Assessment of risk to human health associated with aflatoxins in the Kenyan dairy value chain

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A thesis submitted in fulfilment of requirements for the award of degree of Doctor of Philosophy in Veterinary Public Health of The University of Nairobi.

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

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To

THE ALMIGHTY GOD

"I can do all things through Christ who strengthens me" Philippians 4:13

MY BELOVED FAMILY

Your support has been unwavering.

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LIST OF ABBREVIATIONS AND ACRONYMS

AEZ	Agro-ecological Zones
AFB1	Aflatoxin B1
AFB2	Aflatoxin B2
AFG1	Aflatoxin G1
AFG2	Aflatoxin G2
AFLATOXIN	*Aspergillus flavus* Toxin
AFM1	Aflatoxin M1
AI	Artificial Insemination
ANOVA	Analysis of Variance
CAST	Council for Agricultural Science and Technology
DALYs	Disability-Adjusted Life Years
DHS	Demographic Health Survey
EAC	East African Community
EAS	East Africa Standards
ELISA	Enzyme Linked Immunosorbent Assay
EU	European Union
FAO	Food and Agriculture Organization of the United Nations
GDP	Gross Domestic Product
GLMs	Generalized Linear Models
HBsAg−	Hepatitis B Surface-Antigen Negative
HBsAg+	Hepatitis B Surface-Antigen Positive
HBV	Hepatitis B virus

HBV	Hepatitis B virus infection,
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C virus
HCV	Hepatitis C virus
HPLC	High Performance Liquid Chromatography
HPLC-MS/MS	HPLC- Mass Spectrometry/ Mass Spectrometry
HRP	Horse–Radish Peroxidase
IACs	Immunoaffinity Columns
IARC	International Agency for Research on Cancer
ICC	Intra-cluster Correlations
IGF	Insulin-like Growth Factors
IIASA	Institute for Applied Systems Analysis
IITA	International Institute of Tropical Agriculture
ILRI	International Livestock Research Institute
ILRI	International Livestock Research Institute
Immunodipsticks	Lateral Flow Devices
IREC	Institutional Research Ethics Committee
ISO	International Organization for Standardization
KNBS	Kenya National Bureau of Statistics
KS	Kenya Standards
LC	Liquid Chromatography
MALF	Ministry of Agriculture, Livestock and Fisheries
MERCOSUR	Southern Common Market (Argentina, Brazil, Paraguay and Uruguay)

MOALF	Ministry of Agriculture, Livestock and Fisheries
NCPB	National Cereals and Produce Board
OIE	World Organisation for Animal Health
PPB	Parts Per Billion
PPT	Parts Per Trillion
QRA	Quantitative Risk Assessment
RIA	Radioimmunoassay
SD	Standard Deviation
TLC	Thin Layer Chromatography
UHT	Ultra-high-Temperature
UPLC	Ultra Performance Liquid Chromatograph
USA	United States of America
WHO	World Health Organisation

ABSTRACT

Aflatoxins are considered a food safety priority in Kenya in light of the recurrent outbreaks of aflatoxicosis in humans and livestock and a possible role in childhood stunting and immuno-suppression. Nonetheless information is lacking on the health risks posed to public health by aflatoxins in the Kenyan dairy value chain. This study looked into the levels of contamination by aflatoxins along the dairy value chain and developed a quantitative risk assessment model to assess their public health impact.

A survey of 286 farmer households was carried out in Kwale (n=37), Isiolo (n=56), Tharaka-Nithi (n=65), Kisii (n=64) and Bungoma (n=64) Counties, chosen to represent different agro-ecological zones (AEZ). Determination of aflatoxin levels was carried out by use of competitive enzyme-linked immunosorbent assay. Literature review was conducted to determine the impacts of aflatoxin standards on health and nutrition in Sub-Saharan Africa considering the case of Kenya. Finally this study used the World Organisation for Animal Health (OIE) risk assessment framework consisting of release assessment, exposure assessment, consequence assessment, and risk estimation to estimate the risk of liver cancer from aflatoxin exposure.

Overall, 26% of maize, 10% of millet and 11% of sorghum had aflatoxin B1 (AFB1) exceeding the Kenyan limit of 5 ppb. In samples collected during the rainy season, maize from Kisii and Bungoma, (temperate AEZ), had the lowest mean contamination whereas maize from Kwale (sub-humid AEZ) had the highest contamination. Millet and sorghum from Tharaka-Nithi (humid AEZ) and Isiolo (semi-arid AEZ), respectively, had the highest mean contamination ($p<0.05$).

Dairy feed concentrates from farmers had AFB1 levels from less than 1 ppb to 9,661 ppb. The percentages of dairy feeds with AFB1 above Kenyan limits of 5ppb were 73% from farmers, 90% from feed retailers and 62% from feed manufacturers. AFM1 levels in milk were up to 6,999 ppt and the prevalence was lowest in Kwale (3.5%) and highest in Tharaka-Nithi (64.5%). Exposure to AFM1 through milk was estimated at between 0.3 and 1 ng AFM1 per kg body weight per day through the consumption of milk. The annual incidence rates of cancer attributed to the consumption of AFM1 in milk were 3.5×10^{-3} (95% CI: 3×10^{-3}–3.9×10^{-3}), 2.9×10^{-3} (95% CI: 2.5×10^{-3}–3.3×10^{-3}), 1.4×10^{-3} (95% CI: 1.2×10^{-3}–1.5×10^{-3}) and 2.7×10^{-3} (95% CI: 2.3×10^{-3}–3×10^{-3}) cancers per 100,000 in adult females, adult males, children 6–18 years old, and in children less than five years old, respectively. These annual incidence rates are quite low, nonetheless, risk managers should take action based on cumulative exposure from all sources of aflatoxins and hence the need to know the importance of different sources.

The prevention of aflatoxins in dairy feeds would effectively curb the presence of aflatoxin residues in milk and other animal products meant for human consumption. Strategies to reduce aflatoxins in animal feeds include keeping the moisture and temperature of feeds moderately low (<13% moisture; temperature range of 20 - 35^{o}C) to inhibit mould growth, maintaining cleanliness of on-farm equipment, and, where possible, using mould inhibitors or aflatoxin binders. The use of binders in feeds should be further investigated to determine safety and efficacy. The understanding and awareness of the feed manufacturers, retailers, producers (dairy and grains) and consumers on aflatoxins should be improved so that they produce/demand aflatoxin free foods/ feeds.

CHAPTER 1

GENERAL INTRODUCTION

Kenya's dairy industry contributes 3-4% of the national Gross Domestic Product (GDP) and 12% of the agricultural GDP. Kenya has the highest per capita annual milk consumption in the East Africa region at 120 Kg. Milk is produced by large and small scale dairy farmers with the latter contributing 80% of total milk output. Of the total annual cow milk production only about 15% is processed (FAO, 2012). Processing via heat treatment inactivates a number of hazards in milk but is less effective at rendering harmless heat-stable hazards such as drug residues, pesticide residues, heavy metals, and biological toxins such as aflatoxin M1 (AFM1). Aflatoxins pose significant threats to public health and the economies of countries worldwide. Globally, about US$1.2 billion in commerce is lost annually due to aflatoxin contamination, with African economies losing US$450 million each year (IITA, 2013). In Kenya, losses to farmers run into millions of shillings from condemned grains; in 2009, 31,000 and 1,213 bags of contaminated maize were condemned in Mbeere and Bura irrigation scheme, respectively (Nyaga, 2010). Public health impacts arise from outbreaks of aflatoxicosis first reported in 1981, where 12 people died following consumption of suspected aflatoxin contaminated maize (Ngindu *et al*., 1982). The largest human aflatoxicosis outbreak occurred between 2004 and 2005 in the then Eastern Province during a time of acute food shortage; 125 people died out of 317 reported to be sick from consumption of contaminated grains (Lewis *et al*., 2005).

Aflatoxins are secondary metabolites of the fungi *Aspergillus (A) flavus, A. parasiticus* and *A. nomius.* In tropical and sub-tropical regions, the moulds colonize and produce aflatoxins in more than 40 susceptible crops, especially maize and groundnuts, and are also found in dairy products

and traditionally fermented foods (Grace *et al*., 2015). Naturally occurring aflatoxins include aflatoxin B1 (AFB1), AFB2, AFG1 and AFG2. The B and G naming is based on their fluorescence on thin layer chromatography; B for blue and G for green fluorescence (Bennett & Klich, 2003). When lactating cows are fed aflatoxin-contaminated feeds they excrete aflatoxin metabolites M1 and M2 in their milk (Fink-Gremmels, 2008).

Despite recurrent aflatoxicosis outbreaks and government putting in place control measures, grain contamination beyond legal limits has continued to be reported across the country. The acceptable limit of total aflatoxin (B1, B2, G1 and G2) contamination in food and feeds in Kenya is set at 10 parts per billion (ppb) whereas it is 5 ppb for AFB1 (Kenya Standard (KS) East African Standard (EAS) 2:2013; KS EAS 62:2009). A limit of 0.05 ppb AFM1 in milk is recommended by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO).

In order to improve human and animal health through reduction of their exposure to aflatoxins, there is need to focus on the risks for individual consumers and further study the factors contributing to exposure and health impacts. Health risk assessment is a process that contributes to policy development, public health decision making, the establishment of aflatoxin regulations, and research planning (Sherif *et al*., 2009).

Aflatoxin M1 is injurious to human health, however, no risk assessment has been done to estimate the risk to human health posed by aflatoxins in dairy products in Kenya. Risk assessment is the estimation of likelihood, magnitude and uncertainty of population health risks associated with exposures (Sherif *et al*., 2009). To fill this gap, a risk assessment was conducted in four agro-ecological zones (AEZ) in Kenya (semi-arid, temperate, sub-humid and humid). The International

Livestock Research Institute granted ethical approval for the study (ILRI-IREC2013-09). The overall objective of the study was to assess the risk to human health associated with aflatoxins in the Kenyan dairy value chain. Specific objectives were:

i. To quantify aflatoxin levels in foods and feeds along the dairy value chain in Kenya
ii. To determine risk factors associated with aflatoxins along the dairy value chain in Kenya
iii. To assess the risk to human health due to aflatoxins in the dairy value chain in Kenya
iv. To identify best options to reduce the risk to human health posed by aflatoxins in the dairy value chain in Kenya.

The output of this study was a quantitative estimation of the magnitude of possible adverse health effects in humans from consumption of aflatoxins in the dairy value chain.

CHAPTER 2

GENERAL LITERATURE REVIEW

2.1 Introduction

Mycotoxins in food raise public health concerns due to their adverse health effects. Health effects vary from acute illness and death, following exposure to large doses over a short period, to chronic effects, notably cancer (Lewis *et al*., 2005; Williams *et al*., 2004). Besides health effects, mycotoxins impact negatively on the economy when heavily contaminated food products are destroyed affecting trade and domestic food security (WHO, 2005). This affects the livelihoods of people dependent on the mycotoxin susceptible crops.

Mycotoxins are naturally occurring secondary fungal metabolites. Secondary metabolites are not directly required by the fungi for growth and survival; they are often structurally heterogeneous low-molecular-mass molecules (Brakhage, 2013). Mycotoxin producing fungi are ubiquitous in occurrence, contaminating grains, seeds and forage. To date, more than 400 mycotoxins have been identified and these are classified into groups based on their structural similarities (Bennett & Klich, 2003). Five groups (aflatoxins, fumonisins, ochratoxins, trichothecenes, and zearalenone) are recognized as the most important agricultural mycotoxins based on their known and suspected effects on human and animal health (Shephard, 2008a).

Aflatoxins are important in Kenya due to their widespread occurrence in foods and feeds beyond recommended limits. Occurrence is reported in maize, millet, sorghum, peanut, concentrate feeds and milk countrywide. Human aflatoxin exposure was estimated at 66 (maize), 1 (millet), and 0.5 (sorghum) ng/kg of body weight/day. From milk, exposure was estimated at 0.2 ng/kg of body weight/day for a 60 kg adult consuming 0.4 litres per day of milk (Sirma *et al*., 2018).

Kenya's dairy industry plays an important economic role for feed manufacturers and traders, milk producers, milk processors and traders. Smallholder dairy farmers produce 80% of the 6.2 billion litres of milk in Kenya. In Sub-Saharan Africa, Kenya has the highest per capita milk consumption at about 120 kg annually compared to an average of 25 kg for Sub-Saharan Africa (MALF, 2013). Dairy value chain development can be hampered by aflatoxins and Kenya's dairy sector is no exception. To characterize the extent of the aflatoxin problem and its trends in the dairy sector, further information is needed (Atherstone *et al*., 2016).

2.2 Aflatoxins

Aflatoxins are a group of highly toxic compounds synthesized mainly by the moulds *Aspergillus flavus, A. parasiticus* and *A. nomius* (Busby & Wogan, 1984). Studies on aflatoxins and their contamination began in the early 1960s after they were suspected to have caused a disease in turkeys in Great Britain, initially named turkey "X" because of its mysterious nature. This was associated with deaths of 100,000 turkey poults near London, England, after they had been fed on a peanut meal from Brazil that was contaminated by mycotoxins produced by *A. flavus*. Post mortem results demonstrated liver damage (Sargeant *et al*., 1961). The cause of the disease was then named AFLATOXIN, an acronym coined as follows: The first letter "A" for the genus *Aspergillus*, the next 3 letters "FLA" for species *flavus* and the noun toxin meaning poison. Naturally occurring aflatoxins include aflatoxin B1 (AFB1), AFB2, AFG1 and AFG2. The B and G refer to their fluorescence on thin layer chromatography: B for blue and G for green fluorescence (Bennett & Klich, 2003). *A. flavus* produces B toxins only, whereas *A. parasiticus* and *A. nomius* produce both B and G toxins. Aflatoxin M1 (AFM1) and M2 are hydroxylated metabolites of AFB1 and AFB2, respectively produced by lactating cows fed on aflatoxin-contaminated feeds (Busby & Wogan, 1984).

Aflatoxin B1 is metabolised in the body to form metabolites including AFQ1, AFM1, AFP1, AFB1-8,9-endo-epoxide, and AFB1-8,9-exo-epoxide. The latter binds to DNA to form AFB1–N7-Guanine adduct which confers mutagenic properties to AFB1. The endo and exo-epoxides can also undergo non-enzymatic hydrolysis to form AFB1 dialdehydes. The dialdehydes do not bind to DNA but with primary amine groups, e.g. lysine forming Schiff bases and protein adducts present in plasma or serum such as aflatoxin–albumin (Wild & Turner, 2002). The aflatoxin-albumin adduct has been used as a biomarker for individual exposure over a long period (usually more than three weeks). Other biomarkers include urinary aflatoxins (AFM1, AFB1-N7-Guanine, AFP1, AFQ1 and AFB1-mercapturic acid) (Wild & Gong, 2010). AFB1 is a more potent liver carcinogen than other naturally occurring aflatoxins because they are poorer substrates for epoxidation (epoxidation is a chemical reaction which converts carbon–carbon double bond into epoxides) (Wild & Turner 2002).

2.2.1 Aflatoxins occurrence in foods and feeds

Maize samples tested for aflatoxins during the largest recorded outbreak of aflatoxicosis in the former Eastern Province in 2004 showed contamination levels in the range of 1 to 46,400 ppb (Lewis *et al*., 2005). Daniel *et al*., (2011) followed up with a comprehensive assessment of maize aflatoxin levels in that province during the years 2005 to 2007 following the outbreak. The survey found 41% (2005), 51% (2006) and 16% (2007) of the samples had aflatoxin levels above 20 ppb, the then Kenyan regulatory limit. A survey of aflatoxins in maize in western Kenya reported overall contamination of 49% of the samples while 15% of the samples tested above regulatory limits (Mutiga *et al*., 2015). In a survey of peanut contamination from Busia and Homabay Districts, aflatoxin was detected at levels ranging from 0 to 2688 ppb and 0 to 7525 ppb, respectively (Mutegi *et al*., 2009).

In a survey to determine AFM1 contamination of milk from towns in Kenya, Kang'ethe & Lang'at, (2009) collected 613 milk samples from former Districts of Nyeri, Uasin Ngishu, Machakos and Nakuru. Over 70% of the milk from the various outlets tested positive for AFM1; 20% (from medium scale dairy farmers), 35% (from large scale dairy farmers) and 31% (from market outlets) of milk exceeded the maximum allowable limits of 50 ppt (FAO/WHO). In a survey of marketed milk (raw, pasteurised, UHT milk, yoghurt and lala) in Nairobi over a period of one year, more than 50% of 291 sampled milk exceeded EU limits of 50 ppt (Lindahl *et al*., 2018). Another survey in Nandi County to determine aflatoxin levels in 67 breast milk samples found contamination of 56.7% of the samples with levels ranging from 0.003 to 3.7 parts per trillion (Sirma, 2013). Detection of aflatoxins in breast milk indicates an early exposure of children to aflatoxins and more so in weaning foods mostly comprising porridge made from maize, millet or sorghum mixed with milk. Aflatoxin exposure in children is associated with impaired growth (stunting).

Testing of 830 animal feeds from four urban centers of Kenya between 2006 and 2007 showed widespread contamination of feeds. Eighty-six per cent of farmer sourced feed samples were positive for AFB1 and 67% of these exceeded 5 ppb limits. Eighty-one per cent of feed millers' feeds and 87% of feeds from agrochemical shops were contaminated. Of these, 58% (feed millers) and 66% (agrochemical shops) of the contaminated samples were above Kenyan limits (Kang'ethe & Lang'at, 2009). Okoth & Kola, (2012) screened 72 feed samples for aflatoxin total content from retail shops in Nairobi between 2006 and 2009. All the feeds tested positive with levels ranging from 5.13 to 1123 ppb. Ninety-five per cent of the feeds tested above 10 ppb. They found no significant difference in the level of contamination among dairy meal, cotton based oil-seed cake or sunflower based oil- seed cake. Chronic livestock exposure to aflatoxins reduces productivity (reducing milk yields by up to 25%), and growth rates (Atherstone *et al*, 2016**)**.

2.2.2 Aflatoxin impact on human beings

2.2.2.1 Aflatoxicosis

Aflatoxin poisoning in humans depends on a number of factors including toxin levels, individual susceptibility, age, gender and duration of exposure (Hussein & Brasel, 2001). Several outbreaks in Kenya are associated with famine and chronic malnutrition. Aflatoxicosis manifests as vomiting, abdominal pains, pulmonary oedema, fatty infiltration of the liver, and necrosis of the liver (IARC, 2016).

In Kenya, several outbreaks of acute aflatoxicosis have been recorded in humans (1981, 2001, 2004, and 2005). Most were in the former Eastern Province, an area endemic for aflatoxicosis. In 1981, 12 out of 20 patients with acute hepatitis died following aflatoxin poisoning in Machakos District. Maize sampled from two families, among whom eight members died from the poisoning, had aflatoxin levels as high as 12,000 ppb (Ngindu *et al*., 1982). The 2004 outbreak was the most severe reported in Kenya and resulted in 317 cases and 125 deaths. During that outbreak, a direct relationship was found between aflatoxins in foods and aflatoxins biomarkers in serum from clinical cases (Azziz-Baumgartner *et al*., 2005). The outbreak was the result of widespread aflatoxin contamination of locally grown maize (Nyikal *et al*., 2004). Based on the outbreak, consumption of foods contaminated with 5,000 ppb or above of aflatoxins was associated with fatality while daily consumption of 1,000 ppb was linked to aflatoxicosis with or without fatality. From these figures, the intake of total aflatoxins resulting in a risk of fatality was estimated to be 1 mg/day, or in excess of 20 ppb body weight/day in adults (Wild & Gong, 2010). However, these deaths occurred in chronically under-nourished people. Interestingly, one report found a laboratory technician consumed a large amount in an attempt to commit suicide, without ill effect on more than one occasion (Park & Stoloff, 1989).

2.2.2.2 Carcinogenicity

Naturally occurring aflatoxins are classified as group 1 human carcinogen, meaning there is clear evidence of carcinogenicity (Pitt *et al*., 2012). Exposure to low doses over prolonged periods can cause hepatocellular carcinoma (HCC) or liver cancer (IARC, 2002). Internationally, HCC ranks fourth amongst most common causes of death from cancer (Yang *et al*., 2019). It is estimated to have been responsible for nearly 746,000 deaths in 2012 (9.1% of all cancer deaths that year) and is found more in men than in women. In Kenya, during that year, 1,120 new liver cancer cases and 1,037 cancer mortalities occurred (Ferlay *et al*., 2015). The risk of HCC is greater in individuals exposed both to chronic hepatitis B or C virus (HBV and HCV) and aflatoxins. These risk factors are prevalent in Sub-Saharan Africa, Southeast Asia, and China where Most HCC cases occur. In East Africa, it is estimated that 11.3% and 1.7% of the population are chronically infected with HBV and HCV, respectively (Parkin, 2006).

Globally, 14% and 19% of all HCC cases are attributable to aflatoxin exposure (Liu *et al*., 2012). The Joint FAO/WHO Expert Committee on Food Additives arrived at estimates of AFB1 potency to be 0.3 cancers per year per 100,000 persons per ng AFB1 kg^{-1} body weight per day in hepatitis B positive individuals. In hepatitis B negative individuals, the potency was 30 times lower (Shephard, 2008b).

Aflatoxins are also implicated in the aetiology of liver cirrhosis and hepatomegaly. A study in Kenya reported that the prevalence of hepatomegaly increased in children with higher aflatoxin exposure (Gong *et al*., 2012). This is consistent with the liver being the key target organ for aflatoxin toxicity (Gong *et al*., 2016).

2.2.2.3 Effects on the Immune System

The link between aflatoxin exposure and immunomodulation has been well investigated in cell models and animals including observations of farm animals (IARC, 2002; Pierron *et al*., 2016; Williams *et al*., 2004). The immunomodulation happens through suppression of immune function mainly through cell-mediated immune responses. Aflatoxin exposure reduced T or B lymphocyte activity, impaired macrophage/neutrophil effector functions, modified synthesis of inflammatory cytokines, suppressed natural killer cell-mediated cytolysis, decreased resistance to infectious diseases, induced reactivation of chronic infection, decreased immunity to vaccination, and impaired immune function in developing animals (Jiang *et al*., 2008).

2.2.2.4 Growth impairment

Susceptibility to aflatoxins appears to be greatest in young children, whose exposure begins *in utero* and peaks when children are weaned to homemade cereal based preparations which are mostly contaminated with aflatoxins (Owaga *et al*., 2011). An association between aflatoxins and growth impairment has been demonstrated in children in Benin and Togo (Gong *et al*., 2002; 2004). In Kenya, children consuming cereals with high aflatoxin levels were found to be more wasted than those fed cereals with lower aflatoxin levels in Kisumu District (Okoth & Ohingo, 2004). Childhood stunting and underweight are common in Kenya whereby nationally 4%, 11% and 26%, of the children under the age of 5 years are wasted, underweight and stunted, respectively (DHS, 2015). However, cross-sectional and cohort studies cannot prove causation as there may be confounding due to other factors that are related both to aflatoxin exposure and stunting. Randomised controlled trials are considered gold standard in proving causation. A cluster randomised controlled trial in Eastern Kenya found reducing aflatoxin exposure had no effect on child linear growth (Hoffmann *et al*., 2018). A study among Nepalese children aged up to

36months found no significant association between chronic aflatoxin exposure among the children and growth impairment (Mitchell *et al*., 2017).

Various hypotheses have been put forward on the potential mechanism of aflatoxin effect on growth. One theory postulates that aflatoxins cause immune suppression, which increases susceptibility to infectious diseases, and thus affects growth directly or results in compromised intestinal integrity through altered barriers (Wild & Gong, 2010). Another hypothesis postulates that aflatoxins induce growth retardation by interrupting insulin-like growth factors (IGF) pathway through liver toxicity (Gong *et al*., 2016). IGF facilitates the growth promoting effects of growth hormone. A study in Kenya reported an inverse association between IGF protein levels and AFB-albumin adducts in children aged between six and seventeen years (Castelino *et al*., 2015).

2.2.3 Aflatoxin impact on livestock

Poorly stored, homemade dairy concentrates are suspected to be the main source of aflatoxins ingested by livestock (Lanyasunya *et al*., 2005). However, smallholder farmers rely more on commercially produced feeds than home compounded feeds which if stored under high humidity are also likely to be contaminated with aflatoxins. The impact of aflatoxins in animals is species and dose dependent and is also influenced by a variety of other factors including age, breed, sex, nutrition, activity and some stresses. Of the domestic species, poultry, swine and cattle are of greatest economic concern in terms of aflatoxicosis. In all species, aflatoxicosis manifests as general unthriftiness, reduction in weight gains, reduced feed conversion efficiency, lowered immunity, and lowered production (Robens & Richard, 1992). Dietary levels of aflatoxin (in ppb) generally tolerated are; ≤50 in young poultry, ≤100 in adult poultry, ≤50 in weaned pigs, ≤200 in finishing pigs, <100 in calves, <300 in cattle (Atherstone *et al*., 2016). Ruminants are less

vulnerable to aflatoxins as their ruminal flora of microorganisms is capable of degrading aflatoxins (Bhat *et al*., 2010).

