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MAPPING AFLATOXIN RISK FROM MILK CONSUMPTION USING BIOPHYSICAL AND SOCIO-ECONOMIC DATA: A CASE STUDY OF KENYA

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ABSTRACT

This research reports a mapping of aflatoxin risk in the milk value chain in Kenya using a geographic information systems (GIS) approach. The objective was to spatially locate regions at risk by taking into account biophysical and socio-economic factors such as humidity and rainfall, dairy cattle density, maize production and travel time to urban centres. This was combined with historical data of aflatoxin outbreaks obtained from literature search and geo-referenced. Median values for the datasets were then used to define the thresholds. Criteria-based mapping using Boolean overlays without weighting was implemented in the ArcGIS v10.3 platform. Areas of convergence were overlaid with regions of historical outbreaks to come up with likely locations of aflatoxin risk and target sample surveys to these areas. Higher resolution maize production and consumption data would be desirable to ensure more accurate results. The process followed in this project ensures an evidence-based and replicable methodology that can be used in other regions and with different crops. Feed and milk samples collected in the different categories identified support that this approach can be used to guide sampling and regional studies. The research also discusses the strengths and limitations of the approach.

Keywords: mycotoxins, Kenya, GIS, risk maps, aflatoxins, East Africa, dairy consumption, dairy products

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INTRODUCTION

Aflatoxins are the most researched mycotoxins that contaminate agricultural products, especially associated with maize and groundnuts [1]. They occur mostly in the tropics and are produced by *Aspergillus* moulds when environmental conditions are favourable. Toxins occur under drought conditions, when plants are more vulnerable to colonization by *Aspergillus*, but are also associated with post-harvest storage conditions that allow high humidity [2]. Aflatoxins are regarded as an important food risk in many African countries. In 2004, in the worst known outbreak, 125 people out of 317 affected died in a region of eastern Kenya [3]. Chronic exposure to mycotoxins can lead to liver cancer, and risk is greatly increased among hepatitis B sufferers [4].

Livestock are also susceptible to acute and chronic mycotoxicosis and milk may be contaminated if animal feed or fodder is contaminated with mycotoxins. Rapid development of smallholder dairying in Kenya and new cattle feeding practices, such as higher levels of concentrates, create potential for new risks from mycotoxins. Kenya has more than two-thirds of the dairy cattle population in eastern and southern Africa, and the milk consumption is also the highest in the region [5]. The country has more than 600,000 smallholders, each with between one and three cows, who currently produce 80% of Kenya's milk, more than three quarters of which is sold through the informal sector [6]. There have been no comprehensive studies of the health risk posed by mycotoxins in the dairy value chain in Kenya, or the link between intensification and risk, and this lack of evidence hinders development of appropriate policy and risk management.

Cross-disciplinary approaches to disease epidemiology are becoming more important. Geographic information system (GIS)-based mapping can help predict areas at high risk for disease occurrence, which can help in targeting surveillance and interventions to areas at highest risk. Risk mapping has been widely used in infectious disease epidemiology and there have been some applications to mapping the risk of aflatoxins. Monyo [7] carried out a study to determine the occurrence and distribution of aflatoxins in groundnuts and maize in Malawi. This study captured global positioning system (GPS) coordinates of all grain samples and combined these with long-term climatic data to produce a pre-harvest aflatoxin risk map. In another study involving aflatoxins, Jaime-Garcia and Cotty [8] used geostatistics to determine whether geographical location significantly influenced the extent to which cottonseed became contaminated with aflatoxins and to identify areas with the greatest contamination problems. Weather variables were used together with contamination data to determine relationships and kriging, an interpolation method that generates an estimated surface from a scattered set of points, was used to make estimations in un-sampled areas. Surface maps of aflatoxin contamination of cottonseed showed areas with recurrent high aflatoxin contamination while other areas showed low contamination. These changing spatial patterns of contamination were explained by differences in factors such as precipitation across seasons within the regions.

The studies presented above highlight the importance of incorporating GIS into epidemiological studies. Using time-based data helps extract information from spatial data; analytical outputs can also be displayed as layers. Data about disease incidence, including location, can be incorporated easily in a GIS for comprehensive analyses [9]. The results presented here were part of a multi-disciplinary study carried out to assess the risk of aflatoxins in the feed-dairy chain in Kenya to human health as well as economic costs. Risk of aflatoxins in the feed-dairy chain was mapped to provide an overview of potential risk "hot spots" and to guide more detailed fieldwork and sampling.

