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SURVEY OF INFORMAL MILK RETAILERS IN NAIROBI, KENYA AND PREVALENCE OF AFLATOXIN M1 IN MARKETED MILK

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ABSTRACT

Aflatoxins are toxic by-products of fungi contaminating maize and other crops; they can be carried over into milk, meat and eggs when livestock eat aflatoxin-contaminated feed or fodder. People who consume such animal products are exposed to the toxins. To assess the aflatoxin contamination status in marketed raw milk and associated risk factors in peri-urban Nairobi, we conducted a census of raw milk retailers in Dagoretti Division, Nairobi, Kenya. Structured questionnaires were filled in by face-to-face interviews with all retailers who agreed to participate in this study. Small portions of milk were purchased from each respondent and tested for aflatoxin M1 (AFM1) using competitive enzyme-linked immunosorbent assay (cELISA) tests. Geographic coordinates of each eligible retailer were recorded. A total of 350 milk retailers were visited and 344 were plotted on a map. Of these, 250 retailers answered the questionnaire and a milk sample from each of 200 retailers underwent cELISA analysis for AFM1. Four types of businesses were found: kiosks (71%), dairy shops (21%), street or mobile vendors (3%) and grocery stands (1%); for 4% the business type was not identified. Milk was mainly sourced directly from dairy farms (59%) or from intermediate distributors (35%). Most retailers sold less than 20 litres per day of raw milk. The mean daily milk consumption of the milk retailers' households was 940 ml for adults and 729 ml for children. Although 58% of retailers had heard about aflatoxins and the majority of them agreed that aflatoxins could be present in milk, only 29% believed that "milk safety cannot be solely judged by sight or taste" and only 6% that "milk is not completely safe even after boiling". The cELISA test found the mean concentration of AFM1 was 128.7 parts per trillion (ppt) (median=49.9; 95% confidence interval=3.0–822.8) with a maximum of 1675 ppt. Overall, 55% of samples exceeded the European Union maximum level of 50 ppt and 6% exceeded the recommended maximum level of the United States Food and Drug Administration of 500 ppt. Compared to milk from street vendors, a significantly higher amount of AFM1 was detected in milk from kiosks and dairy shops, especially when the milk was sourced from farms without an intermediate distributor. Our findings indicate the need to better understand and manage aflatoxin in milk in Nairobi.

Key words: Aflatoxin M1, Kenya, milk, dairy value chain, milk retailers, Dagoretti, mycotoxin, informal milk marketing

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INTRODUCTION

Aflatoxins are toxic by-products of fungi, mainly *Aspergillus flavus* and *A. parasiticus*, which contaminate maize and other crops in areas with hot and humid climates. Aflatoxin contamination is a food safety risk and a potential barrier to trade; concern about aflatoxins has recently increased among national and regional policy communities. For example, aflatoxins were the main hazard in border rejection notifications in the European Union in 2012 [1, 2], leading to establishment of monitoring or alert systems on aflatoxin-sensitive foods in exporting countries [3]. Kenya has been a hotspot for aflatoxin-related deaths, and the most severe aflatoxicosis outbreak ever reported occurred here in 2004 [4].

Around 20 different aflatoxins have been described and among these, aflatoxin B1 is the most carcinogenic and commonly detected in food [5]. Animals are exposed to aflatoxin B1 through consumption of feed. Common feed ingredients such as maize, groundnuts and oilseeds are prone to contamination. In addition, mouldy crops considered unfit for human consumption may be used for livestock feeds [6]. In both humans and animals, aflatoxin M1 (AFM1) is a major metabolite of aflatoxin B1 and it is transferred into milk. It is heat stable and persists in final dairy products for human consumption after processing and heating [7].

In common with many other African countries, consumption of maize in Kenya is high, making up 36% of the daily caloric intake [8] and maize consumption attracts significant attention as an important route for aflatoxin exposure. Although the risk from milk is generally perceived as less important than maize [9], milk consumption in Kenya is also high. Some estimates are as much as 145 litres per person per year, which is almost five times as high as that in other African countries [10]. Almost all dairy product consumption in Kenya is in the form of liquid milk [11] and the predominant use in households is making tea followed by feeding children [12]. Even though the amount of aflatoxin carried over into milk from feed is much lower than the amount dairy cows consume in feed, aflatoxin intake from milk could be enough to have health impacts, especially in susceptible groups such as pregnant women and young children [5, 13].