2.2.4 Economic impact of aflatoxins

Three classes of economic impacts from mycotoxins have been identified, namely; effects on animal health, effects on human health, and market losses (Wu, 2006). Human and animal health losses include morbidity and mortality and increased veterinary and medical costs. Other impacts include reduced production, cost of control measures and investments in mycotoxin research. Market effects include market losses due to rejection of foods/feeds above limits set for local and international markets (Wu, 2007).Compliance with stringent regulations for mycotoxins can result in economic losses arising from increased costs for trade (Jaffee *et al*., 2005; Otsuki *et al*., 2001). In 2001 it was estimated that there could be an annual loss of USA$ 670 million by African food exporters of cereals and dried fruit, in trying to meet strict European Union (EU) aflatoxin standards (Otsuki *et al*., 2001). For instance, Kenyans intending to access EU market for trade in nuts and cereals intended for direct human consumption must meet set limit of 2 ppb AFB1 which is lower than recommended limit of 5 ppb for Kenya.

Currently agricultural methods for controlling these mycotoxins in crops are not widely available or affordable. As a result, the fact that export markets impose strict standards, whereas domestic markets are not able to enforce standards means that higher quality crops tend to supply export markets whereas lower quality crops can be sold in domestic markets.

2.3 Legal limits

Aflatoxin regulation levels are set at parts per billion (ppb) or parts per trillion (ppt) ranges apart from in countries where there is zero tolerance. The acceptable levels in various commodities varies by country and whether the product is intended for human or animal consumption. In 2003,

at least 99 countries worldwide had mycotoxin regulations for food and/or feed including for aflatoxin B1 or the sum of aflatoxins B1, B2, G1 and G2 (FAO, 2003). However, different nations have adopted different limits, and the permitted levels may vary depending on the product and its use. For example, the US Food and Drug Administration authority have recommendations for feed depending on species.

Kenya has adopted East Africa Standards for maximum allowable limits of aflatoxins in foods and feeds. The acceptable limit of total aflatoxin contamination in cereals and feeds is 10 parts per billion (ppb; µg/kg) (Kenya Standards (KS)-East Africa Standards (EAS) 62:2009; KS EAS 2:2013). There are no official Kenya limits for milk. Previous studies have reported prevalence compared to the FAO/WHO limit of 50 ppt (ng/kg) (Kang'ethe & Lang'at, 2009).

2.4 Detection methods

There are various methods for detecting and quantifying aflatoxins in agricultural food crops, feed and samples from human and animal subjects, which can be classified as:

1. Chromatographic methods
    a. Thin Layer Chromatography
    b. High Performance Liquid Chromatography
    c. Gas Chromatography
2. Spectroscopic Methods
    a. Fluorescence Spectrophotometry
    b. Frontier Infrared Spectroscopy
3. Immunochemical Methods
    a. Radioimmunoassay (RIA)
    b. Enzyme Linked Immunosorbent Assay (ELISA)
    c. Lateral Flow Devices (Immunodipsticks)
    d. Immunosensors

Chromatographic methods such as TLC and HPLC are official analytical techniques. These methods use immunoaffinity columns (IACs) for sample extraction and clean-up before HPLC analysis. The development of multi-analyte HPLC-MS/MS methods has enabled analytical chemists to combine analytical steps with a confirmatory test by measuring the mass spectrum of the HPLC peak. The highly specific nature of mass spectrometry eliminates need for extract purification. However, chromatographic facilities especially those coupled with mass spectrophotometers are expensive to build, require highly trained personnel, and generally have a low throughput unless staff numbers are large and spare instruments are available (IARC, 2012).

Rapid screening methods including ELISA, fluorometric methods, lateral flow devices, and a range of tests give a yes/no result for contamination above or below a set control level. These have been developed for situations where quick decisions are required, such as at granaries, silos, and factories (IARC 2012). ELISAs have the advantage of not requiring sample extract purification and can handle many samples in a single experiment. Its disadvantages include cross-reactivity with related mycotoxins, matrix interference problems, possible false positive/negative results and confirmatory LC analysis required (Pascale and Visconti, 2008).

CHAPTER 3

GENERAL METHODOLOGY, DESCRIPTION OF THE STUDY AREAS AND FARM CHARACTERISTICS

3.1 Introduction

Weather conditions affect occurrence of fungal species, and subsequently the production of mycotoxins. Aflatoxins, produced by *Aspergillus* species, contaminate crops in hot and humid regions of the world (Reddy *et al*., 2010). In recognition of variability of aflatoxin contamination in food and feeds across agro-ecological zones (AEZs), this study was conducted in Counties representing different AEZs in Kenya. AEZs approach for choosing study areas takes into account various factors such as temperature, soil type, humidity and altitude which affect occurrence of aflatoxins (IIASA/FAO, 2012). In Benin, China, Kenya and Nigeria studies showed differing distribution of aflatoxin producing *Aspergillus* in soils from different AEZs (Cardwell & Cotty, 2002; Donner *et al*., 2009; Okoth *et al*., 2012; Zhang *et al*., 2017). In Cameroon, aflatoxin content of broiler feeds varied based on AEZ (Kana *et al*., 2013). Previous AFM1 prevalence surveys in Kenya have been purposive, focusing on dairy production systems in urban and peri- urban regions (Kang'ethe & Lang'at., 2009; Kang'ethe *et al*., 2010). Milk from dairy farms in these regions has a high likelihood of containing AFM1 residues due to use of commercial feeds such as dairy meal which are prone to contamination by AFB1 (Lanyasunya *et al*., 2005). The risk of aflatoxin contamination and human exposure to aflatoxins through contaminated food is likely to be different across AEZs. These AEZs have a range of climatic conditions, cropping systems, storage practices and food consumption patterns that differ. This chapter presents data collected from dairy households across four AEZs in Kenya. Specifically, the data is on cow population and breeds, average milk yield per cow per lactation, average milk price per litre at the various stages of the

dairy value channel, and types of dairy feeds. Data collected was fed into a risk assessment model for determining health risks of aflatoxins in the dairy value chain in Kenya.

3.2 Materials and Methods

3.2.1 Agro-ecological zones

A map showing agro-ecological zones (AEZs) in Kenya guided selection of study sites (Figure 3-1). The potential AEZs were arid, semi-arid, humid, sub-humid and temperate zones based on International Institute for Applied Systems Analysis (IIASA) and FAO global agro-ecological zoning methodology (IIASA/FAO, 2012). Because this study targeted farmers growing cereals and keeping dairy cattle, no site was selected from the arid zone. Instead two study sites were selected from the temperate zone, as it is most favourable for crop and dairy farming. The study sites selection involved listing of Counties, locations and sub-locations falling under each of the AEZ of interest (semi-arid, humid, sub-humid and temperate). Numbers were assigned to the areas and the randbetween function of Microsoft Excel® used to select a site randomly based on their number.

The study sites selected were Lonkopito sub-location, Isiolo County in the semi-arid zone; Karongoni sub-location, Tharaka-Nithi County in the humid zone; Waa sub-location, Kwale County in the sub-humid zone; East Sang'alo sub-location, Bungoma County and Gitare sub-location, Kisii County, both temperate zones. The randomly selected sub-locations for the temperate, semi-arid, humid zone may not be typical for the AEZs as they were located close to the edge of the AEZs.

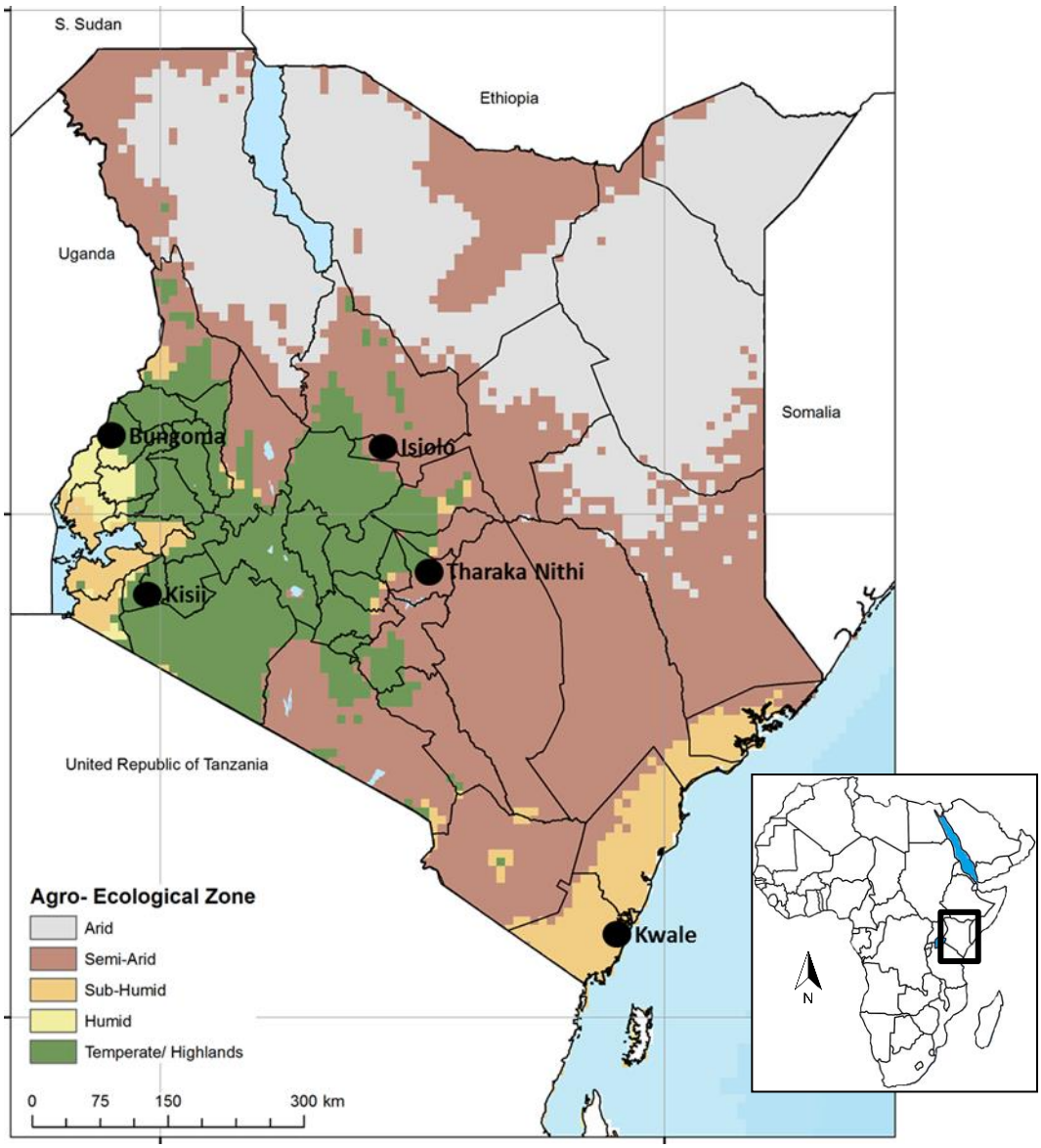

Figure 3-1 Spatial distribution of study sites according to agro-ecological zones in Kenya (IIASA/FAO, 2012; Map produced by ILRI 2013)

3.2.2 Description of the study areas based on Kenya County guide 2014-2015

3.2.2.1 Kwale County

Physical and Agro-Climatic Conditions

Kwale County is located in the south coast of Kenya and covers an area of approximately 8,270 km^2. The County borders the Republic of Tanzania to the southwest, Taita Taveta County to the

west, Kilifi County to the north, Mombasa County to the northeast, and the Indian Ocean to the east. It has four major topographical features, namely the coastal plain, the foot plateau, the coastal uplands and the Nyika plateau. Distribution of soil types in the County is dependent on the topography. In the coastal plain, soils are predominantly sand and loamy; coastal uplands range from sand to loam, loam to clay and shallow to deep and are heavy textured; in the foot plateau, soil varies from loamy-to-sandy, and from shallow to deep and well-drained; at Nyika plateau, soils are developed on gritty sandstones, shale and basement system rocks. Kwale County has a monsoon type of climate; it is hot and dry from January to April while June to August is the coolest period of the year. Rainfall comes in two seasons i.e. short rains are experienced from October to December while the long rains run from March-June/July. Rainfall amounts range between 400mm and 1,680 mm per annum. The average temperature of the County is 24.2°C. Agro-ecologically the County is a sub-humid zone.

Population and economic activity

Kwale County is home to an estimated 649,931 people with a population density of 79 people per km^2 and an annual growth rate of 2.6%. Children aged 14 years and below constitute 47.2% of the population, people aged 15 to 64 years constitute 49.4% of the population while those aged 65 years and above constitute 3.4% (Kenya National Bureau of Statistics (KNBS) 2009).

Agriculture is the main economic activity. Most Kwale farmers practise mixed farming whereby they rear livestock and grow crops. Crops grown include maize, beans, mangoes and vegetables. The County has 5,324 dairy cattle and 201,006 beef cattle (MALF, 2016).

3.2.2.2 Bungoma County

Physical and Agro-Climatic Conditions

Bungoma County is located in Western Kenya and borders the Republic of Uganda to the west and three Counties, Busia County to the southwest, Kakamega County to the southwest and southeast and Trans Nzoia County to the northeast. The County occupies an area of approximately 3,032 km^2. Generally, the County is flatland suitable for agriculture and livestock rearing. It has good land and soil with gently sloping terrain making it one of the most arable lands in Kenya. The soils are suitable for maize farming. Bungoma County receives bimodal type of rainfall with the average annual rainfall ranging from 1200mm to 1800mm per annum. Most of the rain falls in the months of April-May and July-August. Temperatures range between 15^0C and 30^0C. The coldest months are July, August and September. The County has two agro-ecological zones: humid and temperate/highland zones.

Population and economic activity

Bungoma County has a population size of 1,375,063 with a population density of 453.5 people per km^2 and an annual growth rate of 4.3%. Children aged 14 years and below constitute 45.9 % of the population, people aged 15 to 64 years constitute 51.4% of the population while those aged 65 years and above constitute 2.3% (KNBS, 2009).

Main economic activity in the County is Agriculture with sugar cane and maize farming being major crops grown. Number of dairy cattle in the County is 129,758 and beef cattle is 252, 657 (MALF, 2016).

3.2.2.3 Kisii County

Physical and Agro-Climatic Conditions

Kisii County is located to the south east of Lake Victoria and is bordered by six Counties namely Narok to the south, Migori to the west, Homa Bay to the north west, Kisumu to the north, Bomet

to the south east and Nyamira to the east. It covers an area of approximately 1,302 km^2. Kisii County is characterized by a hilly topography with ridges and valleys. Seventy five percent of the County has red volcanic soils, which are rich in organic matter. The rest of the County has clay soils with poor drainage, red loams and sandy soils. Kisii County exhibits a highland equatorial climate resulting into a bimodal rainfall pattern with average annual rainfall of 1500m with the long rains falling between March and June while the short rains are received from September to November. The months of July and January are relatively dry. The maximum temperatures range between 21°C – 30°C while the minimum temperatures range between 15°C – 20°C. The County has three agro-ecological zones comprising the humid, sub-humid and temperate/highland zones.

Population and economic activity

Kisii County has a population size of 1,152,282 and a population density of 874.7 people per km^2 with an annual growth rate of 2.75%. Children aged 14 years and below constitute 45% of the population, people aged 15 to 64 years constitute 51.6% of the population while those aged 65 years and above constitute 3.4% (KNBS 2009).

Agriculture is the main economic activity of Kisii County comprising growing of crops including tea, bananas, maize and coffee and dairy farming. Their dairy cattle size is at 167,931 and beef cattle totalling 112,502 (MALF, 2016).

3.2.2.4 Tharaka-Nithi County

Physical and Agro-Climatic Conditions

Tharaka-Nithi County is located in the central region of Kenya and borders the Counties of Embu to the south and south west, Meru to the north and north east, Kirinyaga and Nyeri to the west and Kitui to the east and south east. The total area of the County is approximately 2,662.1 km^2;

including the shared Mt Kenya forest, which is estimated to have 360 km^2 in Tharaka-Nithi County. Well drained and fertile, deep red loam soils characterize the soils of the County. Rainfall in the County ranges from 2,200 mm in Chogoria forest to 500 mm in Tharaka. Temperatures range from a minimum of 11°C to a maximum of 25.9°C. The County has three agro-ecological zones: semi-arid, temperate/highland and humid zones.

Population and economic activity

Tharaka-Nithi County has 365,330 people with a population density of 138 people per km^2 and an annual population growth rate of 1.8%. Children aged 14 years and below constitute 39.1% of the population, people aged 15 to 64 years constitute 55.5% of the population while those aged 65 years and above constitute 5.3% (KNBS 2009).

Agriculture is the main economic activity in the County. Major produce grown in high altitude areas include coffee, tea and horticultural crops while low altitude areas which are extensively dry are known for keeping livestock such as cattle, goats, sheep and honey production. Dairy cattle total 68,924 whereas beef cattle are 97,301 (MALF, 2016). Millet, sorghum and cassava also do well in the lowland areas.

3.2.2.5 Isiolo County

Physical and Agro-Climatic Conditions

Isiolo County is located in the upper eastern region of Kenya. It borders seven Counties namely Garissa to the east, Wajir to the northeast, Meru to the southwest, Samburu to the east, Marsabit to the northwest, Kitui to the southwest and Tana River to the southeast. It approximately covers an area of 25,700 km^2. The County has mostly sandy and saline soil with low water holding capacity, making it difficult to engage in agricultural activities. Rainfall ranges from 150 mm to

650 mm per annum typical of arid and semi-arid lands in Kenya. Temperatures range from a minimum of 12.0°C to a maximum of 28.0°C.

Population and economic activity

The population of Isiolo County is at 143,294 with a population density of 5.66 people per km^2 and an annual growth rate of 1.45%. Children aged 14 years and below constitute 44% of the population, people aged 15 to 64 years constitute 52% of the population while those aged 65 years and above constitute 4% (KNBS 2009).

Livestock keeping contributes to the economy of the area. The cattle tally for the area is 252 dairy and 213,413 beef (MALF, 2016).

3.2.3 Sample size determination

Multi-stage cluster random sampling was selected for this study. The sampling methods were designed with reference to Veterinary Epidemiologic Research (Dohoo et al., 2009). Dohoo *et al.* (2009) defined cluster as a natural or convenient collection of study objects with one or more characteristics in common. To achieve multistage sampling, County, Location, Sub-location and villages were selected in that order. Farmer households within the villages comprised the sampling frame and a list of these was developed. In each Sub-location interviews were undertaken for farmers, milk traders, feed retailers and milk consumers (Figure 3-2). From expert opinion and observation a representative map of the dairy value chain was derived.

To determine farmer sample size, the formulae below was used using an expected prevalence of 72% (the prevalence of aflatoxin M1 in milk from farmers in urban centres in Kenya (Kang'ethe & Lang'at, 2009), 95% level of confidence and a 10% precision level.

$$n=\frac{Z\alpha^2 pq}{L^2}$$

(Where n= sample size; Z α^2= Z statistic for a level of confidence; *p*= expected prevalence; *q*= 1-*p;* L= precision)

$$n = \frac{(1.96)^2\,(0.72)\,(0.28)}{(0.1)^2} = 77$$

The sample size was adjusted to account for clustering at Sub-location and village level using the formula below:

$$DesignEffect = 1 + ICC(K - 1)$$
$$ICC = Intra - clustercorrelation$$
$$K = Averageclustersize$$

Design effect is the ratio of variance obtained from taking the clustering and stratification by region into account to the variance that would have been obtained if a comparable-sized, simple random sample had been drawn from the population. Clustering at Location level was adjusted using an ICC of 0.05 and an average cluster size of 15 yielding a design effect of 1.7. Clustering at village level was adjusted using an ICC of 0.2 and an average cluster size of 8 yielding a design effect of 2.4. To get the final sample size, the 77 was multiplied with the design effect of 1.7 to give 134. The 134 was then multiplied with the design effect of 2.4 to give a final adjusted sample size of 321. The 321 was divided equally across the study Counties giving a sample size of 64 farmers per County. To achieve this, it was purposed to visit eight villages per Sub-location and in each village eight farmers were interviewed. Sampling targeted cereal producers and livestock keepers who were selected randomly using randbetween function of Microsoft Excel®. Sampling frame included farmers who fit the criteria in selected villages. Local administrators and extension officers assisted in constructing the sampling frame.

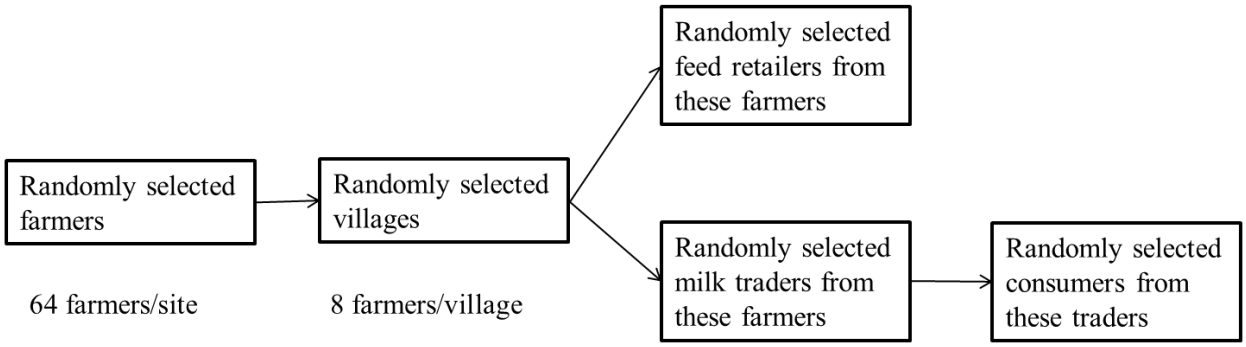

Figure 3-2 Sampling approach in the study areas

3.2.4 Questionnaire preparation and administration

Questionnaires were developed based on previous questionnaires by the International Livestock Research Institute (ILRI) covering the subject matter. The process involved consultation with experts in value chain analysis. The questionnaires were quantitative and structured (Appendix 1). Structured questionnaires are designed to capture information about study subjects and their environment (Dohoo *et al*., 2009). Questions were mostly close-ended but included a few open-ended questions. The open-ended questions captured numerical data as values e.g. mean monthly income and age. Administration of questionnaires was through in-person interview. To assist with the administration of questionnaires, trained enumerators were recruited.

Questionnaires were pre-tested with dairy farmers at Dumboini and feed and milk traders at Uthiru, Centres close to the University of Nairobi's Upper Kabete Campus in Nairobi. Questionnaires were written in English, if need be, a local translator was recruited to translate to local dialect. The translator would then give the answer in English to the interviewer for recording. Through pre-testing, the questionnaires were refined before printing a final version. In general, the questionnaires were designed to obtain data on:

- Household characteristics (Household (hh) size, age of household head (hhh), gender of hhh, marital status of hhh, education level of hhh, primary activity of hhh, monthly income, importance of dairy as a source of income).
- Farming characteristics of the study areas (livestock number, types of feeds, milk production).
- Knowledge, practices and perceptions related to aflatoxins in milk.
- Milk production per lactation and breed (local or cross).

Approximation of milk production was by calculating the area (triangle OBC); a product of lactation length (OC) and milk production at calving (OB) divided by two as illustrated in Figure 3-3 (Njuki *et al*., 2011).

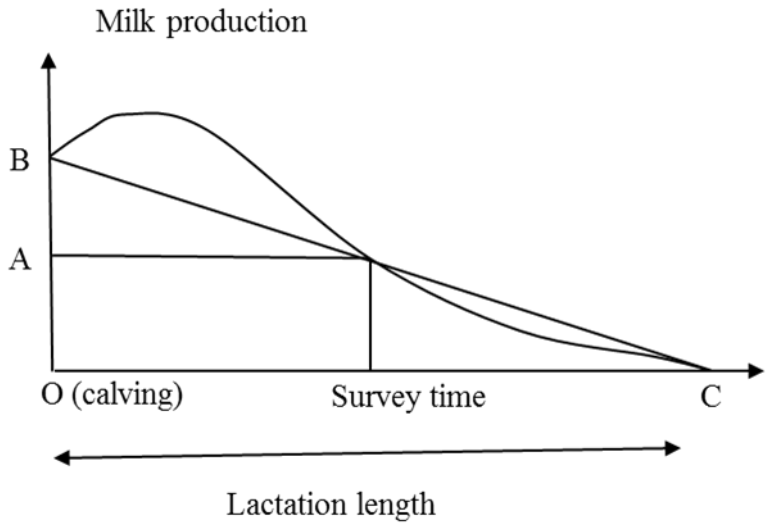

Figure 3-3 Approximation of the level of milk production by calculating the area (triangle OBC)

3.2.5 Data analysis

Stata® 13 (StataCorp LP, Texas, USA) was used for statistical analyses. Shapiro test was done to test normality of data. To compare proportions and measure associations for categorical data, Chi Square Test and Fisher's Exact Test were used. For comparison of two or more than two means, t-

test and ANOVA were used, respectively. Significance was reported at 95% confidence interval. Histograms were developed to assess if data was normally distributed.

