents were shown pictures of ugali whose amount had been pre-determined. Respondents would point at the picture corresponding to their intake per day. A cup of milk was taken to contain 300ml of milk. Per household consumption of ugali or milk was calculated by dividing the total ugali/milk consumed by the total number of households. Per capita consumption was derived from total consumption of ugali/milk divided by the total population.

3.6.4 Evaluation of household perception on consumption of milk from cows fed on mouldy feed

To understand household perceptions on consumption of milk from cows fed on mouldy feed, the respondents were asked whether they considered milk to be safe from cows fed on mouldy feed.

3.6.5 Evaluation of household perception on human health risks associated with consumption of mouldy maize and mitigation measures

To assess household(hh) perception on human health risks associated with consumption of mouldy maize, hh respondents were asked questions aimed at establishing whether according to them consumption of mouldy maize can cause health problems and if yes which ones. They were also asked to explain how moldy grain can be made safer for consumption.

3.6.6 Evaluation of knowledge on recognition of spoilt grains

Knowledge on recognition of spoilt grains was assessed by asking the respondents to state the criterion they use to identify spoilt grains.

3.6.7 Evaluation of farming practices

Parameters relating to farming practices were assessed by interviewing farmers and recording their responses. The practices evaluated included: mode of harvesting, mode of drying, storage, and disposal of spoilt produce.

3.7 Enzyme-linked Immunosorbent Assay (ELISA) screening tests

3.7.1 Determination of Aflatoxin B1, B2; G1 and G2 in cereals

3.7.1.1 Grain sample preparation

Maize, sorghum and millet samples were ground using a grinder (Grindomix® GM200 knife mill, Retsch GmbH, Germany) at 8000 rpm for 40 seconds to obtain flour.

3.7.1.2 Sample Extraction

All the reagents were brought to room temperature before use.

Two grams of the representative sample was weighed and put in a screw top glass vial. To the sample, 25 ml of methanol/distilled water (70/30; v/v) was added and mixed thoroughly for 10 min at room temperature (RT) using a shaker. The extract was then filtered through Whatman filter paper No. 4 and the filtrate diluted 1:6 in phosphate buffered saline containing 500 µl/l Tween-20 (PBS-Tween) and analysed for aflatoxin by a competitive ELISA as described below.

3.7.1.3 Aflatoxin screening using competitive ELISA (Ridascreen® test Kit)

Sufficient numbers of antibody coated microtiter wells were inserted into the micro-well holder for all standards and samples to be run in duplicates. Into each well, 50 µl of standard solutions or extracted sample, 50 µl/well of enzyme conjugate and diluted antibody solution were dispensed in duplicates. This was mixed using a shaker for 30 seconds and the plates incubated at room temperature in the dark for 30 minutes to facilitate interaction between the toxins and the antibody.

The plates were thereafter washed three times in PBS-Tween, using Wellwash®, allowing three minutes for each wash. Substrate solution was then added at 100 µl/well and the plates incubated for 15 minutes in the dark for colour to develop. The reaction was stopped by adding 100 µl/well of 1 mol/L H_2SO_4 and the absorbance read at 450 nm in an ELISA plate reader (Labsystems Multiskan® PLUS, Labsystems, Helsinki, Finland).

3.7.2 Determination of Aflatoxin M1 in milk

3.7.2.1 Concentration of Aflatoxin M1 in milk

Five millilitres of milk sample was warmed and centrifuged for 15 minutes at 3500 rpm. To defat the milk, the upper cream layer was removed using a Pasteur pipette. Two point five millilitre of the defatted milk was transferred to a test tube and 5 ml of ethyl acetate (88.11g/mol; melting point -83.6^{o}C) added to it. This was vortexed for 1 minute and the

mixture centrifuged at 3500 rpm for 15 minutes at room temperature. Three millilitre of ethyl acetate layer was then transferred into a clean test tube and evaporated to dryness using a stream of nitrogen. The sample was then diluted with 250 µl of the sample dilution buffer and 30 µl of 70% methanol water. This was vortexed and then analysed for aflatoxin with competitive ELISA as described below.

3.7.2.2 Screening for Aflatoxin M1 using competitive ELISA (Ridascreen® test Kit)

Sufficient number of antibody coated micro-titre wells were inserted into the microwell holder for the standards and samples to be run in duplicates. A hundred micro litres of the standard solutions and prepared sample were added to separate duplicate wells and the plates incubated for half an hour at room temperature in the dark to allow interaction of the toxins and the antibody. The plates were subsequently washed three times in PBS-Tween, using Wellwash® with an interval of 3 minutes per wash. To the washed plates, a hundred micro litres of diluted enzyme conjugate was added, mixed using a shaker for 30 seconds and incubated for 15 minutes at room temperature in the dark.

The plates were then washed in three times in PBS-Tween, using Wellwash®. A hundred microliters of substrate/chromogen was added to each well then mixed using a shaker for 30 seconds. The mixture was incubated for 15 minutes at room temperature in the dark. To stop the reaction, 100 µl of stop solution (1 mol/L $H_2SO_{4)}$ was added to each well then mixed gently by shaking the plate manually and the absorbance read at 450 nm using ELISA micro plate reader (EMax® Endpoint ELISA Microplate Reader, USA).

3.7.3 Determination of Aflatoxin M1 in urine

3.7.3.1 Screening for Aflatoxin M1 using competitive ELISA (Helica® test Kit)

Any debris or precipitate was removed from the urine sample by centrifugation at 3000 rpm for 10 minutes. An aliquot of both the standards and samples were diluted 1:20 (50 µl

sample/standards: 950 µl distilled water). A mixing well was placed in a micro-well holder for each standard and sample to be tested and an equal number of antibody coated micro titre wells in another. Two hundred micro litres of assay buffer were dispensed into each mixing well. To each appropriate well containing the assay buffer, a hundred micro litres of diluted standard and sample were added. They were mixed using a shaker for thirty seconds. A hundred micro litres of the mixture was transferred from each mixing well to a corresponding antibody coated micro titre well. This was mixed using shaker for 30 seconds and incubated at room temperature for an hour.

The plates were then washed three times in PBS-Tween, using Wellwash®. A hundred micro litres of conjugate was added to each well and incubated at room temperature for 15 minutes in the dark. Subsequently the plates were washed three times in PBS-Tween, using Wellwash@. A hundred micro litres of the substrate reagent was then added to each microwell and incubated at room temperature for 15 minutes. A hundred micro litres of stop solution was added to each microwell using a multi-channel pipette. Optical density (OD) of each microwell was read with a microtiter plate reader at an absorbance of 450 nm and readings recorded.

3.8 Aflatoxin determination using High Performance Liquid Chromatography (HPLC)

Validation of the ELISA aflatoxin results was done using HPLC. Thirty percent of the milk and cereal samples screened using ELISA were randomly selected for HPLC using the Rand Between function of Ms Excel®. The HPLC equipment was made by Shimadzu® Japan equipped with a fluorescence detector: RF20A, gradient pump: LC 20AT, column oven: CTO 10ASVP, a degassing unit, and an auto-sampler: SIL 20 AHT.

3.8.1 Determination of aflatoxin M1 in milk using HPLC

The method used was based on ISO standard 14501:2007 and Evira 8556/1.

3.8.1.1 HPLC Model

The requirements for HPLC analysis of AFM1 included: flow rate of 0.8 ml/min, fluorescence excitation at wavelength of 360 nm, fluorescence emission at wavelength of 420 nm, injection volume of 20 ul, mobile phase of acetonitrile:water (25:75), detector RF-A, florescence detector LOD 0.001 ug/kg LOQ 0.002 ug/kg

3.8.1.2 Preparation of the sample

The test sample was warmed in a water bath between 35°C and 37°C. The sample was then centrifuged at 2000 g for 15 minutes and 50 ml of skimmed milk sample collected.

3.8.1.3 Immunoaffinity column preparation

A disposable syringe of 50 ml was placed on top of Immunoaffinity Column (IAC) with an adapter and the column connected to a vacuum manifold.

3.8.1.4 Extraction and purification of sample

Fifty millilitres of the test sample was added into a 50 ml syringe and allowed to pass through the IAC at a rate of 2 ml/min while controlling the volume flow by using vacuum system. The 50 ml syringe was then replaced by a 10 ml syringe barrel. The column was washed with 10ml water by allowing it to pass through the column at a flow rate of 2 ml per minute. The column was then blown completely dry after washing. After drying, the column system was disconnected and aflatoxin eluted from the column by passing 4ml pure acetonitrile in about 60 seconds through the column using 10 ml syringe. The volume flow rate was controlled by means of a syringe plunger. The eluate was collected in a conical tube and the volume evaporated to dryness by blowing a stream of nitrogen over it.

The sample was then reconstituted using 400 µl of 10% acetonitrile.

3.8.1.5 HPLC: Pump setting, chromatographic performance and calibration curve of aflatoxin M1

The eluent was pumped at a constant flow rate of 0.5 ml/minute through the HPLC column.

The stability of the chromatographic system was checked by repeatedly injecting a fixed amount of aflatoxin M1 standard working solution until stable peak heights were achieved.

Calibration curve was obtained by plotting the obtained peak height for each standard working solution against the mass of aflatoxin M1 injected. The volumes of standard solutions injected in sequence into the HPLC loop contained: 0.05ng, 0.10ng, 0.20ng and 0.40ng.

3.8.1.6 Calculation of results

Aflatoxin M1 concentration was calculated with the formula:

$C=A \times (V/M)$

C= concentration of aflatoxin M1 in sample (µg/kg)
A=the concentration of aflatoxin M1 in the analyzed sample solution (ng/ml)
V=volume of sample soaked into the column (50ml) or the weight of the milk powder (5 g)

The results were corrected with the recovery test and given in µg/kg with three decimals.

3.8.2 Aflatoxin determination in cereals using HPLC

3.8.2.1 HPLC Model

The requirements for HPLC analysis of total aflatoxins included: injection volume of 20 µl, flow rate of 1ml/min, runtime of 35 min, fluorescence excitation at wavelength of 363 nm, fluorescence emission at wavelength of 440 nm, mobile phase: acetonitrile:water (20:80), analytical column: waters Nova pak 3.9×150 mm; 4.6 um, detection limit of 0.01 ppb, and fluorescent detector RF 20A.

3.8.2.2 Immunoaffinity column preparation

A disposable syringe of 20 ml was placed on the top of Immunoaffinity Column (IAC) (Romer labs®, USA) with an adapter and the column connected to a vacuum manifold. Storing solution was dropped off from the column but not completely.

3.8.2.3 Sample extraction and purification

Twenty grams of the representative sample was weighed and 80 ml of acetonitrile water added, this was shaken for 2 hours in a horizontal shaker. The extract was filtered using water suction and the total volume measured using a volumetric flask.

Nine millilitres of the filtrate was transferred into a silanized round bottom flask. This was evaporated to dryness using a rota vapour. The extract was diluted with PBS solution by adding 20 mls of PBS.

For the IAC clean-up step, the PBS extract was filtered into 20 ml syringe using a Whatman filter paper No. 4. The solution was allowed to drop at a rate of 1-3 ml/min. Once all the solution was dropped, the column was washed still with 15 ml of PBS buffer.

In preparation for elution of the sample, silanized test tube was placed under the column and 3 ml of methanol added into the syringe. The methanol was left to stand in the column for a few seconds then let to drop down.

The eluate was then let dried completely under a stream of nitrogen. Into the dried sample was added 200 µl Trifluoroacetic Acid (TFA), vortexed and mixture let to stand at room temperature for 30 minutes. Eight hundred microlitres of acetonitrile water (30:70) was added into it and filtrated into a brown vial. The sample was then taken to run in the HPLC column. The calibration curve was then plotted and sample quantified as µg/kg.

3.9 Data Management and Analysis

3.9.1 Data Entry and Cleaning

Using Microsoft Excel® 2007 template, data was entered, cleaned and coded for analysis.

3.9.2 Statistical Data Analysis procedures

Analysis was performed using IBM SPSS software version 19.

Descriptive statistics were done to get frequencies and charts.

