LEVELS OF AFLATOXINS IN HUMAN BREAST MILK AND HOUSEHOLD MAIZE. THE CASE OF MOTHERS ATTENDING MCH MAKINDU HOSPITAL MAKENI

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF REQUIREMENTS FOR THE DEGREE OF MASTER OF PUBLIC HEALTH OF KENYATTA UNIVERSITY.
DECLARATION

This Thesis is my original work and has not been presented for a degree in any other University.

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Date
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Declaration by supervisors

"We confirm that the work reported in this thesis was carried out by the candidate under our supervision"

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Date
9/5/2012
DEDICATION

I dedicate this work to my family and friends, for the encouragement, love, care and support during the entire period of the study.

I specially acknowledge the support of the Drug Metabolism and Pharmacology and Toxicology Unit of the Department of Pharmaceutical Sciences and the participation of the MCH and Family Planning Department, for their support during the execution of this study in their respective institutions. I would like to thank all my laboratory workers for their time and cooperation. The support of the MCH and Family Planning Department has been hard to accomplish.

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DEFINITION OF OPERATIONAL TERMS

1. Poverty Refers to lack of capacity to choose quality food.

2. Jaundice Refers to liver proliferation, characterized by yellowness in the body and mostly the mucous membranes.

3. Service provider Refers to personnel deployed in provision of mother child health services.
# ABBREVIATIONS & ACRONYMS

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
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<tr>
<td>AFM$_1$</td>
<td>Aflatoxin M$_1$ 4-Hydroxyaflatoxin B$_1$</td>
</tr>
<tr>
<td>AFB$_1$</td>
<td>Aflatoxin B$_1$ 6-Methoxydifurocoumarone</td>
</tr>
<tr>
<td>AFB$_2$</td>
<td>Aflatoxin B$_2$ Dihydroaflatoxin B$_1$</td>
</tr>
<tr>
<td>AFG$_1$</td>
<td>Aflatoxin G$_1$ Dihydroaflatoxin G$_1$</td>
</tr>
<tr>
<td>AFG$_2$</td>
<td>Aflatoxin G$_2$ Dihydroaflatoxin G$_2$</td>
</tr>
<tr>
<td>MOH</td>
<td>Ministry of Health</td>
</tr>
<tr>
<td>ROK</td>
<td>Republic of Kenya</td>
</tr>
<tr>
<td>MOA</td>
<td>Ministry of Agriculture</td>
</tr>
<tr>
<td>ASAL</td>
<td>Arid and Semi Arid Land</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for Social Sciences</td>
</tr>
<tr>
<td>PPT</td>
<td>Parts per Tririon</td>
</tr>
<tr>
<td>PPB</td>
<td>Parts per Billion</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas Liquid Chromatograph</td>
</tr>
<tr>
<td>HPLC</td>
<td>High Performance Liquid Chromatography</td>
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<tr>
<td>ELISA</td>
<td>Enzyme Linked Immuno Sorbent Assay</td>
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DON - Deoxynivalenol

DALY - Disability Adjusted Life Years

MSF - Medicine San Frontiers France, Paris

GPS - Global Positioning System
ABSTRACT

Aflatoxin poisoning is a public health, and social economic problem. It is of great importance world wide and especially in the developing countries where 4.5 billion people are affected. Minute levels of aflatoxins in food may cause severe poisoning in human beings and animals. Aflatoxicosis can be acute or chronic exposure, characterized by acute liver damage, cancer, teratogenic, immunosuppression, kwashiorkor, retarded growth rate, nutritional modification diarrhea and even death. In this study the levels of aflatoxins M1 and B1 in 113 human breast milk and maize samples, were determined using the Enzyme Linked Immuno Sorbent Assay (ELISA) method. A cross-sectional study among lactating mothers, visiting maternal child health clinic (MCH) in Makindu hospital, Makueni District was carried out. Milk samples from lactating mothers was collected using sterile zinc free plastic containers. Each mothers household was visited and a maize sample collected. Information on the socio-economic and demographic data was collected using an open ended questionnaire administered to the lactating mothers. Data analysis was carried out by the aid of SPSS software upon acquisition of descriptive statistical tools e.g mean, median, range, standard deviation, frequencies and percentages. The results indicated that out of the 113 human breast milk samples tested 92(82%) were AFM1 positive with levels of 5-559ppt. Maize samples had levels of AFB1 of 5-260ppb, were mouldy, insect infested and fragmented, factors that enhance aflatoxins contamination. Multiple logistic regression was used to explore the relationship between multiple, risk factors and aflatoxin in maize and human breast milk levels. A Chi square test showed a relationship between MCH mothers household maize aflatoxins and aflatoxins in mothers breast milk, $x^2 = 80 \text{ df 5 P=0.001}$. The study points to an underlying problem of high levels of AFM1 and AFB1 that requires the action of policy makers. The study provides a baseline data on the aflatoxin M1 levels on human breast milk that may be used as a biomarker by public health intervention policy programmes. The study may also enhance future comparison and estimation of trends on aflatoxicosis. Results may assist designation and implementation measures against aflatoxicosis in Makueni District.
CHAPTER ONE: INTRODUCTION

1.1 Background

Aflatoxicosis is a condition that occurs after consumption of aflatoxins in humans and animals which may be acute or chronic. The human exposure globally has been estimated as 4.5 billions most of them living in the developing countries (Egmond et al., 2005). Aflatoxins are mycotoxins produced by certain fungi especially, *Aspergillus flavus* and *Aspergillus parasiticus*, which grow on food crops under favourable conditions. Mycotoxins literally mean poison from fungi. "Aflatoxin" is derived from "A" for *Aspergillus* and “Fla” for the species “Flavus” and the word toxin (Egmond et al., 2005). There are about 300 mycotoxins known but those of most concern, based on their toxicity and occurrence, are aflatoxin, deoxynivalenol (DON) or Vomitoxin, zearalenone and fumonism. Aflatoxins are one of the most potent and toxic substances that occur naturally. There are four different types namely aflatoxins, B1, B2, G1 and G2 with Aflatoxin B1 (AFBI) the most toxic, carcinogenic, hepatoxic, mutagenic and immunosuppressants (Wagacha et al., 2008).

Aflatoxin M1 (AFMI) is a metabolite of AFBI excreted in milk of lactating animals and humans (Polychronaki et al., 2006) following consumption of AFBI contaminated maize, milk, including mothers milk, animal products such as meat, eggs and peanuts. Susceptibility to a aflatoxicosis depends on the species, age and nutritional status of humans and animals. Young members of the human and animals are usually more susceptible to the acute effects of the disease (Polychronaki et al., 2006). Adverse effects on the human and animals may be expressed as liver damage, gastro intestinal dysfunction, anaemia, reduced feed consumption, reduced reproduction, immune suppression, decreased milk and egg production, retarded growth and development (A.Gurbay et al., 2010). Infant immune system development may be a key predictor of morbidity and mortality from disease (Mahdavi et al., 2010).

The favourable conditions of mycotoxins are temperatures between 24 and 35°C, moisture content exceeding 7%, latitude between 40°N AND 40°S of the equator, pH range of 4-8, and relative humidity of 70%. Drought stress increases the number of aspergillus spores in the air (Reddy et al., 2007).
Maize is the most notorious food regarding aflatoxicosis outbreaks in Kenya. Harvesting and storage under hot damp or poor ventilation/aeration conditions are important factors for its contamination with aflatoxins, inadequate pest control and the poor selection of the varieties to plant (vulnerability to drought stress) may also contribute to food contamination by aflatoxin.

Makueni District of Eastern Province of Kenya is within the arid and semi-arid land (ASAL) districts of the country where maize, beans, green grams, millet and sorghum are grown and are aflatoxin prone grains. The ASAL districts are food deficient and maize is brought into the districts from as far as Uganda, Tanzania and relief foods where contamination may be due to improper handling during transporting, storage in traders stores, in the consumers homes, or from maize harvested within the victim’s districts but is handled poorly at harvesting or during storage. The permissible levels of aflatoxin in the USA is 0 ppb (Williams et al., 2004) while in Kenya it is 20ppb. Human exposure to aflatoxin M₁, at levels of nanograms per day occurs mainly through consumption of aflatoxin M₁ contaminated milk, including lactating mothers’ milk and animal products such as meat and eggs (Mahdavi, et al., 2010).

1.2 Problem Statement
Aflatoxin poisoning remains a severe problem in many developing countries, mainly due to poverty (Williams et al., 2004). Exposure of infants to aflatoxin M₁ (AFMI) and of mothers to aflatoxin B₁ has been linked to major health implications. Aflatoxin M₁ levels have been linked to underweight, retarded growth rate and malnutrition in children (Polychronaki et al., 2006). The nutritional status of children determines their health. Acute Aflatoxicosis was reported with 125 deaths, 300 hospitalized (KMOH, 2004) where 60% of the affected were children. 2005, 2006 and 2007 cases have been reported (Wagacha et al., 2008). Aflatoxin M₁ assessment in human breast milk in Kenya has not been carried out while children continue suffering from aflatoxicosis health impact. Agap is existing in knowledge on biological exposure to aflatoxin poisoning in humans in developing countries. Data only available from a small number of countries.
(Polychronaki et al., 2006). There are gaps in current knowledge on what levels of aflatoxins cause acute and chronic aflatoxins poisoning after consumption of contaminated maize. Human health effects and impacts of aflatoxins have not been fully documented in Kenya. Surveillance and food monitoring, analytical methods on aflatoxins are not readily available in most government hospitals. (Lewis et al., 2005). Fetal and early childhood environment, including the nutritional status of the mother and the infant are considered critical for growth and risk of disease in later life of the child. AFM$_1$ exposure from human breast milk may play an etiology in kwashiorkor, neonatal susceptibility to infection, compromised response to prophylactic immunizations in children, a common characteristic in Makindu Hospital as reported by the Disease Surveillance Committee in Makindu Hospital.

1.2 Justification

Public health is a philosophy of intervention aimed at protecting and promoting the health of the population. In accordance to the WHO definition, health is complete social, physical, emotional and spiritual well-being of an individual and not merely the absence of disease. Aflatoxin poisoning contravenes the health of an individual and particularly children who are a fountain of a society. It causes liver cancer, gastrointestinal diseases, anaemia, retarded growth, immuno system suppression and several other malfunctions of the body. Children are more susceptible to effects of aflatoxins. Aflatoxin M$_1$ assessment in human breast milk in Kenya has not been well documented and could be used as Biomarker for aflatoxicosis in Makueni District in Eastern Province, Kenya where it has been frequently causing high levels of morbidity and mortality (KMOH, 2004). This creates a gap in knowledge on the prevalence and related factors on aflatoxins in maize and human breast milk in MCH Makindu hospital. There is no comprehensive data set from which to evaluate the extent and severity of human exposure to aflatoxin in Kenya. There is need to fully elucidate the community response to prevention and control of aflatoxicosis through mother to child transmission of AFM$_1$ via the breast milk. (Mahdavi et al, 2010, Gurbay et al., 2010). Results from this study will improve disease surveillance, encourage collaborations and participation of health workers, agricultural sector and the community. The results will be important source of reference for future researchers. This will enhance and strengthen application
of health sector reforms policy and strategies to realize effective prevention and control of aflatoxicosis in Makueni District and other affected areas in Kenya. Information on prevalence and causal factors on aflatoxicoses is of prime importance in Kenya, there is dearth of knowledge on the extent of mothers breast milk aflatoxins contamination and its consequences and particularly among lactating mothers attending MCH Makindu hospital. The findings of this study will bridge the existing knowledge gap.

1.4 Research Questions

1. What is the prevalence and levels of AFM1 in human breast milk among lactating mothers attending MCH Clinic in Makindu Hospital?
2. What are the levels of aflatoxin in maize samples collected from the households of the mothers?
3. What is the relationship between the aflatoxin in human breast milk and maize samples from the mothers households?
4. What are the factors associated with levels of aflatoxins in human breast milk and maize.