To assess risk factors associated with aflatoxin contamination of cereals, feeds and milk beyond limits, a combination of univariate and multivariate analysis were performed. In univariate analysis, the relationship between the proportion of samples below and above limits and responses (explanatory variables) including knowledge, attitude and practices about aflatoxins was analysed using Chi square and Generalized Linear Models (GLMs) with binomial errors. In multivariable analysis, the factors with p values less than 0.2 in univariate analysis were entered in the multivariable model and GLMs performed with the response variable being presence of aflatoxins below or above limits. In that model, stepwise simplification was used to optimize the model.

3.3 Results

3.3.1 Socio-demographic and economic characteristics

3.3.1.1 Household characteristics

In total 286 farmer households were interviewed, corresponding to a response rate of 89.1% out of a sample size of 321. The distribution of households across the 5 Counties was as follows: Kwale (n=37), Isiolo (n=56), Tharaka-Nithi (n=65), Kisii (n=64) and Bungoma (n=64). Attaining the target of 64 farmers per County was not possible due to a lower number of dairy farmers in the villages than anticipated. In Kwale, Waa Sub-location, only six villages fulfilled the criterion of livestock keeping and, in some villages, less than eight farmers kept dairy cows. In Isiolo, Lonkopito Sub-location, there were only seven villages meaning only 56 farmers were sampled. Respondents were mainly household heads but, in some cases they were spouses, adult children or domestic workers. However, recorded demographic details refer to the household heads only.

Tharaka-Nithi and Kisii had a mean household size of five whereas Kwale, Isiolo and Bungoma had seven each.

3.3.1.2 Age of household heads from the five Counties

Most household heads were above 30 years old with the exception of Isiolo where close to 50% were below that age (Table 3-1).

Table 3-1 Age distribution of household heads in the Counties

County (%)	Age (years)					
	20-29	30-39	40-49	50-59	60-69	70+
Kwale	5.7	11.4	31.4	11.4	17.1	22.9
Isiolo	13.0	35.2	14.8	14.8	13.0	9,3
Tharaka-Nithi	0	11.3	14.5	21.0	38.7	14.5
Kisii	3.3	15.0	15.0	30.0	18.3	18.3
Bungoma	4.8	19.0	27.0	23.8	19.0	6.3
Total	5.1	18.6	19.7	21.2	21.9	13.5

3.3.1.3 Gender of household heads from the five Counties

Male headed homes were the majority in all Counties. Isiolo had the highest percentage of female headed households (Table 3-2).

Table 3-2 Gender distribution of household heads in the Counties

County (%)	Male	Female
Kwale	91.9	8.1
Isiolo	69.6	30.4
Tharaka-Nithi	90.8	9.2
Kisii	93.8	6.2
Bungoma	87.5	12.5
Total	86.7	13.3

3.3.1.4 Marital status of household heads

Of all household heads, 90% were married (Table 3-3). Among the male respondents 96.8% were married and for women 47.4%. Almost half of the women heads were widowed (44.7%).

Table 3-3 Percent distribution of marital status of household heads

County (%)	Single	Married	Divorced	Separated	Widowed
Kwale	8.1	86.5	2.7	0	2.7
Isiolo	1.8	94.6	0	0	3.6
Tharaka-Nithi	0	90.8	0	0	9.2
Kisii	1.6	90.6	0	0	7.8
Bungoma	0	87.5	0	1.6	10.9
Total	1.7	90.2	0.3	0.3	7.3

3.3.1.5 Level of education of household heads

Education and County were significantly associated ($p<0.05$). In Isiolo (80.4%) and Kwale (35.1%) most household heads had no formal education whereas most in Tharaka-Nithi (41.1%) and Kisii (28.6%) had completed primary education. In Bungoma, 31.5% of household heads had completed secondary education (Table 3-4).

Level of education was weakly associated ($p = 0.023$) with age amongst men whereas in women there was no association. Men aged 30 to 49 had attained highest levels of education as compared to men aged 50 and above.

Table 3-4 Percent distribution of education levels of household heads in the Counties

County (%)	Never schooled	Primary incomplete	Primary complete	Secondary incomplete	Secondary complete	Tertiary	University
Kwale	35.1	13.5	32.4	2.7	5.4	10.8	0
Isiolo	80.4	16.1	1.8	0	1.8	0	0
Tharaka-Nithi	10.7	19.6	41.1	10.7	10.7	5.4	1.8
Kisii	3.6	19.6	28.6	8.9	26.8	10.7	1.8
Bungoma	9.3	13	27.8	11.1	31.5	5.6	1.9
Total	27.4	16.6	25.9	6.9	15.8	6.2	1.2

3.3.1.6 Primary activity of household heads

There was an association between County and primary activity of the household head ($p<0.01$;Table 3-5). Crop farming was the primary activity of household heads in Kisii, Tharaka-

Nithi and Bungoma. All respondents from Isiolo relied on animal keeping. Kwale household heads were mostly involved in formal employment (27%) and animal keeping (21.6%).

Table 3-5 Primary activity of household heads summarized by County

County (%)	Crop farming	Animal keeping	Trade in animal products	Trade in agricultural products	Salaried	Business	Unemployed	Retired	Casual labour
Kwale	13.5	21.6	0	13.5	27	16.2	0	5.4	2.7
Isiolo	0	100	0	0	0	0	0	0	0
Tharaka-Nithi	55.4	13.8	4.6	1.5	13.8	9.2	0	1.5	0
Kisii	51.6	4.7	0	0	20.3	14.1	1.6	4.7	3.1
Bungoma	73.4	1.6	0	0	12.5	1.6	0	6.2	4.7
Total	42.3	26.9	1	2.1	14	7.7	0.3	3.5	2.1

3.3.1.7 Income

The mean monthly household income per County was as follows: Bungoma Ksh 16,238; Kwale Ksh 15,442; Tharaka-Nithi Ksh 13,475; Kisii Ksh 13,428; Isiolo Ksh 10,564. In general, mean monthly income was Ksh 13,767.2 (95% C.I. 11,582.5 - 15,952). There was an association between importance of dairy as a source of income and County. Majority across the Counties classified dairy as a minor source of income. The second most common response varied: Kwale and Kisii said dairy was a negligible source of income, Isiolo an only source of income, Tharaka nithi and Bungoma same as other sources of income (Table 3-6).

Table 3-6 Response on classification of importance of dairy as source of income

County (%)	Only source	Major source	Same as other sources	Minor source	No or negligible source
Kwale	0	13.5	18.9	40.5	27
Isiolo	32.1	3.6	0	33.9	30.4
Tharaka-Nithi	0	20	27.7	43.1	9.2
Kisii	0	12.5	17.2	46.9	23.4
Bungoma	0	18.8	28.1	51.6	1.6
Total	6.3	14	18.9	43.7	17.1

3.3.2 Farming characteristics of the study areas

3.3.2.1 Livestock number

The mean number of cattle in Kwale and Isiolo was significantly higher than in the other Counties ($P<0.001$). The mean number of cattle ranged from three to eighteen across Counties (Table 3-7).

Table 3-7 Descriptive statistics of cattle owned by the Counties

County	Minimum	Mean	Maximum	Standard deviation
Isiolo (n=56)	2	9	40	7.9
Kwale (n=35)	1	18	100	19.8
Tharaka-Nithi (n=63)	1	3	13	1.9
Kisii (n=64)	1	3	18	2.6
Bungoma (n=64)	1	3	11	1.9
Total (n=282)	1	6	100	9.5

3.3.2.2 Animal feeding

As regards feeding, Isiolo farmers relied only on extensive grazing on unimproved pastures. The rest of the Counties had a variety of feeds for their livestock with cut and carry forages being the main one (Table 3-8 and Figure 3-4). The proportion of farmers using concentrate feeds in Kisii (67.2%) and Tharaka-Nithi (50.8%) was significantly higher than Bungoma (21.9%) and Kwale (5.4%).

Table 3-8 Summary of types of feeds fed to livestock in the study Counties

Feed type	Bungoma (n=27, %)	Kisii (n=20, %)	Kwale (n=5, %)	Tharaka-Nithi (n=54, %)	Total (n=106, %)
Cut and carry forages [a]	88.9	85	80	57	72
Fibrous crop residues [b]	7.4	20	0	63	38
Banana leaves and stems	14.8	65	0	50	42
Sugarcane (whole crop)	77.8	15	0	1.9	24
Oil meal (sunflower, cotton)	0	0	0	20.4	10
Spoilt grains	11	5	0	0	4

Key: [a] (Napier, natural grass, tree fodder); [b] (Maize stovers and straws)

Figure 3-4 Feeding systems in the study Counties

3.3.2.3 Cereals and feeds storage practice

The storage period for rainy season maize between harvest and the time of sampling was on average three months for all Counties. Tharaka-Nithi County had the longest storage period followed by Kwale and Bungoma; Kisii had the shortest (Table 3-9).

Table 3-9 Summary on average period of maize storage from time of collection

Region	Mean storage period (months)	Standard deviation	Minimum	Maximum
All (n=194)	3.0	2.0	.4	13.6
Kwale (n=26)	3.2	1.5	0.9	8.6
Tharaka-Nithi (n=58)	4.2	2.3	0.4	5.8
Kisii (n=53)	1.7	1.7	0.4	8
Bungoma (n=57)	3.0	1.6	1.7	13.6

Across the regions, farmers bought an average 21.7 kg of dairy meal lasting about 20 to 90 days maximum. The cows were fed an average of 1.6 kg of concentrate feed per day. Most farmers stored the feeds in gunny bags placed in a house on a plank (Table 3-10).

Table 3-10 Summary of quantity of feeds bought by farmers and storage characteristics

Region	Mean quantity bought per purchase (kg)	Preferred storage type	Mean storage (days)	Standard deviation	Min. days	Max. days
All (n=87)	21.7	1=Main house on a plank 2=Main house on floor	18.9	16.4	1	90
Kwale (n=2)	70	1=Raised store house	4.5	3.5	2	7
Tharaka-Nithi (n=28)	22.4	1= Main house on a plank 2=Main house on floor	18.9	12.3	2	45
Kisii (n=43)	20.4	1= Main house on a plank 2=Main house on floor 3=Raised store house	19.7	18.9	1	90
Bungoma (n=14)	20.6	1= Main house on a plank 2=Main house on floor	18.4	17.0	1	60

3.3.2.4 Milk production

Average of farm daily milk production for Kwale and Kisii Counties excluded two large-scale farmers producing a total of 180 and 108 litres, respectively per day. Kisii and Isiolo had the highest and least milk production, respectively (Table 3-11).

Table 3-11 Summary of milk production, consumption and sales in the study Counties

	Kwale (n=24)	Isiolo (n=55)	Tharaka-Nithi (n=60)	Kisii (n=63)	Bungoma (n=64)	Total (n=266)
Mean daily milk production (L)	2.4	1	4	5.4	3.4	3.4
Mean daily household consumption (L)	1.4	1	1.6	3.1	1.4	1.8
Mean daily milk sales (L)	1	0	2	1.7	2	1.4
Mean price per litre	60	NA*	42.3	40	51.8	46.5

*NA = Not applicable

Milk production per cow per lactation, of an average 14 months, did not differ amongst crossbreeds. Milk production per lactation for cows from Isiolo County was lowest (Figure 3-5). The pastoralists from Isiolo County only kept local cattle breeds. Livestock keepers from the other Counties had local and exotic cross-bred cattle.

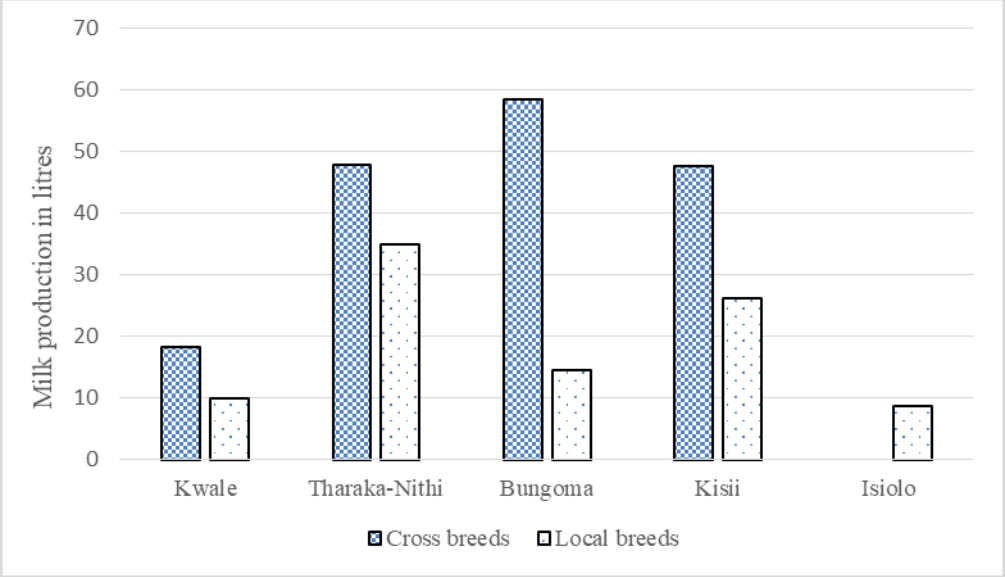

Figure 3-5 Milk production in litres per breed per lactation

3.3.3 Knowledge, attitude and practices towards aflatoxins

Ninety nine percent of the farmers were aware that drinking milk is good for their health. Eighty eight percent believed that they could not get sick from drinking well-boiled milk. Only 12% were aware that aflatoxins could be present in milk (Table 3-12).

Table 3-12 Knowledge, attitude and practices towards aflatoxins in dairy

Do you think… (n=286)	Yes (%)	No (%)	Don't know (%)
a. Drinking milk is good for your health?	99	0.3	0.7
b. Milk safety can be judged by sight?	51	46	3
c. Milk safety can be judged by taste?	81	16	2
c. You worry more about chemicals in milk than about germs?	47	43	10
d. You can get sick from drinking well-boiled milk?	8	88	4
e. Milk from cows fed mouldy feed is unsafe for human consumption?	38	36	26
f. Meat from cows fed mouldy feed is unsafe for human consumption?	34	36	30
g. Aflatoxins can be present in milk?	12	6	82
h. Your customers will pay more for certified aflatoxin free milk?	3	18	79

3.3.4 Risk factor analysis

Univariate analysis for comparison of farms with millet testing above limits and below limits revealed that County, AEZ, knowledge of aflatoxins, household size, practice of whether the household undertake procedures to prevent/mitigate aflatoxin, knowledge that eating mouldy cereal can cause health problem, education and household monthly income were significantly

associated with aflatoxin contamination above or below limits. None of the factors was significantly associated with aflatoxin contamination above or below limits in sorghum.

For maize, the univariate analysis revealed that County, AEZ, knowledge of aflatoxins, knowing that cereals can get mouldy, sorting visibly spoilt maize, knowing a person can get sick from eating mouldy food were significantly associated with aflatoxin contamination above or below limits .

Univariate analysis for comparison of farms with milk testing above limits and below limits revealed that County, AEZ, knowledge of aflatoxins, knowledge of moulds are harmful to human and animal health, practice of whether the household undertake procedures to prevent/mitigate aflatoxin, knowing cereals can get mouldy, knowledge of aflatoxins can be present in milk, education, household size, feeding commercial feeds and feeding homemade feeds were significantly associated with aflatoxin contamination above or below limits.

Table 3-13, Table 3-14 and Table 3-15 show the final multivariate model analysis of possible risk factors for maize, millet and milk contamination with aflatoxins. Significant variables are marked with asterisks indicating their significance levels. High average monthly household income remained as a main risk factor for milk and millet contamination. Mouldy cereals remained as a risk factor for maize contamination with aflatoxins above limits.

Table 3-13 Final multivariable model results for risk factor analysis for maize contamination above limits

Remaining factor	Estimate	Standard Error	P value
Intercept	1.7707	0.2704	5.85e-11 ***
Identification of mouldy cereal	0.9116	0.3357	0.00662 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3-14 Final multivariable model results for risk factor analysis for millet contamination above limits

Remaining factor	Estimate	Standard Error	P value
Intercept	2.399e+01	3.436e+03	0.9944
Sub-humid AEZ	2.532e-01	2.943e+04	1.0000
Temperate AEZ	3.657e+00	1.862e+00	0.0495 *
Household undertaking procedures to prevent aflatoxins	2.150e+01	3.436e+03	0.9950
Income	1.035e-04	5.159e-05	0.0448 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 3-15 Final multivariable model results for risk factor analysis for milk contamination above limits

Remaining factor	Estimate	Standard Error	P value
Intercept	-1.358e+00	3.219e-01	2.46e-05 ***
Semi-arid AEZ	-1.704e+00	6.640e-01	0.01030 *
Sub-humid AEZ	-2.323e+00	1.062e+00	0.02873 *
Temperate AEZ	-1.636e+00	5.226e-01	0.00175 **
Income	2.085e-05	8.624e-06	0.01562 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

3.3.5 Highlights of the milk industry in the study areas

The milk industry in the study Counties was composed of both formal and informal value chains (Figure 3-6). Farmers selling their milk directly to consumers in the neighbouring farms or to hawkers/milk shops characterized the informal milk value chain. In the formal milk value chain farmers and hawkers took milk to milk cooling centres whereby it was picked by milk processors.

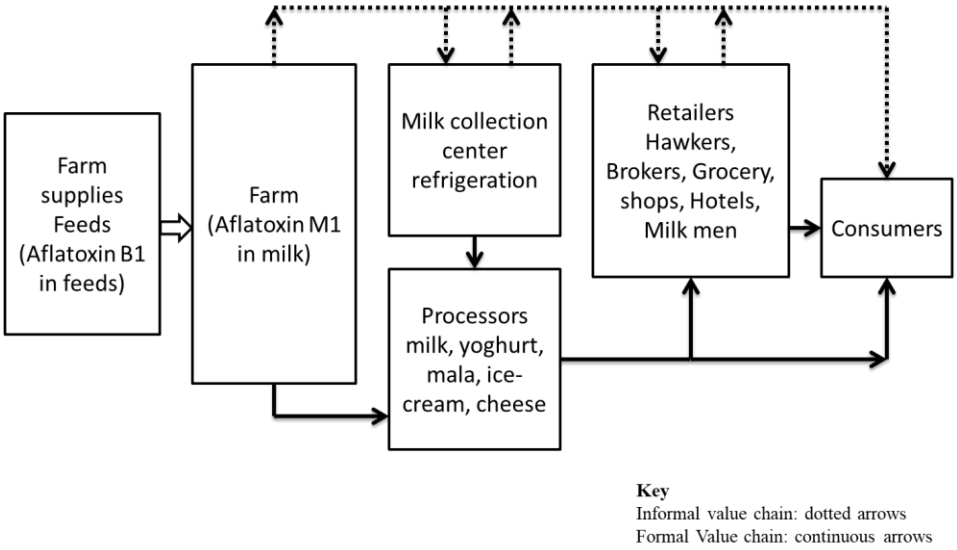

Figure 3-6 Formal and informal milk value chain map of the study areas

3.4 Discussion

This chapter aimed to describe the socio-demographic and farming characteristics for the dairy sector in the five Counties (Isiolo, Kwale, Tharaka-Nithi, Kisii and Bungoma) specifically looking at their farming characteristics, milk industry, and knowledge, attitudes and practices related to aflatoxins in milk. In total 286 farmer households were interviewed. The households composed of an average of five members in Tharaka-Nithi and Kisii Counties. Kwale, Isiolo and Bungoma had an average of 7 members per household. Those average household composition are higher than the Kenyan rural household size of 4.4 people (DHS, 2015). The higher figures could be because, as dairy farmers, the sampled households were richer and older thus could support the many members. Literacy levels of the household heads were low across the Counties as only about a quarter of the heads had completed primary education. Most respondents from Isiolo County had no formal education; the remoteness of the area coupled with nomadic lifestyle meant the prospects of getting educated were low.

Households in all the five Counties engaged in agriculture as their main source of income. All farmers except those from Isiolo and Kwale practised mixed livestock and crop farming. Dairy was classified as a minor source of income by the households apart from in Isiolo where it was regarded as the only source. The Counties did not differ much in their mean average monthly income. High average income was a risk factor for contamination of millet with aflatoxins. Farmers with higher income are more likely to purchase more millet and with longer storage there was an increased chance of contamination.

Dairy farms in Isiolo and Kwale Counties had, on average, thrice the number of cattle as Tharaka-Nithi, Kisii and Bungoma. However, the cattle were dual purpose but mainly for beef. Smallholder farmers in Tharaka-Nithi, Kisii and Bungoma kept low yielding cross breeds. This accounted for the low daily milk production per cow of approximately three and a half litres. That yield was comparable to that achieved by small-holder dairy farmers in Timau producing 4-8 litres per cow per day (Karuga, 2009). A few medium to large scale farms, who reared high potential yielding dairy cows, were seen in Kisii and Kwale. High income was a risk factor for milk contamination with aflatoxins. High income increases the purchasing power for high milk yielding cows and need for commercial feeds thus increased chance of feeding aflatoxin contaminated feeds which ends up in milk. This effect was more in humid areas then temperate zones.

The most common cattle feed was cut and carry forages comprising Napier grass, natural grass and tree fodder. Other feeds depended on local crops found in the areas and their seasonality. For example, sugar cane and bananas are common in Kisii and Bungoma, respectively. Thus, Kisii farmers fed banana leaves and stems to their cattle and Bungoma farmers' sugarcane. Maize stovers and straws were fed to cattle after harvesting maize. In some farms, they let the cattle into the farms after harvesting maize. Concentrates were fed to lactating animals mainly during the dry

season. This was because most farmers could not afford to purchase concentrate feeds. The poor feeding of the cattle contributed to the low milk yields.

Other value chain actors in the dairy areas included dairy feed manufactures, retailers and animal health service providers. However, farmers preferred to sell their milk directly to brokers, milk shops or local consumers to improve their cash liquidity as the processors were paying a constant rate and on monthly basis. Smallholder farmers were selling their milk at prices ranging from Kshs 42 to 70 per litre. Raw milk sold in Isiolo and Kwale fetched higher prices because of limited supply. The dairy industry in the study areas was hindered by similar factors to those described by Karuga (2009) during a value chain analysis of small-holder farmers in Timau, Laikipia County which is classified as temperate AEZ, similar to Kisii and Bungoma in this study. The constraints included: lack of milk cooling facilities; poor animal feeding systems and poor general husbandry resulting in low yields as most farmers used little or no supplemental feeds; and, high cost of artificial insemination (AI) and animal health services. There is a cultural predilection of keeping low yielding cross-bred dairy cows due to the high cost and poor quality of AI services. The farmers could also be avoiding risk as high producing cattle have higher input requirements. The milk value chains in the study Counties were mainly informal.

Perceptions of farmers towards milk were good in terms of knowledge of benefits and potential hazards. However, close to 90% were not aware of chemical hazards resistant to boiling/ pasteurization that can be present in milk such as aflatoxins. It is important to educate the farmers on good agricultural practices that lead to increased and safe production of milk and beef.

3.5 Conclusion

The dairy industry in Kenya generates approximately Kshs 73 billion per annum equivalent to about 4% national GDP. Smallholder farmers contribute significantly to the total milk production in Kenya. In this study the smallholder farmers in dairy potential areas of Tharaka-Nithi, Kisii and Bungoma had low productivity. Improved extension services to the farmers to support them in keeping improved dairy herds, which are well fed, and kept under good husbandry would go a long way in alleviating poverty in the areas. The farmers would benefit from increased income from dairy and improved health of the people through drinking safer milk.

CHAPTER 4

AFLATOXIN OCCURRENCE IN FOODS AND FEEDS FROM FOUR AGRO-ECOLOGICAL ZONES IN KENYA

4.1 Introduction

Grains including maize, sorghum and millet constitute important staple foods in Kenya. The grains are used as human food and animal feed and are a source of processed foods such as cooking oil and breakfast cereals. In the past Kenya was exporting maize but this has changed in recent years, the country is now a net importer of maize with minimal exports mainly to other East African Community countries (Gitonga, 2016; NCPB, 2015). Besides their nutritive and economic benefits, maize, sorghum and millet form ideal substrates for aflatoxin-producing fungi under conditions of high temperature and humidity. Countrywide there has been reported occurrence of aflatoxins in these commodities (Muthomi *et al*., 2012; Mwihia *et al*., 2008; Okoth & Kola, 2012).

Aflatoxin M1 (AFM1), found in milk, is a toxic metabolite of aflatoxin B1 (AFB1). The transfer of aflatoxins from feed to milk (carryover) is influenced by various nutritional and physiological factors, including feeding regimes, rate of digestion, health of the animal, hepatic biotransformation capacity, and actual milk production. Estimated carryover is between 1 to 2% of the total amount of AFB1 ingested (Fink-Gremmels, 2008). Dairy animals are exposed through feeding on contaminated grains and their by-products (maize bran, maize germ, wheat bran and other grain milling by-products). Other easily contaminated feeds include protein rich supplements (cotton seed cake, sun flower cake, fish meals and other oil seed by-products) (Lanyasunya *et al*., 2005). Lack of awareness on aflatoxins and poverty has contributed to farmers feeding visibly mouldy grains to animals (Kiama *et al*., 2016).