METHODS

Study area

The study covered the whole of Kenya, which is primarily a tropical country, though its climate varies from tropical along the coast to temperate inland to arid in the north and north-eastern parts of the country. Most parts of Kenya have two rainy seasons: the 'long rains', which occur from March/April to May/June and the 'short rains' which occur from October to November/ December. There are four main climatic zones, which can be further subdivided into agro-ecological zones based on temperature and crop suitability (water requirements of leading crops). The Central Highlands and the Rift Valley have fertile soils and an annual rainfall of up to 3000 mm. On average, daytime temperatures range from 21–26 degrees Centigrade. Western Kenya is hot and wet throughout the year with annual rainfall over 1000 mm and average daytime temperatures of 27–29 degrees Centigrade. Northern and eastern Kenya are hot and arid with annual rainfall of less than 510 mm and daytime temperatures of above 30 degrees Centigrade, sometimes soaring up to 39 degrees Centigrade in some desert areas [10].

Generation of data layers

Literature review was conducted to establish the environmental and socio-economic factors that were expected to influence aflatoxin occurrence and that could be spatially mapped. Experts were concurrently consulted and came up with a list of factors that were judged to potentially influence or predict risk of aflatoxin exposure via the dairy chain. The data were categorized into biophysical and socio-economic data. The biophysical data in the list of factors were humidity and temperature whilst the factors in the socio-economic category were dairy cattle density, farming systems and feed resources. Following an initial scoping and qualitative survey, two additional factors (milk and maize consumption) were added since aflatoxin poisoning can only occur after the produce is ingested. The datasets are described in the following section and their data sources are indicated in Table 1.

Generation of the dairy cattle density layer

To create the cattle density map, official cattle numbers were obtained from the Ministry of Agriculture, Livestock Development and Marketing in the form of field reports for the year 2000 by researchers at the International Livestock Research Institute (ILRI). The ILRI researchers then mapped the division-level reports on total cattle numbers as well as figures broken down by breed: grade (dairy animals and crosses) and zebu (beef cattle). To create an up-to-date map of corresponding division boundaries and division-level cattle data, researchers made use of the latest division-level boundaries digitized from District Development Plans. These cattle density figures were later validated using data from the Smallholder Dairy Project (SDP) from 1998 to 2000, which involved a survey of over 3000 households for characterization purposes. This cattle density layer was then classified using the median value into two quantiles representing low and high cattle density in ArcGIS v10.3 [11].

Generation of the farming systems layer

The farming systems map was derived from the global livestock productions map [12] and covered Africa, Asia and Latin America. It was based on land cover, human population density, length of growing period, temperature and elevation data layers. Ten systems were mapped for the developing world using a decision tree which began by distinguishing between landless and land-based livestock production systems using a threshold of 450 people per square kilometre. Similar steps were followed for the subsequent branches of the decision tree until the final classifications were derived [11]. Farming systems data for Kenya were then extracted from the global dataset using the ArcGIS Spatial Analyst extension v10.3 [11].

Generation of the dairy feed resources layer

Several studies have documented the presence of aflatoxin in dairy feeds in Kenya [13, 14]. For this particular study, only cereals in the form of bran, cake and stover were considered because of their relevance to the study. Herrero *et al.*[15], in their system-wide study, looked at six different cereals, namely wheat, maize, barley, rice, sorghum and millet. The formulae for estimating the feed were calculated by considering the following factors: *production of grain conversion factor indicating how much straw is produced compared to crop yield (derived from harvest indices) utilization factor—the fact that cereals are grown in a particular area does not mean that these are actually used as feed resources. Other competing uses are as soil amendments or as fuel for cooking, proportion of the grain that is turned into agro-industrial by-products (bran), proportion of the crop yield giving by-products, for example, oilcakes, dry matter content of fresh straw and energy value of the stover expressed in MJ/MT dry matter* [15].

Total cereal feed resources in the form of dry matter for Kenya were then extracted from the regional layer in ArcGIS Spatial Analyst v10.3 [11]. Classification was done using the median value of the data.

Generation of humidity layer

The CliMond climate dataset [16] consists of gridded historical climate data and some future climate scenario data at 10' or 30' spatial resolution. The underlying historical data were sourced from the Worldclim and the Climate Research Unit (CRU) (CL1.0 and CL2.0) datasets [16]. These data were reformatted, adjusted and recombined to generate all of the required data. The Worldclim dataset was drawn primarily on data from 1961 to 1990, though station records from 1950 to 2000 were used occasionally to fill gaps in records. However, the CRU datasets draw exclusively on data from 1961 to 1990 [16]. The humidity data for Kenya were then extracted from the global dataset using the ArcGIS Spatial Analyst extension v 10.3 [11].

