Aflatoxins and fumonisins contamination of food and feeds in Makueni County

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SAFE FOOD SAFE DAIRY

• OBJECTIVES

• To establish baseline information on mycotoxin contaminants and their human exposure on the bench mark sites

• To expand awareness about potential health hazards related to above contaminants and their management
CHARACTERISTICS of MAKUENI

- Rainfall - Annual 800 - 1200 mm
- Temperatures - 24°-33° in the hot seasons and 18° to 24° during the cold seasons
- Area - 8008 Km²
- Poverty index – 64.1% cf 45.9% Kenyan Average
- Population - 884,527, Density: 110.4 people per Km 2, (Male – 49 %, Female – 51 %)
- Elevation - 1000 to 1600 meters
STRATEGY

• Transect from high to low
• Selected three sub locations
  – Mukuyuni – had incidence of aflatoxin outbreak
  – Unoa – no such outbreak – what good practices?
  – Kaunguni – had many outbreaks
STRATEGY

- Sample all sources of household food prone to aflatoxin and fumonisins exposure – maize, sorghum, millet, milk
- Sample feed, soil
- Estimate the exposure at household and especially in vulnerable groups- children and elderly – children urine, breast milk
- Explain why Makueni had many outbreaks
STRATEGY

• Select representative households
• Establish a sampling frame
  – Growing maize, millet, sorghum
  – Practicing dairy farming (cows, goats)
  – Have at least one child aged less than five years
STRATEGY

• 1412 qualifying households
• Mukuyuni – 124 households (10 villages)
• Unoa – 73 households (15 villages)
• Kaunguni – 96 households (25 villages)
SAMPLES COLLECTED

- Maize - 378 (322 farm and 56 market)
- Sorghum - 193 (184 farm and 9 market)
- Millet - 19 (17 farm and 2 market)
- Milk - 265 (210 cow and 55 goat)
- Urine - 377 (sampled twice -293 +
- Breast milk - 98
- Soil - 292
ANALYSIS DONE

- Maize
- Sorghum
- Millet

- Feed  \(\rightarrow\) Aflatoxin

- Urine
- Milk (cow, goat, breast)  \(\rightarrow\) Aflatoxin M1

- Aflatoxin and fumonisin
ANALYSIS

- Soil - isolation of *Aspergillus* and *Fusarium* species
- Anthropometric measurements – height, weight
- Questionnaire – knowledge, practices, attitudes
## MYCOTOXINS

<table>
<thead>
<tr>
<th>Distribution (Continent)</th>
<th>Toxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa and Asian Sub continent</td>
<td>Aflatoxin</td>
</tr>
<tr>
<td>Australia</td>
<td>Aflatoxin and Fumonisins</td>
</tr>
<tr>
<td>North America</td>
<td>Aflatoxin, Ochratoxin, Zearalenone (ZEN) and Deoxynivalenol (DON)</td>
</tr>
<tr>
<td>South America</td>
<td>Aflatoxin, Fumonisins, Ochratoxin ZEN, DON,</td>
</tr>
<tr>
<td>Eastern Europe</td>
<td>ZEN and DON</td>
</tr>
<tr>
<td>Western Europe</td>
<td>Ochratoxin, ZEN and DON</td>
</tr>
</tbody>
</table>
Mycotoxins

- Contamination of food and agricultural commodities by various types of toxigenic moulds (fungi) is a serious and a widely neglected problem.
- It has been estimated by FAO that worldwide approximately 25% of the crops get contaminated by moulds and are affected by mycotoxins (CAST 1989; Rice and Ross 1994), and the estimated loss extends to billions of dollars (2010 -2.3 million bags condemned equivalent to $86m)
Mycotoxins are ubiquitous and produced by several fungi, particularly by many species of *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps*, and *Alternaria* etc.

They are secondary metabolites from fungi with unclear functions.

Over 400 known mycotoxins have been identified today with a potential of 30,000 different metabolites.
## RESULTS - MILK

<table>
<thead>
<tr>
<th>County</th>
<th>Sub location</th>
<th>% Positive</th>
<th>Mean (ppt)± SD</th>
<th>Range ppt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Makueni</td>
<td>Mukuyuni (n=73)</td>
<td>84.9</td>
<td>26.1 ±37.2</td>
<td>0.002 to 193.3</td>
</tr>
<tr>
<td></td>
<td>Unoa (n=88)</td>
<td>89.8</td>
<td>16.6 ±3.4</td>
<td>0.005 to 273.8</td>
</tr>
<tr>
<td></td>
<td>Kaunguni (n=72)</td>
<td>91.7</td>
<td>12.8 ±1.0</td>
<td>0.84 to 35.8</td>
</tr>
</tbody>
</table>
RESULTS – MILK/URINE