Aflatoxins are products of the moulds: *Aspergillus flavus, A. parasiticus* and *A. nomius* while infesting crops and forage. Aflatoxins that occur naturally include aflatoxin B1 (AFB1), aflatoxin B2, aflatoxin G1 and aflatoxin G2 (IARC, 2002). Aflatoxins and other mycotoxins in crops and animal source foods pose urgent agricultural related health problems globally (Grace *et al*., 2015). They are classified as a class 1 human carcinogen known to cause liver cancer (IARC, 2002). In Africa, liver cancer is a serious health problem, with reported 8.19 cases per 100,000 inhabitants (Wu *et al*., 2011). Observational studies have shown an association between stunting and aflatoxin exposure (Khlangwiset *et al*., 2011). Stunting is common in Sub-Saharan Africa, where also food-borne aflatoxin exposure is high. Aflatoxin exposure in livestock is linked with reduced feed intake, reduced feed conversion efficiency and reduced weight gain (Khlangwiset *et al*., 2011). Besides health impacts, negative economic impacts are felt when food products contaminated above legal limits are condemned (Wu, 2008).

Warm and humid tropical climate experienced in East Africa favours the growth of aflatoxigenic moulds in crops. In order to reduce human and livestock exposure, the Government of Kenya has set limits for aflatoxins in food and feed as 10 parts per billion (ppb) for total aflatoxin whereas that of AFB1 is 5 ppb (EAC, 2005). During the largest aflatoxicosis outbreak in Kenya, levels up to 100 times the legal limit were found in home-grown maize (Lewis *et al*., 2005). That sparked campaigns to combat aflatoxin contamination in maize in Kenya. However, it is important to highlight other sources of aflatoxin exposure from foods such as groundnut, millet, sorghum and milk. This study was undertaken to determine the occurrence of aflatoxins in maize, millet, sorghum, milk and animal feeds sourced from four agro-ecological zones (AEZs) in Kenya.

4.2 Materials and Methods

4.2.1 Study sites selection

Study sites were randomly chosen from a map of agro-ecological zones in Kenya (IIASA/FAO, 2012). Study sites comprised one County each from the semi-arid, humid and sub-humid zones and two Counties in the temperate zone, being an area with high maize growing and dairy keeping activity. Study Counties included Isiolo (semi-arid), Tharaka-Nithi (humid), Kwale (sub-humid), Bungoma (temperate), and Kisii (temperate) (Figure 3-1).

4.2.2 Sample size calculation

Household sample size was calculated using an expected prevalence of 72% (Kang'ethe & Lang'at, 2009), 95% level of confidence and a 10% desired precision level (Dohoo *et al*., 2009). Calculated sample size of 321 was divided by five to give a sample size of 64 farmer households per County. To achieve this, eight villages were sampled per Sub-location and within each village, eight farmers sampled. Targeted farmers were randomly selected from a sampling frame that listed all the farmers who fit the criteria of growing cereals and keeping livestock.

4.2.3 Sampling

Sampling was carried out in two phases representing dry and rainy seasons in order to account for seasonal variation in aflatoxin contamination. Dry season samples were collected in February and March 2014 whereas rainy season ones were collected in July and October 2014 (Figure 4-1). During dry season sampling it was expected that the households would be in a cereal growing period and that they would have previous season cereals stored for at least three months. Rainy season sampling was planned to coincide with the cereal harvest period and, therefore, assuming availability of freshly harvested cereals.

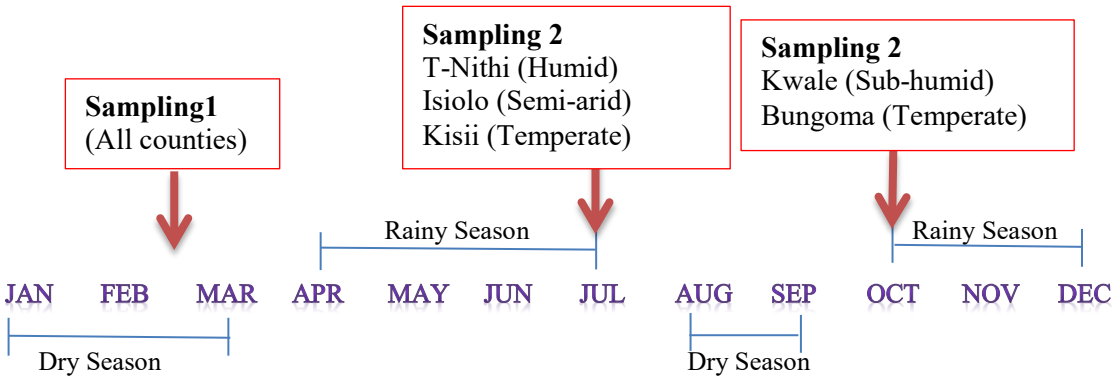

Figure 4-1 Sampling periods based on seasonal calendar for the study Counties

Approximately 500g each of millet, sorghum, maize and feeds were sampled in brown paper bags per household using a scoop sterilized in sodium hypochlorite. Samples were drawn from the top, middle and bottom of bags of pre-mixed grains. Samples were also taken from local feed retailers supplying the households. Feed samples were also obtained from feed manufacturers supplying the retailers.

Raw milk samples were collected from farm-bulked milk, which was mixed manually, and a maximum of 300ml collected. Samples were transported in cool boxes and stored at 4°C (grains and feeds) and at -20 ºC (milk). Sample analysis was conducted at the Department of Public Health, Pharmacology and Toxicology, University of Nairobi and the Biosciences eastern and central Africa–ILRI mycotoxin laboratory, Nairobi.

4.2.4 Aflatoxin analysis

4.2.4.1 Determination of aflatoxin B1 in maize, sorghum, millet and feeds

Romer Series II Mill (Romer Labs Inc., 1301 Stylemaster Drive Union, MO 63084) was used to grind samples in preparation for aflatoxin extraction. Eighty percent acetonitrile was used to extract AFB1 from 5g of ground samples following manufacturer's instructions. For cereals the ratio of sample to extraction solvent was 1:5 (w/v) whereas for feeds 1:100 (w/v) (Figure 4-2).

Aflatoxin quantitative detection was carried out using Helica Low Matrix AFB1 competitive enzyme-linked immunosorbent assay (ELISA) kit (Helica Biosystems Inc., Santa Ana, CA - Catalogue Number 981BAFL01LM-96). The kit had a limit of detection of ≤ 1ppb and a specificity of 100%. An aflatoxin specific antibody optimized to react with AFB1 was pre-coated to a polystyrene micro-well by the manufacturer. Diluted standards or samples were added to the antibody-coated micro-wells and incubated for 30 minutes. Micro-well contents were decanted and non-specific reactants were removed by washing with phosphate buffer saline-tween. One hundred µl/well of horse–radish peroxidase (HRP)-conjugated AFB1 were added and incubated for 30 minutes. Aflatoxin from the extracted sample and the HRP-conjugated AFB1 compete to bind with the antibody coated to the micro-well. After incubation, the well contents were again decanted and non-specific reactants were removed by washing. Enzyme substrate was added (100 µl/well), incubated for 15 minutes and the reaction was stopped by adding stop solution (100 µl/well). The optical density was measured at 450 nm using a micro-plate reader (Labsystems Multiskan® PLUS, Helsinki, Finland). The intensity of the colour was directly proportional to the amount of bound conjugate and inversely proportional to the concentration of aflatoxin in the sample or standard. To ensure samples tested were within the lower and upper standards in a given assay they were re-diluted and the assay repeated. Aflatoxin standards ranged between 1 ppb and

20 ppb. Samples that tested above 20 ppb in a given assay were re-diluted and the assay repeated so that it fell within the standard range. The final reading took into account the dilutions applied.

Figure 4-2 Aflatoxin extraction using 80% acetonitrile

4.2.4.2 Determination of aflatoxin M1 in milk

Quantification of AFM1 was done according to manufacturer's recommendations using Helica AFM1 ELISA quantitative kit (Helica biosystems, inc, Santa Ana, CA 92704, USA, Catalog no 961AFLM01M-96). The kit had a limit of detection of 2ppt and a specificity of 100%. Thawed milk samples were prepared by centrifuging at 2000g for five minutes to induce separation of upper cream layer. The upper cream layer was removed using a Pasteur pipette and the lower plasma used in the assay.

An aflatoxin specific antibody optimized to react with AFM1 was pre-coated to a polystyrene micro-well by the manufacturer. Two hundred micro litres of standards or samples were added to the antibody-coated micro-wells and incubated at ambient temperature for two hours. Micro-well contents were decanted and non-specific reactants removed by washing with phosphate buffer saline-tween. One hundred µl/well of horse–radish peroxidase (HRP)-conjugated AFM1 were

added and incubated for 15 minutes. Aflatoxin from the extracted sample and the HRP-conjugated AFM1 compete to bind with the antibody coated to the micro-well. After incubation, the well contents were again decanted and non-specific reactants removed by washing. Enzyme substrate was added (100 µl/well), incubated for 15 minutes and then the reaction was stopped by adding stop solution (100 µl/well). The optical density was measured at 450 nm using a micro-plate reader. Samples tested were maintained within the lower (0 ppt) and upper limits (100 ppt) of standards in a given assay by re-dilution and repeat of assays.

4.2.5 Statistical analyses

Data entry was done in Microsoft Excel® 2010 and analyzed using a combination of R® (version 3.1.3) and Stata® 13 (StataCorp LP, Texas, USA). Aflatoxin concentrations' data being skewed, non-parametric tests were applied including calculation of geometric means as well as arithmetic means. Non-detectable samples were assigned the value of 1 ppb when calculating geometric means. Chi Square Test and Fisher's Exact Test were used to compare proportions of aflatoxin contamination amongst Counties and AEZs. In analysis of variance of aflatoxin levels amongst Counties and AEZs Kruskal–Wallis rank sum test was. Wilcoxon sign-rank test was used to compare the means of aflatoxin contamination of samples collected during the dry and rainy seasons. Significance was reported at 95% confidence interval.

4.2.6 Ethical approval

Ethical approval for the study was acquired from the International Livestock Research Institute (ILRI) (approval number ILRI-IREC2013-09). All participants were informed about the purpose of the study and gave their informed consent to participate before sampling was carried out.

4.3 Results

The survey was based on a total of 286 farmer households. Distribution across Counties was as follows: 37 from Kwale, 56 from Isiolo, 65 from Tharaka-Nithi, 64 from Kisii and 64 from Bungoma. The households sampled in Kwale and Isiolo were less than the targeted 64 because in Kwale County, Waa Sub-location only six villages kept livestock and within the villages farmers were less than 8. In Isiolo County, Lonkopito Sub-location the villages were seven in total thus only 56 farmers could be sampled.

4.3.1 Contamination levels of cereals

A total of 205 millet, 164 sorghum and 497 maize samples were collected and analysed. Most samples were home grown except those obtained from Isiolo (semi-arid AEZ) which were market-sourced. Overall, 26% (maize), 10% (millet) and 11% (sorghum) of the samples had AFB1 beyond Kenya limits of 5 ppb (Figure 4-3). Sorghum sampled during the rainy season from humid ($p<0.01$) and semi-arid ($p<0.05$) zones had significantly higher aflatoxin contamination levels compared to dry season samples. Dry season maize as compared to rainy season maize from the temperate zone had significantly higher aflatoxin contamination levels ($p<0.05$).

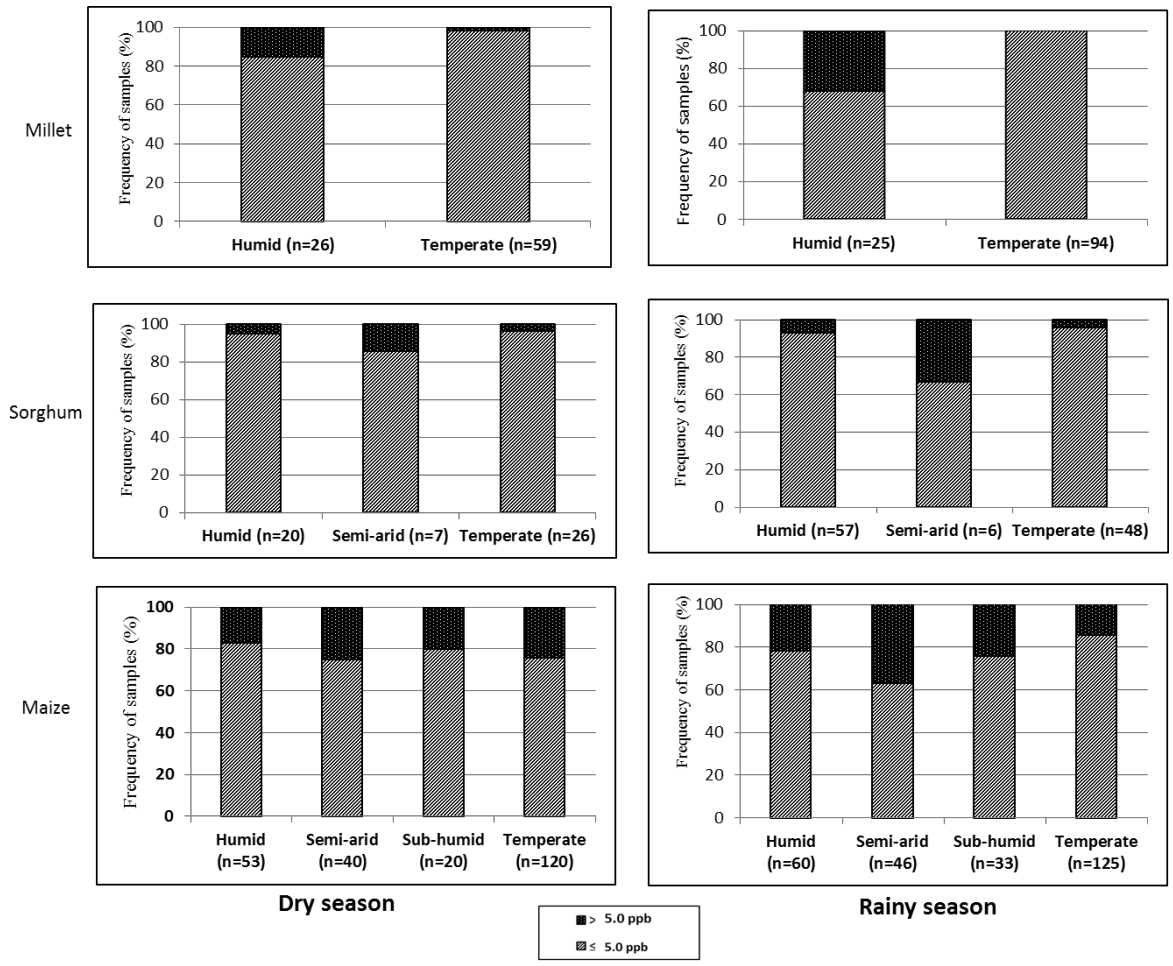

Figure 4-3 Percentage of samples in the two categories of levels of aflatoxins plotted against agro-ecological zones

4.3.1.1 Contamination levels of cereal samples collected during the dry season

The levels of aflatoxins ranged from 1 to 1,658.2 ppb (millet), 1 to 23.1 ppb (sorghum) and 1 to 1,137.4 (maize) ppb (Table 4-1). Mean aflatoxin levels did not significantly differ among AEZs. Millet samples from Tharaka-Nithi (Humid AEZ) had the highest proportion contaminated above Kenya legal limits of 5ppb AFB1 in foods ($p<0.05$).

Table 4-1 Levels of aflatoxin contamination in samples collected during the dry season

Region	Range (ppb)	Mean (ppb) Arithmetic mean	Mean (ppb) Geometric mean	Percent detected	Percent exceeding 5 ppb
Millet					
Kwale (sub-humid; n=1)	N/A	1.4	1.4	100	0
Tharaka-Nithi (humid; n=26)	<1.0 – 1658.2	66.2	1.3	69.2	15.4
Kisii (temperate; n=35)	<1.0 – 3.0	0.5	0.5	77.1	0
Bungoma (temperate; n=24)	<1.0 – 13.8	0.9	0.4	75	4.1
Sorghum					
Isiolo (semi-arid; n=7)	<1.0 – 11.9	2.0	1.0	57.1	14.3
Tharaka-Nithi (humid; n=20)	<1.0 – 23.1	1.5	0.4	85	5
Kisii (temperate; n=1)	N/A	0.7	0.7	100	0
Bungoma (temperate; n=25)	<1.0 – 12.3	0.9	0.4	84	4
Maize					
Kwale (sub-humid; n=20)	<1.0 – 19.2	3.5	2.1	95	20
Isiolo (semi-arid; n=40)	<1.0 – 1137.4	67.3	2.7	50	25
Tharaka-Nithi (humid; n=53)	<1.0 – 774.7	23.9	1.3	75.4	17
Kisii (temperate; n=63)	<1.0 – 371.5	8.9	1.6	77.8	25.4
Bungoma (temperate; n=57)	<1.0 – 39.3	3.5	1.3	71.9	22.8

N/A: not applicable

4.3.1.2 Contamination levels of cereal samples collected during the rainy season

Aflatoxin levels in the samples ranged from 1 to 152.3 ppb (millet), 1 to 91.7 ppb (sorghum) and 1 to 536.8 ppb (maize) ppb (Table 4-2). Tharaka-Nithi (humid AEZ) had significantly higher mean of aflatoxins in millet than Counties from the temperate zone (p=0.004). Sorghum samples from Isiolo (semi-arid AEZ) had the highest mean aflatoxin contamination as compared to the rest. Kwale (sub-humid AEZ) Maize had the highest mean aflatoxin contamination.

Comparison of proportions testing above the 5 ppb limit showed maize from the semi-arid region had the highest proportion, humid and sub-humid zones moderate proportion while temperate zone the least. Sorghum from the semi-arid zone had a significantly higher proportion testing above the Kenyan legal limits ($p<0.05$).

Table 4-2 Levels of aflatoxin contamination in samples collected during rainy season

Region	Range (ppb)	Mean (ppb) Arithmetic mean	Mean (ppb) Geometric mean	Percent detected	Percent exceeding 5 ppb
Millet					
Tharaka-Nithi (humid;	<1.0 – 152.3	10.9	2.3	64	32
n=25)	<1.0 – 3.0	0.1	0.8	21.1	0
Kisii (temperate; n=52)	<1.0 – 2.9	0.6	0.5	97.6	0
Bungoma (temperate; n=42)					
Sorghum					
Isiolo (semi-arid; n=6)	<1.0 – 12.8	3.8	1.5	100	33.3
Tharaka-Nithi (humid;	<1.0 – 17.9	1.2	1.0	33.3	7
n=57)	<1.0 – 16.4	0.9	1.1	10.5	5
Kisii (temperate; n=19)	<1.0 – 91.7	3.5	0.4	96.5	3.4
Bungoma (temperate; n=29)					
Maize					
Kwale (sub-humid; n=33)	<1.0 – 394.1	28.9	2.8	97	24.2
Isiolo (semi-arid; n=46)	<1.0 – 120.7	9.6	2.2	97.8	37
Tharaka-Nithi (humid;	<1.0 – 536.8	23.6	1.4	88.3	21.7
n=60)	<1.0 – 102.6	4.0	1.3	46.8	17.7
Kisii (temperate; n=62)	<1.0 – 217.6	7.9	0.8	81	11.1
Bungoma (temperate; n=63)					

4.3.2 Levels of aflatoxins in feeds from feed manufacturers, feed retailers and farmers

A total of 277 feeds were sampled from households (n=144), feed retailers (n=31) and feed manufacturers (n=102). Samples from feed manufacturers were sourced from the following Counties: Mombasa (supplies Kwale County), Meru (supplies Tharaka-Nithi County), Bungoma (supplies Bungoma County) and Nakuru (supplies Kisii and Bungoma Counties). The dairy feeds comprised of dairy meal, pollard, maize, maize germ, maize bran, rice germ, rice bran, wheat pollard, wheat bran, young stock, calf meal, calf pellet, sorghum, cotton seed, sunflower and pyrethrum mix and home-made concentrates.

The mean (geometric) AFB1 concentrations in feeds were 9.8 ppb (feed manufacturers), 25.6 ppb (feed retailers) and 13.7 ppb (farmers). Farmers' feeds from Tharaka-Nithi had the highest geometric mean (Table 4-3). Overall, home produced dairy feeds had lower AFB1 geometric means (0.4 ppb in dry season, n=18; 18.9 ppb in rainy season, n=4) than purchased feeds (7.0 ppb in dry season, n=41; 25.3 ppb in rainy season, n=20).

Table 4-3 Prevalence of AFB1 in farm feeds in the selected Counties in Kenya

County	AEZ	N	prev. >1ppb (%)	prev. >5ppb (%)	prev. >10ppb (%)	prev. >20ppb (%)	prev. >50ppb (%)	prev. >100ppb (%)	amean (ppb)	median (ppb)	gmean (ppb)
Kwale	Sub-humid	2	100.0	50.0	0.0	0.0	0.0	0.0	3.7	3.7	3.7
T. Nithi	Humid	57	94.2	88.5	84.6	57.7	36.5	7.7	349.9	22.9	29.9
Kisii	Temperate	40	100.0	100.0	100.0	87.5	75.0	0.0	55.0	26.3	9.5
Bungoma	Temperate	19	75.0	25.0	18.5	16.7	8.3	0.0	16.5	0.4	0.8

AEZ: agro-ecological zone; N=number of feed samples; prev.: prevalence (%); amean: arithmetic mean; gmean: geometric mean, T.Nithi: Tharaka-Nithi; 1ppb is the limit of detection.

Only 5% of the local retailers' feeds tested below Kenyan limits of 5 ppb AFB1 (Table 4-4).

Table 4-4 Prevalence of AFB1 in feeds from feed retailers in the study sites

County	Number of samples	prevalence >5 ppb (%)	range (ppb)	amean (ppb)	median (ppb)	gmean (ppb)
Tharaka-Nithi	15	86.7	<1-1198	115.4	20.3	19.1
Kisii	10	100.0	9-310	76.9	48.6	46.6
Bungoma	6	83.3	<1-103	47.2	52.8	19.7
All	31	90.3	<1-1198	89.8	42.3	25.6

N: number of samples; amean: arithmetic mean; gmean: geometric mean; 1ppb is the limit of detection

All feeds from sampled Meru County's feed manufacturers exceeded Kenyan limits of 5 ppb AFB1 (Table 4-5).

Table 4-5 Prevalence of AFB1 in feeds from different feed manufacturers encountered in the study sites

Feed source-County	Number of feed samples	Feed market-County	AEZ of County where feed is fed to cattle	prevalence (>5ppb)	range (ppb)	amean (ppb)	median (ppb)	gmean (ppb)
Mombasa	7	Kwale	Sub-humid	28.6	<1-51.7	9.8	2.9	3.0
Meru	9	T. Nithi	Humid	100.0	14-4682	875.7	162.5	175.0
Nakuru	76	Kisii, Bungoma	Temperate	59.2	<1- 252.9	31.8	8.5	7.3
Bungoma	10	Bungoma	Temperate	70	<1-204.7	75.0	53.5	16.2
All	102	---	---	61.8	<1-4682	109.4	11.7	9.8

N: number; amean: arithmetic mean; gmean: geometric mean; 1ppb is the limit of detection

Farmers' feeds from Tharaka-Nithi County (humid AEZ) that were collected during the rainy season had higher AFB1 concentration (up to 9,661 ppb) compared to dry season feeds as shown by the Wilcoxon rank sum test at 95% level of confidence (Table 4-6). In the dry season, Bungoma County had the highest AFB1 levels with a quarter testing above 40 ppb.

Table 4-6 Prevalence of AFB1 in farmers' feeds during dry and rainy seasons in Kenya

County	AEZ	samples (Season)	range (ppb)	amean (ppb)	gmean (ppb)	samples (Season)	range (ppb)	amean (ppb)	gmean (ppb)	p-rs
Kwale	Sub-humid	1(dry)	NA	0.8	0.8	2(rainy)	4-5	4.9	4.9	0.1213
T. Nithi	Humid	20(dry)	<1-28	13.2	8.6	52(rainy)	<1-9661	477.3	37.6	0
Kisii	Temperate	30(dry)	<1-68	19.9	5.1	16(rainy)	11-344	138.1	88.7	0.4757
Bungoma	Temperate	11(dry)	<1-85	22.3	3.0	12(rainy)	<1-81	12.1	1.5	0.9278

AEZ: agro-ecological zone; amean: arithmetic mean; gmean: geometric mean; p-rs: two-sample Wilcoxon ranksum test at 95% confidence; T. Nithi: Tharaka-Nithi; 1ppb is the limit of detection

Figure 4-4 shows occurrence of AFB1 in farmers' feeds.