Generation of the maize consumption layer

Maize consumption data were sourced from provincial reports for 2002 to 2006. The data were represented as total kilogrammes consumed per district per year [17]. The data were

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then classified into two classes using the median value of the data into two quantiles within the ArcGIS v 10.3 platform [11].

Generation of the milk consumption layer

Increased milk consumption will likely increase aflatoxin exposure by this route. A milk consumption study was carried out by the SDP in 2000 in Nairobi and Nakuru to assess household dairy consumption and demand patterns [18]. In that study, a questionnaire covering various aspects of consumption and expenditure was administered to 210 randomly selected households, and the data is available at the sub-location level, which is a lower administrative level than the maize consumption data.

Determination of locations of historical outbreaks

Temporal and spatial data of reported historical occurrences/outbreaks of aflatoxicosis were obtained in existing literature. Whereas a few of the reported cases were already geo-referenced in the reports, the vast majority had been reported at the level of administrative units. These were then converted into geo-referenced point locations with X and Y coordinates within ArcGIS v 10.3 [11], using centroids as the reference locations.

Mapping approach

Spatial data for each of the factors described above were collated and spatial resolution of all the datasets resampled to 1 km pixel size. For those criteria with discrete values, for example the farming systems map, knowledge of the specific farming system with higher risk for aflatoxin contamination was required. In the case of this study, the intensive farming system was treated as the most likely system for occurrence of aflatoxins. All the datasets were then transformed into binary maps (0, 1) where 0 = low and 1= high. For each criterion, the following reclassifying algorithm in ArcGIS was applied:

Reclassify (in_raster, reclass_field, remap, {missing_values}):

Where *in_raster* is the input criteria being transformed and *reclass_field* is the field denoting the values to be reclassified.

Each of the criteria were considered to have the same weight and therefore there was no scoring or ranking. 'Criteria-based' mapping using Boolean overlays without weighting was then implemented in the ArcGIS v 10.3 platform [11]. This method took the input layers and added them together in an additive un-weighted overlay model.

Sampling and aflatoxin testing

To test if our risk categories would be reflected in aflatoxin levels in milk and feed, samples were collected for analysis. Three districts were randomly selected from each of the high-risk/historical outbreak, low-risk/historical outbreak and high-risk/no outbreak categories, and from each of these, three villages [19]. On arrival at each village, three farmers were randomly requested for milk and feed samples, giving a final sample size of 81.

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If farmers gave oral and written consent to participate in the study and to provide samples, feeds and bulk milk were sampled. In addition, the farmer was interviewed using a simple structured questionnaire on the mode of feeding, storage (mode and time) and feeding system. A sample of about 300 g of concentrates or feed grains was collected from each household. Samples were collected from the top, middle and bottom of the bag containing feed concentrates or grains using a feed sampler, to get representative samples from each bag. A sample of about 20 ml of milk was collected in a 50 ml tube from each household. Milk samples were placed in a cool box for transport and later frozen whereas feed samples were kept at ambient temperature.

Toxins were extracted with 70% methanol from a ground sample. Feeds (concentrates and grains) were analysed using competitive enzyme-linked immunosorbent assay (ELISA) kits for aflatoxin B1 (Low matrix ELISA, Helica Biosystems Inc, Santa Ana, CA). The ELISA was performed according to the manufacturer's instructions [20]. Competitive ELISA for aflatoxin M1 (Low matrix ELISA, Helica Biosystems Inc, Santa Ana, CA) was performed following the instructions of the manufacturer.

Statistical analyses were done using STATA 13.0 (StataCorp LP, Texas, USA). The proportion of positive samples and the proportion of samples exceeding recommended levels of the World Health Organization and the Food and Agricultural Organization of the United Nations (5 ppb for feeds and 50 ppt for milk) [20–22] were compared between the different categories using chi squared test and the levels of aflatoxins in log (n+0.1) analysed for differences between the categories using t-test.

RESULTS

Determination of risk factors

The dairy cattle density map classification results are shown in Figure 1, where the areas with high dairy cattle density are shown in dark brown colour and are the areas hypothesized to carry a higher risk for aflatoxin in milk due to higher numbers of dairy cattle. The median value used for dairy cattle density was 610 animals per square kilometre. The data show that the areas with high cattle density largely cover the central and western Kenya highlands.