1.5 Broad Objectives

To detect aflatoxin levels in human breast milk and maize from mothers’ attending MCH clinic Makindu Hospital and determine factors associated with aflatoxins contamination in maize.

1.6 NULL HYPOTHESES

1) Human breast milk and maize from lactating mothers attending Mother Child Health clinic Makindu hospital are not contaminated with detectable levels of aflatoxins.

2) There is no relationship between aflatoxin levels in human breast milk and household maize samples from mothers attending MCH Makindu hospital.
1.6.1 Specific Objectives

1) To determine the prevalence and levels of aflatoxins M1 in human breast milk among lactating mothers attending MCH Clinic in Makindu hospital with their children.

2) To establish the levels of aflatoxins B1 in maize samples collected from the households of the mothers.

3) To determine the relationship between levels of aflatoxins in human breast milk and maize samples from the mothers households.

4) To establish factors associated with aflatoxin levels in maize.

1.6.2 Significance and Anticipated Output

Results from this study will be used by policy makers to formulate interventions on aflatoxicosis via breast milk. The findings from this study will also be used as a biomarker on aflatoxicosis in Makueni district. Results will form a basis from which to evaluate the extent and severity of children exposure to AFM1 from human breast milk. The findings of this study will address this information gap and hence facilitate policy making in health matters. The NGOs, Agricultural Sector, MoH, and other researchers may use the findings to research on appropriate mitigation measures on aflatoxicosis.

1.6.3 The Rationale for Assessing AFM1 in Human Breast Milk

The aflatoxin once absorbed into the human body they are excreted in milk as metabolites in high detectable levels which provides a tool for assessing their concentrations in the human body which reflects the human exposure.

Furthermore, breast milk is the major vehicle for excretion of the AfM1 in lactating mothers. Continuous monitoring of these substances in human milk would provide data which would detect trends in levels of exposure, especially in countries where Aflatoxicosis is abundant and people have a lower lifespan due to health implications. In developed countries, such a body of monitoring data is available and the newly generated data will be used as baseline data. In addition to providing a measure of the human exposure can enhance mitigation measures.
1.6.4 The Rationale for Collecting Maize Samples

Most cereals are subject to Aflatoxin contamination either in the field and/or in the store. Small quantities of these residues are persistent in nature and have the ability to accumulate and concentrate in food chains. Food is thus considered to be the most important source of Aflatoxins in the human body. Other sources like inhalation and absorption through the skin also contribute to the body burden. Foods especially maize have been found to have the highest Aflatoxin concentrations. In addition to investigating the source of Aflatoxins in human breast milk through ingested food, the data obtained would also demonstrate the environmental pollution by Aflatoxins.
CHAPTER TWO: LITERATURE REVIEW

2.1 Overview of Aflatoxicosis

Aflatoxicosis is the poisoning that occurs from ingesting of aflatoxins in humans and animals. Two forms of aflatoxicosis have been identified as acute severe intoxication, resulting in direct liver damage and subsequent illness or death, and the symptoms of severe intoxication include haemorrhagic necrosis of liver, bile duct proliferation (jaundice), edema, and lethargy, (Egmond et al., 2005). Chronic subsymptomatic exposure causes a range of consequences such as liver cirrhosis, immunosupression, rye’s syndrome, cancer, mutagenicity (Lewis et al., 2005). Aflatoxins are a family of fungal toxins produced mainly by two Aspergillus species which are especially abundant in areas of the world with hot, humid climates. Aspergillus flavus, which is ubiquitous, produces B aflatoxins. A parasiticus, which produces both B and G aflatoxins, has a more limited distribution. These fungi liberate aflatoxins in grains and oil crops stored with a moisture content of more than 13.5% and 10% respectively, under conditions of 65-70%, relative humidity and a temperature between 10 and 40°C (Lewis et al., 2005).

2.2 Overview of AFM1 in Human Breast Milk

Maternal to child exposure of aflatoxin M1 in breast milk is an under evaluated risk factor from dietary exposure to aflatoxin B 1. Fetal and early childhood environment, including the nutritional status of the pregnant mother and the infant, are considered critical for growth and risk of disease in later life. Many people in developing countries are not only malnourished but also chronically exposed to high levels of toxic fungal metabolites (mycotoxins) (Navas et al., 2005). Aflatoxins were initially isolated and identified as the causative toxins in Turkey as X disease (necrosis of the liver) in 1960 when over 100,000 turkeys died in England (Lanyasunya et al., 2005). There are four generally recognized. Aflatoxins designated as B1, B2, G1 and G2. The metabolites, M1 & M2 are found in greater quantities than G1, greater than G2, greater than B2. However, aflatoxin B1 is the major mycotoxin produced by most species under favourable conditions giving metabolites of AFM1. AFM1 has the same effects as AFB1 but less potent (Lewis et al., 2005)
Aflatoxins have been reported to be associated with a Reye-like Syndrome in Thailand, New Zealand, Czechoslovakia, the United States, Malaysia, Venezuela, Europe (Gurbay et al. 2010). Humans who ingest mycotoxin-contaminated foods eliminate variable amounts of the toxin in bodily fluids or accumulate them in tissues. For this reason, contamination of human breast milk presents a potentially serious health hazard (Navas et al., 2005). The occurrence of aflatoxin in human tissues or fluids is a problem, particularly in tropical or subtropical countries. Aflatoxin M1 (AFM1) has been detected in breast milk, cord blood, and maternal blood in African countries (Sudan, Ghana, Kenya, Nigeria, Sudan, Gambia), in the Guangxi region of China, (Navas et al., 2005), in Turkey (Gurbay et al., 2006), and in Australia and Thailand. Aflatoxin contamination also poses a serious prenatal health hazard because it can cross the human placental membrane and may be concentrated by the developing feto-placental unit. Although the interaction between dietary aflatoxin intake and exposure of mother, fetus, and newborn infant is very complex, depending on the physiological status of the mother and on food composition, the presence of aflatoxin B1 (AFB1) and its metabolites in human blood and breast milk presents a serious health hazard.

Aflatoxin M1 (AFM1) is the principal hydroxylated aflatoxin metabolite in the milk of dairy cows fed a diet contaminated with aflatoxin B1 (AFB1). It is also present in the milk of nursing mothers who consumed foodstuffs with AFB1 (Polychronaki et al., 2006). The fact that milk, the basic food of infants, can be contaminated with AFM1, a highly toxic substance, has been a matter of considerable concern. There are few papers on the occurrence of AFM1 in human milk in the world (Keskin et al., 2009). While the percentages of contaminated samples in France, Italy, Australia and Zimbabwe were 0 (0/42), 0.4% (1/231; 0.194 ng ml-1), 15% (11/73; range, 0.028-1.031 ng ml-1) and 11% (6/54; range 0.014-0.05 ng ml-1), respectively, in Thailand and Sierra Leone AFM1 was found in 45% (5/11; range, 0.039-1.736 ng ml-1) and 31% (35/113; range, 0.2-99 ng ml-1), respectively, of the samples. Egypt and the United Arab Emirates also had high incidence and levels, 55% (66/120; range, 0.02-2.09 ng ml-1) and 91% (127/140; range, 0.053-3.4 ng ml-1), (Navas et al., 2005)
2.3 Global Exposure To Aflatoxins

Aflatoxins are present in the food chain. They have been found in human cord blood and apparently can enter the developing fetus in humans and animals. In addition, aflatoxins have been found in human breast milk (Polychronaki et al., 2006, Gurbay et al., 2006), cow's milk and dairy products (Navas, et al., 2005) and infant formula. Not only has exposure to aflatoxins been implicated in hepatocellular carcinoma, hepatic failure, encephalopathy and Reye's syndrome, such exposure may also affect health and well being of the fetus and neonates. Thus, it has been postulated that intra-uterine and neonate exposure from human breast milk AFM1, contaminated food products may play an etiology in Kwashiorkor, neonatal susceptibility to infection and jaundice, immunosuppression, childhood infections, malignant disease and compromised response to prophylactic immunizations in children. Aflatoxins have been demonstrated in human cord blood and sera of women immediately after birth. This demonstrated that transplacental transfer and concentration of aflatoxin by the feto-placental unit (Williams et al., 2004).

2.4 Aflatoxin Maize Poisoning In Kenya

In 1978 the Government Chemist in Kenya had collected 195 human food samples and 12 animals feed samples for analyses. On the human food, 52 samples had beyond 150 ppb of aflatoxin, while 1 of the dog food samples had 300 ppb (Wagacha et al., 2008). In 1982 it was reported that 12 people, several dogs and doves died in Machakos District, Eastern Province, after consuming aflatoxin-contaminated maize. In 1991, 172 samples of maize flour for human food were analyzed for aflatoxin, ochratoxin A and Zearalenone. Ochratoxin was the most prevalent (50-5000 ppb), aflatoxin 0.4 -20 ppb and Zearalenone 2500-5000 ppb were reported. In 1993 survey of small - holder poultry farms in the peri-urban areas around Nairobi, 35.6 % of 90 samples of compounded poultry feed collected were found positive for aflatoxins (Wagacha et al., 2008). In 1995, 40 samples of maize flour packed in 90kg bags, 58 samples of a popular brand named Ugali' and 74 samples of another popular brand named ‘Jogoo’ (both packed by major milling companies in Kenya) were collected from Nairobi area. All brands contained aflatoxin B1 and B2 (0.2 – 20mg), ochratoxin A (50 – 1,5000mg), and zearalenone (2,500 – 5,000mg) (Wagacha et al., 2008). This data provides a warning that Kenyan
consumers, who take on average 0.4gms of maize and maize products per day, are at great risk of exposure to these mycotoxins even from apparently ‘good’ quality maize flour.

2.5 Situation of Aflatoxicosis in Makueni District

Aflatoxins contaminate staple foods in Makueni district Kenya, particularly maize as a result of hot, humid storage conditions that promote fungal growth. High exposure to aflatoxins occurs throughout childhood in the region, suggesting that growth and development could be critically affected (KMOH 2004). Makueni District of the Eastern Province in Kenya is within the arid and semi-arid land (ASL) districts of the country. These are characterized by prolonged dry spells with scanty rainfall Aflatoxin B₁ is potent when it contaminates food chains. The potency was illustrated by an outbreak of aflatoxin poisoning in Kenya from January – July 2004, which resulted in 125 recognized deaths and hospitalization of over 300 others across various districts like Makueni, Kitui, Machakos, and Thika. Makueni had the largest proportion of morbidity and mortality. Samples tested had more than 20 ppb of aflatoxin (Lewis et al., 2005). The chronic districts like Makueni had aflatoxin level of more than 8000 ppb. It is uncontestable fact that mycotoxins pose a serious health risk to both livestock and human being world wide particularly in the tropics due to high temperatures, moisture and poverty.