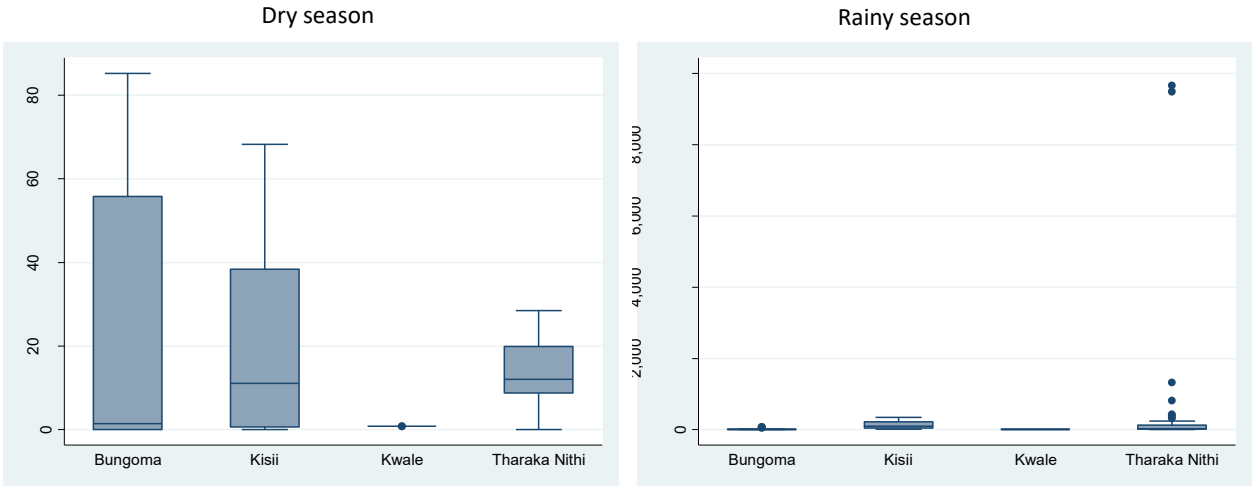

Figure 4-4 Prevalence of AFB1 in farmers feeds in the dry season and rainy season

4.3.3 Aflatoxin M1 occurrence in milk

In total, 512 samples from 282 farmers were collected and analysed. Of these, 39.7% were positive and 10.4% had levels exceeding allowable WHO/FAO limit of 50 ppt (Table 4-7). Comparisons of concentrations of aflatoxins between milk collected during dry season and wet season showed the following: Isiolo County wet season milk had higher AFM1 levels ($p=0.02$) whereas Bungoma County dry season milk had higher AFM1 levels ($p<0.001$) (Table 4-8). In the dry season, Kwale County had the highest median (>200 ppt AFM1), followed by Kisii County with an AFM1 median above 100 ppt.

Table 4-7 Prevalence of AFM1 in milk from farmers in Kenya

County	AEZ	N	prev. >2ppt (%)	prev. >5ppt (%)	prev. >20ppt (%)	prev. >50ppt (%)	prev. >100ppt (%)	range (ppt)	amean (ppt)	median (ppt)	gmean (ppt)
Kwale	Sub-humid	36	3.5	3.5	3.5	2.0	3.5	<2-485	20.5	1.0	1.0
Isiolo	Semi-arid	56	53.7	40.7	14.8	5.6	1.9	<2-820	14.1	2.9	3.3
T.Nithi	Humid	64	64.5	46.8	40.3	25.8	14.5	<2-6999	152.0	2.1	13.8
Kisii	Temperate	63	27.1	20.8	16.6	10.4	4.2	<2-230	15.8	1.0	3.0
Bungoma	Temperate	64	16.6	11.9	7.1	4.8	0.0	<2-230	12.8	1.5	3.6

AEZ: agro-ecological zone; N: number of milk samples; prev.: prevalence (%); amean: arithmetic mean; gmean: geometric mean; T.Nithi: Tharaka-Nithi; 2ppt is the limit of detection

Table 4-8 Prevalence of AFM1 in milk during dry and rainy seasons in the study sites in Kenya

County	AEZ	N (season)	range (ppt)	amean (ppt)	gmean (ppt)	N (season)	range (ppt)	amean (ppt)	gmean (ppt)	p-rs
Kwale	Sub-humid	30(dry)	<2-256	11.2	1.1	29 (rainy)	<2-485	17.7	1.2	0.7580
Isiolo	Semi-arid	56 (dry)	<2-70	4.1	1.7	54 (dry)	<2-820	25.4	2.5	0.0471
T. Nithi	Humid	64 (dry)	<2-358	32.6	7.6	62 (rainy)	<2-6999	167.6	9.1	0.7435
Kisii	Temperate	63 (dry)	<2-216	13.8	2.4	48 (rainy)	<2-465	20.7	2.5	0.1402
Bungoma	Temperate	64 (dry)	<2-230	16.7	4.2	42 (rainy)	<2-86	6.3	1.6	0.0002

AEZ: agro-ecological zone; N: number of milk samples; amean: arithmetic mean; gmean: geometric mean; p-rs= two-sample Wilcoxon ranksum test at 95% confidence; T. Nithi=Tharaka-Nithi; 2ppt is the limit of detection

4.4 Discussion

This study investigated the occurrence of AFB1 (in cereals and feeds) and AFM1 (in cow milk) in samples from five Counties representing four AEZs in Kenya in the rainy and dry seasons. Other factors not assayed in this study that affect colonization and production of aflatoxins by *Aspergillus* in grains include stress of the host plant during planting, type of *Aspergillus* strains present in the soils and agricultural practices. The sampling method used aimed to collect a representative sample, however, the very heterogeneous distribution of aflatoxin in grains, challenges in sampling and variability of results can affect the reliability of results.

Most millet and sorghum samples were within acceptable Kenyan limits apart from a few like one millet sample that had over 1600 ppb, which is 320 times above the legal limit. Millet and maize from the humid and sub-humid zones had consistently higher mean levels in both rainy and dry seasons as compared to samples from the temperate zone. High humidity in these zones favours colonization and production of aflatoxins in grains (Reddy *et al*., 2010). Concentration of AFB1 in millet were comparable to levels found in Ethiopia (arithmetic mean of 1.12 ppb) and in Nandi (temperate AEZ) in 2011-12, when the arithmetic mean was 7.9 ppb and a reported range of 0.1–6.4 ppb (Chala *et al*., 2014; Sirma *et al*., 2015). The Nandi study reported a much wider range of sorghum contamination (0.2–210.1 ppb) from market sourced samples than for this study a difference that could have been due to source of samples (Sirma *et al*., 2015). The Ethiopia study reported a much higher mean level (29.5 ppb) of AFB1 in sorghum (Chala *et al*., 2014). This current study is amongst a few in East Africa reporting on aflatoxin contamination in sorghum and millet. Considering aflatoxins were found in levels above Kenya legal limits there's need for further studies to evaluate the role of sorghum and millet in contributing to aflatoxin exposure in East Africa.

In this study, a quarter of the maize tested had aflatoxin contamination above allowable Kenyan limits. The high contamination levels are consistent with those found in Nairobi market maize whereby only 17% of the maize were found fit for human consumption (Okoth & Kola, 2012). Regionally, Kwale County (sub-humid AEZ) maize collected during the rainy season had the highest mean level of aflatoxin followed by maize from Isiolo County (semi-arid AEZ) collected during the dry season. Across Counties, Isiolo County rainy season maize had the highest proportion testing above Kenya limits. Since Isiolo and Kwale Counties produce little maize, if any, imported grains from neighbouring Counties could contribute to their exposure to aflatoxin. Poor transport and storage of the grains increases likelihood of contamination with aflatoxins (Kaaya & Kyamuhangire, 2006). Isiolo County maize collected during the rainy season had almost double the mean aflatoxin in maize from Tharaka-Nithi County, which is one of their main sources of maize. A study within Makueni County, a semi-arid area that has had a previous outbreak of aflatoxicosis, found contamination of slightly more than a quarter (35.5%) of the locally grown maize exceeding legal limits (Mwihia et al., 2008). This is comparable to the high levels found in maize from Isiolo County. It is, therefore, important for traders and millers to improve the quality of their cereals by testing for aflatoxins. Maize from temperate regions had a relatively low mean contamination as compared to that from the semi-arid, humid and sub-humid zones. A previous survey in the region found 2% (Bungoma) and 8% (Kisii) of maize collected from local mills to be contaminated above Kenya limits (Mutiga et al., 2015), results that were much lower than in the present study. In the temperate region, unfavourable climatic conditions for the fungal growth and, to a lesser extent, presence of low-aflatoxin-producing L strains of *Aspergillus* found in their soils could have contributed to the low levels (Okoth *et al*., 2012).

The results of AFB1 in dairy feeds and AFM1 in cattle milk from rural villages and urban centres reported in this study are comparable to earlier reports from Kenyan urban and peri-urban areas (Kang'ethe & Lang'at, 2009; Kang'ethe *et al*., 2007) but higher than Ethiopian reported ones (Gizachew *et al*., 2016). Farmers' feeds had lower geometric mean AFB1 than feeds from feed retailers. This could be explained to be due to lower initial aflatoxin contamination of home-made feeds or poor storage practices of the manufactured feeds along the dairy feed value chain. Feed retailers' feeds had higher AFB1 concentration as compared to feed manufacturers' feeds, possibly due to contamination or multiplication of *Aspergillu*s fungi along the dairy feed value chain. Tharaka-Nithi and Kisii Counties had higher proportions of milk exceeding the 50 ppt AFM1 WHO/FAO limit probably due to their higher proportion of dairy breeds and a corresponding higher proportion of farmers who fed dairy concentrates to cattle. High AFB1 concentrations in dairy feeds have been shown to reduce milk production by up to 25% (Guthrie & Bedell, 1979) and cause a decrease in feed conversion efficiency and reproduction efficiency. Farmers in Bungoma County may have grazed their cattle on natural pastures with reduced concentrate feeding during the rainy season resulting in low AFM1 concentrations in the milk.

4.5 Conclusion

A substantial proportion of samples exceeded recommended limits for the relevant commodities raising concern of chronic exposure to aflatoxins given their grave impact on human and animal health. Moreover, there are uncertainties on the actual safety levels, evident from different regulations of mycotoxins in different regions (FAO, 1997a). There is need to focus on the risks for individual consumers from susceptible foods and a study of the factors contributing to exposure and health impacts. Risk and economic assessments of aflatoxins on human and animal health would provide this information that is lacking in East Africa.

CHAPTER 5

RISK ANALYSIS FOR LIVER CANCER FROM AFLATOXIN EXPOSURE THROUGH MILK

5.1 Introduction

Globally, many countries have set limits for aflatoxins in food and feed in order to protect their markets and promote good health for their people. In Kenya, outbreaks of aflatoxicosis have occurred from human dietary exposure through susceptible foods such as maize, groundnuts, millet, sorghum and dairy products.(Nyikal *et al*., 2004). Occurrence of aflatoxin B1 (AFB1) in foods/feeds and aflatoxin M1 (AFM1) in milk has been reported above Kenyan regulatory limits of 5 ppb AFB1 in foods/feeds (levels for dairy are not specified) and the European Union (EU) recommended limits of 50 ng/kg AFM1 in dairy (Kiarie *et al*., 2016; Mutegi *et al*., 2009; Mutiga *et al*., 2015; Senerwa *et al*., 2016; Sirma *et al*., 2016). Aflatoxin positive foods are freely marketed in informal markets in Kenya where there is low compliance to standards and inadequate regulatory enforcement.

Aflatoxins are poisonous fungal by-products produced by the *Aspergillus* species. Aflatoxins cause varied health effects from acute to chronic illness, notably liver cancer which is positioned number four among leading causes of cancer deaths worldwide (Yang *et al*., 2019). Liver cancer incidence is more in men than in women with a standardized incidence rate of 15.3 per 100,000 among men and 5.4 per 100,000 among women (IARC, 2016). In the USA, among children, the incidence of primary malignant liver cancers is approximately 1 per 100 million (Guo & Zhang, 2013). In Kenya, an estimated 1120 new liver cancer cases and 1037 liver cancer deaths occurred in 2012 (Ferlay *et al*., 2015). Worldwide, amongst primary liver cancers, hepatocellular carcinoma (HCC) occurs the most and between 14% and 19% of the cases annually are attributable to aflatoxin

exposure (Liu *et al*., 2012). Besides aflatoxicosis other factors that increase the risk of developing the disease include hepatitis B virus (HBV) infection, hepatitis C virus (HCV) infection, alcoholism, smoking, and hereditary conditions (Mutuma *et al*., 2011). In eastern/south-eastern Asia and Africa, HCC rates are particularly high because of concurrent infections with hepatitis virus and aflatoxin exposure.

Risk analysis process entails the quantification of risk, the modeling of identified risks and making of decisions from those models (Vose, 2002). Risk analysis can be performed based on the Codex Alimentarius Commission or the World Organization for Animal Health (OIE) guide (FAO, 1999; OIE, 2010). In OIE, risk analysis has four components of hazard identification, risk assessment, risk management and risk communication. The risk assessment component of the analysis estimates the risks associated with a hazard and can be done qualitatively or quantitatively (OIE, 2010). In this study risk assessment was performed to quantify the risk of HCC in children and adults exposed to aflatoxins in milk. Risk assessment outputs are beneficial as they provide necessary information to guide food regulators and scientists in making risk management decisions and undertaking of processes, such as the setting of standards/limits for mycotoxins in foods (Shephard, 2008b). For Kenya, the estimated population risk for HCC from AFB1 exposure through maize is at 11 cancers/year per 100,000 (maize from urban markets) and 29 cancers/year per 100,000 population (maize from rural markets). Similar estimates for the risk of liver cancer from consumption of AFM1 in milk from Kenya are lacking. Additionally, there are no official Kenyan limits for AFM1 in milk (Sirma *et al*., 2018), previous studies have reported levels based on FAO/WHO standards of 500 ppt or EU standards of 50 ppt (EU, 2006). The carcinogenic potency for AFM1 has been calculated to be 10% of the potency of AFB1 based on the induction of HCC in AFB1-treated rats versus AFM1-treated rats (Cullen *et al*., 1987). More than 50% of

marketed milk in Nairobi city and 10% of rural milk samples were found to contain AFM1 at levels above the EU standards (Lindahl *et al*., 2018; Senerwa *et al*., 2016). The common and high occurrence of aflatoxins in milk in Kenya warrants the need for quantifying its health risks. This study presents a quantitative risk assessment to estimate aflatoxin exposure from cow's raw milk in rural Kenya and its contribution to the risk of HCC, using the scenario tree depicted in Figure 5-1.

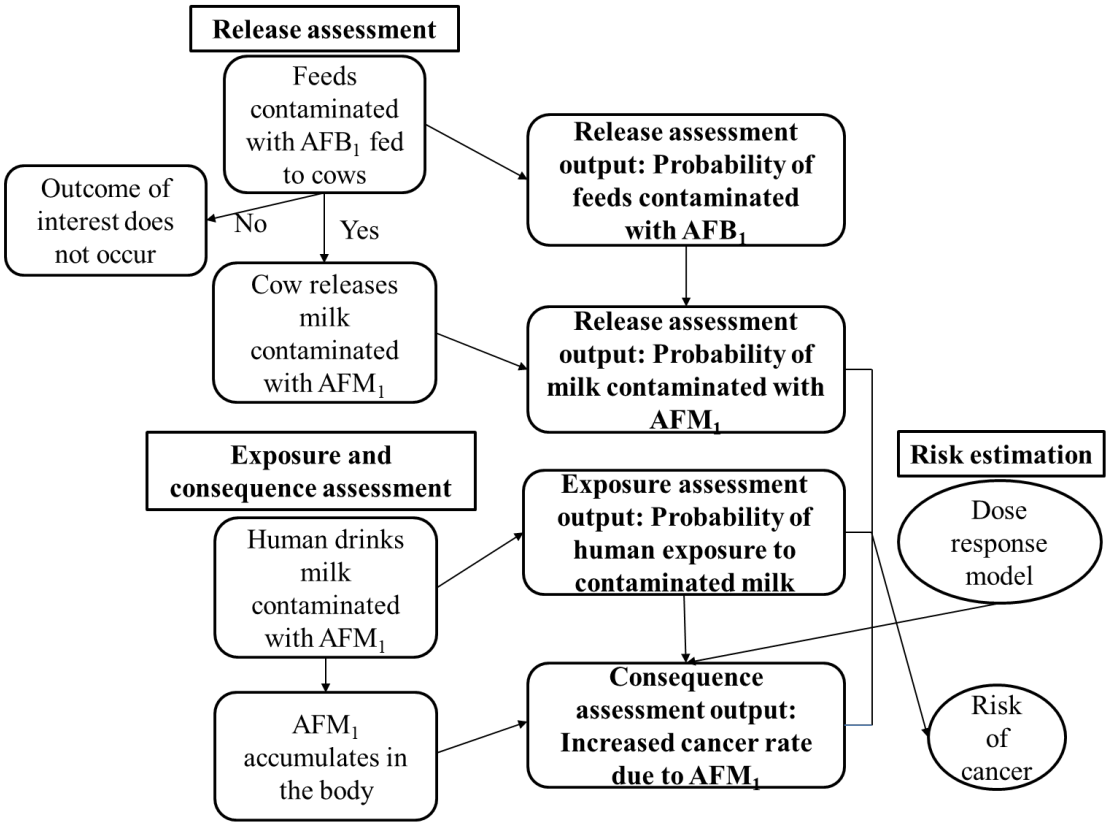

Figure 5-1 A scenario tree for estimation of risk for liver cancer for humans following consumption of AFM1 contaminated milk

5.2 Materials and Methods

5.2.1 Study Sites and Household Selection

This study was based on data from a cross-sectional study in five Counties representing four agro–ecological zones (AEZs) in Kenya. The Counties selected were: Isiolo (semi-arid AEZ), Tharaka-

Nithi (humid AEZ), Kwale (sub-humid AEZ), Bungoma (temperate AEZ), and Kisii (temperate AEZ). Household size calculation yielded 321 dairy cattle farms, which were divided equally among the five Counties resulting in a sample size of 64 farmers per County.

5.2.2 Aflatoxin Analysis

Aflatoxin M1 testing was done using competitive enzyme-linked immunosorbent assay (ELISA) method following manufacturer's instructions. Accuracy, precision, and linearity of each ELISA plate reading was evaluated based on a regression coefficient (r2) calculated from a calibration curve made from calculated values of standards provided by the manufacturer. The standards had concentrations of 0, 5, 10, 25, 50 and 100 ng/kg with a given limit of detection of 2 ppb. ELISA plate reading with an r^2 of less than 0.95 was repeated.

To validate results for animal feeds, two samples each from categories of low (0–5 µg/kg), medium (5.1–20 µg/kg) and high levels (20.1–10,000 µg/kg) were tested using a Shimadzu Nexera X2 ultra performance liquid chromatograph (UPLC) fitted with a prominence fluorescence detector (RF-20A XS). Validation samples had included in them two reference materials with known concentrations (5 and 32 µg/kg obtained from the Office of the Texas State Chemist). Any UPLC reading with off range reference material concentration was repeated. In order to check for agreement of results between ELISA and UPLC, correlations tests were done.

5.2.3 Quantitative Risk Assessment

A quantitative risk assessment (QRA) was performed based on the OIE framework comprised of four steps namely: release assessment, exposure assessment, consequence assessment, and risk estimation (OIE, 2010). To make the QRA model, the four QRA steps were incorporated in a Microsoft Office Excel with an @RISK software version 6.0 (Palisade Corp, Ithaca, NY, USA) included as an add-in to analyze the QRA data (Table 5-1). The QRA data were disaggregated by

agro–ecological zones, gender, and age groups in order to understand the health risks of sub-populations among rural farming households.

Table 5-1 Parameters used in the risk model

Risk Assessment Step	Name	Distributions
Release assessment	AFM_1 occurrence in milk in extensive rearing without concentrates	Risk Beta (Number of positive + 1, Number of sample–Number positive + 1)
Release assessment	AFM_1 occurrence in milk in intensive rearing without concentrates	Risk Beta (Number of positive + 1, Number of sample–Number positive + 1)
Release assessment	AFB_1 occurrence in feed in extensive rearing with concentrates	Risk Beta (Number of positive + 1, Number of sample–Number positive + 1)
Release assessment	AFB_1 occurrence in feed in intensive rearing with concentrates	Risk Beta (Number of positive + 1, Number of sample–Number positive + 1)
Release assessment	AFM_1 occurrence in milk in extensive rearing with concentrates	Risk Beta (Number of positive + 1, Number of sample–Number positive + 1)
Release assessment	AFM_1 occurrence in milk in intensive rearing with concentrates	Risk Beta (Number of positive + 1, Number of sample–Number positive + 1)
Exposure assessment	Frequency of milk consumption (rate)	Risk Duniform (bootstrap of raw data)
Exposure assessment	Whether milk was consumed that day	Risk Binomial (1, rate)
Exposure assessment	Volume of milk consumed, if consumed	Risk Duniform (bootstrap of raw data)
Exposure assessment	AFM_1 status in milk	Risk Binomial (1, occurrence of AFM_1)
Exposure assessment	AFM_1 levels in milk	Risk Duniform (bootstrap of raw data)
Exposure assessment	Body weight	Risk Normal
Exposure assessment	Hepatitis B prevalence	Risk Binomial (1, hepatitis B prevalence)

5.2.4 Release Assessment

Release assessment involved the description of the biological pathways necessary for an activity to 'release' pathogenic agents into a particular environment (OIE, 2010). The release assessment step was based on data on type of farming (either intensive or extensive), feeding of maize-based feeds, number of farms, lactating animals on concentrates and those without, total milk produced, and levels and occurrence of aflatoxins in feeds and milk. Aflatoxin occurrence in feeds and milk was modelled as beta distribution.

5.2.5 Exposure Assessment

Exposure to AFM1 was determined as a product of milk consumed per day and the concentration of AFM1 in milk divided by individual body weights (Equations (1) and (2)). Data on milk consumption per day was collected using 24-hour and 7-day dietary recall. Assumptions of body weights included: 60 kg (standard deviation (SD) of 5) for adult males, 55 kg for adult women (SD of 4), a range of 25–50 kg for children aged 6–18 years, and a range of 5 to 25 kg for children less than 5 years of age. Normal distribution was applied on adults' body weights whereas for children's weight uniform distribution was used due to their high variability. Aflatoxin exposure distribution was simulated using the Monte Carlo statistical method. In Monte Carlo simulation many scenarios or iterations are produced from random sampling of each probability distribution (Vose, 2002).

$$AFM1(\mu g) = Milk\frac{consumption}{day(L)}\ x\ Concentration(\frac{\mu g}{L}) \quad (1)$$

$$AFM1\ intake\ per\ kg\ body\ weight = \frac{AFM1(\mu g)}{Body\ weight(kg)} \quad (2)$$

5.2.6 Consequence Assessment

In the consequence assessment step, the link between specified AFM1exposure and HCC is described based on cancer potency (Shephard, 2008a). Cancer potency is an increase in annual HCC incidence rate per unit change in aflatoxin exposure which varies across populations by hepatitis B virus (HBV) status. In hepatitis B surface-antigen positive (HBsAg+) individuals, potency has been estimated to be 0.3 cancers per year per 100,000 population per ng AFB1 per kg body weight per day. In hepatitis B surface-antigen negative (HBsAg−) individuals, the potency was 0.01 cancers per year per 100,000 population per ng AFB1 per kg body weight per day (Shephard, 2008a). A prevalence rate of 13% HBsAg+ was assumed based on an estimate range of 11% to 15% in Kenya (Liu & Wu, 2010).

5.2.7 Risk Estimation

Results from release, exposure and consequence assessments were integrated to give a risk estimate. The annual incidence rate (expressed as cancers per year per 100,000 population) for HCC from AFM_1 exposure was obtained as the product of the exposure data and an average carcinogenic potency (Equation 3) (Shephard, 2008b). A Monte Carlo simulation was performed with 5000 iterations to come up with possible distributions of risk. On each iteration, the @RISK software sampled values from each probability distribution and combined them according to the Excel model.

$$Probability\ of\ cancer\ per\ 100{,}000\ population = AFM1\ intake\ per\ kg\ body\ weight\ x\ Dose\ response \quad (3)$$

5.3 Results

5.3.1 Release Assessment

Five hundred and twelve milk and 144 feed samples were analyzed in total. Seventy three per cent of the feeds and ten per cent of the milk samples tested above Kenyan limits of 5 µg/kg AFBI and

EU limits of 50 ng/kg, respectively. Milk samples from the humid agro–ecological zone (AEZ) were most likely to exceed the EU limits ($p < 0.05$). Table 5-2 compares AFM1 levels in milk from cows fed with or without concentrates/maize-based feeds.