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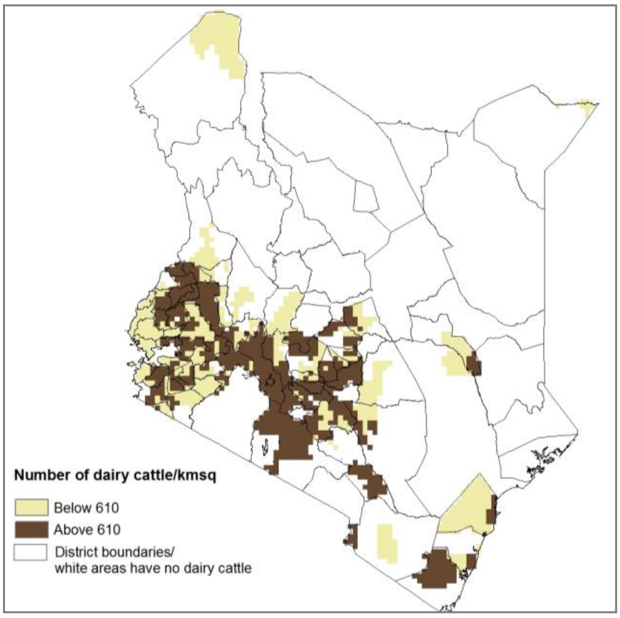

Figure 1: Map showing dairy cattle density in Kenya.
(Source: Ministry of Livestock and Agriculture Development [23])

The areas with intensive farming systems are shown in Figure 2 as the dark green areas. The rationale for including farming systems as one of the risk factors for aflatoxin contamination is that livestock in intensive systems may be at a higher risk of dietary exposure to aflatoxins than livestock kept in extensive systems because the former are more likely to be receiving nutrient-dense feed containing maize or groundnut products, which are more likely to be contaminated with aflatoxins [14]. In Kenya, large parts of the country are covered by pastoral or agro-pastoral systems, and intensive livestock systems are mainly in the central and western areas (Figure 2).

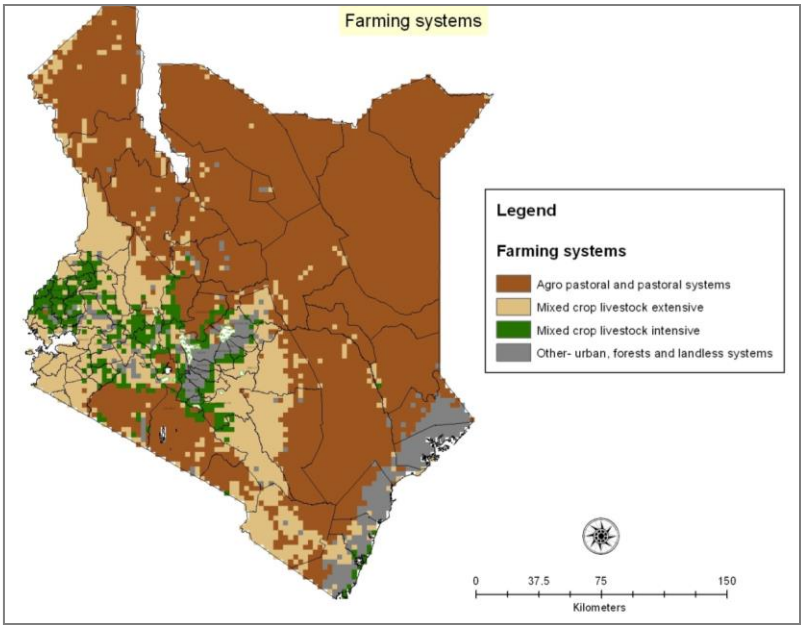

Figure 2: Map showing farming systems in Kenya
(Derived from Robinson *et al.* [12]**)**

The map with total cereal feed resources is shown in Figure 3. This map has been classified, using the median value of the data, into two quantiles showing areas with low and high total cereal feed resources. The median value for total annual feed was 0.1 MT per year hence the dark brown areas are the areas above the median. Areas where more cereal feed resources (bran, cake and stover) in dry matter form are given to animals were considered to be more at risk.