Chi-square tests were used to check whether respondents in the various household characteristics were distributed evenly across the three study sub-locations.

Analysis of Variance (ANOVA) was used to compare the means of aflatoxin levels between the study sub-locations for the different sample types.

Linear regression was used to determine association between different household practices and outcome of Aflatoxin exposure.

Relative Risk (RR) was calculated to determine the likelihood of urine testing positive for aflatoxin in children who were stunted, underweight or wasted.

Correlations were done to determine strength of association between HPLC test results and ELISA.

CHAPTER 4

RESULTS

4.1 Socio-demographic characteristics of study participants

This survey was based on a total of 261 households (Table 4). Population of households covered was 1529, with 594, 563 and 372 people coming from Laboret, Chepkongony and Kilibwoni sub-locations, respectively. Mean household size was six for each of the study site. A total of 243 children under age of 5 had their anthropometric measures taken.

Table 4 Population of sampled households and the average family sizes

Site	Targeted households	Sampled households	Population from which households were sampled	Average family size
Laboret	121	101	594	5.88
Kilibwoni	76	63	372	5.90
Chepkongony	118	97	563	5.80
Total	315	261	1529	

The targeted household number of 317 was not met as some of the originally included households could not be interviewed either due to relocation or declination to be included in the study. Some replacements were done for households which could not be reached. The percentage replacements done were 13.9%, 9.5% and 17.5% for Laboret, Kilibwoni and Chepkongony respectively.

Household characteristics are summarized in Table 5. There was a significant difference ($p<0.01$) in the percentages of people with different education levels across the sub-locations. The proportion of persons from Kilibwoni with primary education is greater than those from

Chepkongony which in turn does not significantly vary from the proportion in Laboret ($p<0.05$). A significantly higher percent (1.4%) of people have university education from Kilibwoni compared to the other sites.

Table 5 Household Characteristics

Characteristic	Laboret (%)	Kilibwoni (%)	Chepkongony (%)	Test statistic (p value)
Gender	(n=594)	(n=372)	(n=563)	
Male	48.3[a]	55.1[b]	52.4[a,b]	0.104
Female	51.7[a]	44.9[b]	47.6[a,b]	
Age structure	(n= 591)	(n=371)	(n=557)	
<1 years	5.9[a]	3.8[a]	5.0[a]	
2 – 5	21.2[a]	20.5[a]	18.1[a]	
6 – 18	32.3[a]	35.3[a]	37.2[a]	0.586
19 – 35	25.4[a]	25.9[a]	27.6[a]	
36 – 60	13.9[a]	13.5[a]	11.0[a]	
61+	1.4[a]	1.1[a]	1.1[a]	
Dependency (years)	(n=591)	(n=371)	(n=557)	
Dependents (0-14;65+)	52.8[a]	52.0[a]	54.9[a]	0.638
Productive population (15-64)	47.2[a]	48.0[a]	45.1[a]	
Education	(n=579)	(n=359)	(n=557)	
Primary	54.6[a,b]	60.4[b]	53.7[a]	
Secondary	18.3[a]	11.7[b]	17.1[a]	
Post-secondary	1.6[a]	2.5[a,b]	5.0[b]	0.000
University	.0[a]	1.4[b]	.2[a]	
Below school admission age	24.4[a]	22.3[a]	22.6[a]	
None	1.2[a]	1.7[a]	1.4[a]	
Mean livestock no. per household				
Cattle	3.8	3.8	3.0	
Sheep	2.0	0.9	1.5	0.272
Goats	0.1	0.3	0.2	

Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the .05 level.

4.2 Household income

Main source of livelihood for the three sub-locations was from livestock/fish/milk sales (Fig. 6). The percentage of households earning from fruits and vegetable sales, remittances and other sources were significantly different across the sites ($p<0.05$). Mean asset ownership value across the three sub-locations was Kenyan shillings 595,436.01; Kilibwoni had the highest mean value at Kshs 805,152.38, then Laboret at Kshs 620,668.32 and lowest Chepkongony at Kshs 432,955.67.

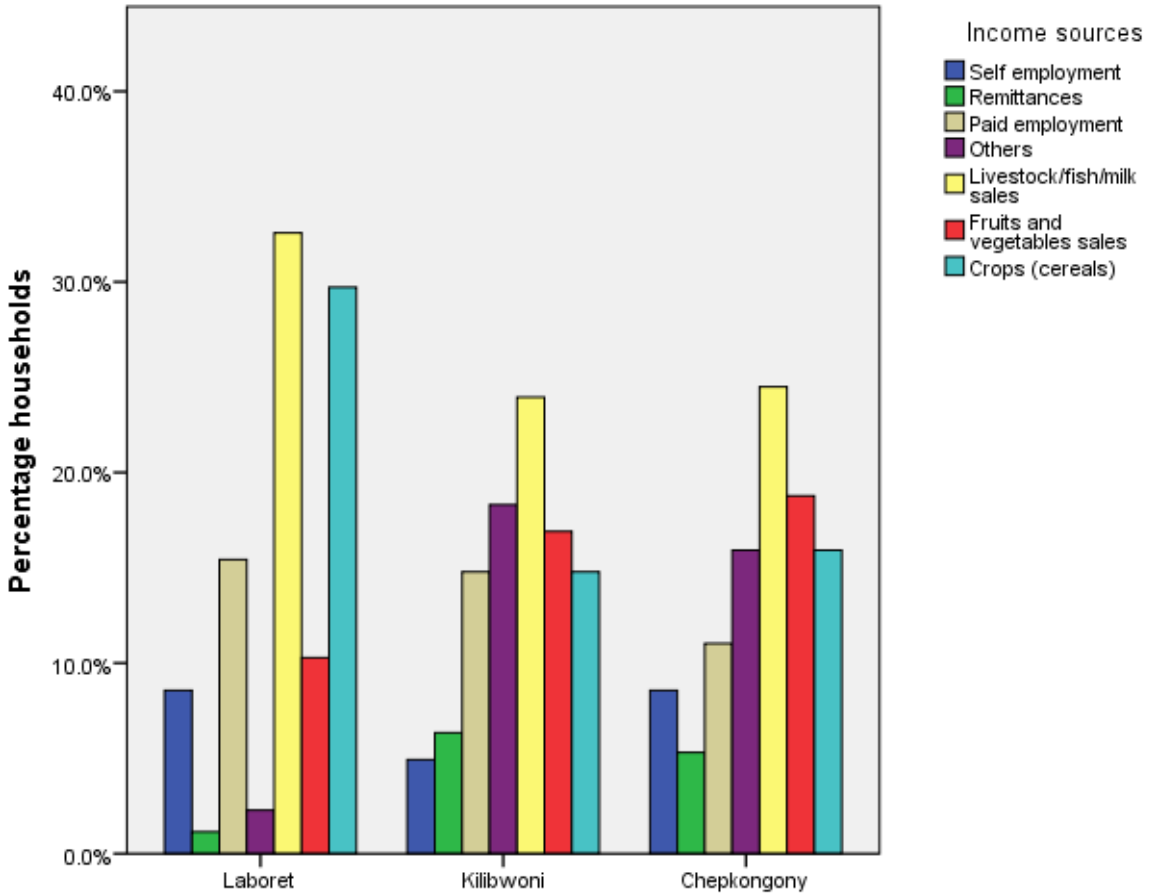

Figure 6. Various sources of income for the study sub-locations

4.3 Food Consumption

4.3.1 General food consumption by children

The percent frequencies of consumption of various preparations of maize, milk and sorghum by the different age groups is presented on Table 6-8. Amongst children 1 year old and less, there was a significant difference in the percent consuming maize porridge (p=0.012) and fresh milk (p=0.000) across the study. Percentage of the 1 year old and less from Kilibwoni consuming maize porridge and fresh milk was the least compared to those from Laboret and Chepkongony (Table 6 and 7). Ugali made from sorghum was the least popular food amongst all age categories in comparison to the other food preparations (Table 8).

Table 6 Percentage consuming various maize preparations amongst different age categories

Age Categories	Ugali			Uji			Githeri		
	Laboret	Kili-bwoni	Chep-kongony	Laboret	Kili-bwoni	Chep-kongony	Laboret	Kili-bwoni	Chep-kongony
<1	51.4[a]	35.7[a]	53.6[a]	74.3[a]	28.6[b]	60.7[a]	25.7[a]	7.1[a]	17.9[a]
1.0 – 5	96.0[a]	98.7[a]	95.0[a]	68.8[a]	63.2[a]	58.4[a]	58.4[a]	76.3[b]	69.3[a,b]
>5.0	98.4[a]	97.2[a]	97.4[a]	49.7[a]	52.0[a]	50.7[a]	64.3[a]	93.6[b]	85.0[c]

Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the .05 level.

Table 7 Percentage consuming various milk preparations amongst different age categories

Age Categories	Fresh			Mursik			Others		
	Laboret	Kili-bwoni	Chep-kongony	Laboret	Kili-bwoni	Chep-kongony	Laboret	Kili-bwoni	Chep-kongony
<1	60.0[a]	35.7[a]	85.7[b]	8.6[a]	7.1[a]	3.6[a]	42.9[a]	42.9[a]	14.3[b]
1.0 – 5	85.6[a]	94.7[b]	90.1[a,b]	29.6[a,b]	34.2[b]	19.8[a]	65.6[a]	21.1[b]	41.6[c]
>5.0	77.3[a]	94.0[b]	88.6[c]	51.5[a]	43.1[b]	46.7[a,b]	75.2[a]	20.3[b]	53.7[c]

Table 8 Percentage consuming various sorghum preparations amongst different age categories

Age Categories	Ugali			Uji		
	Laboret	Kili-bwoni	Chep-kongony	Laboret	Kili-bwoni	Chep-kongony
<1	0[a]	0[a]	14.1[a]	100[a]	100[a]	100[a]
1.0 – 5	4.5[a,b]	0[b]	20.4[a]	95.5[a]	100[a]	98[a]
>5.0	1.6[a]	0[a]	45.2[b]	98.4[a,b]	100[b]	92.5[a]

4.3.2 Household maize consumption

Main source of household consumed maize was from own farming (Figure 7); this was consumed as ugali by a majority (Table 6). On average maize flour consumption was 1.64 kg per household per day across the study (Table 9). That translates to approximately 300 g of ugali per person per day taking into account the average household size of six.

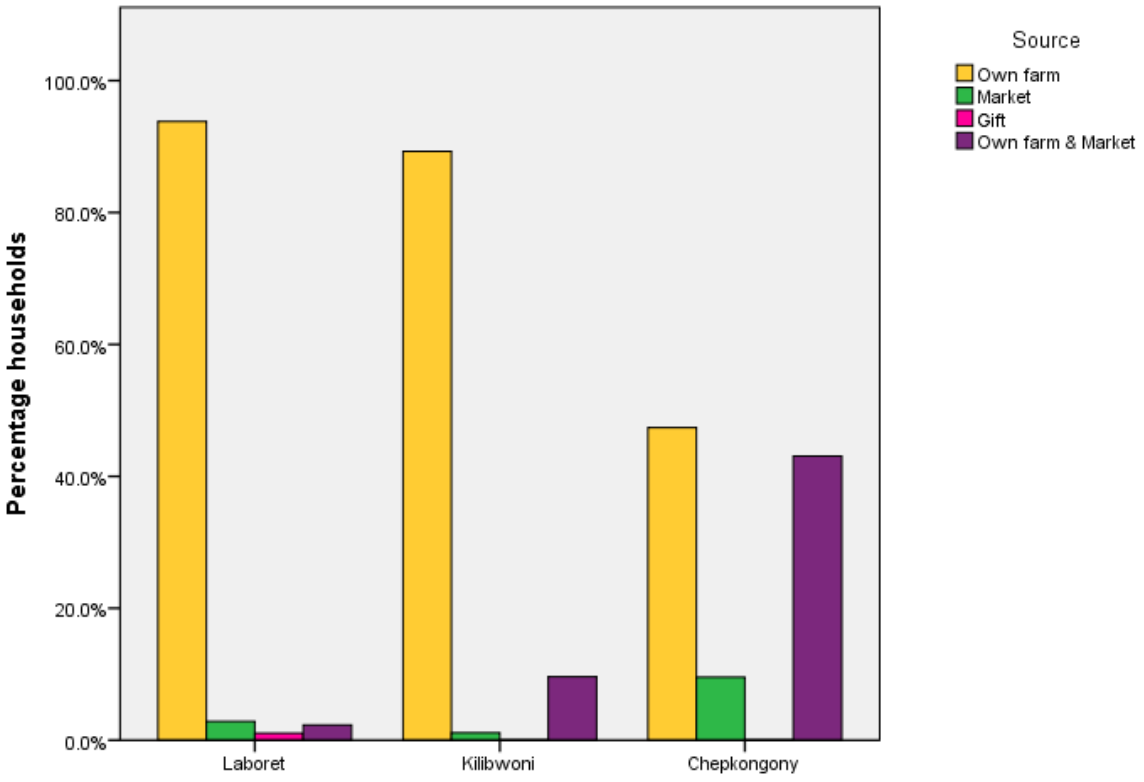

Figure 7. various sources of household maize and their percent frequencies in Laboret, Chepkongony and Kilibwoni study sub-locations

Table 9 Household and per capita consumption of maize flour in study sub-locations

Sub-locations	Total consumption (kg/day)	Household consumption (kg/household/day)	Per capita consumption (kg/person/day)
Laboret	144.82	1.43	0.26
Kilibwoni	117.08	1.86	0.33
Chepkongony	157.67	1.63	0.30

4.3.3 Household milk consumption

The chief source of household milk was from own farming (Fig. 8) and was consumed at an average of 0.5 Litres per day per person across the sub-locations. The household and per capita consumption of fresh whole milk is presented on Table 10.