Table 5-2 Comparison of AFM1 levels in milk from cows fed with or without concentrates/maize-based feeds from four agro-ecological zones in Kenya

Agro-Ecological Zone	Mean AFM_1 Levels in Milk from Cows Fed with Concentrates or Maize Based Feeds	Probability of Samples Exceeding EU Limits (50 ng/kg)	Mean AFM_1 Levels in Milk from Cows Not Fed Concentrates or Maize Based Feeds	Probability of Samples Exceeding 50 ng/kg
Semi-Arid	n/a	-	8.3 ($n = 53$)	0.04
Sub-Humid	370.7 ($n = 2$)	*	4.7 ($n = 30$)	**
Humid	52.9 ($n = 67$)	0.46	10 ($n = 21$)	**
Temperate	34.6 ($n = 47$)	0.13	21.3 ($n = 41$)	0.08

* All samples were above 0.05 ng/g; ** all samples were below 0.05 ng/g.

5.3.2 Exposure Assessment

Across the agro–ecological zones milk was consumed at an average of 0.4 liters daily. Temperate region had the highest average milk consumption compared to the rest of the AEZs ($p < 0.01$; Table 5-3).

Table 5-3 Summary statistics of cow milk consumption in liters per day across agro–ecological zones (AEZs) in Kenya

AEZ	Mean	Median
Semi-Arid ($n = 200$)	0.2	0.2
Sub-Humid ($n = 112$)	0.3	0.2
Humid ($n = 192$)	0.3	0.3
Temperate ($n = 416$)	0.5	0.4
Total ($n = 920$)	0.4	0.3

Exposure estimates for AFM1 ranged from 0.3 to 1 ng/kg of body weight per day. Children five years old and below had the highest exposure estimate at 1 ng/kg of body weight per day (95% CI: 0.6–1.4). Adult females followed at 0.4 ng/kg of body weight per day (95% CI: 0.2–0.5). Adult males and children aged 6–18 years old both had a mean exposure estimate of 0.3 ng/kg of body weight per day (95% CI: 0.1–0.5).

5.3.3 Consequence Assessment and Risk Estimation

The annual incidence rates among adult males was estimated at 2.9×10^{-3} (95% CI: 2.5×10^{-3}–3.3×10^{-3}) cancers per 100,000 whereas among the adult females category the estimate was 3.5×10^{-3} (95% CI: 3×10^{-3}–3.9×10^{-3}) cancers/year per 100,000 (Figure 5-2 to Figure 5-5). Among children aged 6–18 years old incidence rates were 1.4×10^{-3} (95% CI: 1.2×10^{-3}–1.5×10^{-3}) cancers/year per 100,000 whereas among children less than five years old the estimates were 2.7×10^{-3} (95% CI: 2.3×10^{-3}–3×10^{-3}) cancers per year per 100,000 (Figure 5-6 to Figure 5-13). Most categories from the humid AEZ had higher annual incidence rates as compared to the other AEZs (Table 5-4).

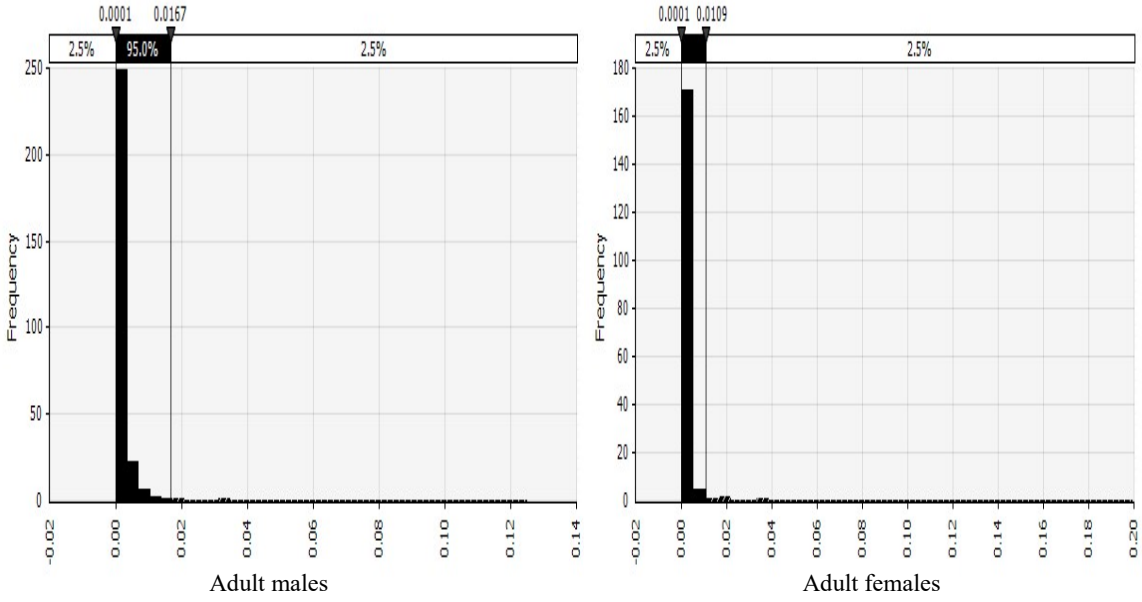

Figure 5-2 Probability distribution of risk of cancer in adult males and females from a semi-arid AEZ in Kenya.

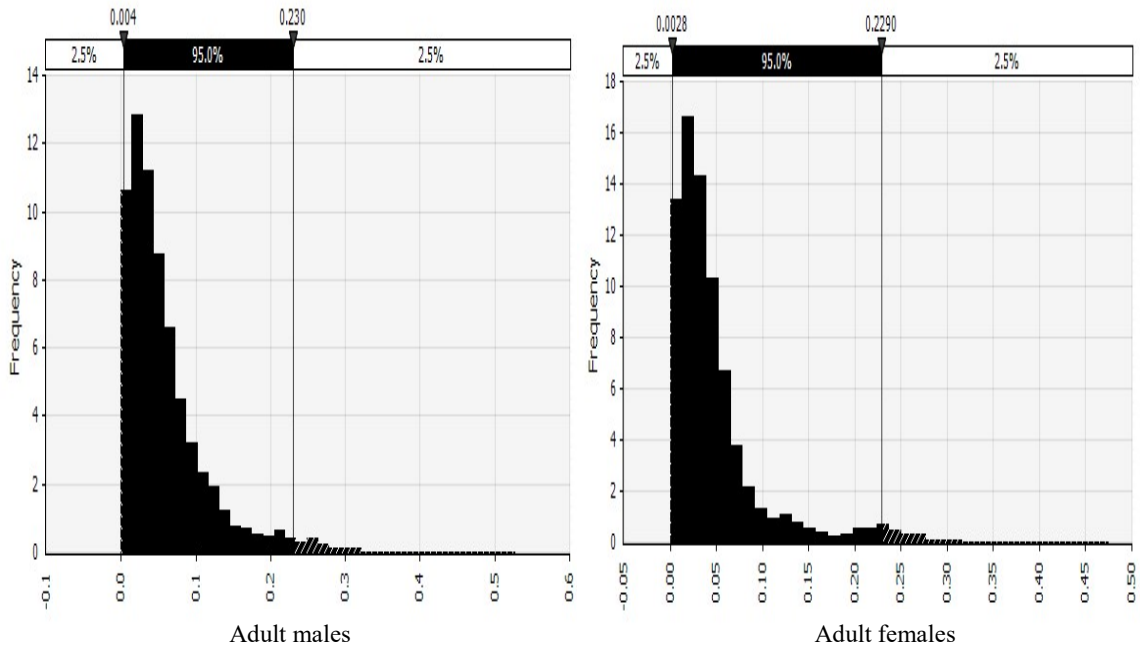

Figure 5-3 Probability distribution of risk of cancer in adult males and females from a sub-humid AEZ in Kenya

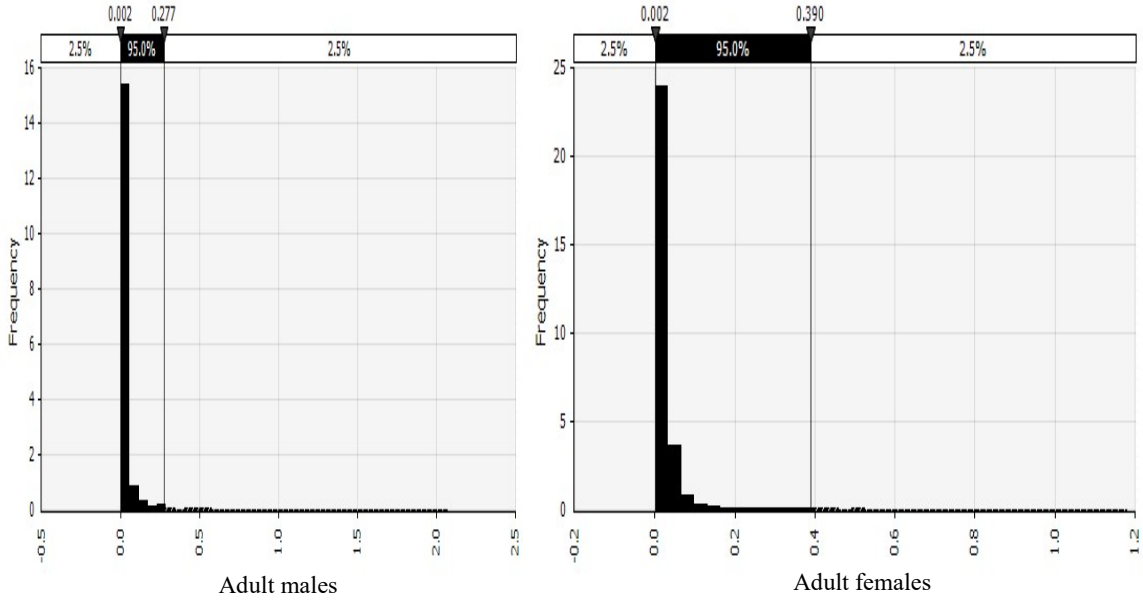

Figure 5-4 Probability distribution of risk of cancer in adult males and females from a humid AEZ in Kenya

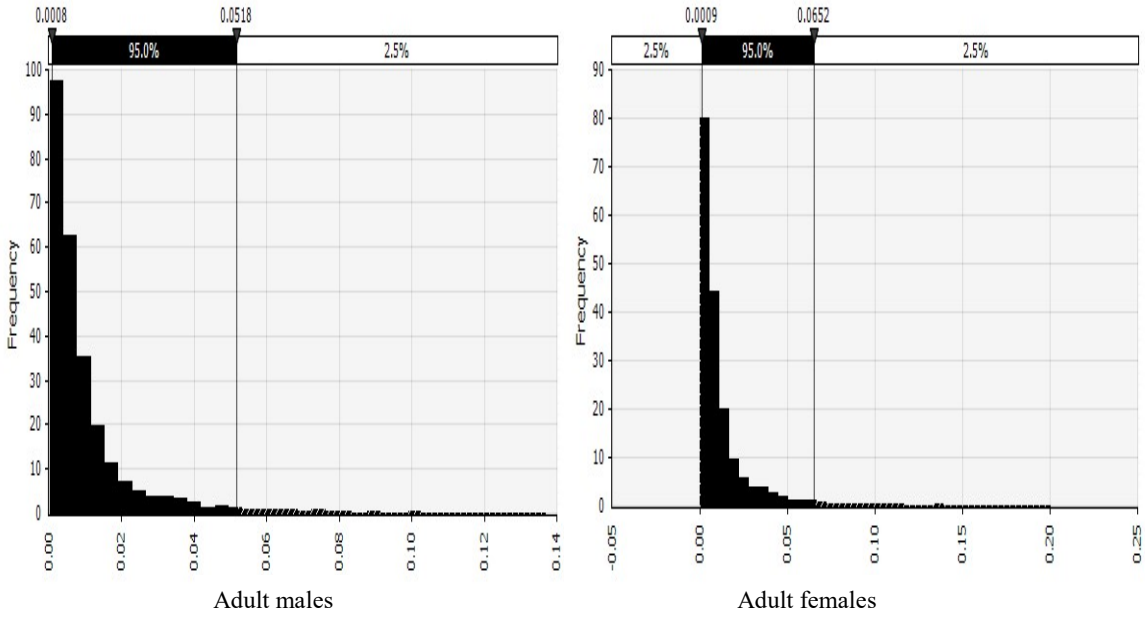

Figure 5-5 Probability distribution of risk of cancer in adult males and females from a temperate AEZ in Kenya

Table 5-4 Estimated annual hepatocellular carcinoma (HCC) incidence rate per 100,000 among different sub-populations (95% confidence intervals)

AEZ	Adult Male	Adult Female	Child 6–18 Years	Child <5 Years
Semi-arid	4×10^{-5} (3×10^{-5}– 5×10^{-5})	5.9×10^{-3} (4.2×10^{-3} –7.5×10^{-3})	2×10^{-5} (2×10^{-5}–3×10^{-5})	1×10^{-4} (8×10^{-5}–1×10^{-4})
Sub-humid	3.2×10^{-3} (2.3×10^{-3}– 4×10^{-3})	1.7×10^{-3} (1.2×10^{-3} –2.1×10^{-3})	5×10^{-6} (4×10^{-6}–6×10^{-6})	1.3×10^{-2} (9.2×10^{-3}–1.7×10^{-2})
Humid	3.3×10^{-3} (2.3×10^{-3}– 4.2×10^{-3})	2×10^{-4} (1×10^{-4} – 3×10^{-4})	2.7×10^{-3} (1.9×10^{-3}–3.4×10^{-3})	2.3×10^{-3} (1.6×10^{-4}–2.9×10^{-3})
Temperate	1.3×10^{-3} (9×10^{-4}– 1.7×10^{-3})	3×10^{-4} (2×10^{-4} – 4×10^{-4})	7×10^{-4} (5×10^{-4}–9×10^{-4})	2.4×10^{-3} (1.7×10^{-3}–3×10^{-3})
All	2.9×10^{-3} (95% CI: 2.5×10^{-3}–3.3×10^{-3})	3.5×10^{-3} (95% CI: 3×10^{-3}–3.9×10^{-3})	1.4×10^{-3} (95% CI: 1.2×10^{-3}–1.5×10^{-3})	2.7×10^{-3} (95% CI: 2.3×10^{-3}–3×10^{-3})

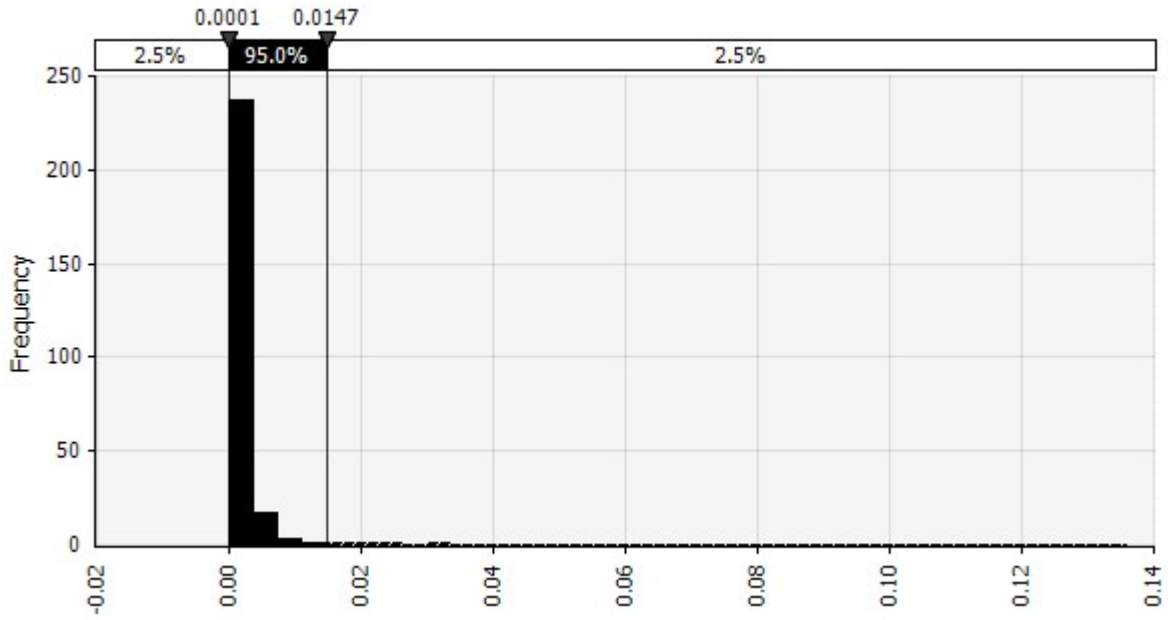

Figure 5-6 Probability distribution of risk of cancer in children 6-18 years old from semi-arid agro-ecological zone in Kenya.

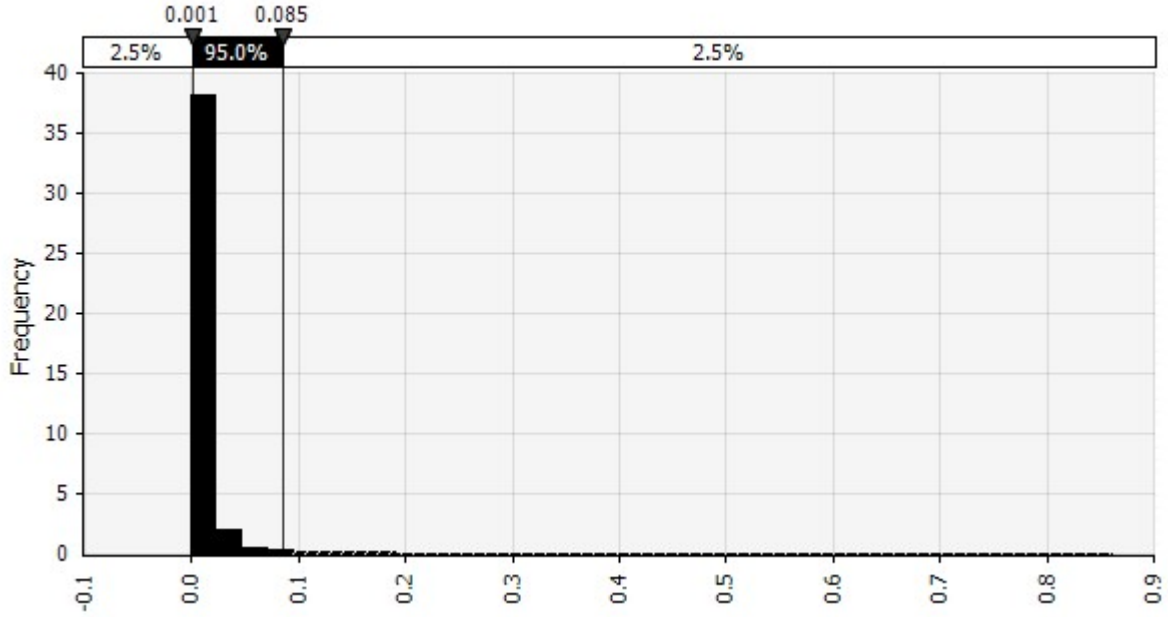

Figure 5-7 Probability distribution of risk of cancer in children below five years old from semi-arid agro-ecological zone in Kenya.

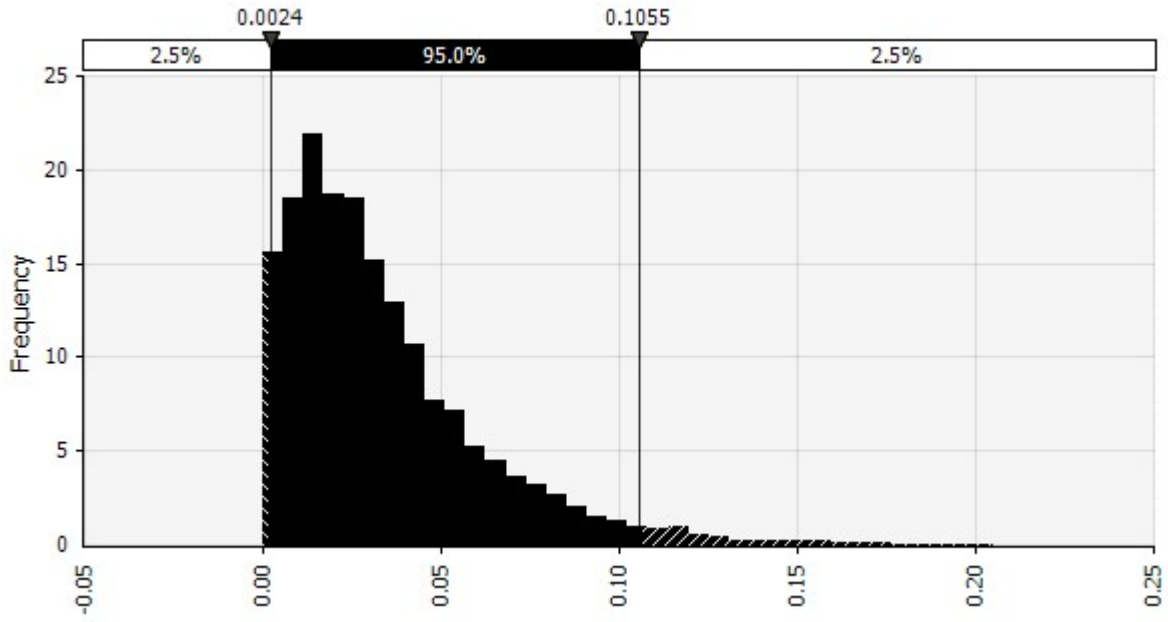

Figure 5-8 Probability distribution of risk of cancer in children 6-18 years old from sub-humid agro-ecological zone in Kenya

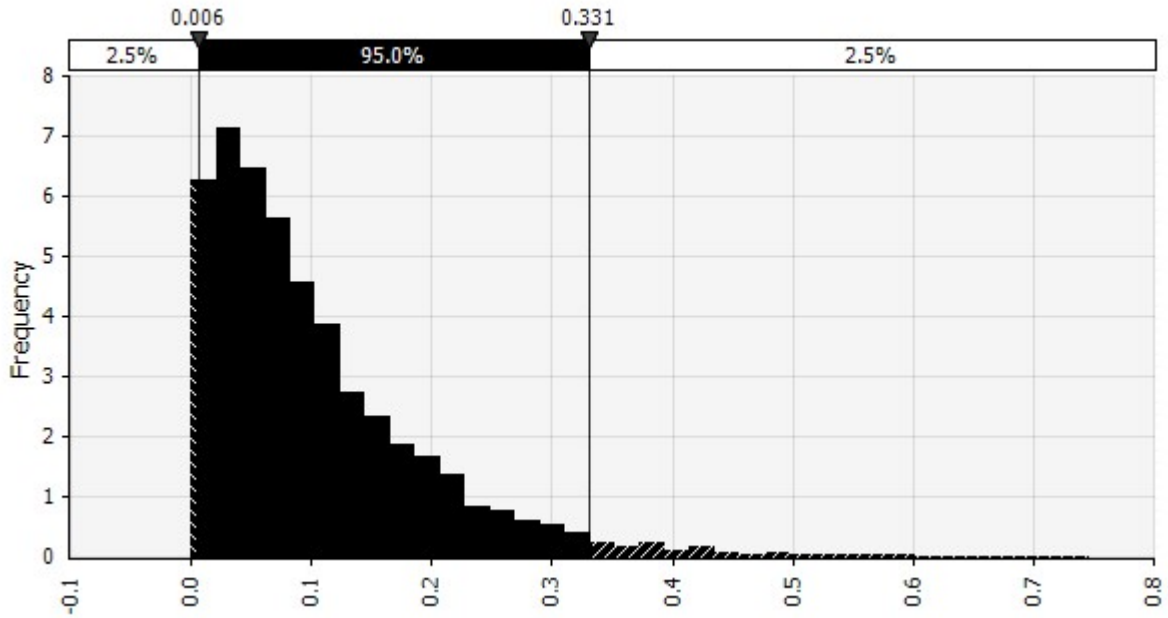

Figure 5-9 Probability distribution of risk of cancer in children below five years old from sub-humid agro-ecological zone in Kenya

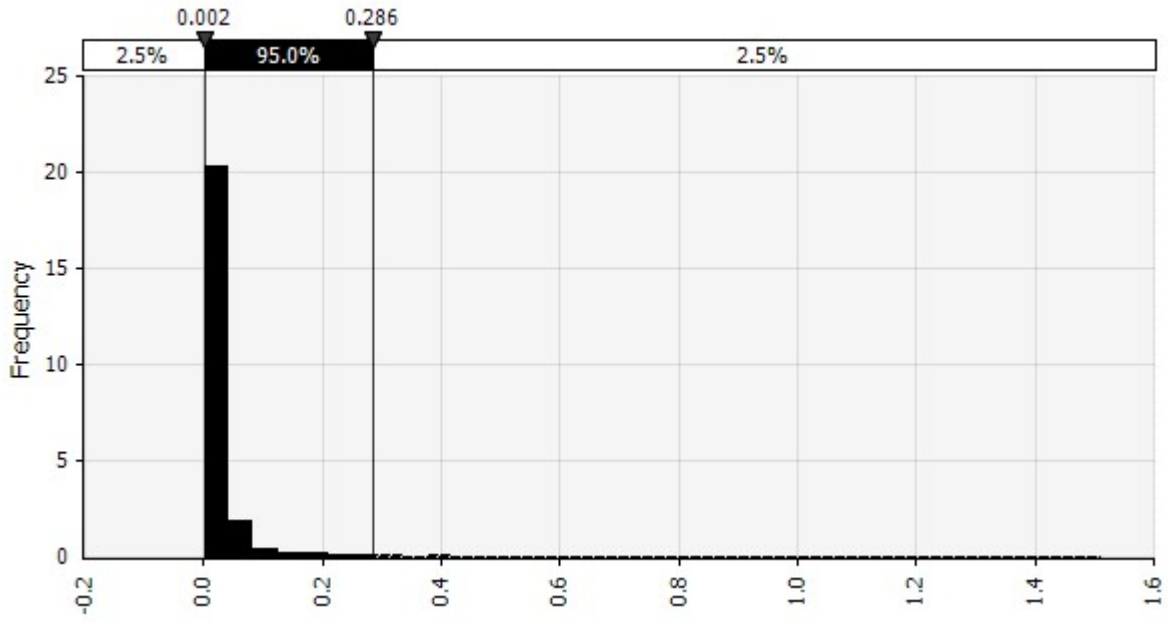

Figure 5-10 Probability distribution of risk of cancer in children 6-18 years old from humid agro-ecological zone in Kenya