Most of the milk sold in Kenya, as in other parts of East Africa, is distributed through the informal dairy sector [11, 14]. The lack of basic official data on marketing routes and

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related milk retailers and outlets makes it difficult to understand the hazards in milk (including aflatoxins) and creates challenges in implementing food safety surveillance and interventions. This study was conducted to bridge this knowledge gap by providing information on the informal milk distribution system and the prevalence and levels of AFM1 in unprocessed raw (neither boiled nor pasteurized) milk marketed in a densely populated low-income area in Nairobi.

Milk retailers might represent people with higher exposure to hazards in milk since the easy access may cause them to consume more. Aflatoxin contamination in milk occurs in dairy farms and the most effective method to control AFM1concentration in milk is by reducing aflatoxin B1 contamination of cattle feed through the application of Good Agricultural and Storage Practices [15]. Given that the problem is created on farm, and that consumers lack means to identify and reject AFM1-contaminated milk, milk retailers have not been considered important for aflatoxin management. However, it is possible that aflatoxin risk can be reduced through information from retailers to consumers [16] or farmers. Therefore, this study aimed additionally to assess milk consumption in milk retailers' households and their knowledge about aflatoxin in milk.

MATERIALS AND METHODS

Study design and participants

The study site was Dagoretti Division in West Nairobi. Dagoretti holds 10.5% (329, 577) of Nairobi's population and covers 5.6% (38.6 km^2) of Nairobi's surface area according to the 2009 national population census [17]. The majority of the residents live in low-income informal settlements [18].

All milk retailers selling unprocessed raw milk in the study site were targeted. Since the total number and location of the milk retailers was unknown, eligible respondents were identified by visiting all the visible retail outlets and vendors in the study site and ascertaining if they sold unprocessed raw milk, with the aim of producing as complete a census of milk retailers as possible. Interviews and sample collection were conducted between December 2013 and February 2014.

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Interviews

All identified milk retailers were informed of the purposes of the study and asked for their consent. Milk retailers who agreed to participate were administered a structured questionnaire by face-to-face interviews, which asked about attributes of the respondents, business practices, hygiene awareness and family members' milk consumption. Detailed information on their business practice was obtained by asking about the amount, price and source of milk for selling for the latest week. For these questions, the respondents were asked not to include pasteurized/packaged milk. Milk consumption data was collected for three specific household members: a man, a woman and a child. The 'man' and 'woman' had to be adults aged 18 years and above. A child of six months to three years of age was considered as "child" and in case there was no child under three years of age, the youngest child below 18 years in the family was considered. Knowledge of aflatoxin was assessed with seven questions shown in Table 1.

Samples

Milk was purchased from the eligible milk retailers who agreed to sell their milk for research purposes. The samples were carried in sterilized 50-ml plastic tubes in a cold storage box to the laboratory and stored at –20°C until analysed.

Aflatoxin assays

Aflatoxin M1 competitive enzyme-linked immunosorbent assay (cELISA) kit (Helica Biosystems Inc., Fullerton, CA, USA) was used for quantitative detection of AFM1 in the samples, following the manufacturer's instructions. The optical densities of the kit standards were used to create an equation to estimate the aflatoxin levels of the samples from the optical densities of the test.

Spatial data

The geographical locations (global positioning system coordinates) of all the eligible retail outlets were recorded by GARMIN eTrex (Garmin International, Inc., US). A distribution map was derived by marking the location of each visited retailer and used to visually analyse the spatial distribution of aflatoxin contamination.

Statistics

The continuous data from the questionnaires and AFM1 levels were analysed descriptively using appropriate statistics, including arithmetic mean, median, and 2.5th

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and 97.5th percentiles. The data for AFM1 level were strongly right-skewed and Box-Cox transformation was used to find the transformation parameter, lambda. According to the lambda, data were log-transformed and the median and the 95% confidence interval (CI) were calculated using the parameters solved by maximum likelihood estimation in fitdist function in statistical program R.