- Cow milk 7.4% exceed 50ppt (Mukuyuni)
- Goat milk 90.9% positive, none exceeded 50ppt
- Human breast milk (n=98), 86.7% positive
- Urine aflatoxin M1 (n=377), 79.3% positive; range 0.302 – 10415.1 ppt, mean 910.6ppt SD±1772.3
# RESULTS – AFLATOXIN (MAIZE, SORGHUM & MILLET)

<table>
<thead>
<tr>
<th>Commodity</th>
<th>% positive</th>
<th>% ≥ 10 ppb</th>
<th>Mean ppb</th>
<th>Highest ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home (322)</td>
<td>80.4</td>
<td>29.5</td>
<td>26.4</td>
<td>279.2</td>
</tr>
<tr>
<td>Market (56)</td>
<td>91.1</td>
<td>49.0</td>
<td>37.4</td>
<td>288.7</td>
</tr>
<tr>
<td>Sorghum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home (184)</td>
<td>83.2</td>
<td>34.5</td>
<td>9.3</td>
<td>264.2</td>
</tr>
<tr>
<td>Market (9 )</td>
<td>100</td>
<td>33.3</td>
<td>9.5</td>
<td>33.4</td>
</tr>
<tr>
<td>Millet</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home (17)</td>
<td>82.4</td>
<td>17.6</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>Market (2)</td>
<td>100</td>
<td>50</td>
<td>7.9</td>
<td>14.4</td>
</tr>
<tr>
<td>Feed</td>
<td>100</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Commodity</td>
<td>Source</td>
<td>% positive</td>
<td>% ≥ 2ppm</td>
<td>Mean ± SD ppm</td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------------</td>
<td>------------</td>
<td>----------</td>
<td>---------------</td>
</tr>
<tr>
<td>Maize</td>
<td>Home (285)</td>
<td>91.9</td>
<td>29.9</td>
<td>1.31 ± 2.19</td>
</tr>
<tr>
<td></td>
<td>Market (49)</td>
<td>94.2</td>
<td>38.2</td>
<td>2.14 ± 3.05</td>
</tr>
<tr>
<td>Sorghum/Millet</td>
<td>Home (198)</td>
<td>97.1</td>
<td>60.8</td>
<td>2.19 ± 1.81</td>
</tr>
<tr>
<td></td>
<td>Market (11)</td>
<td>100</td>
<td>36.4</td>
<td>1.84 ± 1.90</td>
</tr>
<tr>
<td>Feed</td>
<td></td>
<td>100</td>
<td>100</td>
<td>3.1 ± 0.28</td>
</tr>
</tbody>
</table>
FUNGAL ISOLATION-MAIZE

![Bar chart showing fungal isolation frequencies.]

- **Aspergillus**: Highest frequency
- **Fusarium**: Moderate frequency
- **Penicillium**: Lower frequency
- **Trichoderma**: Lowest frequency
- **Others**: Very low frequency

The bar chart indicates the isolation frequency of different fungal species in maize.
# Distribution and characteristics of *Aspergillus Section Flavi* in Makueni

<table>
<thead>
<tr>
<th></th>
<th>Kaunguni</th>
<th>Unoa</th>
<th>Mukuyuni</th>
<th>Number producing sclerotia %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Isolates</strong></td>
<td>41</td>
<td>20</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td><strong>A. flavus</strong></td>
<td>95</td>
<td>94</td>
<td>67</td>
<td>(N=61 ) 71</td>
</tr>
<tr>
<td><strong>Strain</strong></td>
<td>S</td>
<td>S</td>
<td>S</td>
<td></td>
</tr>
<tr>
<td><strong>A. parasiticus</strong></td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>(N=4 ) 67</td>
</tr>
<tr>
<td><strong>A. tamarii</strong></td>
<td>0</td>
<td>0</td>
<td>26</td>
<td>0</td>
</tr>
</tbody>
</table>
Toxigenic versus Atoxigenic Aspergillus
• Among all known mycotoxins present in feed, aflatoxin (AFB1) has the most significant impact to the dairy industry.
• Because little of the AFB1 consumed is degraded by rumen and the resulting metabolite (aflatoxicol) is as toxic as AFB1,
• That ruminants have little protection against this toxin
Aflatoxins – Effects in Cattle

• AFB1 and AFM1 (metabolite) are found in feeds and milk, respectively. Dairy cattle will produce milk contaminated with AFM1 after consuming feeds contaminated with AFB1.

• The AFB1 is rapidly absorbed in the digestive tract and primarily metabolized by liver enzymes, converting it to AFM1, which is then excreted in milk and urine. AFM1 is less toxic than AFB1.
EFFECTS in CATTLE

- Clinical signs seen in cattle fed contaminated feeds - chronic exposure
- Target organ is the Liver
  - Reduced weight gain
  - Reduced feed conversion efficiency
  - Reduced milk production (33 % Masri et al 1969)
  - Decreased feed intake
  - Reduced fertility (2%, Gutherie 1979)
  - Increased susceptibility to diseases
EFFECTS - Poultry

- Poultry are the most susceptible to aflatoxin among the livestock with ducks being the most among poultry.