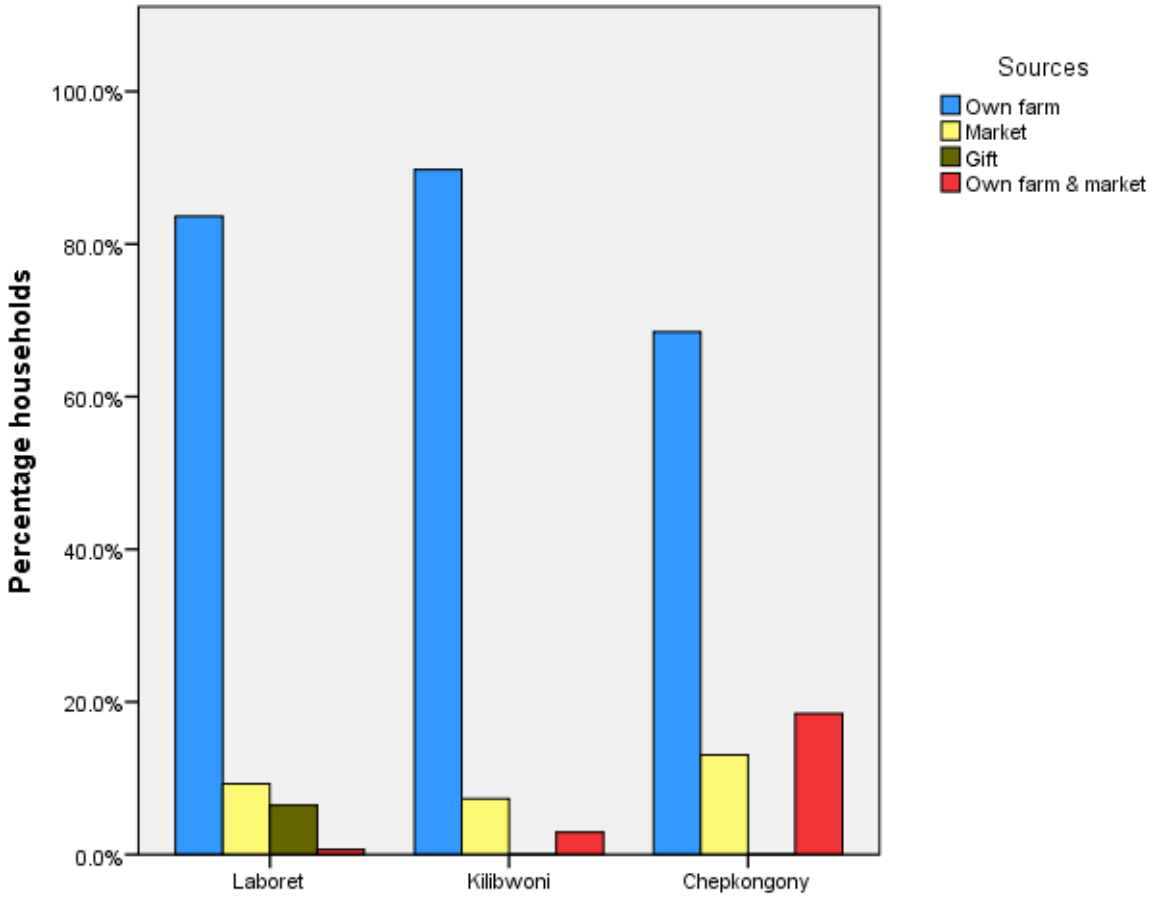

Figure 8. various sources of household milk and their percent frequencies in Laboret, Kilibwoni and Chepkongony

Table 10 Household and per capita consumption of fresh milk in study sub-locations

Sub-locations	Total consumption (Litres/day)	Household consumption (Litres/household/day)	Per capita consumption (Litres/person/day)
Laboret	252.23	2.50	0.55
Kilibwoni	161.79	2.57	0.48
Chepkongony	235.29	2.43	0.48

4.4 Knowledge, attitudinal and behavioral responses related to aflatoxin

4.4.1 Knowledge on recognition of spoilt grains

Colour change as the number one indicator of spoilt maize in the three sites was identified by 68.3%, 60.3% and 90.7% of the respondents from Laboret, Kilibwoni and Chepkongony respectively (Table 11). Of those, 7.2% (5/69), 52.6% (20/38) and 56.8% (50/88) from Laboret, Kilibwoni and Chepkongony respectively identified colours elaborated by *Aspergillus* i.e. black, brown, green or a combination of any of the three.

Table 11 Indicators of spoilt grains as per the respondents in the study sites

Criteria	Site		
	Laboret (n=101) %	**Kilibwoni (n=63) %**	**Chepkongony(n=97) %**
Colour change	68.3	60.3	90.7
Rotten	48.5	52.4	9.3
Bitter taste	3	6.3	5.2
Mouldy	6.9	7.9	15.5
Smell	1	6.3	19.6
Insect damage	8.9	11.1	22.7
Sprouting	2	4.8	3.1

4.4.2 Knowledge on risks associated with consumption of mouldy grains

The mentioned health risks associated with consumption of mouldy grains are presented in Table 12. Cancer as a health risk was identified by a small percentage of the respondents with none mentioning it from Kilibwoni.

Table 12 Risks associated with consumption of spoilt grains

Risk	**Laboret (n=10) %**	**Kilibwoni (n=63) %**	**Chepkongony (n=97) %**
Stomach upset	24.8^{a}	46.0^{b}	$33.0^{a,b}$
Aflatoxicosis	$12.9^{a,b}$	22.2^{b}	5.2^{a}
Cancer	6.9^{a}	$.0^{b}$	1.0^{b}
Death	2.0^{a}	19.0^{b}	2.1^{a}

Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the .05 level.

4.4.3 Knowledge on mitigation of aflatoxins

Responses on methods of mitigating aflatoxin effects in grains were varied with more than half from Chepkongony suggesting that mouldy maize be milled and mixed with good maize to make it safe (Table 13).

Table 13 Perceived methods of making mouldy grains safe for consumption

Action	Laboret (n=17)	Kilibwoni (n=32)	Chepkongony (n=11)
	%	%	%
Sorting	5.9[a]	3.1[a]	18.2[a]
Use of preservatives	35.3[a]	15.6[a]	9.1[a]
Early harvesting	17.6[a]	0[a]	0[a]
Milling and mixing with good grain	11.8[a]	43.8[b]	54.5[b]
Proper drying	35.3[a]	28.1[a]	9.1[a]
Proper storage	11.8[a]	9.4[a]	0[a]
Timing of cultivation	23.5[a]	15.6[a]	0[a]

Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the .05 level

4.4.4 Household perception on consumption of milk from cows fed on mouldy feed

Majority of the households felt that consumption of milk from cows fed on mouldy feed was safe (Table 14). The percentage that perceived the milk was safe was much lower in Laboret (51.5%) compared to Kilibwoni (76.2%) and Chepkongony (72.2%). The percentage not knowing the safety of that milk significantly varied across the study areas (p=0.000).

Table 14 Perception on consumption of milk from cows fed on mouldy feed

Safety	Laboret (n=101)	Kilibwoni (n=63)	Chepkongony (n=97)
Yes (%)	51.5[a]	76.2[b]	72.2[b]
No (%)	13.9[a]	20.6[a]	14.4[a]
Don't Know (%)	34.7[a]	3.2[b]	13.4[c]

4.4.5 Household practices related to aflatoxins

Significant difference was found in the storing, shelling and drying practices for grains (p=0.000). A greater percentage (58.8%) from Chepkongony preferred to store grains in granary with iron sheets whereas none stored in a crib. The percentage storing in thatched granary could not be differentiated across the sub-locations. Farming in Laboret was highly mechanized with 75% shelling their maize using machines (Table 15). On options for disposing spoilt grains, majority used it as animal feed or for making busaa (Table 16).

Table 15 Households practices about aflatoxins

Practice	Laboret (%)	Kilibwoni (%)	Chepkongony (%)
Store grains in a crib (n=15)	26.7[a]	73.3[b]	0[c]
Store grains in a granary with iron sheet (n=80)	22.5[a]	18.8[a]	58.8[b]
Store grains in thatched granary (n=18)	38.9[a]	33.3[a]	27.8[a]
Store grains in a bag (n=74)	47.3[a]	23.0[b]	29.7[b]
Shell grains manually by pounding (n=72)	5.6[a]	26.4[b]	68.1[c]
Shell grains by machine (n=104)	75[a]	25[b]	0[c]
Dry on ground on cob (n=23)	0[a]	73.9[b]	26.1[c]
Dry on ground no canvas (n=14)	35.1[a]	21.4[a]	42.9[a]
Dry on ground with canvas (n=182)	50[a]	17.6[a]	32.4[c]

Comparison of the percentages per row for each column is presented by the superscript letters; columns having same letter do not differ significantly from each other at the .05 level

Table 16 Percentage households disposing their spoilt grains using various options

Disposal option	Laboret (n=101)	Kilibwoni (n=63)	Chepkongony (n=97)
Animal feed	67.3[a]	81.0[a,b]	84.5[b]
Busaa	23.8[a]	22.2[a]	29.9[a]
Give away	1[a]	0[a]	0[a]
Throw away	4[a]	4.8[a]	10.3[a]
Leave it in the shamba	13.9[a]	9.5[a]	28.9[b]

4.5 Determination of aflatoxin levels using ELISA

4.5.1 Levels of total aflatoxin (B1, B2, G1, G2) in maize

A total of three hundred and seven maize samples were analyzed in total. Of these, 39 samples were from market and posho mills. More than 60 percent of total household samples across the study tested positive for aflatoxins with a range of 0.17 – 5.3 ppb (Table 17). Market samples were all within the acceptable limits of 10 ppb. However, more than half of the samples tested positive (Table 18).