Associations between variables in the questionnaire and the AFM1 level in the milk samples were analysed using the Wilcoxon rank-sum test to validate the risk factors associated with AFM1 contamination in milk. The evaluated variables were shop type, source of retailed milk, amount of milk retailed, selling price of milk, number of customers, respondents' awareness of hygiene and position of the respondent (owner or employee). The Steel-Dwass test was used for multiple comparisons. A nominal significance level of 5% was used for all statistical tests. All analyses were performed using the statistical program R (http://www.r-project.org).

Ethical statement

Written or verbal informed consent was obtained from all study participants who also received a written copy of the informed consent form, including the aims of the study and contact information. The study design and tool underwent ethical review (Approval number 2013–09) by the International Livestock Research Institute ethical review board.

RESULTS

A total of 350 eligible milk retailers were visited; geographical coordinates were recorded for 344 milk retailers. Of these, 250 retailers completed the questionnaire. Milk samples from 200 retailers underwent cELISA test for AFM1 detection.

Distribution system of unprocessed raw milk

We classified the milk retail outlets in Dagoretti into four types: kiosks (70.9%) were small shops selling a variety of foods and hard goods; dairy shops (21.1%) sold exclusively dairy products; street or mobile vendors (2.9%) delivered milk to households; and grocery stands (1.4%) sold vegetables, fruits and (occasionally) milk in small huts. For 13 retailers (3.7%), the business type could not be classified into any of the four groups. In the kiosks and grocery stands, milk was stored at room temperature in transparent plastic jugs of approximately three-to-four-litre capacity and displayed in

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front of the shops so that customers could recognize it on sale from outside. The dairy shops, called "milk bars", kept their milk in refrigerated tanks. The mobile vendors transported and sold their milk outdoors in metal or plastic containers. Overall, 65.7% of the milk retailers bought the milk directly from dairy farms, not from milk processing plants or large co-operative milk outlets. The milk retailers reported selling an average of 41.9 litres of raw milk per day (median=15.0; 2.5th percentile=3.0; 97.5th percentile=150.0) at an average of 58.4 Kenya shillings per litre (median=60.0; 2.5th percentile=48.0; 97.5th percentile=75.0) (Table 2). Overall, 54.0% (135/215) of survey respondents were female. Women constituted 53.9% (96/178) of the milk retail outlet owners, 54.2% (26/48) of employees and 63.6% (7/11) of family members working in the retail outlet.

Aflatoxin M1 concentration in milk

The mean concentration of AFM1 in the study samples was 128.7 parts per trillion (ppt) (median=49.9; 95% CI=3.0–822.8) with maximum of 1674.9 ppt. Overall, 55.0% exceeded the European Union maximum level of 50 ppt and 6.0% exceeded the United States Food and Drug Administration maximum level of 500 ppt [19]. Compared to milk from street vendors, higher AFM1 was detected in milk from dairy shops (p=0.04) and kiosks (p=0.06) (Figure 1). All of the samples with more than 500 ppt of AFM1 were from kiosks and dairy shops. Milk samples of the retailers sourcing their milk directly from farms showed relatively higher (p=0.08) AFM1 level (mean =157.1 ppt, median=65.7, 95% CI=4.6–936.6) than those of the retailers sourcing their milk from intermediate distributors or traders (mean=75.1 ppt; median=35.9; 95% CI=2.3–553.6). The number of sources of milk for sale, the amount of milk being retailed, the number of customers, the respondents' knowledge of hygiene, and the position of respondents (owner or employee) were not associated with the AFM1 levels.