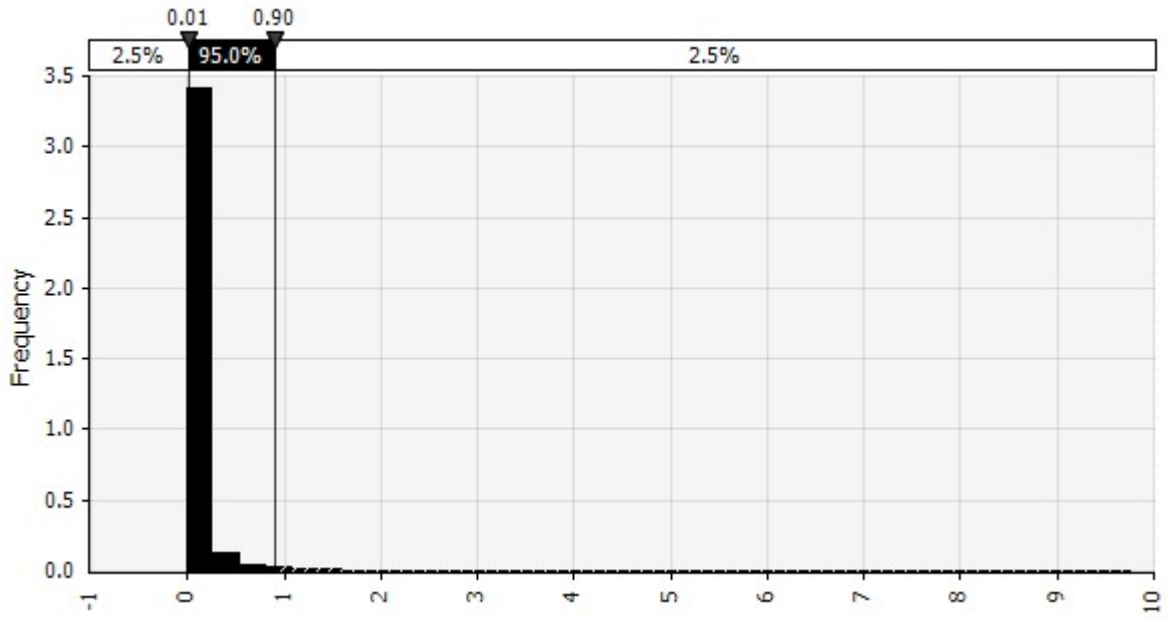

Figure 5-11 Probability distribution of risk of cancer in children below 5 years old from humid agro-ecological zone in Kenya

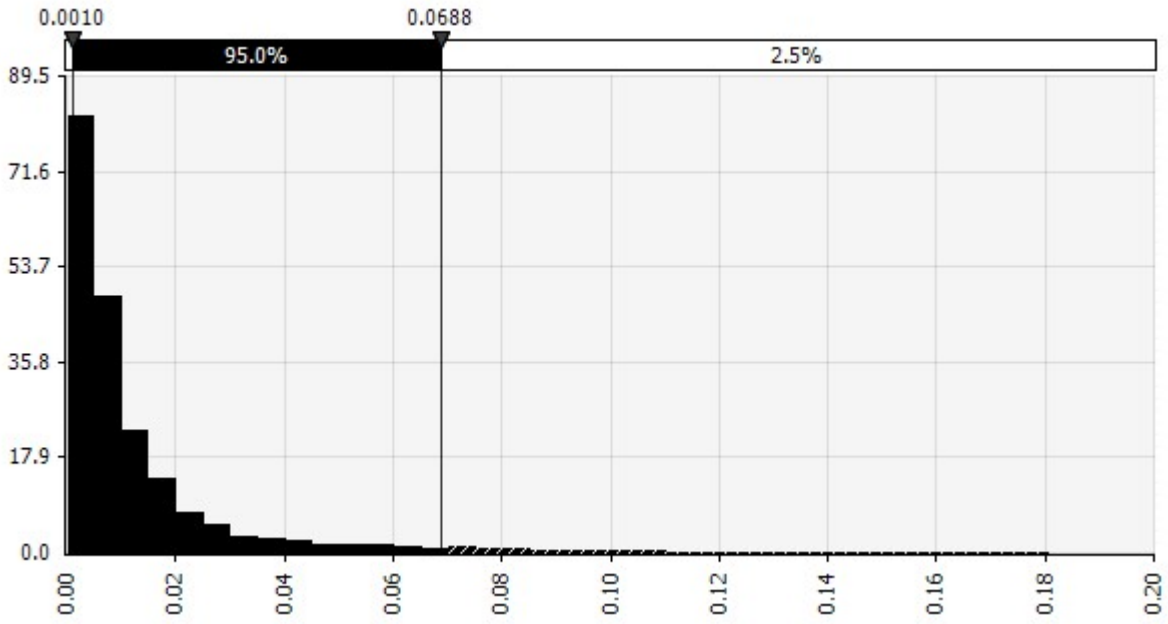

Figure 5-12 Probability distribution of risk of cancer in children 6-18 years old from temperate agro-ecological zone in Kenya

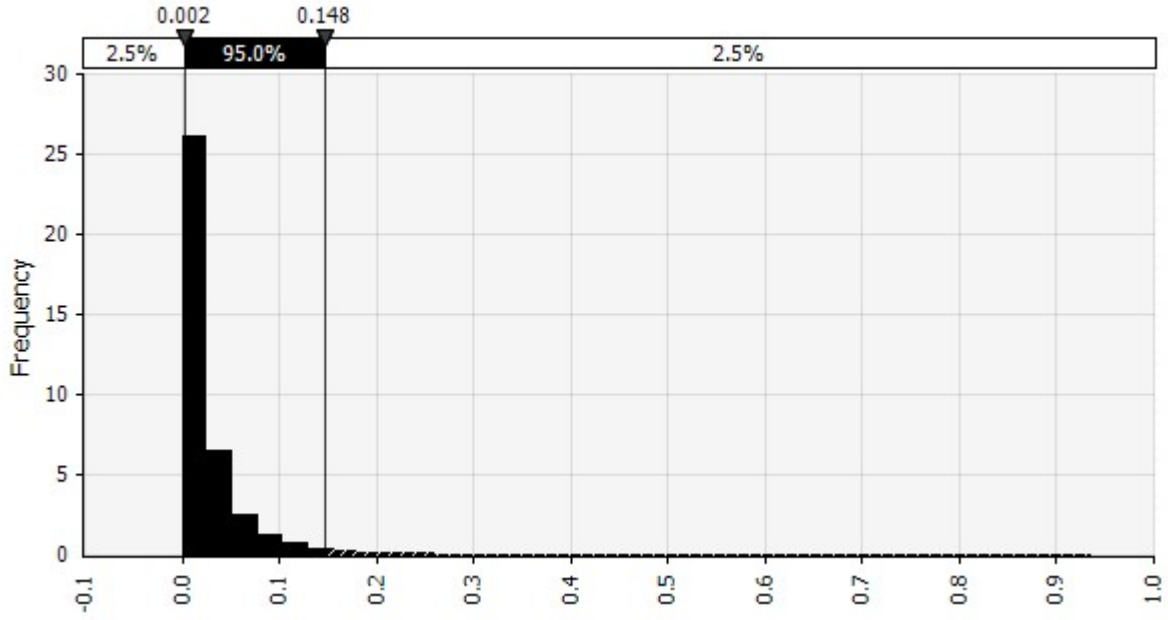

Figure 5-13 Probability distribution of risk of cancer in below five years old from temperate agro-ecological zone in Kenya

5.4 Discussion

Risk assessment is the process of estimating the magnitude and the probability of a harmful effect on individuals or populations from specified agents or activities (Liu & Wu, 2010). This study conducted a quantitative risk assessment of hepatocellular carcinoma (HCC), taking into consideration hepatitis B virus prevalence in Kenyan rural populations and aflatoxin exposure through milk in four AEZs. Most Kenyans consume milk as boiled in tea and porridge (Kiama *et al*., 2016b). The boiling or pasteurization of milk does not eliminate or inactivate aflatoxins, as they are heat stable (Kuboka *et al*., 2019). Generally, the amount of AFM1 carried over into milk varies from less than 1% to 2% of the dose of AFB1 ingested by the cow (Fink-Gremmels, 2008). In this study, 73% of the feeds had AFB1 beyond Kenyan regulatory limits. Milk from cows fed concentrates or maize-based feeds had higher AFM1 levels compared to those not fed. Commercial feeds are much more likely to be contaminated with aflatoxins than hay or fodder stored at the farms (Lanyasunya *et al*., 2005). AFM1 exposure estimate (0.3 to 1 ng/kg of body weight) is similar to the amount reported in Argentina of 1.22 ng/kg body weight (Signorini *et al*., 2012).

Worldwide the standardized annual incidence rate for liver cancer is 15.3 per 100,000 among men and 5.4 per 100,000 among women (IARC, 2016). In this study, based on the levels of AFM1 and the consumption of milk in rural Kenya, and assuming a 10-fold lower carcinogenicity than AFB1, the calculated annual incidence rate was 0.00294 and 0.00347 per 100,000 in males and females, respectively. These translate to a contribution of as little as 0.02% and 0.06% to the global incidence rates. Incidence rates for HCC reported in this study are comparable to estimates for Gambia (Shephard, 2008b) but relatively lower than those reported from a risk assessment in Kenya based on aflatoxin exposure from groundnuts (Wambui *et al*., 2016) and maize (Shephard, 2008b). The Shephard (2008b) study reported an incidence rate of 29.2 and 11 cancers per year

per 100,000 population from maize collected from rural markets and commercial markets, respectively. The relatively higher incidence rates reported are likely due to a focus on maize, in which much higher aflatoxin levels are reported compared to milk in Kenya, and the higher carcinogenicity of AFB1. In addition, that study assumed a higher prevalence of hepatitis B (25%) than the 13% that was used in this study. Although liver cancer incidence is reported as more common in females than in males, this assessment found almost matching annual HCC incidence rates for both gender which may indicate that exposure to AFM1 through milk occurs equally in both genders. However, this study did not consider other possible differences, such as different HBV prevalence in men and women, exposure to other carcinogens such as alcohol, or risk factors such as obesity. Estimates for children did not take into account different base rates in this population, as there was no information on this for Kenya.

Higher annual HCC incidence found in the humid AEZ is consistent with higher AFM1 contamination in milk, maize, millet and sorghum and feeds in this zone. High humidity supports mould growth in foods and feeds and possible aflatoxin production. The control of aflatoxins in dairy feeds would significantly reduce the carryover of aflatoxins to milk and other animal products intended for human consumption. Key interventions to reduce aflatoxins in animal feeds include keeping moisture and temperature of feeds moderately low (<13%) to inhibit mould growth, keeping equipment used on-farm clean, and, where possible, using mould inhibitors or binders (Lanyasunya *et al*., 2005).

The calculated risk of HCC in this study is based on an assumption that the carcinogenicity of AFM1 is 10 times lower than of AFB1. This assumption is based on rather weak evidence from animal trials: If carcinogenicity is higher in humans, then the relative contribution of AFM1 would be higher. Furthermore, concerns over aflatoxin in milk are not only related to cancer cases but

also to the risks of stunting and immunosuppression in young children. There may be more risks with AFM1 in milk products than shown by this risk assessment. The risk assessment method used follows the OIE method as opposed to the Codex Alimentarius risk assessment, which is suitable for microbiological risks (OIE, 2010). Another method is the margin of exposure approach, which has been used successfully for other dietary carcinogens (Lachenmeier *et al*., 2012; Lachenmeier & Rehm, 2015), but this requires more information on benchmarking doses. However, it is unlikely that another method would have given final estimates of completely different magnitudes. While the risk assessment here was based on data from rural Kenyan farmers, the estimate of the risk was of the same magnitude as the estimates done by Ahlberg *et al*., (2018) for urban populations.

5.5 Conclusions

In conclusion, this study demonstrated for the first time that AFM1 is likely to contribute to a small proportion of HCC cases occurring in rural Kenya. Despite the relatively low annual HCC incidence rates from exposure through milk, there is still reason for risk managers to take action due to the cumulative exposure from all sources of aflatoxins. In addition, the prognosis for liver cancer is very poor, with an overall ratio of mortality to incidence of 0.95 (Ferlay *et al*., 2015). It is hoped that the risk estimates provided here will guide the Kenyan authorities in setting legislative levels for AFM1 in milk and milk products.

CHAPTER 6

THE IMPACTS OF AFLATOXIN STANDARDS ON HEALTH AND NUTRITION IN KENYA

6.1 Introduction

One wonders whether there is a trade-off between food security and food safety on looking at a scenario where people are struggling to find food for their daily needs, and governments are relying on relief food to supply food for the poorest. The safety and quality of such foods may sometimes be compromised. In Africa, most livestock and livestock products are produced by small-scale farmers, many of them women, who sell mainly through informal markets and have limited resources, information and capital (Grace *et al*., 2015). These factors can constrain their ability to provide safe food. Safety is always determined by a compromise between the objectives of using limited resources most effectively (minimising cost) and of achieving the highest levels of safety (minimising risk); absolute safety, or 'zero risk', is not a realistic goal in any human domain (Black & Niehaus, 1980).

Kenya is an east African country with a large smallholder and informal sector and has experienced well-documented food safety problems (Oloo, 2010). Food safety in Kenya, is the responsibility of multiple agencies coordinated by the Ministry of Health. The Kenya Bureau of Standards hosts secretariats of technical committees, numbering about 30, that develop standards for food and agricultural products. The East African Community (EAC) is working with Member States to harmonise standards. Intergovernmental organizations including Codex Alimentarius Commission, established jointly by the FAO and WHO, and the OIE have a role to protect the

health of consumers and facilitate trade through development of international standards for food and feed. Member countries are expected to domesticate these standards for improved food safety.

Aflatoxins are considered a food safety priority in Kenya mainly due to high-publicity outbreaks in which dozens of people died (Lewis *et al*., 2005). Aflatoxins are toxic fungal byproducts produced mainly by *Aspergillus flavus* mould. Aflatoxins are carcinogenic and strong association has been found between aflatoxin exposure and immunosuppression and stunting (Khlangwiset *et al*., 2011; Leroy, 2013). Aflatoxins commonly contaminate maize and groundnuts, which are staples in many African Countries. Aflatoxins may also be present in animal-source foods especially in dairy products. Aflatoxin M1 (AFM1) present in milk is a metabolite of Aflatoxin B1 (AFB1) found in animal feeds. The quantity of AFM1 excreted in milk is typically only around 1-2% of the total amount of AFB1 ingested (Fink-Gremmels, 2008). The presence of aflatoxin residues in poultry eggs, meat and organs may not be a problem in developing Countries because very small amounts are carried over (Grace & Unnevehr, 2013). Because of aflatoxin's potential for harm, globally countries have set regulations for aflatoxins, especially AFB1, and there are also recommendations by FAO/WHO; however, the allowed levels vary between different Countries (Egmond *et al*., 2007).

This chapter discusses food and feed safety standards for aflatoxins in different Countries and their development. It then presents a case study based on this research where an assessment was carried out on the aflatoxins present in different foods and development of a quantitative risk assessment to assess aflatoxins' public health impact. Considering if the standards were to be strictly applied, the public health hazards of consuming aflatoxins are discussed in relation to the impacts on availability of key nutrients. Conclusions are drawn about tradeoffs between food safety and nutrition security, and appropriate food safety standards in Kenya.

6.2 Materials and methods

Literature review was done to identify standards for aflatoxins in food and feed in Africa and globally. Information was synthesized on the levels of aflatoxins in feed tolerated by different animal species. Findings from aflatoxin prevalence surveys in food and feed were summarized (Chapter 4) and the data used to generate estimates of the health impact of aflatoxins in cereals consumed by Kenyans using the quantitative risk assessment model. The health impact of removing all food which exceeded the permitted standards for aflatoxins was assessed using a naïve model in which all food removed for non-compliance translated into the number of adults with that food removed from their diet.

6.3 Results

6.3.1 Literature review of standards for aflatoxins in food and feed

The FAO conducted international surveys on standards for aflatoxins in 1997 and 2003 (Egmond & Jonker, 2005; FAO, 1997b). In 2003, the most common standards for aflatoxins were: 4 ppb for total aflatoxins in food, 2 ppb (1-20ppb) AFB1 in food and 0.05 ppb (not detectable to 15 ppb) for AFM1 in milk. Since then additional reviews have emerged on aflatoxin standards. The analysis of literature revealed, in general, several interesting features of standards:

i. A lack of uniformity. Aflatoxin standards may specify food in general, specific foods, feed in general and specific feeds. Moreover, standards may be for total aflatoxins (B1, B2, G1, G2), AFB1 only or, in the case of milk AFM1 only.

ii. Standards have become more common: In 1997, 77 countries had specific regulations for mycotoxins in different foods and feeds, 13 countries had general provisions, while about 50 countries did not have data (FAO, 1997b). By 2004, 99 countries had mycotoxin regulations (Egmond & Jonker, 2005).

iii. Standards have become more rigorous: A study in 2009 of 11 regions and countries found that two had not changed legislation on aflatoxins but nine had changed to lower the legal limits and/or to extend the number of food categories covered.

iv. Countries with more aflatoxin problems tend to have laxer standards; for example, in tropical countries, the average limit for aflatoxins in feed is 54.5 ppb (0-300) and in non-tropical countries 26.3 ppb (1-200). Similarly, the Texas State is one of the USA states most prone to aflatoxins and has laxer standards than other states.

v. Some countries show zero tolerance: In dealing with hazards ubiquitous in nature, such as fungi and fungal toxins, and given ever increasing ability to detect molecules in miniscule amounts, zero tolerance is usually considered not a sensible approach.

vi. Countries and nations that share strong food trade relations tend to have similar regulations on allowable levels of aflatoxins in maize: in most of top 20 trade relationships, importing and exporting country have the same aflatoxin standard for maize (Wu & Guclu, 2012).

Additional trends were found when focusing only on standards with fewer foods.

i. Little relation between standards and consumption: For example, USA has both one of the world's highest milk per capita consumption levels and also the most lenient standard for aflatoxins in milk. Similarly, five countries have per capita maize consumption greater than 100 kg per year, but standards are either absent or lenient (Table 6-1).

ii. Little relation between standards and vulnerability: The same is seen for countries with high hepatitis B prevalence, a major contributor to the development of liver cancer after aflatoxin exposure (Shephard, 2008a). Among countries with prevalence of 15% or higher, one country reports there are no standards; 3 countries do not report standards; one reports lenient standards; and only one relatively strict standards.

Table 6-1 Aflatoxin standards for countries with per capita maize consumption greater than 100 kg per person per year

Country	Maize kg/person/year	Aflatoxin standards status	References
Lesotho	167	• No standards reported	
Malawi	131	• 5 ppb AFB1 in exported groundnuts	(FAO, 2004)
Zambia	119	• No official standards reported	
Mexico	116	• 20 ppb total aflatoxins in cereals and products • 12 ppb total aflatoxins in corn flour for tortilla	(FAO, 2004)
South Africa	100	• 5 ppb AFB1 in food • 10 ppb total aflatoxins in food • 0.05 ppb aflatoxin M1 in milk	(FAO, 2004)

Table 6-2 Aflatoxin standards for different species (all feed types)

Species	Range (ppb) of standards reported	Average ppb	Levels generally tolerated	Reference
All	5-300 (n=22)	48		
Pigs	0-300 (n=31)	40	≤50 in weaner ≤200 in finishing pigs	IARC, 2012
Cattle	0-300 (n=31)	41	<100 in calves <300 in cattle	IARC, 2012
Sheep and goats	5-75 (n=19)	26		
Dairy	0-75 (n=27)	19		
Poultry	0-300 (n=29)	33	≤50 in young ≤100 in adult poultry	IARC, 2012
Duck	10-10 (n=1)	10		
Turkey	10-10 (n=1)	10		
Rabbit	10-10 (n=1)	10		
Trout	10-10 (n=1)	10		

Compared to other foods, standards for aflatoxins in milk are often stricter because milk is targeted to children and infants, who are considered more vulnerable to toxins. Regulations for AFM1 existed in 60 countries at the end of 2003 (FAO, 2004). Fifty ppt was the most common limit, present in the European Union (EU), European Free Trade Association (EFTA) and candidate EU countries, but some other countries in Africa, Asia and Latin America also apply this limit. Another

common limit of 500 ppt is applied in the United States, several Asian and European countries, and it occurs most frequently in Latin America, where it is also established as a harmonized MERCOSUR limit.

Standards for livestock feed show much more variation than standards for food and milk. This study found:

i. Wide variation in standards: There is wide variation on species and type of feed covered (Table 6-2;Table 6-3).

ii. Weak relation between standards and aflatoxin vulnerability: Many trials have been carried out to investigate aflatoxin toxicity in domestic animals (Applebaum at al., 1982; Yueming *et al*., 2003). This study found that, overall, feed standards do not bear a strong relation to the levels generally found to be tolerable (Table 6-2). For example, monogastric animals (poultry and pigs) are generally more susceptible than ruminants (cattle, sheep and goats) because the rumen microbial flora can break down aflatoxins. Yet, standards for aflatoxins in monogastric feeds may be similar or more lenient than those of ruminants (Table 6-2: Table 6-4).

iii. Lack of risk targeting. Low risk feeds are regulated similarly to high risk; and feeds that are intended only as part of diet are regulated similar to feeds that are intended to comprise the whole of the diet. Only a small number of countries (e.g. Canada and the United States) have regulations which allow contaminated feed to be directed towards more resistant species.

Table 6-3 Aflatoxin standards for different feed types (all species)

Feed type	range ppb	average ppb
Low risk feeds (n=8)	5-50	20
Complementary/concentrates (n=12)	5-30	23
Complete/combined/mixed (n=51)	25-100	25
All feeds (n=29)	20-100	29
Straight/cereal (n=5)	20-200	82
High risk feeds: Corn/cottonseed/peanut/copra (n=25)	5-300	85

Source: our analysis

Table 6-4 Kenya regulations for aflatoxins in foods and feeds

Food	Total aflatoxins ppb	AFB1 ppb
Foodstuff (maize, groundnuts, millet)	10	5
Dairy feeds	10	5

Kenya was one of only five African countries to report standards on aflatoxins in 2003. Since then the Kenyan standards have been reviewed whereby the acceptable limits for total aflatoxins contamination in foods and feeds (dairy cattle feed) is 10 ppb (Kenya Standards-East Africa Standards 2:2005; KS 62: 2009). The current standards for feeds are in the process of revision to East African standards thus harmonizing requirements in the East African Community. When this process is complete, Kenyan standards will likely match East African Standards which specify aflatoxin limits in poultry feeds; total aflatoxins limit of 50 ppb and aflatoxin B1 limit of 20 ppb for adult poultry feed and for young poultry maximum limit for aflatoxin total is 50 ppb and 10 ppb for aflatoxin B1.

Although Kenya has one of the highest per capita milk consumptions in Sub-Saharan Africa, Kenyan standards contain no separate limits for milk. Discussions with stakeholders revealed that some assumed that the 10 ppb standards prevail while others assumed that the EU standard of 50 ppt apply (Table 6-5).

6.3.2 Levels of aflatoxins in feed and food in Kenya

This study reports levels of aflatoxins in foodstuffs including maize, millet, sorghum and milk and animal feeds collected from four agro-ecological zones in Kenya (Kwale, Isiolo, Tharaka-Nithi, Kisii and Bungoma). More than 50% of animal feeds tested above limits (Table 6-5).