Table 17 Aflatoxin in maize in the various sub-locations

Sub-location	Percentage positive	Range (ppb)	Mean	Test statistic
Laboret (n=106)	67.9	0.18 – 3.6	1.05	0.144
Kilibwoni (n=60)	73.3	0.19 – 5.3	1.10	
Chepkongony (n=102)	65.7	0.17 – 3.2	0.83	

Table 18 Percentage positive, range and mean of total aflatoxins in market maize from study sites

Sub-location	Percentage positive	Range (ppb)	Mean
Laboret (n=12)	66.7	1.2 – 1.8	1.608
Kilibwoni (n=13)	69.2	0.7 – 2.1	0.841
Chepkongony (n=14)	71.4	0.6 – 2.8	1.153

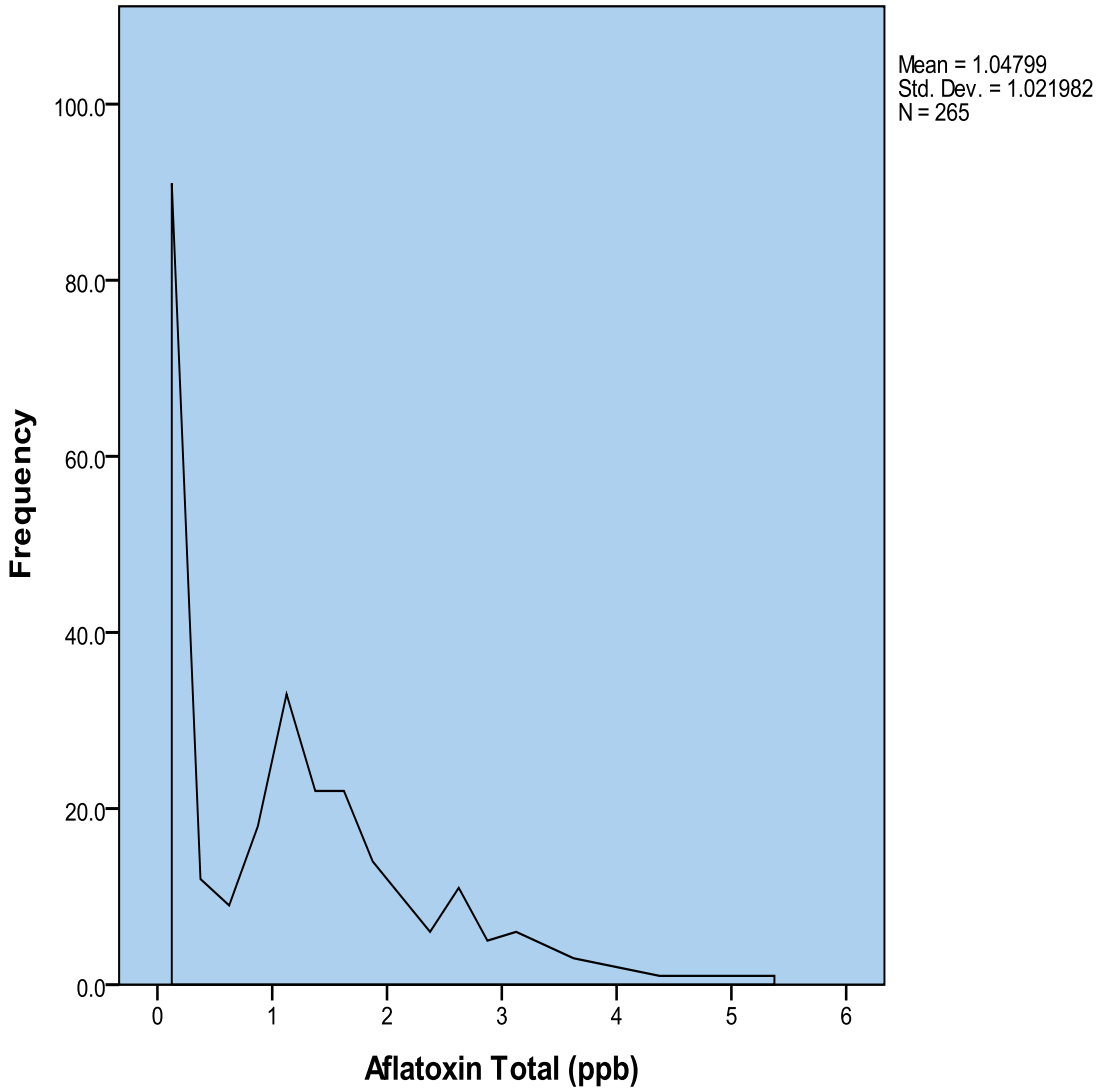

Figure 9. Distribution of total aflatoxin in maize in Nandi County

4.5.2 Levels of total aflatoxin in sorghum and millet

Levels of aflatoxin in sorghum analyzed ranged from 0.15 to 210.1 ppb. Kilibwoni had the highest percent (45.5%; 10/18) of samples exceeding maximum tolerance limit of 10 ppb set by KEBS (Table 19). Thirty nine millet samples were analyzed in total with levels ranging from 0.14 to 6.4 ppb. None of the samples exceeded the 10 ppb limit (Table 20). Five samples had sorghum and millet combined, they all tested positive for aflatoxins with highest sample having 11.1 ppb aflatoxin.

Table 19 Summary of total aflatoxin results in sorghum in the study sub-locations

Sub-location	Range (ppb)	Mean	Percent below 10 ppb	Percent exceeding 10ppb
Laboret(n=18)	1.9 – 210.1	48.36	36.4	27.3
Kilibwoni(n=22)	0.15 – 170.8	20.62	27.3	45.5
Chepkongony(n=22)	0.21 - 74	8.91	36.4	27.3

Table 20 Levels of total aflatoxin in millet

Sub-location	Percentage positive	Range	Mean
Laboret (n=14)	92.9	0.14 – 2.9	1.47
Kilibwoni (n=9)	100	0.42 - 2	1.07
Chepkongony (n=16)	87.5	0.45 – 6.4	1.47

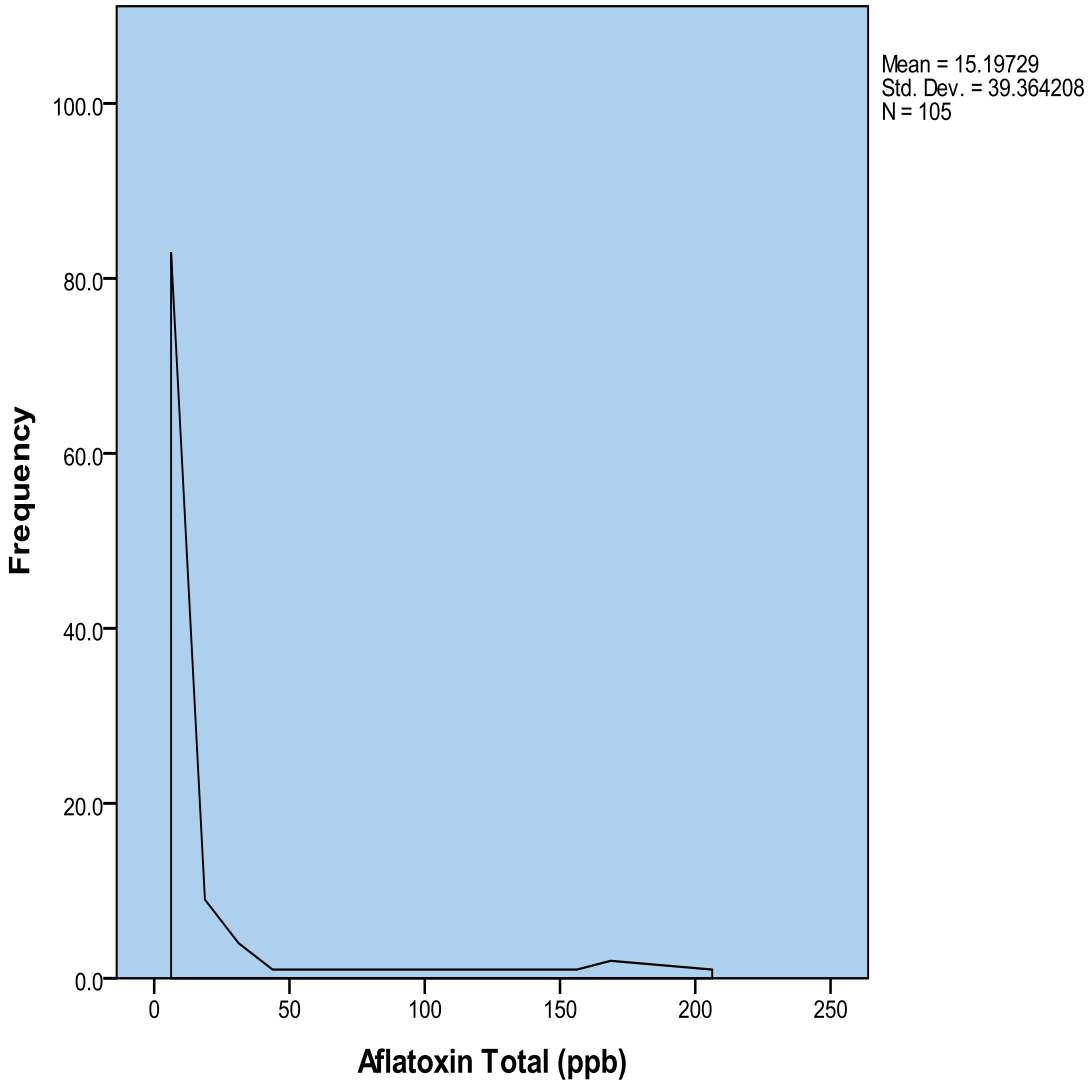

Figure 10. Distribution of total aflatoxin in millet and sorghum in Nandi County

4.5.3 Levels of aflatoxin M1 in cow milk

A total of two hundred and sixty four raw fresh milk samples were analyzed. Of these, samples from Chepkongony had the highest percent positives (55.7%). However, none of the samples tested exceeded the maximum allowable limit for FAO/WHO of 50 ppt (Table 21).

Table 21 Summary of aflatoxin M1 results in fresh cow milk

Sub-location	Percentage positive	Range (ppt)	Mean	Test statistic
Laboret (n=103)	50.5	0.002– 22.6	1.29	0.716
Kilibwoni (n=65)	49.2	0.002 – 22.6	1.73	
Chepkongony (n=97)	55.7	0.05 – 23.1	1.31	