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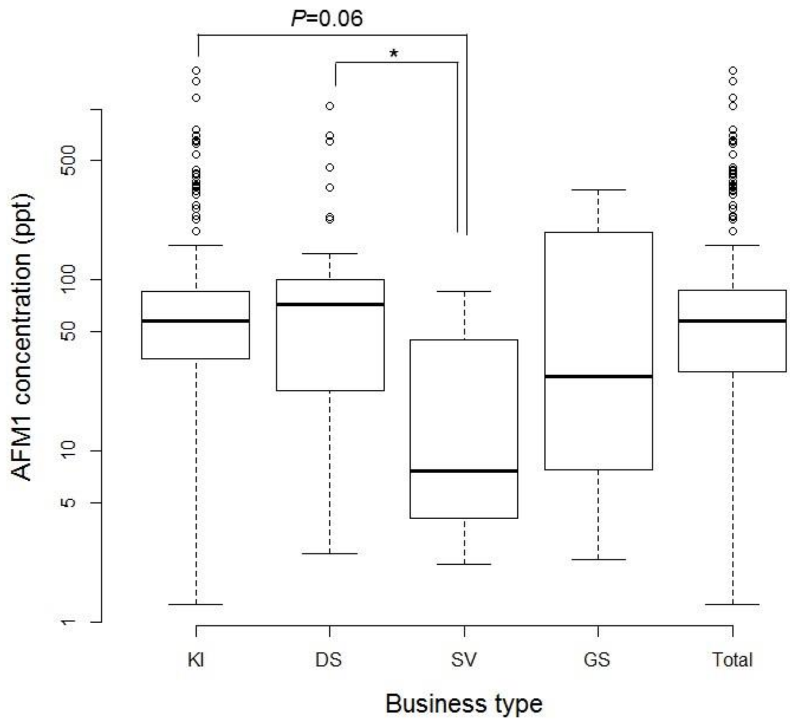

Figure 1: Aflatoxin M1 concentration in the milk samples, by business type. KI, kiosk (n=142); DS, dairy shop (n=36); SV, street vendors (n=8) and GS, grocery stand (n=4). ****p*<0.05**

Distribution of milk retailers

Milk retailers were found to be present over most of the study site, with no strong geographical concentrations. There were no visually evident geographical differences in AFM1 level (Figure 2).

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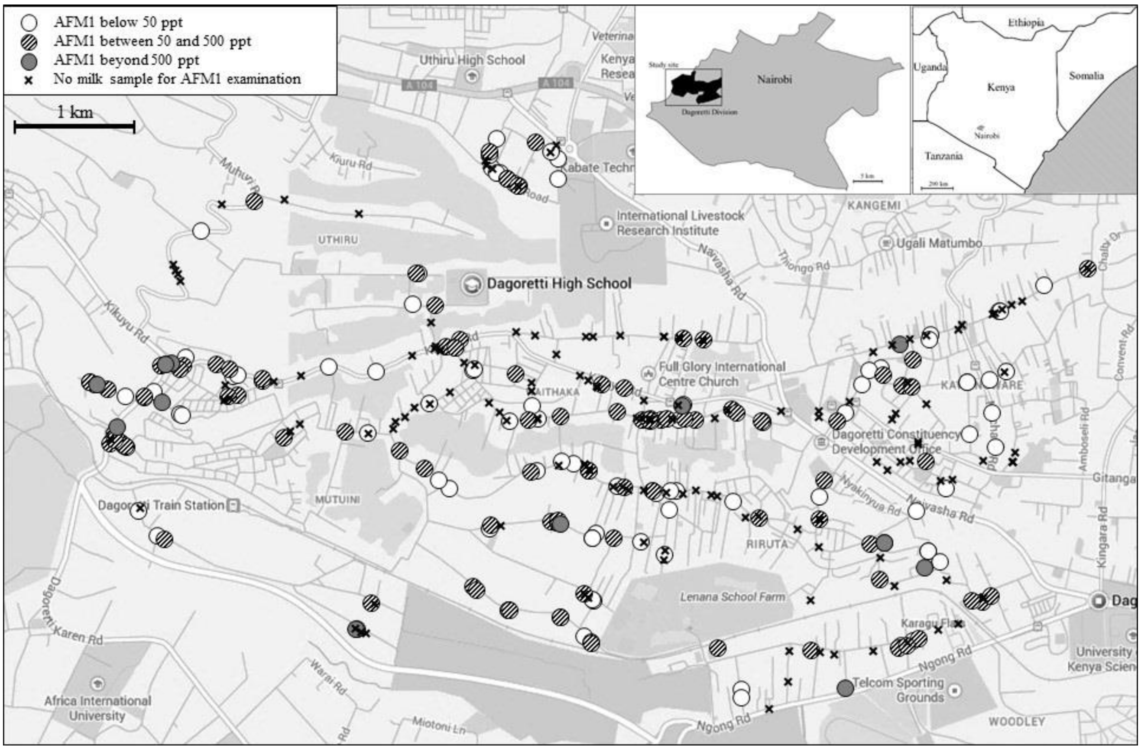