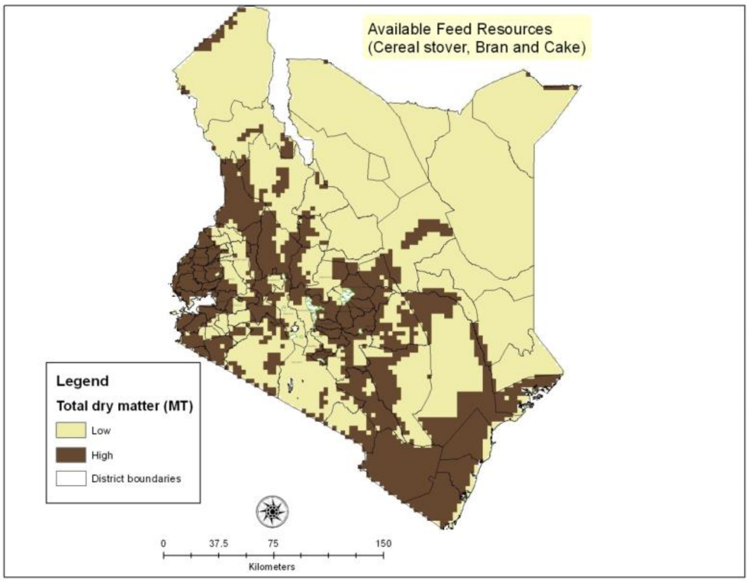

Figure 3: Map showing total cereal feed resources in Kenya (Source: Herrero *et al.* [15])

The map showing average relative humidity classified using the median is shown in Figure 4. The fungi producing aflatoxins grow better in conditions of warmer temperature and higher humidity [24, 25]. Milani [25] further states that whereas there are many factors involved in mycotoxin infectivity, such as biological factors, harvesting, storage and processing conditions, climate is the most important. In this study, relative humidity was used to represent areas of climate suitability for aflatoxins. Initially, temperature data had been included as a risk factor, but were later removed upon realising that when the same classification methods were used, the areas were completely overlaid with those of relative humidity. Therefore, there was no extra information gained from the temperature data and only the humidity layer was used in the final risk map. A median value for relative humidity was 0.34.

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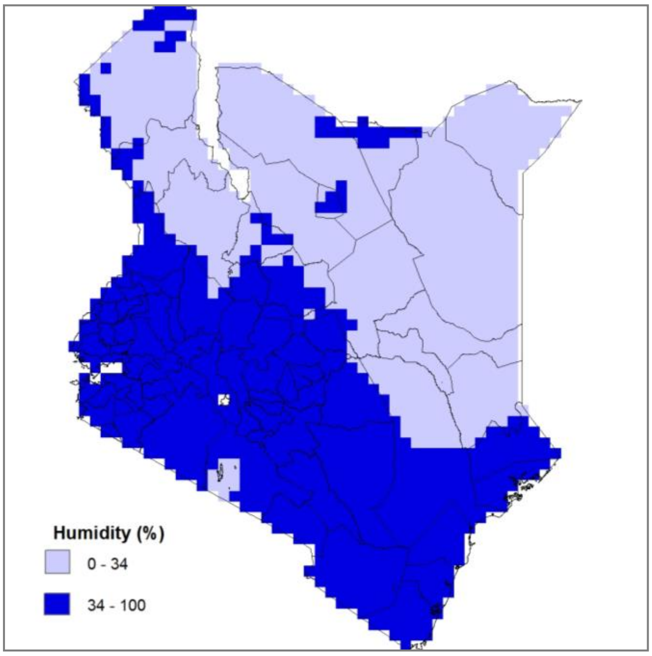

Figure 4: Map showing average relative humidity for Kenya (Kriticos *et al.* [16])

The map showing maize consumption, classified using the median value in kilogrammes per district per year, is shown in Figure 5. The median value was 38,869,209 kilogrammes per district per year and the areas that have high maize consumption are shown in a dark colour. Maize, groundnuts and cottonseed are the crops most prone to contamination and visibly spoiled maize is sometimes fed to livestock [26]. In regions where there is a higher rate of maize consumption, there is a subsequent higher risk that contaminated maize will enter the dairy chain.