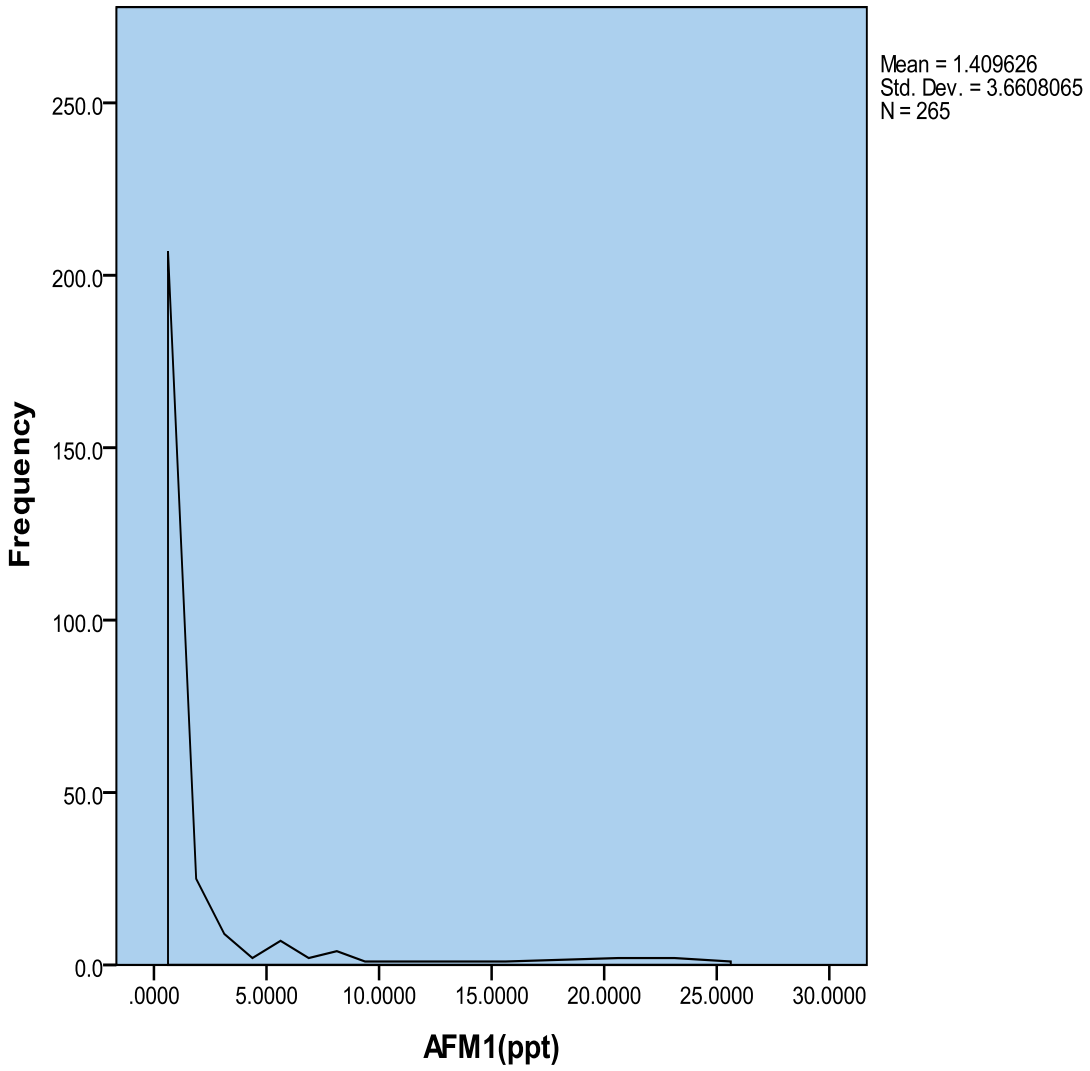

Figure 11. Distribution of Aflatoxin M1 in cow milk in Nandi County

4.5.4 Levels of aflatoxin M1 in human milk

A total of 67 human milk samples were collected and analyzed, the distribution of aflatoxin M1 across the sub-locations is shown in Fig. 11. Table 22 gives the percentage positives, range and mean in Laboret, Kilibwoni and Chepkongony sub-locations.

Table 22 Summary of aflatoxin M1 results in human milk in study sub-locations

Sublocation	Percentage positive	Range (ppt)	Mean	Test statistic
Laboret (n=23)	73.9	0.003–3.4	0.75	0.533
Kilibwoni (n=14)	57.1	0.132–3.7	0.56	
Chepkongony (n=30)	43.3	0.146–2.4	0.46	

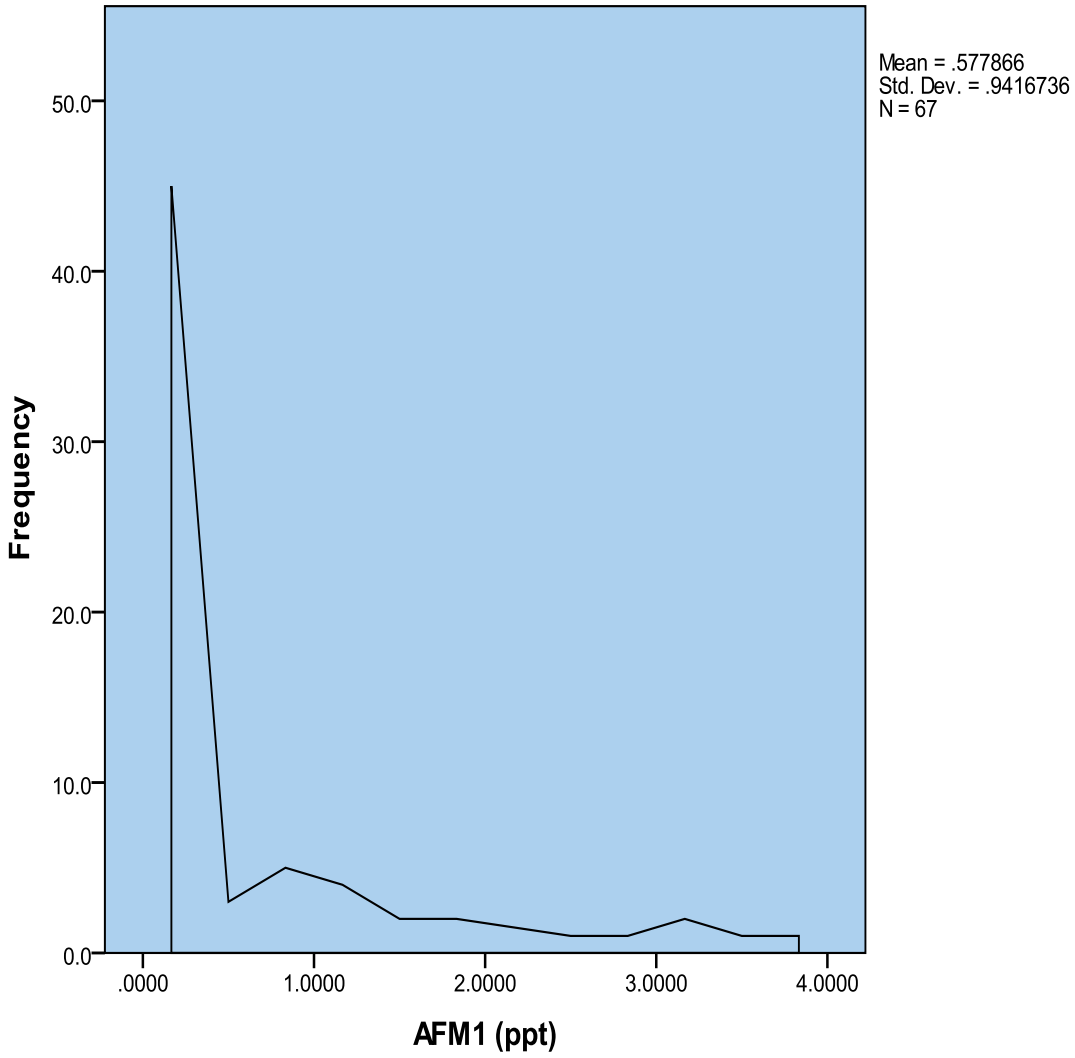

Figure 12. Distribution of AFM1 in human milk in Nandi County

4.5.5 Levels of aflatoxin M1 in urine

Table 23 gives the percentage positive, range and mean of aflatoxin M1 determined in urine of children. A total of 245 children had their urine collected and analysed.

Table 23 Summary of aflatoxin M1 results in urine of children below 5 in study sub-locations

Sub-locations	Percentage positive	Range (ppt)	Mean	Test statistic
Laboret (n=98)	94.9	2.1 – 6089.5	659.56	0.178
Kilibwoni (n=63)	88.9	10.6 – 7022.5	512.33	
Chepkongony (n=84)	88.1	4.9 – 637	356.52	

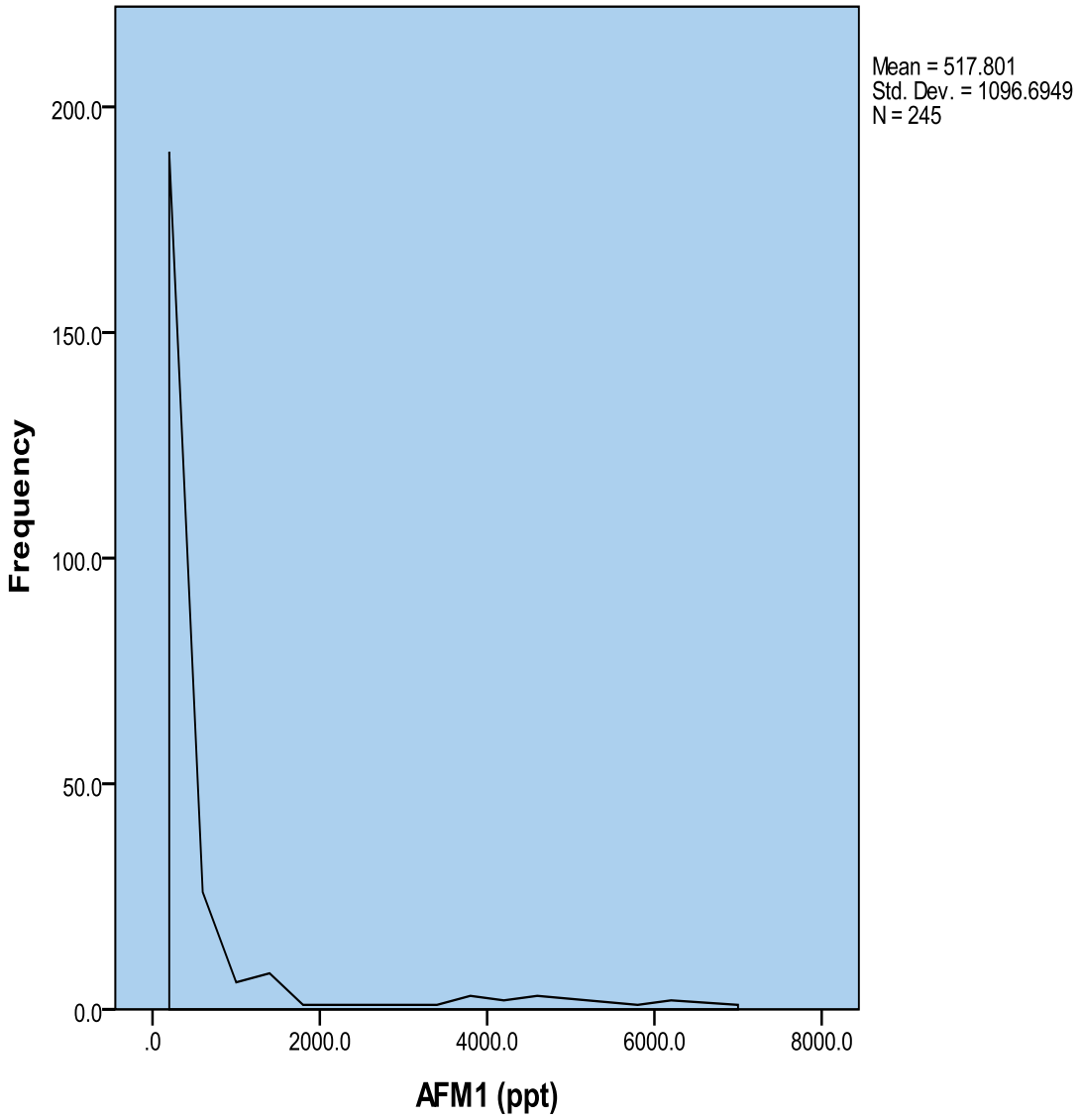

Figure 13. Distribution of aflatoxin M1 in urine of children less than 5 years in Nandi County

4.6 Nutritional status of children

Nutritional status of children under five years as indicated by height for age, weight for age and weight for height Z scores is presented on Tables 24, 25 and 26 respectively. Laboret had the highest percent of stunted children (44.7%) whereas Chepkongony had the highest percentage of children who were extremely stunted (19.2%). However, the proportions of children stunted, wasted and underweight did not differ significantly from each other across the study sites ($p>0.05$).

Table 24 Prevalence rates for stunting

Sub – locations	**Height –for–age (HAZ)**		
	Percentage below -3SD	Percentage below -2SD	Mean Z- score (SD)
Laboret(n=38)	15.8	44.7	-0.88
Kilibwoni(n=32)	15.6	31.2	-1.45
Chepkongony(n=26)	19.2	30.8	-0.49

Table 25 Prevalence rates for being underweight

Sub-locations	**Weight-for-age (WAZ)**			
	Percentage below-3SD	Percentage below-2SD	Percentage above +2SD	Mean Z- score (SD)
Laboret(n=38)	0	0	7.9	0.15
Kilibwoni(n=32)	3.1	9.4	9.4	-0.3
Chepkongony(n=26)	3.8	3.8	7.7	0.1

Table 26 Prevalence rates for wasting

Sub-locations	**Weight–for–height (WHZ)**			
	Percentage below-3SD	Percentage below -2SD	Percentage above +2SD	Mean Z- score (SD)
Laboret(n=38)	2.6	2.6	31.6	1.39
Kilibwoni(n=32)	3.1	3.1	21.9	0.83
Chepkongony(n=26)	3.8	11.5	30.8	0.61

4.7 Factors associated with levels of aflatoxins

There was no significant association between various practices on grain handling or processing and aflatoxin contamination (Appendix 6).