Poverty plays a role on human exposure to mycotoxins. Poor people may lack the capacity to choose the quality of food they eat. Often, poor peasant farmers sell the more attractive better quality food for their harvest, and retain the poor quality one for own consumption by their families. Generally poor people have poor access to useful information. The ignorant may continue consuming harmful substances due to lack of information. Poverty may compromise people’s capacity to implement the innovations necessary to protect food from exposure to mycotoxins. Food insecurity out of poverty may compel non informed farmer to harvest a crop and store it under unsuitable weather and storage conditions, in the rush to plant another crop to secure food supplies in the ensuing periods. (Lewis et al., 2005)
2.6 Control and Prevention of Aflatoxicosis

It requires a vast, complex and expensive environmental manipulation designed to reduce contamination in agricultural commodities which are difficult to control. (Williams et al., 2004). Removing or destruction of the toxin will reduce adverse effects by physical, chemical and biological methods to prevent the growth of mycotoxin producing fungi. To eliminate or reduce the toxin levels, degrade, detoxify the toxins in foods and feeds is through use of chemicals such as acids, alkalis, aldehydes, oxidizing agents and gases (Reddy et al., 2007). However most of these chemicals are not relatively safe for human feed and thus prevention is still the best method to reduce mycotoxin production along the entire food chain (Wagacha et al., 2008)

2.7 Aflatoxin Intervention Strategies

Intervention strategies to reduce exposure to mycotoxins can be undertaken at the individual or community level. Individuals can change their diets to avoid risk foods such as maize. Physical sorting of contaminated grains or nuts could also be useful. The use of chemicals oltipraz and chlorophyllin could reduce exposure to aflatoxin. Good agricultural practices such as rotating crops, irrigation, eliminate draught stress, controlling weeds, cultivating mould resistant stocks and introducing by-controls such as known mycotoxigenic fungal strains. Post harvesting measures include drying rapidly by mechanical means and keeping crops dry, sorting out contaminated maize and nut by physical means, by colour and washing with water. Chemical methods of detoxification include ammunition processes (Egmond et al., 2005)

2.8 Treatment of Aflatoxicosis

Treatment of aflatoxicosis can only be symptomatic because of the immunological and nutritional status which affects different body functions with consequences of several health implications. Research has reviewed aflatoxicosis as one of the six top WHO risk factors which account for 43% of the disability adjusted life years (DALY) in the developing countries where short life span is prevalent (Williams et al., 2004).
2.9 Aflatoxins Pharmacokinetics

Aflatoxins cause poisoning after ingestion when plasma levels increase within a given time to reach a peak concentration. The peak concentration depends on the amount ingested, rate of absorption, volume of distribution and the rates of aflatoxin clearance from plasma by kidneys, sweat and milk excretions. The rapid decline in plasma concentrations that occur after the rate of absorptions declines is due to excretion (Reddy et al., 2007)

2.9.1 Absorption of Aflatoxin

The phases that are readily distinguished include absorption, distribution, and elimination, and can be described using pharmacokinetic models. Aflatoxin is rapidly distributed in the well perfused tissues such as the heart, kidney, liver and other tissues. Aflatoxin is absorbed from the gastrointestinal tract, the lungs and the skin. The absorption of aflatoxin is influenced by anatomical, physiological and biochemical factor. The second most important root of absorption is by the lungs. Pulmonary inhalation of aflatoxin present in dusts for example from maize millers and maize handlers and other gaseous constitutes the major route of industrial exposure. A third route, and relatively rare, is through the skin. Aflatoxin from various sources may be absorbed at different rates (Cigic et al., 2009)

2.9.2 Distribution of Aflatoxins in The Body

Aflatoxin has been detected in most organs and tissues; however, there is evidence that it is concentrated in blood plasma, kidneys, lungs, uterus, placenta and the brain. This is thought to be due to the excretion of the aflatoxin metabolites through the different organs and tissues (Cigic et al., 2009)

2.9.3 Aflatoxins in the placenta

Studies conducted in several species have shown that aflatoxin crosses the placenta and it is taken up by the fetal tissues. (Navas et al., 2005). The aflatoxin level in newborn is correlated with the amount ingested by the mother. The human placenta acts as a barrier to aflatoxin diffusion when the aflatoxin concentration in maternal blood exceed to high
levels, however, the degree to which the placenta acts as a barrier vary from one species to another and in some species it causes abortion. In others it is teratogenic.

2.9.4 Aflatoxins in Plasma
Aflatoxin in plasma is not bound by protein or any other constituent of plasma. The concentration varies according to the level of intake and physiological factors. In general, however the fasting plasma concentration of healthy adults is roughly equal to that in the food taken. These values are based largely with young or middle aged healthy adults (Williams et al., 2004)

2.9.5 Aflatoxins Metabolism
Aflatoxin B1 is metabolized to aflatoxin M1 secreted in the human breast milk. Metabolic activation of aflatoxin B1 to the 8, 9-epoxide leads to binding to glutathione, DNA, and serum albumin. In the DNA aflatoxin B1 forms a major adduct, aflatoxin-N7-guanine, following metabolic epoxidation. Aflatoxin M1, P1 and Q1 are also aflatoxin B1 metabolites but aflatoxin M1 is the major metabolite (Anamika and Waliyar 2009)

2.9.6 Aflatoxins Excretion
The major route of aflatoxin excretion is by the kidneys; however it is also excreted in small amount by the sweat glands, lactating breast, semen and gastrointestinal tract (Faisal et al., 2010)

2.9.7 Toxicity of Aflatoxins
The possible role of aflatoxin influencing human health has been researched mainly in relation to its role as a carcinogenic. However the pivotal role of the immune system incidence is severe and outcome of infectious diseases has been reported (Williams et al., 2004). It affects the micronutrients which affects the epidemiology of many diseases and health risks in humans and animals.

2.9.8 Underweight and Nutritional Related Epidemiology
Underweight is the single most contributory risk factor to the burden of disease worldwide contributing to 14.9% of DALYs. Aflatoxin has been implicated to
underweight in children in Benin and Togo and to the condition of Kwashiorkor. Autopsy evidence from children in Nigeria found aflatoxin in tissues after postmortem death due to malnutrition (Polychronaki et al., 2006)

2.9.9 Analysis of AFM1 Levels

Quantitative and qualitative analysis for human exposure to aflatoxins can be done through human breast milk analysis. From these studies, the exposure rate in human breast milk, retrospective case studies can then help to determine neonates and infants reported mortality and morbidity rate in study area. There is no comprehensive data set from which to evaluate the extent and severity of biological exposure of humans in developing countries. Direct measurement of human biological exposure to aflatoxin is available from only a small number of countries (Mahdavi et al., 2010).

Analysis of the four major aflatoxins B12, B2, G1 and G2 named according to their fluorescent colours blue and green may be carried out using Thin Layer Chromatography (TLC), Gas-Liquid Chromatography (GLC), High Pressure Liquid Chromatography (HPLC) and mass spectrophotometer may also be used but, the equipments are expensive, labour intensive and not suitable for wide spread use. (Keskin et al 2009).

The method of Enzyme Immuno Sorbent Assay (ELISA) is direct, sensitive and specific for aflatoxin testing and gives rapid results within 30 minutes. However the ELISA method is also expensive. (Mahdavi et al., 2008)
CHAPTER THREE: MATERIAL AND METHODS

3.1 Study Area

This study was conducted in Makueni District of Eastern Province in Kenya which is within the Arid and Semi-Arid Land (ASAL). Recurrent aflatoxin outbreaks have occurred in Makueni District. Most of the cases are attended to at Makindu District Hospital. The district is characterized by hot and humid climate due to prolonged dry spells. These factors favour aflatoxin growth, causing contamination of staple foods like maize, sorghum, beans and green gram. The ASAL districts are food deficient and poverty is evident and therefore maize is brought into the district from other districts countries for sale and sometimes as donations and relief foods.

Makueni district is 80% semi arid. Rainfall in the district, which is generally scarce, varies with altitude. Average annual rainfall ranges slightly over 1000mm in the highlands to slightly below 500mm in the low lying south and South East parts of the district. The rainfall pattern is bimodal with significant differences in distribution during different years. There are two rain seasons: long rains season from March to April and short rain season from November to December. The temperature in this district varies considerably with the altitude. To the North it is usually cool while in the low-lying areas of the South it is usually hot. The district has an average temperature of 24.1°C. Generally the district experiences high temperatures during daytime and low temperatures during nights. During the dry periods i.e. between May and October, the lower parts of the district experiences severe heat. However the Northern part especially on hilltops have low temperatures. This is due to the forests and windy conditions that exist in this area. Most of the wind blow towards the hills while less is felt low-lying plains to the South. This explains the high humidity. The Southern parts of the district experience high evapotranspiration rate. There is normally a long dry spell between the months of June and October during which extreme heat is experienced in the low lying parts of the district causing high evapotranspiration.
3.1.1 Location and Size
Makueni district, which was curved out of Machakos district, is one of the ten districts that form the Eastern Province. It borders Kajiado district to the West, Taita Taveta to the South East, Kitui to the East and Machakos district to the North. Makueni district lies on the co-ordinates of 1°35 South and longitude 37°10 East and 38°30 East. The district has an area approximately 7263 sq.kms ranging from 100km wide in the North and less than 20km wide to the South. There are seven divisions in the district namely Wote, Kilome, Kibwezi, Mbooni, Makindu Matiliku and Mtitu Andei.

3.1.2 Health issues in Makueni District
Although accurate information on cause of diseases and deaths is lacking, studies have shown that child diseases and mortality is multifactor. The most common causes of morbidity in Makueni district include Pneumonia, Malaria, Neonatal Tetanus, Malnutrition, Diarrhea and increasingly HIV/AIDS (Lewis et al., 2005). Child diseases and deaths are commonly as a result of several risk factors including unhygienic and unsafe environments. Table 3.1 below shows top ten causes of mortality in Makueni District.

Table 3.1 Top Ten Causes of Mortality in Makueni District

<table>
<thead>
<tr>
<th>SERIAL NO.</th>
<th>DISEASE</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Malaria</td>
<td>40</td>
</tr>
<tr>
<td>02</td>
<td>Diseases of respiratory Tracy Infection</td>
<td>28</td>
</tr>
<tr>
<td>03</td>
<td>Diseases of the skin(including ulcers)</td>
<td>5</td>
</tr>
<tr>
<td>04</td>
<td>Tuberculosis</td>
<td>5</td>
</tr>
<tr>
<td>05</td>
<td>Malnutrition</td>
<td>6</td>
</tr>
<tr>
<td>06</td>
<td>Pneumonia</td>
<td>5</td>
</tr>
<tr>
<td>07</td>
<td>Menengitis</td>
<td>2</td>
</tr>
<tr>
<td>08</td>
<td>Haemorrhage</td>
<td>2</td>
</tr>
<tr>
<td>09</td>
<td>Neonatal Sepsis</td>
<td>6</td>
</tr>
<tr>
<td>10</td>
<td>Respiratory tract infection</td>
<td>1</td>
</tr>
</tbody>
</table>

Source Makueni district MOH report (2007)
3.2 Study Design
A combination of both descriptive cross sectional study and analytical study was conducted in Makindu District Hospital, MCH Department.

3.3 Variables

3.3.1 Independent Variables.
The independent variables for the lactating mothers attending MCH clinic in Makindu hospital were age, occupation, marital status, religion, and level of education, levels of aflatoxins, maize moulds, maize fragmentation, and maize insect infestation.

3.3.2 Intermediate Variable
Levels of aflatoxins in mothers' household maize.

3.3.3 Dependent Variables
Dependent variables are levels of aflatoxins in human breast milk.

3.4 Target Population
This study targeted all the lactating mothers living in Makueni District.

3.4.1 Study Population
The study population comprises of all lactating mothers attending Makindu district Hospital Mother and Child Welfare Department (MCH) every month.

3.4.2 Inclusion Criteria
The study includes lactating mothers attending MCH Department who have been living in Makueni District for the past 6 months or more and were willing to participate in the study.
3.4.3 Exclusion Criteria
The study excluded all lactating mothers with a known health condition like terminally ill or had a sick child and those unwilling to participate in the study.

3.5 Sample Size Determination
The sample size was calculated by single cluster using the WHO formula (MSF, 1995)
Since aflatoxicosis prevalence is estimated at 8% for Makueni District (KMOH 2004) and the precision required was 5% the sample size was calculated as below:-

\[ n = \frac{z^2 \cdot p \cdot (1-p) \cdot D}{d^2} \]

where:
- \( n \) = sample size
- \( p \) = expected prevalence for aflatoxicosis in Makueni District which is 8%
- \( q \) = 1 - \( p \)
- \( z \) = standard normal deviation for risk error at 95%CI which is 1.96.
- \( d \) = degree of accuracy which is 0.05.
- \( D \) = Design effect (1)

Therefore \( n = \frac{1.96^2 \cdot 0.08 \cdot 0.92 \cdot 1}{0.05^2} \)

\( n = 113 \) mothers.