Figure 2: Map of the study site. Each mark represents the location of each milk retailer visited in this study. Aflatoxin levels are designated by the marks

Knowledge and milk consumption of milk retailers

Although 58% (145/250) of the survey respondents had heard about aflatoxins and the majority of them agreed that aflatoxin can be present in milk, only 29% believed that milk safety could not be solely judged by sight or taste and only 6% that milk is not completely safe even after boiling (Table 1). The respondents who agreed that diseases can be transferred via milk (70.0%, 175/250) correctly gave examples such as brucellosis (46.9%, 82/175), stomach ache and/or diarrhoea (6.3%, 11/175) and typhoid (2.9%, 5/175), but none mentioned aflatoxicosis.

The survey respondents were asked to report the daily milk consumption of members of their households. All the reported children were between six months and three years old, except a four-year-old and a six-year-old (1/155 each). The mean daily milk consumption was 900.9 ml (median=900.0; 2.5th percentile=0.0; 97.5th percentile=2500.0) among men (n=216), 978.8 ml (median=950.0; 2.5th percentile=0.0; 97.5th percentile=2500.0) among women (n=212) and 728.6 ml (median=600.0; 2.5th percentile=0.0; 97.5th percentile=1830.0) among children (n=155). The most popular forms of milk

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consumption reported for adults were milk in tea and hot milk (76.5% and 19.6%, respectively). For children, the most popular forms were hot milk, milk in tea and milk in porridge (43.1%, 40.4% and 11.5%, respectively).

DISCUSSION

This study was conducted to understand the aflatoxin contamination status in a peri-urban dairy value chain in Kenya. The mean AFM1 concentration in the collected samples was much higher than the maximum levels of both the European Union and Codex Alimentarius standards. The Kenya Bureau of Standards (KBS) has not set a regulatory limit for AFM1 in milk (implying that Codex Alimentarius standard applies) although KBS defines that cheeses shall not have more than 500 ppt AFM1 [14]. Previous reports have shown seasonality of AFM1 level in milk, attributed to the shortage of fresh green forage and more feeding with stored concentrates during the cold months [20, 21]. Because seasonal variation can be influenced by climate, level of development and farming systems, further studies are necessary to clarify its impact on AFM1 concentration in Kenya.

It is possible that kiosks could be focal points for monitoring because these are the main informal routes of raw milk consumed in this region, according to our data. Although this study's coverage rate on street and mobile vendors may have been insufficient because the field work was limited to the daytime, which is outside of the vendors' business hours (before daylight and after dark), those were not likely to be the main sources for the households in the area. According to results from other household interviews conducted in this region, 72% (148/205) of households reported buying milk from kiosks [22]. On the other hand, if we assume that most of the milk retailers in Dagoretti were identified and the average amount of milk sold by retailers in Dagoretti is 41.9 litres a day, then the 350 retailers identified in this division would be selling 5.4 million litres a year. This corresponds to an average consumption of 18.2 litres of milk per person per year in Dagoretti, which is considerably lower than the earlier estimated average of 145 litres per person per year [10]. This implies that only a minority of milk purchases were assessed in this study, or that this category of local low-income urban inhabitants is consuming far less milk than the national average. In addition, the average consumption previously assessed included pasteurized/packaged milk delivered through the formal sector, whereas our study only focused on informal sector milk. The season might have

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influenced the milk sale because the longest dry spell of the year was observed during the study period [23]. Kenya has an erratic supply of cattle feed caused by erratic rainfall, and milk yield in the dry season considerably decreases [24].