Stunted, underweight and wasted children were less likely to have aflatoxins in their urine compared to those who were not (Table 27).

Table 27 Shows association between stunting, underweight, wasting and aflatoxin M1 in urine

	Aflatoxin		**Relative Risk (RR)**
Nutritional indicator	Positive	Negative	
Stunting			
Yes	33	2	0.9
No	56	5	
Underweight			
Yes	3	1	0.7
No	86	6	
Wasting			
Yes	4	0	0.8
No	84	7	

4.8 Human exposure estimates

Exposure estimates for milk and maize flour were calculated based on the average household consumption and aflatoxin levels. Kilibwoni had the highest exposure estimates from maize flour in µg/hh/day (Table 28). Table 29 shows the aflatoxin exposure estimates from milk.

Table 28 Exposure estimates to aflatoxin from maize flour in Laboret, Kilibwoni and Chepkongony

Sub-location	Mean household aflatoxin total (µg/kg/hh)	Household consumption (kg/hh/day)	Exposure estimate (µg/kg/hh/day)	Exposure estimate (µg/kg/person/day)
Laboret	1.13	1.43	1.6	0.3
Kilibwoni	1.22	1.86	2.3	0.4
Chepkongony	0.87	1.63	1.4	0.2

Table 29 Exposure estimates to aflatoxin from fresh milk in Laboret, Kilibwoni and Chepkongony

Sub-location	Mean household aflatoxin M1 (µg/l/hh)	Household consumption (l/hh/day)	Exposure estimate (µg/l/hh/day)	Exposure estimate (µg/l/person/day)
Laboret	0.00129	2.50	0.003	0.0005
Kilibwoni	0.00173	2.57	0.004	0.0007
Chepkongony	0.00131	2.43	0.003	0.0005

The levels in maize flour and milk reflect on the risk of chronic exposure to aflatoxin in the study sub-locations especially considering maize is a staple food and milk is consumed by majority of the households.

4.9 Determination of aflatoxin levels using HPLC

Table 30 Comparison of HPLC and ELISA results

SAMPLE	HPLC			ELISA		
	Positive (%)	**Mean**	**SD**	**Positive (%)**	**Mean**	**SD**
Milk						
Cow (n=4)	100	0.01	0.013	100	1.00	0.00
Breast (n=2)	100	0.00	0.000	100	0.002	0.0003
Maize						
Household (n=61)	19.7	0.26	3.898	67.2	1.048	1.0407
Market (n=10)	80	56.36	88.702	90	27.631	54.4935

5.0 Correlation between ELISA and HPLC tests

There was a positive correlation between aflatoxin results measured by ELISA and HPLC (Table 31). On regression analysis, ELISA test was found to positively predict HPLC outcome (R^2=0.462, P<0.05; see Appendix 2).

Table 31 Correlation matrix between ELISA and HPLC tests

Correlations

		HPLC results	ELISA results
HPLC results	Pearson Correlation	1	.236**
	Sig. (2-tailed)		.005
	N	155	141
ELISA results	Pearson Correlation	.236**	1
	Sig. (2-tailed)	.005	
	N	141	141

**. Correlation is significant at the 0.01 level (2-tailed).

CHAPTER 5

DISCUSSION

In this study, human exposure to aflatoxins was evaluated through analysis of dietary intake of maize, sorghum, millet and milk. Knowledge, attitudes and practices related to aflatoxin were identified, and association between the practices and aflatoxin levels determined. Nutritional status of children under five was also determined, and association determined between stunting, wasting, underweight and aflatoxin M1 in urine.

Mean household (hh) size of six was found to be larger than the national average Kenyan household size of 4.7 for rural areas (KDHS, 2009). The large hh results in enormous pressure on limited resources for meeting health and nutritional requirements (CBS, 2009). Male : female distribution of the hh was at a ratio of 1:1 across the three sub-locations, this distribution was in agreement with CBS (2009) general population estimate of 51% females and 49% males (1 female: 1 male).

Education levels differed significantly across the study sub-locations. Kilibwoni had the highest percentage of population having both primary (60.4%) and university education (1.4%); this could be attributed to high income and asset ownership in the area and thus parents can afford to pay school fees. Across the three sub-locations there was a low transition from primary to secondary. This implies that the majority are dropping out of school after primary education. The low literacy has implications on hh acquisition and understanding of general information related to aflatoxin mitigation. This therefore increases their vulnerability to aflatoxin exposure. In 2009 Kenya Demographic and Health Survey (KDHS), women with little or no education have low access to certain types of media (newspaper, radio and television) as compared to those who had attained secondary and tertiary education (CBS, 2009).

Majority of the respondents in the study relied upon sources of income across the study included livestock/fish/milk sales and crop sales (Figure 6). Farmers should be encouraged to practice cost effective farming in order to reap maximum benefits thus alleviating poverty. Across the study areas, half the population are considered poor. The actual poverty levels are: 50% Nandi North, 54% Nandi Central and 50% Nandi South (Nandi North, Central and South district development plans, 2009).

Most study participants (68.3% Laboret, 60.3% Kilibwoni, and 90.7% Chepkongony) across the study recognized change of colour in the grains as an indicator of spoilt maize. The colour changes observed relate to the growth of certain fungal species (Fusarium and Aspergillus) and possible aflatoxin production and contamination of the grains. Substantial percentages of the respondents (7.2% Laboret, 52.6% Kilibwoni, and 56.8% Chepkongony) were able to state those colours that are produced during growth by *Aspergillus* species. *Aspergillus nomius* produces light green colonies; *A. caelatus* and *A. tamarii,* both being non-aflatoxin producing, produce yellow-brown colonies on 5/2 agar plates (Cotty and Cardwell, 1999). *A. flavus* and *parasiticus* both produce bright orange reverse on AFPA (*Aspergillus flavus* and *Parasiticus* Agar; Pitt *et al*., 1983). *Aspergillus* growing on maize elaborates black, brown, green or a combination of any of the three colours. The practice of hand picking visibly coloured/spoilt grains coupled with density segregation can result in reduction of 70-90% of mycotoxins (Jouany, 2007). However, sorting was recognized by only 5.9%, 3.1% and 18.2% of the respondents from Laboret, Chepkongony and Kilibwoni sub-locations.

Insect damage was identified by a lower percentage of the hh (8.9% Laboret, 11.1% Kilibwoni, and 22.7% Chepkongony) as an indicator of grain spoilage. Damage by insects plays an important role in aflatoxin contamination of grains by reducing the resistance provided by the testa to fungal penetration and also it helps create a microclimate favourable

for growth and production of mycotoxins by fungi during storage (Tuite, 1983). However, some of the farmers mitigated insect infestation and grain damage during storage by applying chemical preservatives (35.3% Laboret, 15.6% Kilibwoni, and 9.1% Chepkongony).

A higher percentage of respondents (24.8% Laboret, 46.0% Kilibwoni and 33.0% Chepkongony) mentioned stomach upset as a primary risk of consuming spoilt grain compared to the other risks. This is generally true for any food substance if consumed when spoilt. Only 6.9% and 1% of all the respondents in Laboret and Chepkongony respectively identified cancer as a risk arising from consumption of mouldy grains. None from Kilibwoni mentioned it. This indicates lack of awareness on mycotoxin exposure and effects arising from consumption of mouldy grains. Wakhisi *et al.*, (2005) found high incidences of esophageal cancer in North Rift valley which is associated with fumonisin exposure. Considering that there has been no past incidences of reported aflatoxicosis in Nandi, combined with low knowledge base among the respondents, there is need to mount campaigns to raise awareness and methods of mitigating aflatoxin contamination of grains. The lack of knowledge was also evident from the response that milk obtained from cows fed spoilt grains presented no health risk (51.5% Laboret, 76.2% Kilibwoni, and 72.2% Chepkongony). The low knowledge about aflatoxin was also reported by Kang'ethe and Lang'at, (2009) in studies in Eldoret, an urban centre near the study sites.

On measures to decontaminate aflatoxin contaminated grains, responses given referred to measures to prevent contamination (early harvesting, proper drying and storage) rather than decontamination of already contaminated grains.

Storage options significantly differed across the sub-locations ($p=0.000$). A greater percentage (54.7%) in Laboret stored maize in bags whereas those in Kilibwoni and Chepkongony preferred to store the maize in cribs and granaries. The granaries and cribs are

raised above ground increasing air circulation thus promoting drying of the grains which minimizes fungal growth (Diener *et al*., 1987).

None of the hh was destroying spoilt grains, rather it was being used or sold as animal feed by most (67.3% Laboret, 81.0% Kilibwoni and 84.5% Chepkongony). If contaminated with aflatoxin, it can lead to poisoning, as aflatoxin can accumulate in animal products (El-Sayed *et al*., 2000). Spoilt maize was also used to make busaa which further increases risk of exposure to aflatoxins should the grains be contaminated.

Over 65% of the maize samples were positive for total aflatoxin across the sub-locations; ranging from 0.18 – 3.2ppb. All were within maximum tolerance limits for KEBS of 10ppb. These results are comparable with a survey conducted by Muthomi *et al*., (2012) who were unable to detect any aflatoxin in maize but were able to isolate *Aspergillus* species that are potential toxin producing. The fact that in this study only low level of aflatoxin were found, consumption of maize thus contaminated could lead to chronic exposure to aflatoxins over time.

Sorghum had highest contamination levels of aflatoxins across the study areas compared to maize and millet (maximum level of 210.1 ppb). Kilibwoni had the highest percent (45.5%) of sorghum samples exceeding KEBS regulatory limits of 10 ppb aflatoxin. The overall mean of 26.0 ppb of aflatoxins in sorghum is much higher than 15.2 ppb found by Kitya *et al*., (2010) in sorghum samples collected from southern Uganda. The high aflatoxin contamination in sorghum could have been due to contamination of market sorghum as majority of the households bought their sorghum from the local markets (69.4% Laboret, 72.6% Kilibwoni and 49.7% Chepkongony). This is further supported by a study by Okoth and Kola, (2012) who found market maize to be a source of chronic exposure to aflatoxins in

Kenya. Millet samples were contaminated with levels (range 0.14 to 6.4 ppb) within KEBS limits of 10 ppb.

Low levels (0.002 to 23.1 ppt) of aflatoxin M1 were detected in cow milk across the study sub-locations. Study conducted in Egypt, Greece and Iran had levels that were comparable to these results where most samples were within regulatory limits (Egypt-El-Sayed *et al*., 2000; Greece –Roussi *et al*., 2002; Iran-Fallah, 2010). Moreover, levels of aflatoxins were reported in fresh milk collected from urban centers in Kenya, where 20% of the milk from dairy farmers had levels exceeding WHO/FAO levels of 50ppt. The high levels of aflatoxins in milk were as a result of urban farmers using commercial feeds which risked being contaminated with aflatoxin than spoilt maize fed to animals in the study sites (Kang'ethe and Lang'at, 2009).

Human milk was also found to be contaminated with aflatoxin with Kilibwoni having the highest proportion of positives (55.7%), overall the levels ranged from (0.003 – 3.7ppt). These levels are generally higher compared with a study done to analyze collected human milk from nursing mothers in Africa, where the levels ranged from 0.00002 to 0.0018 ppt (Somogyi and Beck, 1993). The fact that on average more than 50% of the human milk tested positive indicates a potential risk of chronic exposure to children less than one year.

More than 80% of the urine samples analysed from children under five was positive for AFM1. These results show that children get exposed to aflatoxin at a very young age, a situation which has been linked to impaired growth (Gong *et al*., 2004). The children as well also get weaned to sorghum and/or millet porridge at an early age which increases their exposure considering the high levels detected in the dietary sorghum compared to maize. In this study no association was found between children having aflatoxin M1 in their urine and them being stunted, underweight or wasted (RR=0.9 stunting; RR=0.7 underweight; RR= 0.8

wasting). This is in disagreement with a study by Okoth and Ohingo (2004) who found an association between aflatoxin in children and wasting. The prevalence of nutritional indicators (36.4% stunting, 4.1% underweight and 5.2% wasting) were found to be comparable with the national average of 39% for stunting, 16% for underweight and 7% for wasting in children less than five years of age (KDHS, 2009).