A sample size of 113 lactating mothers attending Mother Child Health clinic Makindu were taken as the study subjects.

3.6.1 Sampling Techniques
Systematic single random sampling technique was used to select study participant. In Makindu hospital, mother and Child health clinic data indicate a monthly register of approximately 500 lactatating mothers. Given that 113 mothers were required, then our sampling technique was to pick the 5th attending the MCH clinic. Therefore for every 5th mother attending MCH department at Makindu Hospital was sampled systematically (Mugenda and Mugenda 1999). However to determine the starting point the 1st mother, was picked at random after which the systematic process started. But if the 5th mother sampled does not meet the criteria, the 6th mother was sampled. The sampling continued
systematically until 113 mothers were all sampled. From the 113 lactating mothers maize from their households were sampled from the source they were currently using for their food. The 113 samples of human breast milk and maize were transported to the University laboratories for AFM1 and AFB1 analysis.

3.6.2 Questionnaires

A questionnaire was administered to the same mothers to obtain information on age, food consumption, demographic data, and marital status. Information to assess the wealth of the mother, occupation, level of education, aflatoxin awareness, cause of aflatoxicosis and religion was obtained. An interview for health providers and a check list on the health facility was carried out. The data was collected through questionnaires (open ended) where information on aflatoxicosis awareness was recorded. There was a review of available data at the health facilities for trends in morbidity and mortality due to Aflatoxicosis.

3.6.3 Procedure for collecting the Human Breast Milk

A selected mother according to the sampling technique was screened for inclusivity. Once the mother fulfilled the inclusion criteria and agreed to participate in the study. She was then requested to sanitise her hands and the breast area around the nipple. One hundred and thirteen lactating mothers who were regularly breast feeding their babies between ages 1-23 months from Makindu Hospital were sampled. The human breast milk was sampled by hand expression and 50 ml was collected in plastic containers, labeled and immediately put in the cool box awaiting to be refrigerated in the MCH freezer at -4°C. Care was taken to avoid contamination both during and after sample collection. Milk samples were transported using cool ice parked box to the laboratories deepfreezer within 12 hrs and were all analysed within 3 weeks.

The milk was thawed at 37°C in the oven, centrifuged at 3000 rpm just before analysis by an Enzyme Linked Immunosorbent Assay (ELISA) method, (Mahdavi et al., 2008).
3.6.4 AFLATOXIN ANALYSIS IN HUMAN BREAST MILK

3.6.5 Test Principle of ELISA Method

The basis of the test is the antigen-antibody reaction. The wells in the microtiter strips are coated with capture antibodies directed against anti-aflatoxin antibodies. Standards or the sample solutions, aflatoxin-enzyme conjugate and anti-aflatoxin antibodies are added. Free and enzyme conjugated aflatoxin compete for the aflatoxin antibody binding sites (Competitive enzyme immunoassay). At the same time, the aflatoxin-antibodies are also bound by the immobilized capture antibodies. Any unbound enzyme conjugate is then removed in washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) are added to the wells and incubated. Bound enzyme converts the colourless chromogen into blue product. The addition of the stop solution leads to a colour change from blue to yellow. The measurement is made photometrically at 450 nm (optional reference wavelength > 600 nm). The absorption is inversely proportional to the aflatoxin concentration level in the sample.

3.6.6 Test Procedure on the Human breast milk

The human breast milk samples remained in the laboratory deepfreezer throughout the analysis period of 3 weeks. On the day of analysis the samples were removed from the laboratory freezer which was under key and lock for safe keeping and placed in the oven to thaw at 37°C before the analysis. The breast milk samples were then treated as below for the extraction of aflatoxin M1 using the aflatoxin M1 assay. The milk sample was thawed at 37°C in the oven, centrifuged at 3000 rpm just before analysis. The
centrifugation process separates fluid milk from the fat in milk. The fluid part of the milk is then analysed as below;

i) Sufficient number of microtiter wells was inserted into the microwell holder for all standards and samples to be ran in duplicate. Standard and sample positions were recorded.

ii) 100 µl of the standard solutions and the prepared human breast milk sample were added to separate duplicate wells. The plate was mixed gently by rocking manually and incubated for 60 min. at room temperature (20-25 °C) in the dark.

iii) The liquid was poured out of the wells by tapping the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. All the wells were 250 µl washing buffer and poured out the liquid again. The washing procedure was repeated twice.

iv) 100 µl of the diluted enzyme conjugate was added and mixed gently by rocking the plate manually and incubated for 60 min. at room temperature (20-25 °C) in the dark.

v) The liquid was poured out of the wells by tapping the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. All the wells were added 250 µl washing buffer and poured out again. The washing procedure was repeated twice.
vi) 50 μl of substrate and 50 μl of chromogen was added to each well and mixed gently by rocking the plate manually and incubated for 30 min. at room temperature (20-25 °C) in the dark.

vii) 100 μl of the stop solution was added to each well and mixed gently by rocking the plate manually and absorbance measured at 450 nm against an air blank. This was read within 60 minutes after addition of stop solution.

3.6.7 INTERPRETATION OF THE RESULTS

Optical density (OD) values were expressed as percentage of the OD of zero (0) standard. A dose response curve was constructed using the five standards. Since the standard were known, the unknown levels of aflatoxins in the milk sample were measured from the standard curve. If a milk sample contained Aflatoxin levels higher than the highest standard (>80 ppt) the milk sample was diluted with 1 part of methanol (100%) in 10 parts of the buffer 1 (1:10 dilution). The microwells were measured optically using an ELISA Reader machine, model Uniskan 11 type 364 Labystems, Finland with an absorbance filter of 450nm and a differential filter of 630nm. The optical densities (OD) of the samples were compared to the ODs of the standards and interpretive results were determined. The absorption was inversely proportional to the human breast milk aflatoxins concentration in the sample. The lowest Limit of detection was 5 ppt for human breast milk.
3.6.8 Maize Sampling

Maize sample which were a representative of the staple food of the mothers were collected from mothers households. Fifty four samples were sampled from mothers visiting MCH makindu hospital out of the 113 mothers from 4 divisions of Makueni district namely Makindu, Kibwezi, Matilku and Mtito/Andei. 59 of the remaining mothers were only sampled for breast milk. Thirteen samples of maize were sampled from Mothers from each of the 4 divisions who attended Makindu hospital MCH clinic. Many of the mothers travel 5 kilometers and more to the MCH clinic. Sampling for all the 113 mothers was not possible due to financial constraints and accessibility to the mothers house holds. Maize was sampled from the household from the maize that was in use for their food. In the present study 54 samples of maize samples were collected from mothers from households parallel to the collection of human breast milk. One (1) kg of dried maize kernels (seeds) were sampled and stored in brown paper bags (not nylon) ready to be taken to the laboratory for aflatoxin analysis. The aim of the study was to evaluate the relative contribution of maize to the occurrence of Aflatoxin in the human breast milk.

3.7 HANDLING, MANAGEMENT AND LABORATORY METHODS MAIZE SAMPLES

3.7.1 Maize analysis

The maize samples remained the laboratory for 3 weeks before aflatoxin analysis. On the day of analysis the samples were removed from the laboratory shelf which was under key and lock for safe keeping. The maize samples were then treated as below for the extraction of aflatoxin B1 using the aflatoxin total assay.
3.7.2 Aflatoxin total Assay for the Maize Samples by ELISA

Direct competitive enzyme linked immunosorbent assay (ELISA) that determines quantitative levels for the presence of total Aflatoxin B1 was used. This method is common for aflatoxin B1 analysis in grains, cereals, nuts, animal feeds and other commodities. The assay has been validated for corn meal, popcorn, soyabeans, milled rice, sorghum, wheat, cotton seed and peanuts. (Keskin et al., 2009)

3.7.3 Principle of ELISA Method of Analysis

Aflatoxins were extracted from a ground sample of maize with 70% methanol in water. The extracted sample and enzyme conjugated aflatoxin was mixed and added to the antibody-coated microwell. Aflatoxins in samples and control standards were allowed to compete with enzyme conjugated aflatoxin for the antibody binding sites. After a washing step, an enzyme substrate was added and blue colour developed. The intensity of the colour was inversely proportional to the concentration of aflatoxins in maize sample. The microwells were measured optically using an ELISA Reader machine, model Uniskan 11 type 364 Labystems, Finland with an absorbance filter of 450nm and a differential filter of 630nm. The optical densities (OD) of the samples were compared to the ODs of the standards and interpretive results were determined.

3.7.4 Test Principle

The basis of the test is the antigen-antibody reaction. The wells in the microtiter strips were coated with capture antibodies directed against anti-aflatoxin antibodies. Standards or the sample solutions, aflatoxin-enzyme conjugate and anti-aflatoxin antibodies were added. Free and enzyme conjugated aflatoxin compete for the aflatoxin antibody binding sites (Competitive enzyme immunoassay). At the same time, the aflatoxin-antibodies were also bound by the immobilized capture antibodies. Any unbound enzyme conjugate was then removed in washing step. Enzyme substrate (urea peroxide) and chromogen (tetramethylbenzidine) were added to the wells and incubated. Bound enzyme converts the colourless chromogen into blue product. The addition of the stop solution leads to a colour change from blue to yellow. The microwells were measured optically using an ELISA Reader machine, model Uniskan 11 type 364 Labystems, Finland with an absorbance filter of 450nm and a differential filter of 630nm. The optical densities (OD)
of the samples were compared to the ODs of the standards and interpretive results were determined. The absorption was inversely proportional to the aflatoxin concentration in the sample.

3.7.5 Aflatoxin Extraction from Maize

Fifty four maize samples were collected from mother’s households and analyzed for aflatoxin B1 using indirect competitive enzyme linked immunoassay.

   i) A known sample weight of 100g -1000g was ground to pass through a 20 micro diameter mesh screen and mixed properly.
   ii) 20g of the ground sample was weight using an accurate balance sartorius, placed in a clean jar or conical flask that could be tightly sealed.
   iii) 100ml of 70/30 methanol/water (70% methanol) was added to the samples and corked, and extracted in a ratio of 1: 5 (w/v). The mixture was shaken for 3 minutes.
   iv) The sample was left to settle, then the top layer of the extract was filtered through a whatman No. 1 filter and the filtrate collected.
   v) The filtrate was adjusted to pH 6-8. Excessive alkaline or acidic conditions may affect the test results and was adjusted before testing.
   vi) The filtrate was ready for ELISA Kit test and quantification of AFB1

3.7.6 INDIRECT COMPETITIVE ELISA ASSAY

3.7.7 Test Procedure of Aflatoxin B1 in Maize

   i) Sufficient number of microtiter wells was inserted into the microwell holder for all standards and samples to be ran in duplicate. Standards and samples positions were recorded.
   ii) 50 µl of the standard solutions were added on prepared samples to separate duplicate wells.
   iii) 50 µl of the diluted enzyme conjugate was added to each well.
iv) 50 µl of diluted antibody solution was added to each well. The plate was mixed gently by rocking manually and incubated for 30 min. at room temperature (20-25° C) in the dark.

v) The liquid was poured out of the wells by tapping the microwell holder upside down vigorously (three times in a row) against absorbent paper to ensure complete removal of liquid from the wells. All the wells were filled with 250 µl distilled water and poured out again. The washing procedure was repeated twice.

vi) 50 µl of substrate and 50 µl of chromogen was added to each well and mixed gently by rocking the plate manually and incubated for 30 min at room temperature.

vii) 100 µl of the stop solution was added to each well and mixed gently by rocking the plate manually and absorbance measured at 450 nm against an air blank by spectrophotometer machine. This was read within 60 minutes after addition of stop solution.