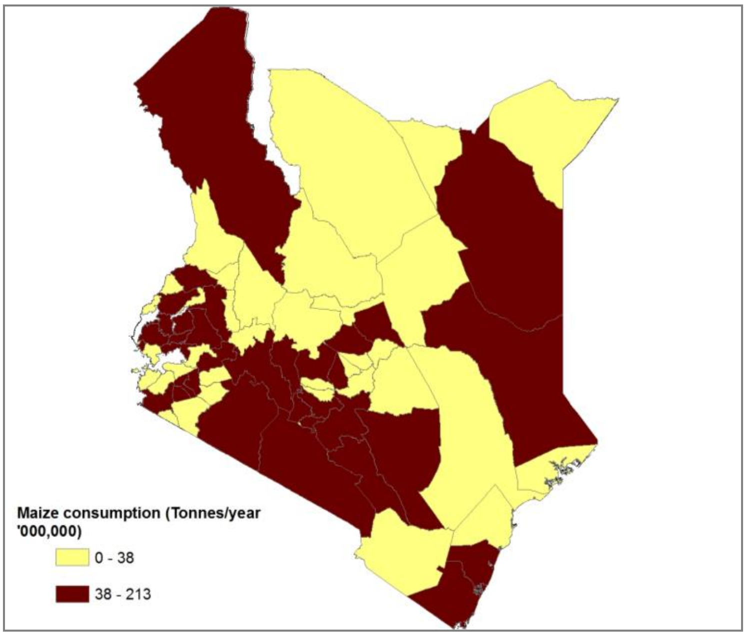

Figure 5: Map showing maize consumption in Kenya (Central Bureau of Statistics [17])

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The map showing milk consumption is shown in Figure 6. The median value of the data was 419,558 litres per square kilometre per year (the dark brown areas in the map are above the median).

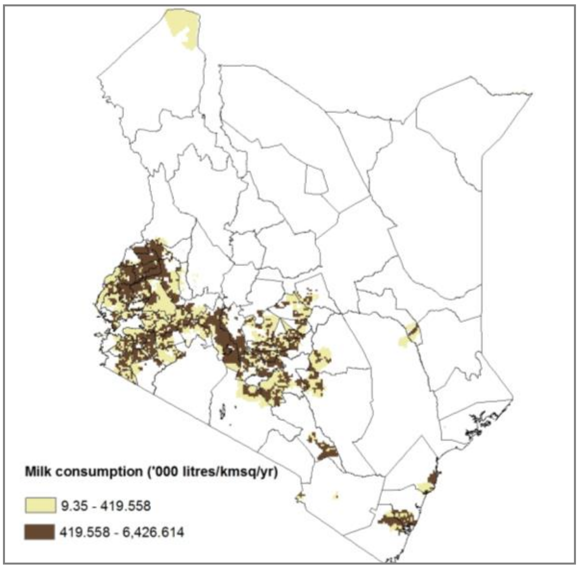

Figure 6: Map showing milk consumption in Kenya (Ouma *et al.* [18])

The map showing locations of historical occurrences of aflatoxicosis is shown in Figure 7. It was generated from past reports, which had information on aflatoxicosis outbreaks in the country. Most reports did not give explicit coordinates of the outbreaks, instead giving information at various administrative units. The mapping therefore was done at two levels: point and polygon levels.

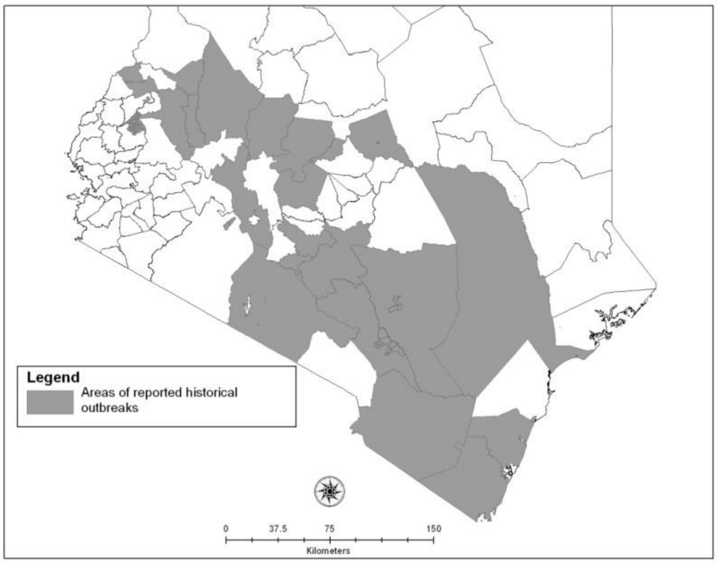

Figure 7: Map showing historical acute aflatoxicosis outbreak areas in Kenya (Source: various reports)

The risk map

The resulting risk map showed administrative areas that met the conditions that had been specified in the methods section, indicating areas that were likely to be at risk of aflatoxin exposure via the dairy chain. These administrative areas were represented at the third administrative or divisional level. The grey areas in the map shown in Figure 8 represent the areas of historical outbreaks, while the hatched red areas represent the 'at risk' areas that were the result of the overlay process. At least 35% of the 'at risk' divisions overlaid with the historical outbreaks districts were targeted for the survey for aflatoxin contamination (Table 2; Figure 8).