A higher percentage of milk samples in this study showed higher aflatoxin levels than farm-level results reported in previous studies [25]. Milk samples from kiosks had more aflatoxin contamination than those from street vendors. If this finding is repeated, it may reflect systematic differences in milk sources or treatments made to milk. For example, addition of water would dilute the aflatoxin levels, whereas addition of contaminated milk powder could increase the levels. Previous studies in Kenya have shown that up to 22% of milk samples were adulterated with added water and sometimes solids such as flour were also added [26]. If adulteration were occurring in this area, it is more likely that the original levels of aflatoxins were diluted and thus the levels measured here are underestimates, and this may need further studies to clarify.

Milk sourced directly from individual farms tended to have higher levels of AFM1. One possible explanation is that sourcing from individual farms appears to be a likely event if farms have higher production, and higher production may be associated with greater use of concentrates, which are more likely to be sources of aflatoxins [27].

This study also indicates that milk retailers may be a subset of the population particularly at risk. The milk consumption by retailers' households in this study was above 900 ml per person per day, which corresponds to more than 300 litres per year and is considerably higher than the reported national average of 145 litres per person per year [10]. Moreover, retailers' knowledge of aflatoxin in milk was not extensive. Based on the data on milk consumption of retailers' households and AFM1 concentration in the milk samples collected in this study, we can roughly estimate the daily exposure to AFM1 from milk to be 93.8 nanograms (ng) per day for children and 120.0 ng per day for adults. These estimates suggest that members of milk retailers' households have an AFM1 intake 1000 times higher than the estimate of the World Health Organization/Food and Agriculture Organization (FAO/WHO) of 0.1 ng per person per day from milk in the African diet [28]. Although no adequate epidemiological studies exist on the dose–response relationships between the intake of AFM1 and acute and/or chronic aflatoxicosis, our data suggest that this poses an important issue for public health,

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particularly for children in milk retailers' households due to their high consumption of milk and high susceptibility [5].

CONCLUSION

Unprocessed raw milk is informally marketed in the densely populated low-income area of Dagoretti in Nairobi. Kiosks are one of the main outlets for such milk in the area. Most milk is contaminated with AFM1 exceeding 50 ppt. The AFM1 concentration in the milk seems to differ by milk retailing route, which may reflect different sources or different treatments along the value chain. Family members of the milk retailers are considered an at-risk group since their milk consumption is high. Our findings indicate the importance of understanding processes which can influence aflatoxin concentration in milk along the value chain, and could orient governmental strategies to ensure supply of safe milk. Even though education of the general public has been impeded by limitations, such as funding and human resources, basic information about aflatoxin and its risk factors should be accumulated and provided.

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Table 1: Attitudes towards milk safety among milk retailers in Dagoretti, Nairobi

Questions	Answer choices	Number	Percent
Drinking milk is good for your health (n=249)	Agree	237	95.2
	Disagree	4	1.6
	Don't know	8	3.2
Milk safety can be solely judged by sight and taste (n=248)*	Agree	170	68.5
	Disagree	71	28.6
	Don't know	7	2.8
Worry more about chemical in milk than germs (n=245)	Worry more about chemical	113	46.1
	Worry more about germs	38	15.5
	Worry about both	15	6.1
	Don't know	79	32.2
Milk is completely safe after boiling (n=244)*	Agree	228	93.4
	Disagree	14	5.7
	Don't know	2	0.8
Diseases can be transferred via milk (n=250)*	Agree	176	70.4
	Disagree	39	15.6
	Don't know	35	14.0
Milk from cows fed mouldy feed is unsafe for human consumption (n=246)*	Agree	121	49.2
	Disagree	40	16.3
	Don't know	85	34.6
Meat from cows fed mouldy feed is unsafe for human consumption (n=248)*	Agree	121	48.8
	Disagree	38	15.3
	Don't know	89	35.9
Customers would pay more for milk if they knew that the shop's milk was safer (n=250)	Agree	100	40.0
	Disagree	99	39.6
	Disagree but customer may increase	3	1.2
	Don't know	48	19.2
Have you ever heard about aflatoxins? (n=250)*	Yes	145	58.0
	No	105	42.0
Aflatoxins can be present in milk (n=247)*	Agree	68	27.5
	Disagree	32	13.0
	Don't know	147	59.5

The total number of the respondents for each question is shown next to the question.