3.7.8 Interpretation of the Results

The average absorbance values for each aflatoxin standard and sample extract dilution (B) and that of the reagent blank (Bo) were calculated. Using these values the percentage inhibition (B/Bo %) for each standard and sample dilution was calculated. A standard response curve used to calculate aflatoxin levels in maize was constructed by plotting aflatoxin standards concentration X-axis and the corresponding percentage inhibition values on Y-axis. The amount of AFB1 in any sample was read off the standard response curve. If a sample contains Aflatoxin levels higher than the highest standard the filtered extract was further diluted in 70% methanol and multiplied by the dilution factor. The lowest limit of detection was 3 ppb for maize.

3.7.9 Data management

Several statistical software packages were used for data management and analysis in order to enhance data quality. This included, SPSS software, Chi square test for categorical variables descriptive statistics for continuous categorical data and correlation analysis for
continuous variables. The data was therefore presented in frequency tables, proportions, bar graphs, relative frequencies, 95% confidence intervals. Data was then analyzed using Chi square test of independence Correlation Coefficient to study the presence or absence of any association between aflatoxin in human breast milk, maize contamination and aflatoxicosis awareness of the lactating mothers.
CHAPTER FOUR: RESULTS

4.0 Introduction

This chapter presents results from the aflatoxins levels detected from 113 mothers breast milk and maize from their households. It also highlights the proportion of aflatoxins M1 in the lactating mothers in MCH Makindu Hospital. Physical contamination on household maize results on fragmentation, insect infestation and moulds are presented. Some of the factors of mothers aflatoxin awareness has been reported.

4.1 SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE STUDY POPULATION IN MCH CLINIC MAKINDU HOSPITAL MAKUENI

Table: 4.1 Distribution of mothers attending MCH clinic in Makindu hospital by age groups.

<table>
<thead>
<tr>
<th>Age of the Mother</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-19</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>20-22</td>
<td>37</td>
<td>31</td>
</tr>
<tr>
<td>23-25</td>
<td>21</td>
<td>18</td>
</tr>
<tr>
<td>26-28</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>29-31</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>32-34</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>35-37</td>
<td>3</td>
<td>2.6</td>
</tr>
<tr>
<td>38-40</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>41-43</td>
<td>2</td>
<td>1.7</td>
</tr>
</tbody>
</table>

4.1.1 Age Of The Lactating Mothers in The MCH Clinic Makindu Hospital

The study indicated that 10% of the respondents were in 17-19 years age group and 31% were in the 20-22 years age group. The 23-25 years age group and 26-28 years age group were both 18%. In the 29-31 years age group were 11%, while 6% were in the 32-34 years age group and 2.6% were in the 35-37 years of age group. The 38-40 years age group and 41-43 years age group were both 1.7%. The mean age of the respondent was
25 years and the range was between 17-43 years age group while the mode was between 20-22 years of age group. The ages are as shown on table 4.1

4.2.0 Prevalence of Afm1 Levels In Human Breast Milk of Lactating Mothers Attending MCH Clinic In Makindu Hospital

The prevalence of aflatoxin M1 levels in human breast milk of the lactating mothers visiting Makindu Sub-District Hospital MCH Department was found to be 82%. The study indicated that most of the mothers’ milk was positive with the toxicant. These findings agree with studies done in Sudan, Nigeria and Egypt (Supranee et al.2003) which reported a prevalence of 80%. The AFM1 findings in Makindu Hospital was high as ideally no aflatoxins levels are acceptable in human breast milk in developed countries. However the allowed levels of aflatoxins human milk is 5 ppt.

Table: 4.2 Distributions of the Levels of Aflatoxin of the Lactating Mothers

<table>
<thead>
<tr>
<th>Levels of Aflatoxin (ppt)</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-49</td>
<td>22</td>
<td>17.6</td>
</tr>
<tr>
<td>50-249</td>
<td>44</td>
<td>35.2</td>
</tr>
<tr>
<td>250-399</td>
<td>47</td>
<td>37.6</td>
</tr>
<tr>
<td>400-550</td>
<td>9</td>
<td>7.2</td>
</tr>
<tr>
<td>&gt;550</td>
<td>3</td>
<td>2.4</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
<td>100</td>
</tr>
</tbody>
</table>

4.2.1 Distribution of the Levels of Aflatoxin M1 in Human Breast Milk of Lactating Mothers Attending MCH Clinic Makindu Hospital

Results in table 4.2 show aflatoxin M1 levels found in the milk of lactating mothers. As shown, 17.6% had 5-49 ppt, 35.2% had 50-249 ppt, 37.6% had 250-399 ppt, and 7.2% had 400-550 ppt while 2.4% had more than 550 ppt. The levels of AFM1 were higher than the allowed levels of 5 ppt in human milk in developed countries. The highest % population of the lactating mothers had more than 250 ppt several times more than the allowed levels of 5 ppt in breast milk.
The mean of the aflatoxin M1 levels in human breast milk was found to be 228.3 ppt, median was found to be 230 ppt, and the standard deviation for all the cases was found to be 150.34 ppt. (table 4.3).

The result in Table 4.3 and figure 4.2 shows that, 18.25% of the lactating mothers visiting MCH Makindu Hospital had no aflatoxins in breast milk. However, 34.92% had between 5-249 ppt aflatoxin M1 and 46.83% had between 250-399 ppt aflatoxin M1. 9.38% had between 400-559 ppt aflatoxin M1 and 2.38% had over 550 ppt aflatoxin M1.

4.2.2 Descriptive Analysis of Aflatoxin M1 in Human Breast Milk of Lactating Mothers Attending MCH Clinic Makindu Hospital.

The mean of the aflatoxin M1 levels in human breast milk was found to be 228.3 ppt, median was found to be 230 ppt, and the standard deviation for all the cases was found to be 150.34 ppt. (table 4.3)

Table: 4.3 Mean and Standard deviation of AFM1 in the human breast milk

<table>
<thead>
<tr>
<th>PARAMETER</th>
<th>MILK AFM1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median for all the cases</td>
<td>230 ppt</td>
</tr>
<tr>
<td>Std deviation for all the cases</td>
<td>150.34 ppt</td>
</tr>
<tr>
<td>Mean</td>
<td>228.3 ppt</td>
</tr>
</tbody>
</table>

4.2.3 AFLATOXIN LEVELS IN HUMAN BREAST MILK

The result in Table 4.4 and figure 4.2 shows that, 18.25% of the lactating mothers visiting MCH Makindu Hospital had no aflatoxins in breast milk. However, 34.92% had between 1-249 ppt aflatoxin M1 and 46.83% had between 250-399 ppt aflatoxin M1. 9.38% had between 400-559 ppt aflatoxin M1 and 2.38% had over 550 ppt aflatoxin M1.
Aflatoxin in human breast milk versus level of education

4.2.4 Relationship between Aflatoxin in Human Breast Milk and Education Level of Mothers Attending MCH Clinic, Makindu Hospital.

A small proportion of about 0.5% had informal education. However, 65% of the respondent had attained primary education, and 34.5% had attained high school education and above. The relationship between AFM1 in human breast milk and levels of education was not significant. \( \chi^2 = 43 \text{ df } 3 \text{ p}=0.532 \) However secondary school and college levels of education, respondents had lower levels of aflatoxin M1 in breast milk as compared to the primary school and lower levels of education (figure 4.14).
4.2.5 Relationship between marital status and Aflatoxins in mothers breast milk

Most of the mothers attending MCH clinic were married (106) as compared to only ( 7 ) who were single parents. A chi-square test to determine the relationship between mothers marital status and aflatoxins in their breast milk was not statistically significant. ( $\chi^2 = 0.59$ df 3 P=0.645).

4.2.6 Relationship between religion and human breast milk

The results indicate that lactating mothers’ religion did not have any effect on levels of aflatoxins in the mothers breast milk. Religion considered were Muslim, Catholic Seventh Day Adventists and Protestants. The relationship between AFM1 in human breast milk and religion was not significant $\chi^2 = 0.98$ df 3 p=0.548

4.2.7 The relationship between aflatoxins in human breast milk and whether they were Working or house wives.

There was no significant relationship between working earning a salary mothers or housewives and levels of aflatoxins in breast milk. $\chi^2 = .99$ df 5 p= 0.322. Many of the mothers were house wives( 75%) and working (25%)
4.2.9 Aflatoxin poisoning cases reported in Makindu Sub-District Hospital
Year 2004-2005

Figure 4.2 shows that aflatoxin cases reported by KMOH, had Makindu Division with the highest number of patients treated at Makindu hospital.

Figure: 4.2 Aflatoxins Cases reported in Makindu Sub-District Hospital

Year 2004-2005
4.3.0 AFLATOXIN B1 IN MAIZE FROM THE MCH LACTATING MOTHERS, MAKINDU.

Table: 4.4 Maize Aflatoxin B1 ppb distribution in 4 divisions

<table>
<thead>
<tr>
<th>DIVISION</th>
<th>AFLATOXIN B1 ppb</th>
<th>PERCENTAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAKINDU</td>
<td>1824.5</td>
<td>33</td>
</tr>
<tr>
<td>MUTITO-ANDEI</td>
<td>1344</td>
<td>24</td>
</tr>
<tr>
<td>KIBWEZI</td>
<td>1316</td>
<td>24</td>
</tr>
<tr>
<td>MATILIKU</td>
<td>1045</td>
<td>24</td>
</tr>
</tbody>
</table>

4.3 MAIZE FROM MOTHERS HOUSEHOLD MCH CLINIC, AFLATOXINS CONTAMINATION

Table: 4.5 Kibwezi maize parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Level of Aflatoxin B1 (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Deviation</td>
<td>74.8</td>
</tr>
<tr>
<td>Average</td>
<td>101.2</td>
</tr>
</tbody>
</table>

4.3.1 Maize Aflatoxin B1 Contamination from Kibwezi mothers household

Maize samples (14) collected from Kibwezi Division were analyzed for aflatoxin B1, insect infestation, and physical appearance. The results in table 4.8 show that standard deviation for aflatoxin B1 levels was 74.8 ppb, the statistical mean was 101.2 ppb.

Table: 4.6 Levels of maize aflatoxin B1 in Kibwezi

<table>
<thead>
<tr>
<th>Range</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-100</td>
<td>AFB1</td>
</tr>
<tr>
<td>101-200</td>
<td>AFB1</td>
</tr>
<tr>
<td>&gt;200</td>
<td>AFB1</td>
</tr>
</tbody>
</table>
4.3.2: Levels of Maize Aflatoxin B1 in Kibwezi

Results from 46.2% of the maize samples collected from Kibwezi were found to contain aflatoxin B1 ranging between 3-100 ppb, 38.5% had between 101-200 ppb and 15.4% had >200 ppb. (table 4.6). Most of the maize had more than the allowed levels 20ppb in animal feed and 10 ppb in humans in some countries in Africa and no aflatoxins in USA (William’s et al., 2004).

4.3.3 MAIZE AFLATOXIN B1 CONTAMINATION FROM MCH MOTHERS IN MAKINDU

4.3.4 Maize Aflatoxin B1 Contamination from mothers Households in Makindu Division

Table: 4.7 Parameters of Aflatoxin B1 in Maize in Makindu Division.

<table>
<thead>
<tr>
<th>Parameters of Aflatoxin B1 in Maize</th>
<th>Levels of Aflatoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>166</td>
</tr>
<tr>
<td>Range</td>
<td>3-260</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>75</td>
</tr>
</tbody>
</table>

4.3.5 Levels Of Aflatoxin B1 In Maize In makindu Division

Results in tables 4.7 and 4.15 show that 13 maize samples collected from Makindu Division, were analyzed for aflatoxin B1, insect infestation, and physical appearance. The standard deviation for aflatoxin B1 levels was found to be 75 ppb, the statistical mean was 166 ppb, and the range was 3-260 ppb.
4.3.6 LEVELS OF AFLATOXINS IN MAIZE FROM MOTHERS HOUSEHOLD IN MUTITU ANDEI DIVISION

Table: 4.8 Parameters of Aflatoxin B1 in Maize in Mutitu Andei Division.