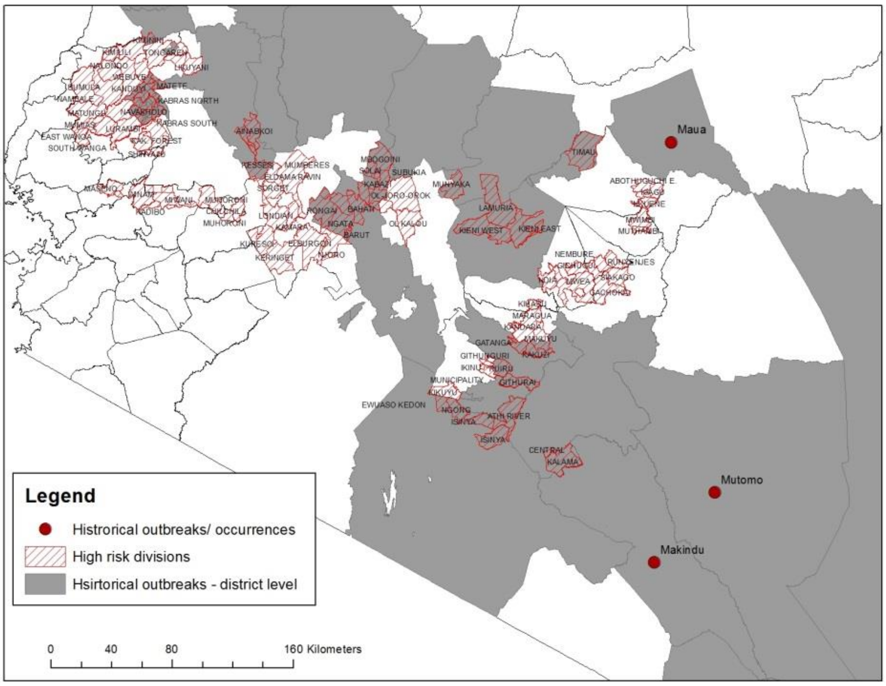

Figure 8: The risk map resulting from overlaying the risk factors

The average aflatoxin B1 level in animal feeds was 9.25 ppb and the average level of aflatoxin M1 in milk was 26.5 ppt (Table 3). There was a significantly higher mean of the logarithmic aflatoxin B1 values in the areas with historical outbreaks compared to those without outbreaks (mean log [aflatoxin B1+0.1] 1.78 and 0.33, respectively, $p<0.001$), but there was no difference in the mean log of aflatoxin M1 when comparing areas with and without outbreaks.

When comparing areas of high risk and areas of low risk, there was a significantly higher mean log (aflatoxin M1+0.1) in high-risk areas compared to low-risk areas (1.82 and 0.37, respectively, $p= 0.01$). There were also significant differences in levels of the feed (Table 3).

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DISCUSSION

This study demonstrates that GIS technology can be useful in integrating diverse datasets, environmental /biophysical and socio-economic, and deriving sensible conclusions about the areas that are most at risk for aflatoxin contamination. Generated maps can then be used to identify areas that should be targeted for surveys to assess the health risk and economic costs of aflatoxins in the dairy value chain, which was done in this study.

The limited number of samples analysed in this study showed higher levels of aflatoxin B1 in areas of historical outbreaks and higher levels of aflatoxin M1 in high-risk areas. There were differences in the proportions of samples exceeding the recommended levels, with higher proportions of samples in the high-risk categories. The prevalence of aflatoxin B1 in feeds and aflatoxin M1 in milk reported in this study is comparable to that of other studies done in Kenya [13, 27, 28]. Although the sample size was limited here, this indicates that a risk map done in this way can be useful as a way of directing sampling and designing studies.

The historical outbreaks of aflatoxicosis were associated with contaminated maize consumption, which could explain the association with higher aflatoxin levels in cattle feeds, which are mainly crops. The risk map focused on aflatoxin exposure through milk, which may be reflected in the higher levels of aflatoxins in milk in those areas.

This study shows a useful approach to identifying risk areas for further studies, but also identifies challenges with the approach and gaps in the knowledge, which will be good to address in the future. Particularly, problems in integrating GIS data from diverse sources have emerged. All the datasets used in the study came from different sources and that made their combination challenging. Some datasets had different spatial coverage since different data collection agencies use different systems of recording. Another major issue in this study was that of data resolution, whereby some datasets had very fine resolution whereas others had coarser resolution and hence less information could be gathered from them. Maize and milk consumption data had very coarse resolution and this affected the overall quality of the final output. The outbreak/occurrences dataset was also of very coarse resolution since most of the information did not have geo-referenced initial outbreaks but referred to broad administrative areas which made the whole process more generalized rather than specific. The issue of currency of data also emerged since datasets like the one on milk consumption were relatively old compared to the rest.