Table 6-5 Summary results of aflatoxin occurrence from various Counties in Kenya

Sample	Levels (ppb)	Limits	Percent exceeding limit	References
Maize from dairy farmers	AFB1 <1 – 1137.4	5 ppb	22	Sirma *et al*., 2016
Millet from dairy farmers	AFB1 <1.0 - 1658.2	5 ppb	6	Sirma *et al*., 2016
Sorghum from dairy farmers	AFB1 <1.0 - 91.7	5 ppb	7	Sirma *et al*., 2016
Milk from dairy farmers	<2 – 6999 ppt AFM1	50 ppt	10	Senerwa *et al*., 2016
Animal feeds (from farmers)	<1 – 9661 AFB1	5 ppb	73	Senerwa *et al*., 2016
Animal feeds (from feed retailers)	<1 – 1198 AFBI	5 ppb	90	Senerwa *et al*., 2016
Animal feeds (from feed manufacturers)	<1 – 4682 AFBI	5 ppb	62	Senerwa *et al*., 2016

6.3.3 Health risk associated with aflatoxins in urban Kenya

This study estimated the health impacts of consuming cereals contaminated with aflatoxins at the levels found in the surveys.

6.3.3.1 Maize

Maize is the most consumed staple in Kenya. Estimates of maize consumption vary from 171 g/person/day to 233 g/person/day (Ranum *et al*., 2014; Ministry of Agriculture, Livestock and Fisheries (MOALF, 2016)). However, the percentage of households consuming this staple is steadily changing owing to production deficit and dietary shifts. Urban households consuming maize flour declined from 86% in 2013 to 78% in 2015 (Onyango *et al*., 2016). The estimated

daily exposure to aflatoxins from maize was 66 ng/kg of body weight/day. That estimate is based on an assumption of a 60-kg adult consuming 233 g of maize per day with mean contamination levels of 17 ng/g (arithmetic mean contamination of maize collected from four AEZs). That exposure estimate is half of the 133 ng/kg of body weight/day estimated for Kenyans consuming commercial flour at about 400 g of maize per and containing mean contamination of 20 ppb (Shephard, 2008a).

Assuming an average age of 40 for hepatocellular cancer (HCC) disease onset (Kew, 2013; Mutuma *et al*., 2011), and disability weights from Salomon *et al*. (2015), the disability-adjusted life years (DALYs) lost per HCC case was estimated to be 51. Using the quantitative risk model, this corresponds to 1416 HCC cases, 1346 deaths and 72,269 DALYs lost per HCC case for Kenya in 2016.

6.3.3.2 Millet and sorghum

Millet and sorghum grow in semi-arid regions of the country and are consumed mainly as flours used in preparation of ugali (thick porridge) and uji (thin porridge). Uji forms part of infant weaner foods and diet for Kenyan children. Table 6-6 shows millet and sorghum production and consumption figures for the years 2015 and 2016. Based on this study 6% and 7% of millet and sorghum, respectively collected from four AEZs in Kenya tested above Kenyan legal limits. Based on an arithmetic mean contamination of 10 ng/g and 2 ng/g for millet and sorghum, respectively in the four AEZs and an assumption of a 60-kg adult, consuming 7 g and 14 g per day of millet and sorghum (MOALF, 2016), respectively resultant aflatoxin exposures would be 1 and 0.5 ng/kg of body weight/day for millet and sorghum, respectively.

Using the quantitative risk model, this corresponds to 25 and 10 HCC cases; 24 and 10 deaths and 1277 and 511 DALYs lost per HCC case for Kenya in 2016 from aflatoxin exposure from millet and sorghum, respectively.

6.3.3.3 Milk

AFM1 was detected beyond FAO/WHO limit of 50 ppt in 10% of farmers' samples from this survey. Based on an arithmetic mean contamination of 35 ppt in the four AEZs and an assumption of a 60-kg adult consuming 0.41 liters per day of milk resultant aflatoxin exposure would be 0.2 ng/kg of body weight/day.

Using the quantitative risk model, this corresponds to 5 HCC cases and deaths each and 255 DALYs lost per HCC case for Kenya in 2016 from aflatoxin exposure in milk.

6.3.4 Effects of removing cereals contaminated with aflatoxins above limits from diets

This study also looked at the implications for the livestock sector on strict application of current standards. The food was considered as discarded because the standards for animal feed are also 10ppb so, if standards are strictly enforced, then contaminated human food cannot be re-directed to animals. Moreover, there are currently no functioning decontamination or bio-fuel plants in Kenya which could potentially make use of contaminated food/feed.

This basic calculation suggested that around 9 million Kenyans would be deprived of the bulk of their diet if standards for cereals were strictly enforced. Moreover, about 3.4 million Kenyans would be deprived of milk which is currently a major source of protein, calcium and other nutrients. In addition, 21 million cattle would be deprived of additional feed. As a rule of thumb, 13kg dry matter of grass plus 3.5 kg of fed concentrates result in an additional 2 kg of milk, so this could lead to another 336,217kg of lost milk.

Table 6-6 Summary of food and feed production and losses arising from aflatoxin contamination

	Food/Feed	Below standards	Kilo tonnes produced	Consumption kg/per capita/year	Discarded kg	Would have fed
Human	Maize	22	3,339	84	734,580,000	8,745,000
	Millet	6	21	2.5	1,260,000	504,000
	Sorghum	7	125	5	8,750,000	1,750,000
	Milk (cow)	10	3733	110	373,300,000	3,393,636
Animal	Feed farmer	73	806	-	588,380	-

6.4 Discussion

"When 5% of your milk fails standards, you have a problem with your milk. When 95% fails standards, you have a problem with your standards" (Blackmore *et al*., 2015). This study vividly illustrated the challenges faced by developing countries, caught between the ever stringent standards and the realities that make compliance impossible for many. Moreover, if standards were to be strictly enforced, the negative impacts of this would be far worse than the current more permissive approach. If standards were strictly enforced, and in the absence of other interventions to replace the staple food discarded, the population would have a maize shortage of 22%; on the other hand, the strict enforcement of standards would save around 1,400 lives a year from averted liver cancer. However, although the mean reduction of maize for the population would be 22%, the poorest would lose more, and the rich would lose less.

Where do un-workable standards come from? A general trend is to adopt standards from international bodies such as International Organization for Standardization (ISO) and CODEX Alimentarius. For instance, Kenya refers to CODEX standards for maximum limits for aflatoxin M1 in milk. However, there is also widespread demand from stakeholders for stringent standards and strict enforcement. This is especially the case after food scares have become increasingly common in emerging economies (Grace & McDermott, 2015). Kenya experienced outbreaks of

aflatoxicosis, which claimed more than 150 lives since 1982 and as a result revised its regulatory limits downwards from 20 ppb to 10 ppb. Regional harmonization can also lead to upwards pressure on standards. According to Humphrey, (2017), the EAC dairy standards are not only strict, inappropriate but also unnecessary and harmful to the small-holder sector which dominates milk production in east Africa.

How can standards be more helpful? In many developing countries, the ability to write standards has gone far beyond the ability to implement them. Importantly, food safety standards should not be developed in isolation from other concerns such as food security. It may be prudent to develop local standards considering local context including aflatoxin levels, consumption patterns, occurrence in various foods and feeds and socio-economic factors. Alternatively, an aspirational approach can be taken whereby it is acknowledged that getting to desired standards takes time and effort. In the case of Kenya, the standards can be grouped as either mandatory or optional e.g. allowing sale of feeds that test up to 100 ppb but recommending an ideal of 20 ppb. Lenient limits for animal feeds is acceptable as they are more tolerant to aflatoxins than humans. Currently in Kenya the limits are the same, which does not incentivize food manufacturers to divert contaminated grains above limits to feed manufacturers. However, food manufacturers who do respect the regulatory limits for their own products can get a higher price for rejected grains on the informal food market than from the feed market, implying higher exposure among relatively poor consumers. Universities and Technical institutions should be incorporated to monitor and evaluate the food safety and quality control situation through research because they have the necessary expertise (Oloo, 2010). This will improve the relationship between standards and consumption or vulnerability. In feeds, recommending inclusion of aflatoxin adsorbents and enzymes as a prevention strategy can be effective. Making standards more accessible would also be useful.

Currently, in Kenya, standards have to be purchased and are often difficult to interpret. In other countries, standards are free to users and widely disseminated.

6.5 Conclusions

This study, drawing on published and unpublished research, has important messages for food and feed standards in Kenya and beyond. In order to impact positively on health and nutrition in Sub-Saharan Africa, standards should be set considering local conditions including use of products/commodities, capacity to enforce regulations, contamination levels of various foods and feed, regional standards and other societal concerns such as food and nutrition security.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 Discussion

Aflatoxins are poisonous compounds produced by certain kinds of moulds, mainly *Aspergillus flavus*. Aflatoxins can be readily measured in foods and feeds, and are classified as B1, B2, G1, and G2. High temperatures and high humidity favour infection of crops with mould and subsequent production of the toxins. Aflatoxins are found in many different crops, but especially maize, which is a key raw ingredient for animal feeds in Kenya. Other factors that increase likelihood of crop infection include drought stress during growth, insect damage to mature grain kernels, and delayed time of harvest. Drying of grains to moisture levels below 13% stops the growth and toxin production of the moulds. Maintenance of moisture levels at 13% or lower during transport and storage also inhibits mould growth. Aflatoxins residues have been traced in meat, milk, and eggs posing food safety risks to the general public. Aflatoxins cause liver cancer in people (specifically, hepatocellular carcinoma (HCC)), and are associated with immunosuppression and stunting in children. In animals, aflatoxins are associated with reduced productivity and growth rate as well as immunosuppression and other health impacts.

Globally it is estimated that aflatoxins contaminate 25% of the world's food supply and approximately 4.5 billion people are exposed to aflatoxin contamination (CAST, 2003). In Kenya, human exposure to aflatoxins has led to deaths due to acute aflatoxicosis and contamination of crops has led to post harvest and economic losses. The basis for this study was to conduct a quantitative risk assessment (QRA) of aflatoxins in the Kenyan dairy value chain to determine the likelihood and impact of HCC from consumption of aflatoxins in dairy. The Quantitative Risk Assessment QRA estimated low annual HCC incidence rates from aflatoxin exposure through

milk. Despite this, there is still need to mitigate and control aflatoxin contamination in the dairy value chain as consumers' demand food that is within maximum allowable toxin limits, aflatoxins exceeding limits are a constraint to trade, aflatoxins in milk potentially have other adverse human health impacts and may impair animal health, and they contribute to cumulative aflatoxin human exposure through various foods. At the same time, given resources are scarce, efforts to manage aflatoxins in milk should be proportionate to their known and potential health burdens.

7.2 Conclusions

This study made the following conclusions:

i. Contamination of food and feed ingredients with aflatoxins was rampant with a substantial proportion of feed samples exceeding the Kenyan recommended limits for aflatoxin B1.

ii. The risk factors that were significantly associated with aflatoxin contamination for cereals were: (1) agro-ecological zones whereby humid zones showed higher contamination of samples (2) knowledge of aflatoxins that determined practices to mitigate aflatoxins at the farm (3) practice of whether the household undertook procedures to prevent/mitigate aflatoxin and (4) household monthly income that determined ability of the farm to purchase more cereals with possible long storage period.

iii. The key risk factors for contamination of milk with aflatoxins included the feeding of commercial feeds and homemade feeds.

iv. Aflatoxin M1 was found to have a likelihood of contributing to a small proportion of hepatocellular carcinoma cases occurring in rural Kenya.

v. The Kenyan standards for livestock feed show weak relation between them and aflatoxin vulnerability by species. The tolerance level for each species is known and should guide standard setting to minimize non-compliance and feed wastage.

7.3 Recommendations

In light of this study findings the following should be considered as strategies to reduce aflatoxin contamination along the Kenya dairy value chain and to mitigate the effects:

i. Enforce feed quality controls especially on the manufacturers to verify that they follow set standards for commercial animal feeds.

ii. Offer extension to key dairy value chain players including dairy farmers, feed manufacturers, millers and retailers on aflatoxin prevention strategies.

iii. Avail grain driers countrywide, where this is cost-effective, and promote their use especially in humid and temperate regions to ensure products attain a moisture level of 13% or lower. This will inhibit mould growth and subsequently aflatoxin production.

iv. Establish clear Kenyan limits for aflatoxin M1 in milk.

v. Update aflatoxin limits in feeds to take into account species susceptibility differences to avoid destruction of feeds due to non-compliance.

vi. Kenya to adopt EAC policy brief No. 8 of 2018 that recommends alternative uses for aflatoxin contaminated foods to safeguard livelihoods.

7.4 Recommendations for further research

There is need for further research in the area of mycotoxins with a view to:

i. Assess co-contamination of foods and feeds with other mycotoxins.

ii. Assess the cumulative health risk assessment of co-occurring mycotoxins in foods and feeds in the dairy value chain.

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APPENDICES

Appendix 1 Mydairy Survey: Producer Questionnaire

Welcome and Introduction: Good morning/afternoon. Thank you for welcoming us. We are researchers from ILRI and University of Nairobi. We are conducting research to learn more about your dairy feeding and milking practices. The findings from this study will benefit you by teaching the best practices in feed and milk handling. The research team will respect your household privacy and confidentiality. We would like your cooperation in this; please indicate by a yes if you agree to participate.

Interview number:	**Sub-County:**
Interviewer name:	
Interview date:	
Village name:	
GPS: Latitude (N/S):	**Longitude (E/W):**
Altitude (m):	
Consent obtained:	***Y/N (if no, request replacement HH from supervisor)***

Socio-demographic characteristics

1. Sex of household head		**2. Sex of respondent**		**3. Respondent's age (years)**
Male – 1 Female – 2		Male – 1 Female – 2		

4. Marital status of household head			
Single – 1 Married – 2	Divorced – 3 Separated - 4	Widowed – 5 No response – 7	

5. Level of education of household head			
Never schooled – 1 Primary incomplete – 2 Primary complete – 3	Secondary incomplete – 4 Secondary complete – 5 Tertiary – 6	University – 7 No response – 8	

6. Please provide the number of people living in your household and their contribution to household income.

No.	**Children ≤ 2years**	**Children 2 - 5 years**	**Children/adolescent ≥ 6 to 18 years**	**Adults (>18 years)**	**Total**
a. Males					
b. Females					
Contribute to hh income full time					
Contribute to hh income some time					

7. What is the primary activity of the head of this household?	

1 = Crop farming 2 = Animal keeping (incl. sales) 3 = Trading in animal products (not own) 4 = Trading in agric. products (not own produce)	5 = Formal Salaried employee 6 = Business – trade / services (non-agric.) 7 = Not working / unemployed 8 = Old/Retired 9 = Casual labour 10 = Other (specify)	
8. What is your household average monthly income in KES?		

FEEDING PRACTICES AND MILK PRODUCTION

How important is dairy as your source of income for the household?

1= Only income source
2= Major income source
3= Same importance as other income sources
4= Minor income source
5= No or negligible income source
6= Don't know

Indicate the **numbers of animals** for the different species kept on the farm

Livestock Species		Number owned by male	Number owned by female	Number owned jointly	Number owned by HH
Cattle	Local				
	Cross*				
	i. Exotic_____				
	ii. Exotic_____				
	iii. Exotic_____				
Goats	Local				
	Cross/ exotic				
Sheep	Local				
	Cross/ exotic				
Poultry	Local				
	Cross/ exotic				
Pig	Local				
	Cross/ exotic				
Donkeys/Horses					

Rabbits				
Dogs				
Other, specify (guinea pig, bees, fish etc				

"Cross" refers to a cross-bred animal which is part-exotic.

Indicate the **number of cattle** owned by age

	Less than 3 months	3months to 1 year	Above 1 year not calved (heifer)	Above 1 year milking	Above 1 year dry	Total
Cows (females)						
Bulls (males)						

Please **list** the **feeds** fed to your **cattle** during the various seasons

Dry season	**Wet season**	**All seasons**

Answer the following concerning **feeds**

Name	Source	Price /Kg	How often	Kg given each time it is fed	How many days is it usually stored on farm	Where is it stored
Branded commercial						
Branded commercial						
Unbranded commercial						
Homemade feeds						
Hay						
Silage						
Grass						
	1= Own farm **2**= neighbour /friend **3**= Shop/market **4**= Other		**1**=Once daily **2**=Twice daily **3**= > twice daily **4**=Weekly **5**= Occasionally			**1**=Raised store house **2**=Main house on floor **3**= Main house on a plank **4**= Cattle shed

						5= Verandah

Do the feeds ever get mouldy **Yes** ☐ **No** ☐

If yes above, which feeds turned mouldy in the last 12 months?

_____

How often did the feeds in Q7 turn mouldy during that year?

_____

What is the approximate amount that turned mouldy that year?

_____

What do you do with **mouldy feeds**? (List in order of priority)

Feed to…	**Dispose**	**Sell to…**
1= Cattle **2**= Goats **3**= Sheep **4**= Pigs **5**= Chicken **6**= Dogs/cats **7**= Other_____	**1**= Shamba **2**= Bury **3**= Burn **4**= Dust pit **5**= Other_____	**1**= Neighbour/other consumer directly **2**= Local market **3**= Feed retailer **4**= Other_____

Please indicate the **total milk** produced on the farm and uses as per yesterday

TOTAL DAILY MILK PRODUCTION (Morning plus evening milk) in litres	Yesterday	Fed to calf	Home consumed	Sold	Price/ Litre

Select up to **3 calves representing 3 breeds**. For each calf, fill a column.

	Calf 1	**Calf 2**	**Calf 3**
Breed			
Indicate age (months)			
Amount of milk fed (L/day), when fed milk			
Age at weaning			
List weaning feeds			

Amount of concentrate feed fed (kg/day) Concentrate feed:			
List common diseases			

Select up to **3 breeds** that are being **milked** currently. For each breed select a cow and fill a column.

		Cow 1	**Cow 2**	**Cow 3**
Breed				
Indicate calving dates of at most the last three calves MM/YY				
Lactation length (Number of months cow is milked from one calving to the next)				
Amount of concentrate feed fed (kg/day)				
Was this cow bred on farm? (Y / N); If yes, at how many months was it first served				
TOTAL DAILY MILK PRODUCTION (Morning plus evening milk) in litre	At Calving (initial milk production)			
	Yesterday			

Do you have low and peak season milk sales? **Yes** ☐ **No** ☐

If yes above, explain

_____

What is your daily production in:

Jan	**Feb**	**Mar**	**Apr**	**May**	**June**	**July**	**Aug**	**Sept**	**Oct**	**Nov**	**Dec**

Do you sell milk to…?

Buyer	Yes/ no	Location (Name/ Village / Town/ City)	Amount (Litres)		Price/Litre		Times sold (per day/week/ month/year)	
			Low season	Peak season	Low season	Peak season	Low season	Peak season
a. Hawker								
b. Milk bar/dairy								
c. Duka/shop								
d. Kiosk/ Kibanda								
e.Supermarket								
f. Neighbour/ otherconsumer directly								

g. Milk processor (e.g. KCC								

In the last 12 months have your cattle showed any the following symptoms

Disease	**Y/N**	**How many cattle**	**How many times**	**Who treated 0= No treatment 1= Vet doctor 2= Animal health specialist 3=CHW 4=Don't know qualification 4=Other____**	**Total direct costs**
In appetence					
Reduced milk production					
Diarrhoea					
Stunted growth					
Weight loss					
Rough hair coat					
Abortion					
Mastitis					
Difficulty in breathing/ coughing					
Cows that were sold or slaughtered due to disease					
Cows that died					
Sick calves in the last 24 months		(Indicate no. out of total born)			
Death of calves in the last 24 months		(Indicate no. out of total born)			

Knowledge, attitudes and practices about aflatoxins

Have you ever heard about aflatoxins? Yes ☐ No ☐

If yes above, explain what you know

_____

Are moulds harmful to human and animal health? Yes ☐ No ☐

If yes above, explain what you know

_____

Does your household undertake any procedures to prevent/ mitigate aflatoxin in foods Yes ☐ No ☐

If yes to above, name them

1._____

2._____

Do you remove visibly spoilt maize before milling	Yes ☐	No ☐
Do you dehusk maize before cooking	Yes ☐	No ☐
Do your cereals ever get mouldy?	Yes ☐	No ☐
Does eating mouldy cereal cause health problems?	Yes ☐	No ☐
Has any member of your family gotten ill following consumption of mouldy food	Yes ☐	No ☐
Has any member of your cattle gotten ill following consumption of mouldy food	Yes ☐	No ☐
Has any member of your family been diagnosed with liver cancer	Yes ☐	No ☐

On average, how much did you spend on health in the last one year?

What do you do with mouldy cereals?(List in order of priority)

Feed to…	**Dispose**	**Sell to…**	**Eat**
1= Cattle **2**= Goats **3**= Sheep **4**= Pigs **5**= Chicken **6**= Dogs/cats **7**= Other_____	**1**= Shamba **2**= Bury **3**= Burn **4**= Pit **5**= Other_____	**1**= Neighbour/other consumer directly **2**= Local market **3**= Feed retailer **4**= Other_____	**1**= Cook **2**= Wash and cook **3**= Mix with good maize **4**= Sort and cook **5**= Muthokoi **6**= Other_____

Record whether you agree or disagree with the following statements *(tick one per row)*:

Do you think…	Yes	No	Don't know	Comment
a. Drinking milk is good for your health?	☐	☐	☐	
b. Milk safety can be judged by sight?	☐	☐	☐	
c. Milk safety can be judged by taste?	☐	☐	☐	
c. You worry more about chemicals in milk than about germs?	☐	☐	☐	
d. You can get sick from drinking well-boiled milk?	☐	☐	☐	
e. Milk from cows fed mouldy feed is unsafe for human consumption?	☐	☐	☐	
f. Meat from cows fed mouldy feed is unsafe for human consumption?	☐	☐	☐	
g. Aflatoxins can be present in milk?	☐	☐	☐	
h. Your customers will pay more for certified aflatoxin free milk?	☐	☐	☐	

DIETARY ASSESSMENT

Rationale: *To determine dietary intake of foods susceptible to aflatoxins by household (hh) members and the most vulnerable children.*

Enumerator:
Interview the person in charge of food preparation in the household (hh). Ask these questions about an adult male, an adult female, an older child (6 to 18 years) and an index child. Index child is the youngest child less than 5 years old that is eating solid foods irrespective of breast-feeding status. If there are no young children in the HH, ask about an older child.

Ask about the pregnancy status of the female respondent_____.

For the **index child**, complete the following information:	
a. Sex	[_] Male [_] Female
b. Age (months)	
c. Is /(was) the child being breast-fed?	[_] Yes [_] No
d. At what age is the child weaned	(months)
e. Which of the following is the child weaned on (circle)	1 = Maize 4 = Groundnuts 2 = Sorghum 5= Milk 3 = Millet

Staples (hh)

Hh member (adult male, adult female, Child 6-18 years, index child)	**Food** **1**= Maize **2**= Sorghum (mtama) **3**= Millet (wimbi) **4**= Groundnuts	**Source** **1**= Own production **2**= Purchased **3**= Borrowed **4**= Food aid **5**= Other	**Description** **1**= Stiff porridge (Ugali) **2**= Loose porridge (uji) **3**= Whole grains **4**= Sauce	**Amount per serving**	Times eaten Per day	Times eaten Per week	Times eaten Per month	Times eaten Seasonal **1**= Christmas **2** =Eid **3**=wedding /funeral/ **4** = Other (specify)

Milk (hh)

Hh member (adult male, adult female, Child 6-18 years, index child)	**Milk preparation** **1**= Fresh (raw) **2**= Fresh (boiled) **3**= Fresh (pasteurised) **4**= Fermented **5**= Powder **6**= In porridge **7**= In ugali **8**= In tea **9**= Other(specify)	**Source** **1**= Own production **2**= Purchased **3** = Food aid **4** = Other_____	**Amount of milk hh** (*cup – equivalent to 300ml*)	**Times drank/eaten** Per day	**Times drank/eaten** Per week	**Times drank/eaten** Per month	**Times drank/eaten** Seasonal **1**= Christmas **2** =Eid **3**=Wedding/funeral **4** = Other _____

Is there a period when **much more or much less** milk is consumed than usual? Yes ☐ No ☐

If yes, please fill in the table below
Enumerator: If the respondent cannot recall the amount for the whole period, ask what the amount per day was then calculate the total from the length of the period

Month	**Reason** **e.g Cows dry**	**Amount consumed** **(Little, Much)**	**Estimated hh consumption (ml)**

Direct observation: good hygienic premises
Enumerator: Take pictures where possible

	YES	**NO**	**Comment**
Animal keeping units			
Enclosed feeding area			
Use of raised feeders or troughs			
Other farms or animal keeping premises next door			
Water available at all times			
Storage conditions: ask to see where the cereals and feeds are and record the following information			
No signs of rodents or pests			
Maize is stored indoors			
Maize is stored raised			

No visibly discoloured kernels in ready to eat maize			
Feed is indoors			
Feed is stored raised			
Feed is adequately covered and stored (Roof and wall covering)			
Storage room is dry			
Visibly mouldy maize on sampling			
Visibly mouldy feeds on sampling			

SAMPLING

Enumerator: Specify samples taken

Sample type	**YES**	**NO**	**Source** **1**= Own production **2**= Purchased
Milk			
Maize			
Sorghum			
Millet			
Groundnut			
Feed 1_____			
Feed 2 _____			
Feed 3 _____			
Feed 4_____			
Other _____			