<table>
<thead>
<tr>
<th>PARAMETERS OF AFLATOXIN B1 IN MAIZE</th>
<th>AFLATOXIN LEVELS (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>89.6</td>
</tr>
<tr>
<td>Range</td>
<td>3 -218</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>82.11</td>
</tr>
</tbody>
</table>

4.3.7: Levels Of Aflatoxin B1 in Mutitu-Andei Division

Maize (15) samples were collected from Mutito-Andei Division and analyzed for aflatoxin B1, insect infestation, and physical appearance. The standard deviation for aflatoxin B1 levels was found to be 82.1 ppb, the statistical mean was 89.6 ppb, and the range was 3-218 ppb. (tables 4.8 and 4.20)

4.3.8 AFLATOXIN B1 CONTAMINATION IN MATILIKU DIVISION

Table: 4.9 Parameters of Aflatoxin B1 in Maize from mothers household in Matiliku Division.

<table>
<thead>
<tr>
<th>MATILIKU PARAMETERS</th>
<th>MAIZE</th>
<th>AFLATOXIN LEVELS (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>3 -271</td>
<td></td>
</tr>
<tr>
<td>Standard deviation</td>
<td>79.6</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td>80.4</td>
<td></td>
</tr>
</tbody>
</table>
4.3.9 Levels of maize Aflatoxin B1 from mothers households in Matiliku Division

Maize (12) samples were collected from Matiliku Division and analyzed for aflatoxin B1, insect infestation, and physical appearance. The standard deviation for aflatoxin B1 levels was found to be 79.6 ppb, the statistical mean was 80.4 ppb, and the range was 3-271 ppb. (table 4.9 and 4.25)
RESULT 4.4.0

4.4.1 AFLATOXINS IN MAIZE FROM MOTHERS HOUSEHOLD AND OTHER FACTORS ASSOCIATED WITH AFLATOXINS CONTAMINATION.

Table 4.10 Makindu Division Physical Appearance of Maize and Aflatoxins in Milk And Maize

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>MOULDY</th>
<th>INSECT DAMAGE</th>
<th>FRAGMENTATION</th>
<th>AFMI</th>
<th>AFB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/29/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M/39/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M/30/06</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>105</td>
<td>28</td>
</tr>
<tr>
<td>M/32/06</td>
<td>Mouldy</td>
<td>+</td>
<td>-</td>
<td>210</td>
<td>56</td>
</tr>
<tr>
<td>M/34/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>0</td>
<td>109</td>
</tr>
<tr>
<td>M/31/06</td>
<td>Mouldy</td>
<td>+</td>
<td>-</td>
<td>288</td>
<td>152</td>
</tr>
<tr>
<td>M/33/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>175</td>
<td>163</td>
</tr>
<tr>
<td>M/27/06</td>
<td>Mouldy</td>
<td>+</td>
<td>-</td>
<td>310</td>
<td>168</td>
</tr>
<tr>
<td>M/35/06</td>
<td>Mouldy</td>
<td>+</td>
<td>-</td>
<td>190</td>
<td>218</td>
</tr>
<tr>
<td>M/37/06</td>
<td>Discoloured</td>
<td>-</td>
<td>-</td>
<td>355</td>
<td>218</td>
</tr>
<tr>
<td>M/36/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>165</td>
<td>226</td>
</tr>
<tr>
<td>M/28/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>375</td>
<td>227</td>
</tr>
<tr>
<td>M/38/06</td>
<td>Mouldy</td>
<td>+++</td>
<td>++</td>
<td>296</td>
<td>260</td>
</tr>
</tbody>
</table>

**KEY**

- - represents no insect damage or fragmentation
- + represents mild insect damage or fragmentation
- ++ represents moderate insect damage or fragmentation
- +++ represents severe insect damage or fragmentation
4.4.2 The Relationship Between Aflatoxin M1 In Human Breast Milk And Aflatoxin B1 In Maize from MCH mothers, Makindu Hospital

The results in table 4.10 indicated that there was significant dependence relationship between the levels of aflatoxin M1 in human breast milk from the lactating mothers attending MCH clinic Makindu hospital and the levels of aflatoxin B1 in maize samples from mothers households. Chi square test showed that it was statistically significant. \( \chi^2 = 80 \), df 5, \( P = 0.001 \). This concurs with past finding in other studies where maize aflatoxin levels have been associated with high levels of aflatoxin B1 in blood (Navas et al. 2005 and Mahdavi et al., 2010), hence high aflatoxin M1 levels in human breast milk. This association could be further explained by the fact that aflatoxins in maize are metabolized to aflatoxin M1 and secreted through the breast milk. Maize is the stable food for most people in Makuwini District.

4.4.3 RESULTS ON FACTORS ASSOCIATED WITH AFLATOXIN LEVELS IN MAIZE AND MCH MOTHERS BREAST MILK IN MAKINDU

The results shown on table 4.10 above outlines factors associated with aflatoxins in human breast milk from mothers attending MCH Clinic Makindu hospital. These are maize fragmentation, moulds, insect infestation, and levels of aflatoxins in milk and maize.
4.10 MAKINDU DIVISION PHYSICAL APPEARANCE OF MAIZE

Figure 4.3 Maize Insect Infestation in Makindu

4.4.4 Insect Infestation of Maize in Makindu

Maize (13) samples collected from Makindu Division show that, 23.1% (3 cases) were not insect infested, 69.2% (9 cases) had mild insect infestation and 7.7% (1 case) had severe insect infestation (tables 4.10 and figure 4.3). A chi-square test to determine the relationship between insect infested maize and the level of aflatoxins in maize was statistically significant ($\chi^2 = 135$, df 5, P = 0.000). High percentage of the maize was insect infested and contaminated with aflatoxins.
Figure: 4.4 Mould physical appearance of maize in Makindu

4.4.5 Maize Moulds in Makindu

Out of 13 maize samples that were collected from Makindu Division, 23.1% (3 cases) were not moldy while 76.9% (10 cases) had moulds (figure 4.4 and table 4.10). Moulds enhance aflatoxin contamination in maize and may contribute to aflatoxins in human breast milk.
4.4.6 Maize Fragmentation in Makindu Division

Results in figure 4.5 and table 4.10 show that 53.8% of maize collected from Makindu Division were found to be normal, 7.7% had mild fragmentation, and 38.5% had moderate fragmentation. This factor of fragmentation of maize enhances aflatoxins contamination.
Table 4.11  KIBWEZI DIVISION PHYSICAL APPEARANCE OF MAIZE AND AFLATOXINS IN MILK AND MAIZE

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>MOULDY</th>
<th>INSECT DAMAGE</th>
<th>FRAGMENTATION</th>
<th>MILK AFMI</th>
<th>MAIZE AFB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/21/06</td>
<td>Normal</td>
<td>-</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M/24/06</td>
<td>Normal</td>
<td>-</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M/26/06</td>
<td>Normal</td>
<td>-</td>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M/18/06</td>
<td>Normal</td>
<td>-</td>
<td></td>
<td>108</td>
<td>22</td>
</tr>
<tr>
<td>M/20/06</td>
<td>Mouldy</td>
<td>-</td>
<td>+</td>
<td>175</td>
<td>45</td>
</tr>
<tr>
<td>M/23/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>250</td>
<td>54</td>
</tr>
<tr>
<td>M/16/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>210</td>
<td>118</td>
</tr>
<tr>
<td>M/22/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>350</td>
<td>128</td>
</tr>
<tr>
<td>M/19/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>405</td>
<td>136</td>
</tr>
<tr>
<td>M/17/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>385</td>
<td>171</td>
</tr>
<tr>
<td>M/25/06</td>
<td>Normal</td>
<td>+</td>
<td>+++</td>
<td>310</td>
<td>172</td>
</tr>
<tr>
<td>M/14/06</td>
<td>Mouldy</td>
<td>+</td>
<td>+</td>
<td>450</td>
<td>220</td>
</tr>
<tr>
<td>M/15/06</td>
<td>Mouldy</td>
<td>++</td>
<td>+</td>
<td>486</td>
<td>250</td>
</tr>
</tbody>
</table>

**KEY**
- - represents no insect damage or fragmentation
- + represents mild insect damage or fragmentation
- ++ represents moderate insect damage or fragmentation
- +++ represents severe insect damage or fragmentation
KIBWEZI MAIZE INSECT INFESTATION

Results in figure 4.6 and table 4.11 shows that out of 14 maize samples collected from Kibwezi Division, 38.5% were normal, 53.8% had mild insect infestation, and 7.7% had moderate insect infestation. Maize infested by insects are prone to be mouldy, hence aflatoxin contamination. A significant relationship was found between the maize insect infestation and the levels of aflatoxins in maize. ($\chi^2 = 220$ df 1 $P = 0.002$). The results showed that insect infested maize had detectable levels of aflatoxins.
MOULD PHYSICAL APPEARANCE OF MAIZE IN KIBWEZI

Results in table 4.11 and figure 4.7 show that out of 13 maize samples collected from Kibwezi Division, 38.5% were not mouldy while 61.5% had moulds. The moulds contributed to the aflatoxins and hence a significant relationship between the aflatoxins levels and the mouldy maize. ($\chi^2 = 235.3$ df 1 $P = 0.001$). Moulds enhance aflatoxins contamination in maize.
KIBWEZI MAIZE FRAGMENTATION

Figure: 4.8 Fragmentation of maize samples from MCH mothers households in Kibwezi

4.4.9 FRAGMENTATION OF MAIZE IN KIBWEZI

Results in table 4.11 and figure 4.8 show, that 30.8% of maize collected from Kibwezi Division were found to be normal, 23.1% had mild fragmentation, 38.5% had moderate and 7.7% had severe fragmentation. Fragmented maize enhances mould growth, hence high levels of aflatoxins contamination.
Table: 4.12 Mutitu-Andei division physical appearance of maize and aflatoxin B1

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>PHYSICAL APPEARANCE</th>
<th>INSECT DAMAGE</th>
<th>FRAGMENTATION</th>
<th>AFLATOXIN IN MILK AND MAIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOULDY</td>
<td></td>
<td></td>
<td>AFB1</td>
</tr>
<tr>
<td>M/42/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>M/49/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>M/51/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>M/52/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>M/40/06</td>
<td>Normal</td>
<td>+</td>
<td>-</td>
<td>19</td>
</tr>
<tr>
<td>M/41/06</td>
<td>Normal</td>
<td>+</td>
<td>+</td>
<td>39</td>
</tr>
<tr>
<td>M/45/06</td>
<td>Mouldy</td>
<td>+</td>
<td>-</td>
<td>65</td>
</tr>
<tr>
<td>M/53/06</td>
<td>Mouldy</td>
<td>++</td>
<td>-</td>
<td>69</td>
</tr>
<tr>
<td>M/47/06</td>
<td>Discoloured</td>
<td>-</td>
<td>-</td>
<td>79</td>
</tr>
<tr>
<td>M/48/06</td>
<td>Mouldy</td>
<td>+</td>
<td>+</td>
<td>122</td>
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<tr>
<td>M/43/06</td>
<td>Mouldy</td>
<td>+</td>
<td>+</td>
<td>165</td>
</tr>
<tr>
<td>M/46/06</td>
<td>Normal</td>
<td>++</td>
<td>++</td>
<td>171</td>
</tr>
<tr>
<td>M/44/06</td>
<td>Mouldy</td>
<td>++</td>
<td>-</td>
<td>196</td>
</tr>
<tr>
<td>M/54/06</td>
<td>Mouldy</td>
<td>++</td>
<td>++</td>
<td>201</td>
</tr>
<tr>
<td>M/50/06</td>
<td>Mouldy</td>
<td>++</td>
<td>-</td>
<td>218</td>
</tr>
</tbody>
</table>

**KEY**
- - represents no insect damage or fragmentation
- + represents mild insect damage or fragmentation
- ++ represents moderate insect damage or fragmentation
Table: 4.13 Mutito-Andei Maize Insect Infestation

<table>
<thead>
<tr>
<th>INSECTS BITES IN MAIZE</th>
<th>CASES</th>
<th>PERCENTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>5</td>
<td>33.3</td>
</tr>
<tr>
<td>Mild (+)</td>
<td>5</td>
<td>33.3</td>
</tr>
<tr>
<td>Moderate (++)</td>
<td>5</td>
<td>33.3</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>100</td>
</tr>
</tbody>
</table>

4.4.10 Mutitu-Andei Maize Insect Infestation

Out of 15 maize samples that were collected from Mtito-Andei Division, 33.3% had no insect infestation, 33.3% had mild insect infestation and 33.3% had moderate insect infestation. (tables 4.13 and 4.12). There was no significant relationship between maize insect infestation and the levels of aflatoxins in maize. ($\chi^2 = 0.05$ df 2 $P = 0.87$)

Fragmentation Of Maize In Mutito-Andei Division

Figure 4. 9 Mutito-Andei Division Maize Fragmentation
4.4.11 Maize Fragmentation in Mutito-Andei Division

Results in table 4.12 and figure 4.9 show that 66.7% of maize collected from Mutito-Andei Division were found to be normal, 20.0% had mild fragmentation, and 13.5% had moderate fragmentation. A chi-square test to determine the relationship between maize fragmentation and aflatoxins in maize was not statistically significant. ($\chi^2 = 0.53$ df 2 $P=0.95$).