The major weakness of this approach is the binary division of the country into high and low risks based on the median. Medians may not always be the best approach for this since, depending on the distribution, they may not be representative and may not capture if there are other clusters. An alternative approach would be to use natural breaks, which can be identified in most GIS software. Natural breaks classes are based on natural groupings inherent in the data [11]. Class breaks identify the best group with similar values that maximize the differences between classes. The features are divided into classes whose boundaries are set where there are relatively large differences in the data

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values [11]. In addition, milk and maize consumption were mapped based on consumption per area, while risk might be more relevant on a per capita basis.

Although the mapping suggested areas of potential high risk for aflatoxins transmitted through milk, there was insufficient data on aflatoxin in milk to ground-truth these results historically. Some of the assumptions used in selecting layers may not be justified. For example, we assumed more intensive farms were at higher risk yet other work from the project [19] shows that farmers who do not use much concentrate may feed cattle mouldy maize residues which could potentially be more contaminated than concentrates.

CONCLUSION

Geographic information systems (GIS) risk mapping was successfully applied to identify geographic areas of potential increased risk in the feed-dairy chain in Kenya. In future studies, it would be desirable to invest more in the data collection to ensure better data compatibility, finer resolution and hence more accurate outputs. It is also desirable to compare estimated risk with actual risk from repeated, probabilistic surveys.

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Table 1: Sources of data

Dataset	Data source
Dairy cattle density	Ministry of Agriculture, Livestock Development and Marketing (2000). Modified after ground-truth study by the Smallholder Dairy Project in 2005
Farming systems	Sere and Steinfeld, Livestock Systems for Africa (2012)
Feed resources	CGIAR Systemwide Livestock Programme (2012)
Humidity	CliMond Version 1.2 (2012)
Maize consumption	Provincial annual reports (2002– 2006)
Milk consumption	Smallholder Dairy Project consumption study (2000)
Reported outbreaks	Various reports

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Table 2: Resulting divisions classified to be both at risk and had a history of outbreaks

Athi River	Ileho	Nalondo	Mwea
Bahati	Kabazi	Nambale	Ndivisi
Bumula	Kabras East	Navakholo	Ngata
Butere	Kabras South	Kikuyu	Ngong
Butula	Kabras West	Kimilili	Njoro
Chepseon	Kakuzi	Kuresoi	Ol-Joro-Orok
East Wanga	Kalama	Lamuria	Ol Kalou
Elburgon	Kamara	Londiani	Shaviringa
Eldama Ravine	Kampi Ya Moto	Lurambi	Shinyalu
Ewuaso Kedong	Kandara	Matete	Sorget
Gatanga	Kanduyi	Matungu	South Wanga
Gichugu	Kasarani	Mauche	Subukia
Githurai	Keringet	Molo	Thika West
Ikolomani North	Kieni West	Mumberes	Tongaren
Ikolomani South	Kihumbuini	Mumias	Ugunja
			Webuye

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Table 3: Levels of aflatoxin B1 (in ppb) in farmers' cattle feed and aflatoxin M1 (in ppt) in farmers' milk, from areas classified as high-risk or low-risk, and with previous or no previous outbreaks

	Mean	Range	Number positives	Number above 5 ppb aflatoxin B1, or 50 ppt aflatoxin M1
Aflatoxin B1 (n=63)	9.3	<0.02-112	58 (92%)	31 (49%)
High-risk areas (n=50)	8.6	<0.02-112	47 (94%)	21 (42%)*
Low-risk areas (n=13)	11.6	<0.02-27	11 (85%)	10 (77%)
Areas with historical outbreaks (n=39)	12.7	<0.02-112	37 (95%)	28 (72%)***
Areas with no historical outbreaks (n=24)	3.6	<0.02-24	21 (88%)	3 (13%)
Aflatoxin M1 (n=80)	26.5	<2-252	56 (70%)	19 (24%)
High-risk areas (n= 54)	33.7	<2-252	41 (76%)	17 (31%)*
Low-risk areas (n=26)	11.6	<2-78	15 (58%)	2 (8%)
Areas with historical outbreaks (n=53)	26.2	<2-105	40 (75%)	14 (26%)
Areas with no historical (n=27) outbreaks	27	<2-252	16 (59%)	5 (19%)

* p<0.05 compared to the category below *** p<0.001 compared to the category below

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