Muriuki and Siboe (1995) found consumption of maize meal in Nairobi to be 0.4 kg/person/day, which is comparable to 0.3 kg/person/day found in this study. This implies there is chronic exposure to high levels of aflatoxins considering an average exposure of 0.4 µg/kg/person/day in maize meal. Milk consumption at an average of 0.5 l/day/person also exposes consumers to a risk of chronic exposure to aflatoxins as it translated to a per person exposure of 0.0006 µg/l/day. Chronic exposure to aflatoxins has adverse health consequences, such as liver disease and hepatocellular carcinomas (Barrett, 2005).

In Kenya currently, chronic exposure to aflatoxins is a real danger (Okoth and Kola, 2012) but this has been overshadowed by incidences of acute exposure (Nyikal *et al*., 2004). There is urgent need to raise awareness on the public health effects of chronic exposure to aflatoxins and for strict monitoring of aflatoxin levels in various susceptible foods be done in the country.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Regarding this study, the following are concluded:

i. The population in Laboret, Kilibwoni and Chepkongony sub-locations comprise of large households and their livelihood depends on livestock/fish/milk and crop sales.

ii. Respondents in the study area had low level of education and a low transition rate from primary to secondary school levels.

iii. Residents of the study areas are on average exposed to aflatoxin levels of 0.4 µg/kg/person/day and 0.0006 µg/l/person/day when they consume 0.3 kg of maize flour and 0.5 litres of milk per day respectively.

iv. Knowledge about aflatoxin is low across the study sub-locations.

v. There is a risk of chronic exposure to aflatoxins by milk and maize consumers in the study areas.

vi. Sorghum was found to have high aflatoxin levels compared to maize and millet and this poses a health risk to consumers.

vii. Majority of the children in the study sub-locations were found to suffer from malnutrition and there was evidence that they are exposed to aflatoxin at a young age from the determined levels in human milk and urine.

6.2 Recommendations

i. Civic education should be conducted in the study areas to raise awareness on aflatoxicosis and mitigation measures.

ii. Stringent monitoring of food meant for human consumption should be done to prevent exposure.

iii. Regulations on aflatoxins limits should be made for infant foods to prevent exposure resulting in stunting.

iv. The Ministry of Agriculture should promote Good Agricultural Practices to prevent grain contamination with aflatoxins.

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Appendix 1: Questionnaire template for household survey

Household Head Name:					
Enumerator Name:		**HHID:**		**DATE:**	**/ /20__**

A. *Household Location*

District	**Division**	**Location**	**Sub-location**
1=Laboret 2= Kilibwoni 3=Chepkongony	1=Kipkarren 2= Kilibwoni 3=Kaptumo	1= Laboret 2= Kilibwoni 3=Kaptumo	1= laboret 2= Kilibwoni 3= Chepkongony

B. Household ID and Characteristics

Members of household	**Name**	**Position in the Household**	**Age**	**Sex**	**Formal education**
1. 2.					
		1=Household Head 2=Spouse of Household Head 3=Child of Household Head 4=Relative of Household Head 5=Other (Specify above)	(Years)	1=Male 2=Female	1 = Primary 2 = Secondary 3 = Post-secondary 4 =University/tertiary 5 = Other

C. Foods consumption

Members of household reference number	**Food item**	**Source(s)**	**Preparations**	**Quantity consumed**	**Frequency of consumption /day**
1. 2.					
	1=Maize 2=Milk 3=Sorghum	1 = Own farm 2. = Market 3. = Food relief 4. = Gift	1= Ugali 2= Uji 3=Muthokoi 3= Mursik 4= fresh milk 5=Sour milk 6=Others(specify)	<u>Units see photograph</u> 1= 2.0kg 2=1.75kg 3= 1.5kg 4=1.25kg 5= 1.0 Kg 6=0.75kg 7= 0.5kg 8=0.25kg 9= Milk/uji/mursik (indicate number of cups taken/day)	Record number of times mentioned

D. Household income by source

Category	Tick	Category	Tick
Crops (grains/seeds) sales		Self employed	
Fruits and vegetables sales		Remittances	
Livestock/fish/milk sales		Other (specify)	
Paid employment			

E. Asset ownership

Asset item	Units (or pairs)	Estimated value KShs*	Asset item	Units (or pairs)	Estimated value KShs*	Main House type	Number of units
Commercial motor veh.			Tractor			Mud / thatch	
Private motor vehicle			Tractor trailer			Mud /iron sheets	
Motor cycle			Tractor plough			Timber /iron sheets	
Bicycle			Tractor harrow			Stone/Iron sheets	
Television			Bullock/donkeys /horse			Wood/thatch	
Radio			Bullock/ donkey plough			Iron sheet	
Private well			Bullock/ donkey harrow			Bricks/iron sheets	
Private borehole			Bullock/ donkey cart				
Water pump			Wheel barrow				
Cultivator			Mobile Phones				
Diesel pumps			Fixed phone				
Water tanks			Other (specify)_____				
Generator							

***To be obtained from market prevailing rates/ or ask community KI**

F. Farming practices

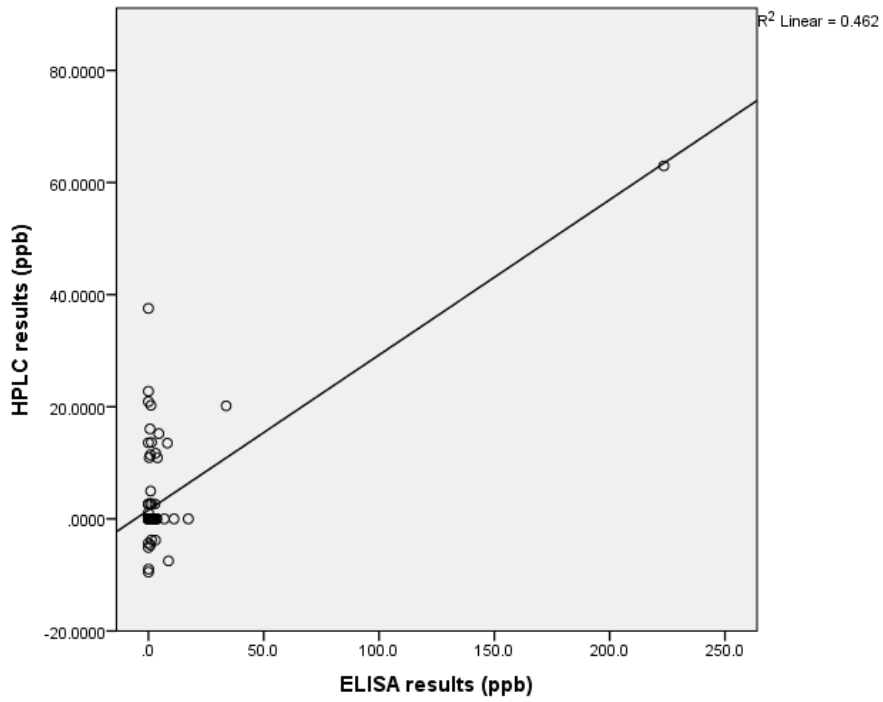
Crop/Commodity	**Storage**	**Shelling**	**Drying**	**Disposal of spoilt grains**
	1. = Crib 2. =Granary/iron sheet 3. = Thatched granary 4. = Bag	1= Hand 2 = Machine 3= Manual by pounding	1. = On ground with canvas 2. = On ground no canvas 3. = On ground on cob	1. = Throw away 2. = Animal Feed 3. =Make busaa 4. = Give away 5= Leave it in the shamba

G. Household Perceptions on spoilt grains and human health risks associated with consumption of mouldy grains

Food Grain	**Criteria for "SPOILT"**			**If consumption of mouldy grain can cause health problems, which ones?**			**If mouldy grain can be made safer, how?**		
	Criterion 1	Criterion 2	Criterion 3	Risk1	Risk 2	Risk 3	Action 1	Action 2	Action 3
1.									
2.									
3.									

H. If you feed mouldy grain or feed to dairy cows is the milk safe to drink? (Yes /No)

Appendix 2: HPLC/ELISA Scatter plot and regression outputs



Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.680[a]	.462	.456	6.6717274

a. Predictors: (Constant), ELISA (ppb)

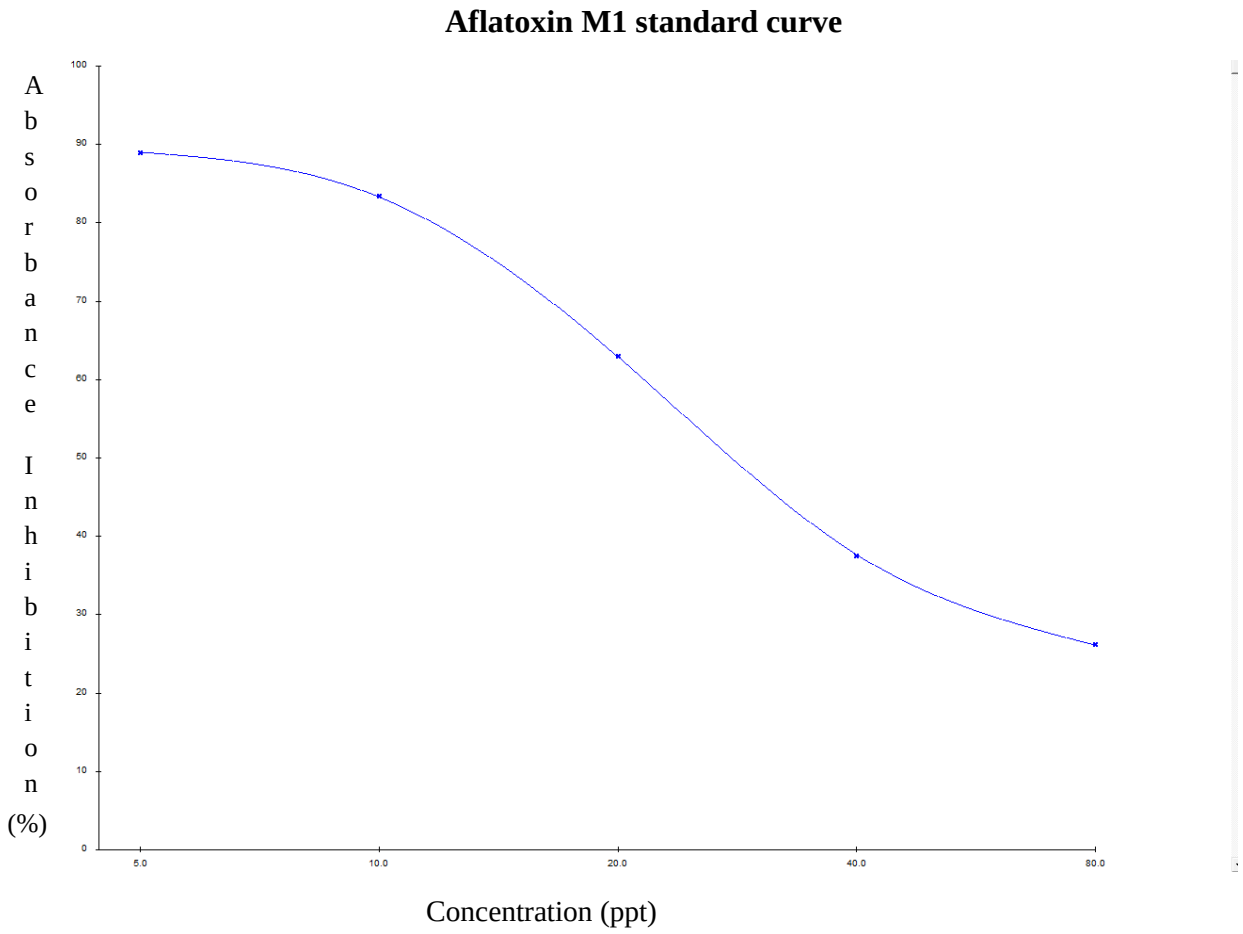
Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.559	.668		2.334	.022
	ELISA (ppb)	.277	.030	.680	9.308	.000

a. Dependent Variable: HPLC (ppb)