Mould physical appearance of maize in Mtitu-Andei

![Diagram showing the percentage of normal and mouldy maize in Mtito-Andei. 53% are normal, and 47% are mouldy.]

Figure: 4.10 Mould physical appearance of Maize in Mtitu-Andei
4.4.12 Maize Mould in Mtito-Andei

Out of 15 maize samples that were collected from Mtito-Andei Division, 47% were not mouldy while 53% had moulds. (table 4.23) A chi-square test to determine the relationship between maize fragmentation and aflatoxins in maize was not statistically significant. \( \chi^2 = 0.74 \) df 3 \( P = 0.83 \).

Table: 4.14 Matiliku Division physical appearance of maize and aflatoxin B1

<table>
<thead>
<tr>
<th>SAMPLE ID</th>
<th>MOULDY</th>
<th>INSECT DAMAGE</th>
<th>FRAGMENTATION</th>
<th>AFLATOXIN IN MILK AND MAIZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>M/1/06</td>
<td>Mouldy</td>
<td>+</td>
<td>+</td>
<td>AFB1 0</td>
</tr>
<tr>
<td>M/12/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>AFB1 0</td>
</tr>
<tr>
<td>M/10/06</td>
<td>Normal</td>
<td>+</td>
<td>-</td>
<td>AFB1 0</td>
</tr>
<tr>
<td>M/3/06</td>
<td>Normal</td>
<td>-</td>
<td>-</td>
<td>AFM1 28, AFB1 105</td>
</tr>
<tr>
<td>M/9/06</td>
<td>Mouldy</td>
<td>-</td>
<td>+</td>
<td>AFB1 29</td>
</tr>
<tr>
<td>M/8/06</td>
<td>Normal</td>
<td>+</td>
<td>-</td>
<td>AFB1 32, AFM1 152</td>
</tr>
<tr>
<td>M/11/06</td>
<td>Mouldy</td>
<td>-</td>
<td>+</td>
<td>AFB1 56, AFM1 250</td>
</tr>
<tr>
<td>M/5/06</td>
<td>Mouldy</td>
<td>-</td>
<td>+</td>
<td>AFB1 95, AFM1 80</td>
</tr>
<tr>
<td>M/4/06</td>
<td>Normal</td>
<td>-</td>
<td>++</td>
<td>AFB1 98</td>
</tr>
<tr>
<td>M/6/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>AFB1 118, AFM1 290</td>
</tr>
<tr>
<td>M/2/06</td>
<td>Mouldy</td>
<td>+</td>
<td>++</td>
<td>AFB1 136, AFM1 110</td>
</tr>
<tr>
<td>M/7/06</td>
<td>Mouldy</td>
<td>++</td>
<td>+</td>
<td>AFB1 172, AFM1 305</td>
</tr>
<tr>
<td>M/13/06</td>
<td>Mouldy</td>
<td>+</td>
<td>+</td>
<td>AFB1 271, AFM1 328</td>
</tr>
</tbody>
</table>

KEY
- - represents no insect damage or fragmentation
- + represents mild insect damage or fragmentation
- ++ represents moderate insect damage or fragmentation
### Table 4.15 Matiliku maize insect infestation

<table>
<thead>
<tr>
<th>INSECT INFESTATION</th>
<th>CASES</th>
<th>PERCENTAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>46.2</td>
</tr>
<tr>
<td>Mild (+)</td>
<td>5</td>
<td>38.5</td>
</tr>
<tr>
<td>Moderate (++)</td>
<td>2</td>
<td>15.4</td>
</tr>
<tr>
<td>Total</td>
<td>14</td>
<td>100</td>
</tr>
</tbody>
</table>

### 4.4.13 Matiliku Maize Insect Infestation

Out of 14 maize samples that were collected from Matiliku Division, 46.2% were not insect infested, 38.5 had mild insect infestation and 15.4% had moderate insect infestation. (table 4.14 and 4.15). There was no significant relationship between maize insect infestation and the levels of aflatoxins in maize from MCH mothers’ households who live in Matiliku. ($\chi^2 = 0.09$ df 2 $P = 0.65$)
Mould physical appearance of maize in Matiliku

Out of 14 maize samples that were collected from Matiliku Division, 38.5% were not mouldy and 61.5% had moulds. (table 4.14 and figure 4.11). There was no significant relationship between maize insect infestation and the levels of aflatoxins maize. ($\chi^2 = 0.09$ df 1 $P=0.91$)
Fragmentation of maize in Matiliku Division

Figure: 4.12 Fragmentation of maize in Matiliku Division

4.4.15 Fragmentation Of Maize In Matiliku Division
Results in figure 4.12 and table 4.14 show that, 30.8% of maize collected from Matiliku Division were found to be normal, 46.2% had mild fragmentation, and 23.1% had moderate fragmentation. There was no significant relationship between maize insect infestation and the levels of aflatoxins maize. ( $\chi^2 = 0.08$ df 2 $P = 0.79$)
4.5 LACTATING MOTHERS ATTENDING MCH-CLINIC AFLATOXICOSIS AWARENESS FACTOR

Table 4.16 Aflatoxicosis Preventive Measures Awareness

<table>
<thead>
<tr>
<th>Percentage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No Knowledge of Aflatoxicosis</td>
<td>6.4</td>
</tr>
<tr>
<td>No Preventive measures</td>
<td>23.1</td>
</tr>
<tr>
<td>Take Preventive measures</td>
<td>70.5</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Lactating mothers’ awareness by symptoms

Figure 4.13 LACTATING MOTHERS AFLATOXICOSIS AWARENESS.
4.5.1 LACTATING MOTHERS AFLATOXICOSIS AWARENESS.

The lactating mothers attending Makindu (MCH) Clinic who responded to the administered questionnaire were 78, while 36 declined answering the questionnaire but gave breast milk samples. Out 78 mothers 52 (65%) were aware of aflatoxin poisoning by answering yes or no method while 26 (35%) did not seem to know what aflatoxin poisoning was. 74 (95%) had knowledge on proper storage of cereals while 4(5%) did not have the knowledge of proper storage of cereals to avoid contamination of aflatoxin. 44 (56.4%) related aflatoxicosis with maize consumption and 34(43.6) related aflatocosis symptoms with witchcraft or herbs, while 41(52.6%) did not relate aflatoxin poisoning symptoms with aflatoxin contamination. Results in figure 4.13 shows that 56% related jaundice (yellowness of the mucous membranes) with aflatoxin poisoning, 21% with swollenness and 23% with other symptoms. On preventive measures as shown on table 4.16 (70%) were aware and 23.1% not aware while 6.4% had no knowledge on aflatoxicosis.

4.5.2 The Relationship Between Aflatoxin M1 in Human Breast Milk and The Level of Awareness Of The Lactating Mothers Attending Mch-Clinic

The results indicate that lactating mothers’ awareness level was 65% while 35% did not know about aflatoxicosis. However, the relationship between AFM1 in human breast milk and aflatoxicosis awareness was not significant. \( \chi^2 = 1.43 \) df 3 p=0.532. These findings support that there was no relationship between aflatoxicosis awareness and the levels of aflatoxin in human breast milk.
4.6 DISCUSSION

AFLATOXIN LEVELS IN MAIZE AND HUMAN BREAST MILK

The data reported here provide evidence for mother to child transmission of aflatoxin M1 through the breast milk. Therefore, there are several matters for concern about child exposure to aflatoxin. AFM1 is a detoxification product of aflatoxin B1 and has been found to be carcinogenic. In the developing countries like Kenya, infants and children are breastfed until 1-2 years of age. Thus, long term exposure to aflatoxin may contribute to long term chronic health problems. Infants and children in the developing countries have many other problems compromising health, such as food shortages due to poverty, malaria, diarrhea, HIV, Kwashiorkor and protein energy malnutrition that make children susceptible to Aflatoxin exposures. Makueni mothers' breast milk was found to be contaminated with aflatoxins.

The maize in Makueni samples had high levels of AFBI, fragmented, insect infested and mouldy. All the factors attributed to heavy contamination hence the high levels of the toxicant. The concern about environmental contamination by aflatoxins both in agriculture and the food industry has led many countries to investigate the magnitude of their own environmental pollution. Residues of this compound have been found at every level of food chains. Human beings are placed at the top of most food chains and it is not surprising that high levels of aflatoxins have been found in human breast milk and samples of maize collected from the households.

As more priorities now being given to this problem in the developing countries more data on aflatoxin residues in mothers breast milk and maize samples are being reported (Navaset et al. 2005). In addition, aflatoxin residue levels have been determined in utero, in infants, placenta, in the semen, and in cord blood of mothers (Navas et al., 2005, Mahdavi., 2010 and Faisal et al 2010). In different countries levels of aflatoxin in human breast milk were reported in Sudan, Zimbabwe and Ghana. In Ghana aflatoxin M1 were found in the range of 16-2075 ppt and aflatoxin B1 in maize and peanut were to range from 130-8218 ppb. In Bangkok aflatoxin M1 in human breast milk was found to range from
5-409 ppt and in Khon Kaen was found to range from 4-372 ppt. All these were high compared to the industrialized countries. Most of the aflatoxin levels in these countries were high compared to the maximum allowable levels in cows drinking milk of not more than 5 ppt in the developed countries (Polychronaki et al., 2006). The aims of the present study was to assess the aflatoxins contamination levels in the human breast milk.

4.7.0 Discussion on the Results of Aflatoxin in Human Breast Milk in Makindu Hospital

Only aflatoxins M1 were analyzed for (purposefully) in the human breast milk collected from the lactating mothers attending Maternal Health Clinic (MCH) in Makindu Hospital-Makueni District. Aflatoxin M1 was detected in 82% (92) milk samples of the mothers and 20% (22) were negative. The median concentration was found to be (230 ppt) of the aflatoxin M1 in the human breast milk samples. The mode of the aflatoxin M1 in the breast milk samples concentration were found to be 0 ≥ 5 ppt (22). The levels of aflatoxin ranging from 0-250 ppt were 35% (44) while the aflatoxin levels ranging from 251-400 were 47% (47) and 400-550 were found to be 9.5% (9) and more than 500 ppt were 2.4% (3). The mean aflatoxin M1 was 228.8 ppt and the standard deviation was 150.3 ppt.