- Clinical signs include:
  - Reduced feed intake
  - Reduced egg production
  - Reduced hatchability of eggs
  - Poor egg shell quality
  - Leg problems – rickets
  - Carcass condemnation - bruising
EFFECTS - Swine

Feeding system in pigs can increased incidences of introduction of aflatoxins- wet feeding systems

Clinical signs in piggery include:-

- Reduced feed intake
- Reduced growth rate
- Lower sow productivity – 800ppb – fewer piglets born live /weaned
- Liver damage
- Vit E (mulberry heart disease) and A deficiency (incoordination and hind legs paralysis)
EFFECTS - Swine

- Depressed immune response – reduced phagocytosis by 36% - in vitro tests with pig cells given 100ng/ml of AFB1, reduced antibody and interleukin production (Liu et al 2002).
EFFECTS - Horses

- Live longer in farms than other livestock
- 300ppb cause death
- Loss of weight,
- Poor body condition
- Reduced fertility
- Liver necrosis
- Immune suppression.
Economic losses - Aflatoxin

Economic losses to a farmer will be due to:

- Reduced Production (milk, eggs, meat, traction)
- Poor fertility
- Increased somatic cell count in milk = poor quality
- Increased susceptibility to diseases
EFFECTS IN HUMAN

• Acute – death (stomach ache, diarrhea, swelling, jaundice)

• Chronic
  • adults (hepatocellular carcinomas)
  • Children – stunting (HAZ), malnourishment (WAZ, underweight), and wasting (WHZ).
METABOLISM OF AFLATOXINS
Aflatoxin in Urine and stunting, wasting and Underweight?

• Likelihood-
  – Underweight (OR 2.4)
  – Stunted (OR 6.9)
  – Wasted (OR 6.8)
FUMONISINS

• Fungi belonging to the genus *Fusarium* are associated with the production of fusariotoxins.

• There are 2 types of toxins produced by these fungi, namely, metabolites that have properties similar to the hormone estrogen such as ZEN (F-2 toxin) and other ones that are the nonestrogenic - trichothecenes.
FUMONISINS

- Produced by *Fusarium vercitiloides* and *proliferatum* especially on maize that has been previously infected during its preharvest stages.

- Has a molecular weight of 721.8 daltons

- 6 different types of fumonisins (FA1, FA2, FB1, FB2, FB3, and FB4) have been reported, wherein the “A” series is the amides and the “B” series possesses a free amine
Human health risks - Fumonisins

- Consumption of fumonisin-contaminated foods by humans has been correlated with increased incidence of esophageal cancer in various parts of South Africa, Central America, China.

- This toxin has also been reported to be immunosuppressive.
Human Health Risks - Fumonisins

• The IARC (International Agency for Research on Cancer) has classified fumonisins under group 2B carcinogens (possibly carcinogenic to humans).

• Suspected risk factors for esophageal and liver cancers, neural tube defects, and cardiovascular problems
• Fumonisins are structurally similar to sphingosine, a component of sphingolipids, which are in high concentrations in certain nerve tissues such as myelin
FUMONISINS - ANIMALS

• Fumonisins are poorly absorbed from the gut with 80% of the dose in ruminants, 82-94% in pigs lost through faeces.
• Kidney and Liver are the major organs where the residues have been detected.
• There is minimal carry over of FB1 in Milk and eggs
Fumonisins - Animals

• Chronic dietary exposure to FB1 (≥50 ppm) is carcinogenic to rodents: hepatocarcinogenic and nephrocarcinogenic in male rats

• In horses, I classical ELEM, is liquefactive necrosis of the white matter, primarily in the cerebrum, which is often evident grossly as cavitation or discoloration

• Hapatotoxicity, The liver is often small and firm, with an increased lobular pattern
FUMONISINS - ANIMALS

- Centrilobular necrosis and moderate to marked periportal fibrosis
- Cardiovascular abnormalities were present in horses with neurologic disease
- In pigs - A decline in feed consumption, respiratory distress and cyanosis and death due to pulmonary edema and hydrothorax
FUMONISINS - ANIMALS

• Acute liver injury is characterized by scattered hepatocellular apoptosis, necrosis and mitosis.
• Fumonisin-induced pulmonary edema appears to result from acute left-sided heart failure
• FB1 decreases cardiac contractility, mean systemic arterial pressure, heart rate and cardiac output, and increases mean pulmonary artery pressure and pulmonary artery wedge pressure
AWARENESS / CONTROL

• Policy workshops  2
• Village workshops 19

• Education level
  – Primary  27.8%, UWEZO education report.
  – GAP- land prep, planting, varieties, weeding, threshing, Drying , selecting, storage