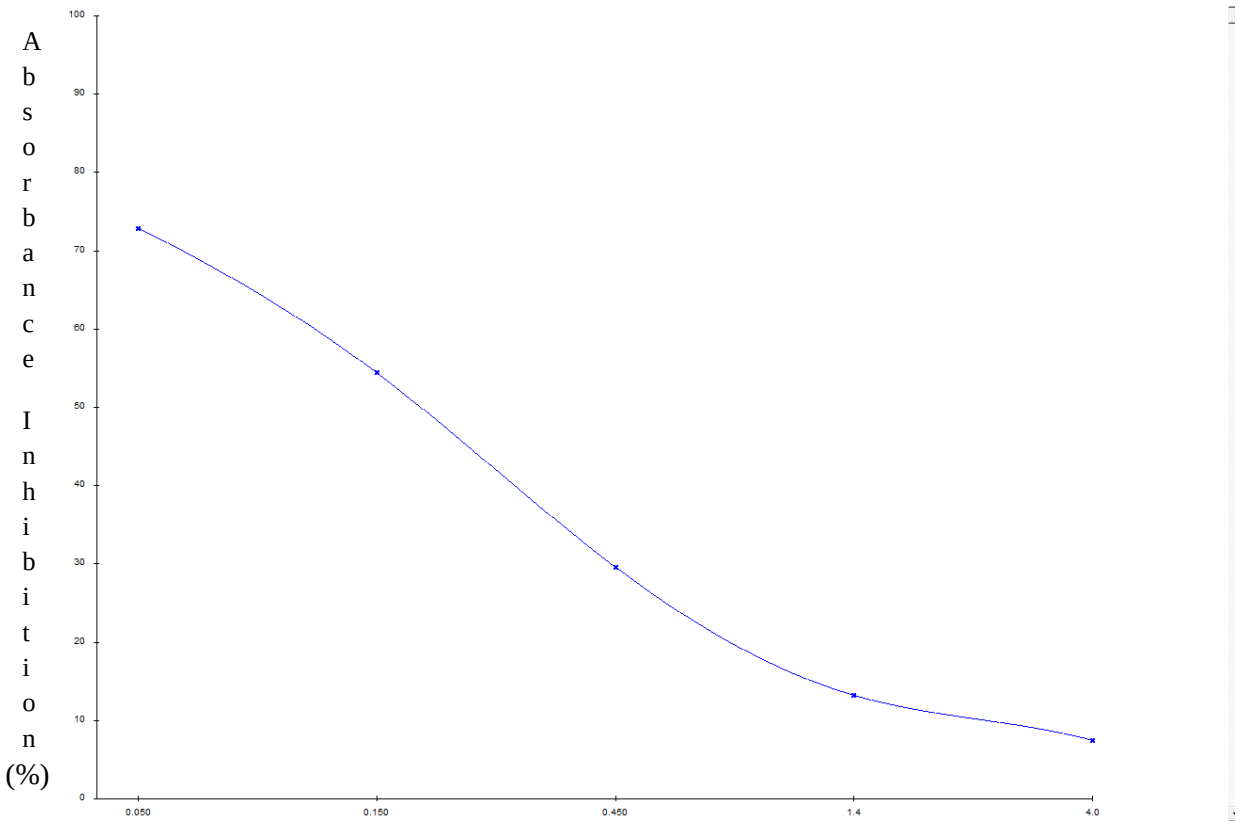
Regression equation (y= bx + a) is **y= 0.277(x) + 1.559**

Appendix 3: ELISA Standards curve



Standards(Std)	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Concentration (ppt)	0.00	5.00	10.00	20.00	40.00	80.00

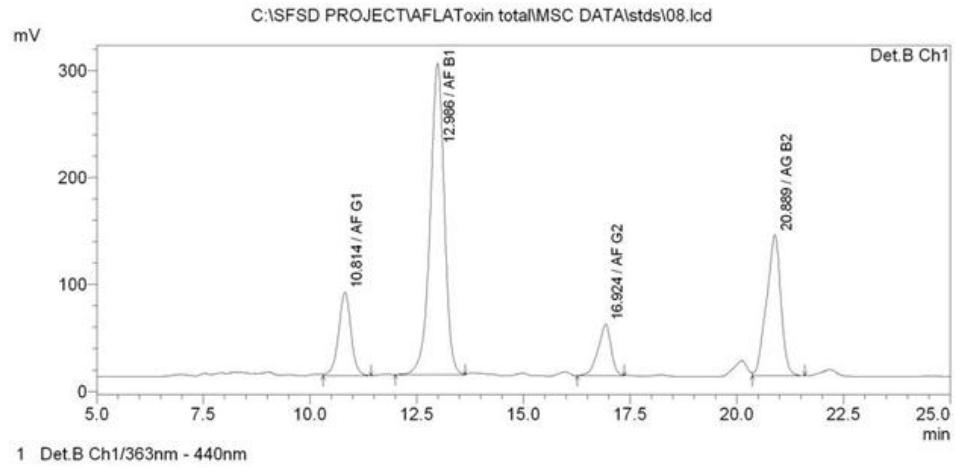
Total Aflatoxins standard curve



Standards(Std)	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Concentration (ppb)	0.00	0.05	0.15	0.45	1.35	4.05

Appendix 4: HPLC Total Aflatoxins standard chromatogram

<Chromatogram>



1 Det.B Ch1/363nm - 440nm

PeakTable

Detector B Ch1 363nm - 440nm

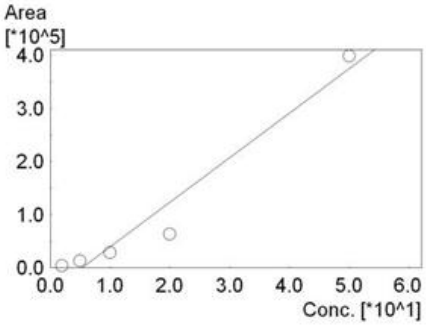
Peak#	Ret. Time	Name	Area	Height	Conc.	Units	at 50%
1	10.8139	AF G1	1569734	77762	199.6638	ng/ml	0.302
2	12.9857	AF B1	6722350	290764	203.9946	ng/ml	0.346
3	16.9240	AF G2	1021430	47891	50.0719	ng/ml	0.322
4	20.8885	AG B2	3078554	131474	50.9602	ng/ml	0.362
Total			12392068	547890			

Appendix 5: HPLC standard curves

==== Shimadzu LCsolution Calibration Curve ====

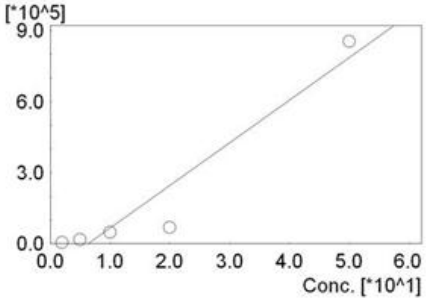
<Calibration Curve>

ID# : 1
Name : AF G1
Quantitative Method : External Standard
Function : f(x)=8384.39*x-44273.4
Rr1=0.9752889 Rr2=0.9511885
MeanRF:3767.82 RFSD:2373.14 RFRSD:62.9844
FitType : Linear
ZeroThrough : Not Through
WeightedRegression : None
Detector Name : Detector B



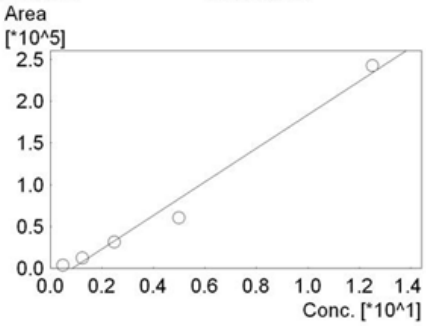
#	Conc (Ratio)	Area	Area
1	2.000	4373.2	4373
2	5.000	13304.6	13305
3	10.000	28326.0	28326
4	20.000	63919.0	63919
5	50.000	398152.8	398153

ID# : 2
Name : AF B1
Quantitative Method : External Standard
Function : f(x)=18054.1*x-115496
Rr1=0.9575992 Rr2=0.9169963
MeanRF:6293.9 RFSD:6067.74 RFRSD:96.4067
FitType : Linear
ZeroThrough : Not Through
WeightedRegression : None
Detector Name : Detector B



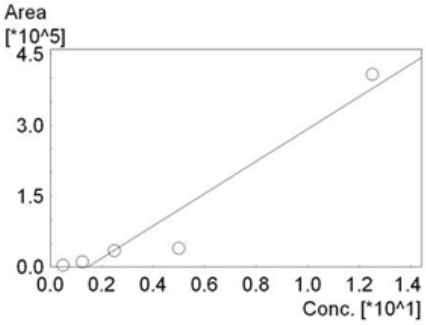
#	Conc (Ratio)	Area	Area
1	2.000	5384.3	5384
2	5.000	17526.2	17526
3	10.000	47227.6	47228
4	20.000	69582.2	69582
5	50.000	853510.9	853511

ID# : 3
Name : AF G2
Quantitative Method : External Standard
Function : f(x)=20077.2*x-17140.7
Rr1=0.9905955 Rr2=0.9812795
MeanRF:12442.8 RFSD:4273.13 RFRSD:34.3423
FitType : Linear
ZeroThrough : Not Through
WeightedRegression : None
Detector Name : Detector B



#	Conc (Ratio)	Area	Area
1	0.500	4031.3	4031
2	1.250	12595.4	12595
3	2.500	31388.2	31388
4	5.000	60691.1	60691
5	12.500	242269.0	242269

ID# : 4
Name : AG B2
Quantitative Method : External Standard
Function : f(x)=34193.1*x-49569.9
Rr1=0.9608864 Rr2=0.9233027
MeanRF:13877.1 RFSD:10851.6 RFRSD:78.1979
FitType : Linear
ZeroThrough : Not Through
WeightedRegression : None
Detector Name : Detector B



#	Conc (Ratio)	Area	Area
1	0.500	3087.2	3087
2	1.250	11135.1	11135
3	2.500	34500.2	34500
4	5.000	39436.7	39437
5	12.500	407691.3	407691

Appendix 6: Multivariable analysis of risk factors for aflatoxins in grains using a linear regression model

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.227[a]	.051	-.010	.976934

a. Predictors: (Constant), Preservation, Bag, Maize cob removed stovers standing handling, Machine harvest, Drying on ground no canvas, Drying on tarmac(no canvas), Drying on ground on cob, Manual by pounding, Hand shelling, Crib storage, Thatched granary, Hand harvest, Drying on ground with canvas, Machine shelling, Granary/iron sheet storage, Stovers stacked in heaps handling

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.710	.329		2.160	.032
	Hand harvest	.053	.498	.012	.107	.915
	Machine harvest	-.732	.765	-.080	-.957	.340
	Stovers stacked in heaps handling	.568	.419	.289	1.358	.176
	Maize cob removed stovers standing handling	.499	.410	.256	1.219	.224
	Crib storage	-.128	.373	-.031	-.342	.733
	Granary/iron sheet storage	-.285	.287	-.139	-.991	.323
	Thatched granary	-.410	.366	-.110	-1.120	.264
	Bag	-.258	.289	-.133	-.895	.372
	Hand shelling	-.005	.205	-.002	-.024	.981
	Machine shelling	.137	.265	.070	.516	.606
	Manual by pounding	-.176	.220	-.089	-.801	.424
	Drying on tarmac(no canvas)	.998	1.025	.063	.973	.331
	Drying on ground with canvas	.081	.264	.035	.307	.759
	Drying on ground no canvas	-.057	.360	-.013	-.159	.874
	Drying on ground on cob	.089	.324	.026	.273	.785
	Preservation	-.085	.180	-.036	-.473	.636

a. Dependent Variable: Aflatoxins