4.8. Prevalence of Aflatoxin M1 in Human Breast Milk of Lactating Mothers Attending Mch Clinic Makindu

The prevalence of aflatoxin M1 detected from the lactating mothers attending the Maternal Child Health Clinic in Makindu Sub-District hospital was found to be 82%. This is comparable to other countries where aflatoxin M1 levels were carried out in Egypt, Bangkok, Ethiopia and Sudan. Thailand and Sierra Leone AFM1 was found in 45% (5/11; range, 0.039-1.736 ng ml⁻¹) and 31% (35/113; range, 0.2-99 ng ml⁻¹), respectively, of the samples. Egypt and the United Arab Emirates also had high incidence and levels, 55% (66/120; range, 0.02-2.09 ng ml⁻¹) and 91% (127/140; range, 0.053-3.4 ng ml⁻¹), respectively. (Mahdavi et al., 2010)
4.9.2 Human Exposure to Aflatoxin

The nutritional and immunologic responses to aflatoxin reviewed above indicate the potential for aflatoxin to affect the immunity and nutritional status of chronically exposed persons. Adult humans are relatively tolerant to aflatoxin, but literature reviews that it’s evident that Aflatoxin affects early growth and some aspects of human immunity and nutrition. Thus, the important questions are how contaminated diets are in developing countries, how much of the ingested dose is significant to human health and nutrition, what are the thresholds for effects on human immunology and nutritional health. AfM1 is a detoxification product that is rapidly excreted, but it may have significant immunologic and nutritional consequences in nursing young (babies and infants).

4.9.3 Aflatoxin M1 concern

Aflatoxins occur worldwide. In Kenya, few cases of aflatoxin poisoning outbreaks were reported to the Ministry of health between 2004 and 2006. The low rate of aflatoxin poisoning outbreaks was thought to be due to lack of reporting and inadequate diagnostic facilities in various health centers to obtain the right diagnosis. Aflatoxin B1 is metabolized to aflatoxin M1 and secreted in the human breast milk. Metabolic activation of aflatoxin B1 to the 8, 9-epoxide leads to binding to glutathione, DNA, and serum albumin. In the DNA aflatoxin B1 forms a major adduct, aflatoxin-N7-guanine, following metabolic epoxidation. Aflatoxin M1, P1 and Q1 are also aflatoxin B1 metabolites but aflatoxin M1 is the major metabolite.

AfM1 is the Hydroxylated mammalian metabolite of AfB1. The significance of AF M1 derives from the observation that aflatoxin B1 contaminated maize results in the appearance of AF M1 in the milk of the lactating mothers, hence infants and children consume the carcinogen.

Since AFM1 is regarded as a carcinogen, though of less activity than Aflatoxin B1, its presence is a particular cause of concern because of the major role of milk in the diet of infants. The young are known to show increased susceptibility to carcinogenic and mutagenic agents.
CHAPTER 5.0: CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

This chapter highlights conclusions of the findings of this study which can be summarized as follows:

i) MCH mothers' breast milk was found to be contaminated with aflatoxins-levels of 228.3 ppt and a prevalence of 82%.

ii) The infant dietary intake of aflatoxin M1 through the mother’s breast milk exceeded the allowed daily intake (ADI) by several folds (mean 228.3 ppt) as compared to 5 ppt allowed levels in milk.

iii) Mothers household maize was contaminated with AFB1 (mean 166 ppb Makindu) compared to allowed levels of 10 ppb in human feed.

iv) There was a relationship between levels of aflatoxins in mothers household maize and aflatoxins in breast milk.

v) Mothers households maize were mouldy, fragmented and insect infested. Factors that contribute to aflatoxin contamination in maize.

vi) MCH clinic mothers’ awareness level was 65% while 35% did not know about aflatoxicosis.

vii) There was no relationship between AFM1 in human breast milk and aflatoxicosis awareness of the mothers attending MCH clinic Makindu.
5.2 RECOMMENDATIONS

In the light of the above conclusions, this study recommends that:

i) NGO's, Ministry of Agriculture, KMOH and other Researchers should quantify the human health impacts and the disease burden due to aflatoxin exposure.

ii) The government should compile an inventory, evaluate the efficacy, and disseminate results on intervention strategies on aflatoxicoses.

iii) The government should develop and augment the disease surveillance, food monitoring, laboratories, and public health response capacity of affected regions.

iv) There is need to develop a response protocol that can be used in the event of an outbreak of acute aflatoxicosis.

v) There is need to enhance control of aflatoxins contamination by national authorities and policy makers.

vi) NGO’s, Ministry of Agriculture, KMOH and other Researchers Create awareness on indiscriminate use of contaminated maize.

vii) The government should equip health facilities in aflatoxicosis stricken regions better to diagnose aflatoxin levels easily.

vii) There is need to incorporate good agricultural practices to prevent maize contamination by aflatoxins.
Further Research

i) The same study should be carried out in other districts to come up with more viable results.

ii) A comparative study to be carried out to investigate whether different climatical conditions affect levels of aflatoxins in human breast milk.
REFERENCES


Keskin, Yaşar; Başkaya, Ruhtan; Karsli, Seher; Yurdun, Türkan; Özyaral, Oğuz (2009). Detection of Aflatoxin M1 in Human Breast Milk and Raw Cow's Milk in Istanbul, Turkey *Journal of Food Protection*, Volume 72, Number 4, April, pp. 885-889(5)


MSF (1995), Medicine San Frontiers, France, Paris


APPENDIX 1

Rahab W. Munenge,
Kenyatta University,
School of Health Sciences,
Department of Public
Health, P.O. Box 43844,
Nairobi.

To the Respondent,

RE: QUESTIONNAIRE

I am a postgraduate student currently taking an MPH degree, at Kenyatta University, and am required to carry out a research as partial fulfillment of the course.

The topic of this research is "Assessment of Aflatoxin M1 levels in human breast milk" at Makindu District Hospital MCH Department. This study seeks to investigate the factors associated with aflatoxin M1 levels in human breast milk that may affect the baby/child in Makueni District, Kenya and to determine the ways of mitigating this problem. The information collected through this questionnaire will be treated as confidential and will only be used for the purpose of the study. I therefore appeal to you to answer the questions as accurately as possible.

Thank you.

Yours faithfully,

Rahab W. Munenge
APPENDIX II

RESPONDENT/INDIVIDUAL QUESTIONNAIRE

Name of the interviewer

Questionnaire No.

Time

Date

Name of the household

Household No.

Dwelling type

Section

Village

6. How many children do you have?

0 1 2 3 4 5 6 7 8 9 and more

7. What is your occupation?

1) Housewife

2) Business woman

3) Teacher

4) Others (specify)

How much do you earn per month?

1) No salary

2) Below 5,000

3) Others (specify)
APPENDIX III - QUESTIONNAIRES

Section A: Demographic information of the respondent/mother

When were you born?
(Age of respondent in years) .................................................................

2. What is the name of the estate or village where you live?
(Specify estate or area) .................................................................................

3. What is your Religion?
1) Catholic
2) Protestant
3) Islam
4) Pentecostal
5) Full gospel
6) Evangelical
7) Redeemed
8) Baptist

4. What is the highest level of education you attained?
1) None
2) Primary
3) Secondary
4) College
5) Others specify,

5. What is your marital status?
1) Single
2) Married
3) Others (specify).

6. How many children do you have?
None 1 2 3 4 5 6 7 8 9 and above.

7. What is your occupation?
1) Housewife
2) Business woman
3) Teacher
4) Others (specify)

8. How much do you earn per month?
1) No salary
2) Below 5,000/=
What is your family's estimated gross income per month?
1) Below 5,000
2) 5,000-9,999
3) 10,000-20,000
4) Above 20,000

1- HEALTH OF THE MOTHER

1. Do you suffer any chronic illness?
   1) Yes  2) No

   If yes which one
   1) hypertension
   2) diabetes
   3) asthma
   4) others (specify)

2. Have you ever heard of aflatoxin poisoning?
   1) Yes  2) No  3) I don't know

   If yes what causes aflatoxicosis?

   .................................................................

3. How does it manifest?
   .................................................................

2- HEALTH OF THE CHILD/BABY

1. Has your baby ever suffered from any of the diseases below:
   (1) Diarrhea
   (2) Vomiting
   (3) Pneumonia
   (4) Measles
   (5) Any other infections

2. How many times has your baby been hospitalized and why?.................
3- **ANTHROPOMETRIC MEASUREMENTS ON CHILD/BABY.**

1. What is the name of the baby or child? .................................................................
2. Date of birth ........................................... month .............................................. and year ..................................
3. Weight at birth ........................................................................................................
4. Weight after every visit .................. to-date ..........................................................
5. Height at birth ...........................................................................................................
6. Height after every visit .................. to-date ..........................................................
7. Baby delivered through ............... (1) normal (2) caesarian (3) forceps
   (4) home delivery (why) ......................... or hospital

4 **SOURCE OF FOOD AND STORAGE**

1. From the shamba
2. Donation
3. Relief food
4. Bought from the market/shops

5. How do you store your food?

1. Outside the house
2. In my sitting room on the floor
3. In a granary
4. Others (specify)

5 **24 HOURS RECALL DIET**

(1) Do you normally eat the following meals?

<table>
<thead>
<tr>
<th>Meal</th>
<th>(1) yes</th>
<th>(2) no</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supper</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snacks</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX IV

Discussion Guide:-

In-Depth Interview for Health Service Providers

Instructions: greet the provider warmly introduce yourself, and explain the purpose of the interview. Explain to the provider that the information she gives will be kept STRICTLY CONFIDENTIAL to protect her identity and will NOT be disclosed to anyone else. Ask her to provide as honest answers as possible. (For each answer provided, whether yes or no, ask why/why not).

1. Have you come across aflatoxin poisoning in the cause of your work?
   a) Yes  b) No

2. Have you ever come across aflatoxin toxin poisoning in babies/children?

3. Do we have several cases of underweight children attending the MCH department in Makindu hospital?
   a) Yes  b) No

4. If yes what do you suggest causes the nutrition related disorder in Makueni District?

5. Agree or disagree
   a) The morbidity burden of the children 0-23 months is high in Makindu hospital?
      1) Yes  2) No

6. Agree or disagree

The listed illnesses are most prevalent in Makindu hospital
   a) Gastro-intestinal tract infections
      1) Yes  2) No
   b) Acute respiratory infections
      1) Yes  2) No
c) Malaria
   1) Yes  2) No

d) Malnutrition
   1) Yes  2) No

e) Kwarshiokor
   1) Yes  2) No

f) Any other illnesses?

7. Agree or disagree

   a) The mortality rate is high for the children aged 0-23 months

      1) Yes  2) No

8. In your opinions what health condition/symptoms do you associate with a aflatoxin poisoning in children 0-23 months?

9. What do you suggest causes aflatoxins in children?
APPENDIX V

The global estimates for the prevalence of under weight, stunting and wasting in children in developing countries:

<table>
<thead>
<tr>
<th></th>
<th>% under weight</th>
<th>% stunted</th>
<th>% wasted</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Africa</td>
<td>27.4% (3.1 million of children)</td>
<td>38.6% (44.6 million of children)</td>
</tr>
<tr>
<td>2</td>
<td>Asia</td>
<td>42.0% (154.1 million)</td>
<td>47.1% (172.8 million)</td>
</tr>
<tr>
<td>3</td>
<td>Latin America</td>
<td>11.9% (6.5 million)</td>
<td>22.2% (12.1 million)</td>
</tr>
<tr>
<td>4</td>
<td>Oceania</td>
<td>29.1% (0.3 million)</td>
<td>41.9% (0.4 million)</td>
</tr>
<tr>
<td></td>
<td>All developing countries</td>
<td>35.8% (192.5 million)</td>
<td>42.7% (229.9 million)</td>
</tr>
</tbody>
</table>

NB. % Below - 2SD of WHO /NCHS reference value

Source: WHO Global database on child growth and malnutrition 1997

Geneva (WHO Programme of Nutrition).
APPENDIX VI: MAP OF MAKUENI DISTRICT

The locations that visit MCH Makindu hospital