*Questions which were included for the scoring of the respondents' knowledge of aflatoxins.

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Table 2: Business structure of milk retailers in Dagoretti, Nairobi, Kenya

		Kiosk (n=184)	Dairy shop (n=44)	Street/mobile vendor (n=8)	Grocery stand (n= 4)	Unknown (n=10)	Total (n=250)
Respondents							
Sex	Man	87(47.3%)	17(38.6%)	4(50.0%)	3(75.0%)	4(40.0%)	115(46.0%)
	Woman	97(52.7%)	27(61.4%)	4(50.0%)	1(25.0%)	6(60.0%)	135(54.0%)
Position	Owner	132(75.9%)	28(68.3%)	6(75.0%)	3(75.0%)	9(90.0%)	178(71.2%)
	Employee	34(19.5%)	10(24.4%)	2(25.0%)	1(25.0%)	1(10.0%)	48(19.2%)
	Owner's family	8(4.6%)	3(7.3%)	0(0.0%)	0(0.0%)	0(0.0%)	11(4.4%)
	Unknown	10(5.4%)	3(7.3%)	0(0.0%)	0(0.0%)	0(0.0%)	13(5.2%)
Age				31.4 (18.2, 30.0, 52.4)*			
Products retailed							
Raw milk only		156(84.8%)	23(52.3%)	6(75.0%)	2(50.0%)	7(70.0%)	194(77.6%)
Boiled milk only		6(3.3%)	2(4.5%)	0(0.0%)	2(50.0%)	0(0.0%)	10(4.0%)
Sour milk only		2(1.1%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	2(0.8%)
Raw and boiled milk		0(0.0%)	1(2.3%)	0(0.0%)	0(0.0%)	0(0.0%)	1(0.4%)
Raw and sour milk		18(9.8%)	13(29.5%)	2(25.0%)	0(0.0%)	3(30.0%)	36(14.4%)
Boiled and sour milk		2(1.1%)	4(9.1%)	0(0.0%)	0(0.0%)	0(0.0%)	6(2.4%)
Raw, boiled and sour milk		0(0.0%)	1(2.3%)	0(0.0%)	0(0.0%)	0(0.0%)	1(0.4%)
Source of milk							
Farm		114 (62.0%)	23(52.3%)	75.0%	1(25.0%)	9(90.0%)	153(61.2%)
Dairy shop		0(0.0%)	2(4.5%)	0.0%	0(0.0%)	0(0.0%)	2(0.8%)
Distributor		65(35.3%)	17(38.6%)	25.0%	2(50.0%)	1(10.0%)	87(34.8%)
Farm and distributor		3(1.6%)	2(4.5%)	0.0%	1(25.0%)	0(0.0%)	6(2.4%)
Milk gathering station		2(1.1%)	0.0%	0.0%	0(0.0%)	0(0.0%)	2(0.8%)
Price of milk (Kenya shillings)		59.0* (50.0, 60.0, 75.0)	56.3* (48.2, 55.0, 70.0)	53.5* (41.4, 52.5, 71.5)	52.5* (45.4, 52.5, 59.6)	58.0* (55.0 60.0 60.0)	58.2* (45.4, 52.5, 59.6)
Retailing amount of milk (l/day)		37.8 (2.0, 15.0, 130.0) *	59.7 (5.0, 30.0, 200.0)*	54.3 (7.25, 30.0, 121.25)*	21.3 (10.0, 20.0, 34.6)*	23.0 (6.1, 17.5, 61.0)*	41.9 (3.0, 15.0, 150.0)*

*mean (2.5th, 50.0 th, 97.5 th percentiles)

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