Aflatoxin B1 and M1 contamination of animal feeds and milk from urban centers in Kenya

*Kang'ethe EK[1], Lang'a KA[2]

1. Department of Public Health, Pharmacology and Toxicology, University of Nairobi, Kenya
2. Division of Public Health, Ministry of Health, Nairobi, Kenya, Nairobi, Kenya

Abstract

Background: Aflatoxin M1 (AFM1) is the principal hydroxylated AFB1 metabolite present in milk of cows fed with a diet contaminated with AFB1and excreted within 12 hours of administration of contaminated feeds.

Objective: This study was initiated to assess the knowledge and practices of urban dairy farmers and feed millers about aflatoxin in feeds and milk, determine the prevalence and quantify the levels of AFB1 and AFM1 in animal feeds and milk respectively from urban environs in Kenya.

Methods: This work was carried out in the Department of Public Health Pharmacology and Toxicology, University of Nairobi, Kenya, between February 2006 and March 2007.

Results: A total of 830 animal feed and 613 milk samples from four urban centers were analyzed for aflatoxin B1 and M1 respectively using competitive enzyme immunoassay. Eighty six percent (353/412) of the feed samples from farmers were positive for aflatoxin B1 and 67% (235/353) of these exceeded the FAO/WHO level of 5µ gKg-1. Eighty one percent (197/243) of the feed samples from feed millers and 87% (153/175) from agrochemical shops were positive, while 58% (115/197) and 66% (92/153) of the positive samples exceeded the FAO/WHO limits respectively.

Seventy two percent (315/439) of the milk from dairy farmers, 84% (71/85) from large and medium scale farmers and 99% (88/89) of the pasteurized marketed milk were positive for aflatoxin M1, and 20%, 35% an 31% of positive milk from dairy farmers, medium and large scale farmers and market outlets respectively, exceeded the WHO/FAO levels of 0.05µ g/Kg-1. Sixty seven percent of the urban smallholder dairy farmers had no knowledge that milk could be contaminated with aflatoxin M1 and neither knew how they could mitigate against this exposure. Feed millers knew about aflatoxin B1 in grains and excretion of aflatoxin M1 in milk, but were not alleviating exposure to animals.

Conclusion: There is need to create awareness and establish routine monitoring of animal feeds and milk to reduce animal and consequently human response.

Key words: Aflatoxin B1, M1, animal feeds, milk, Kenya

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Introduction

Aflatoxins, are secondary metabolites produced by species of *Aspergilus*, specifically *Aspergilus flavus* and *parasiticus* fungi, which are naturally occurring contaminants of food[1] and elaborate the toxins under favourable conditions of temperature, relative humidity/moisture and poor storage conditions[2]. Aflatoxins are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agents in human hepatic and extrahepatic carcinogenesis[3,4]. Toxic effects of aflatoxin B1 (AFB1) in animals are varied due to differences in susceptibility[5]. The toxic dose for cattle has been shown to vary from 300-700ppb with effect on weight gain and dietary intake[6]; depress the immune status[7] and also cause growth impairment[8,9]. Urban dairy farmers in Kenya have been shown to spend nine times more money to purchase commercial feeds than their rural counterparts[10] and are at a higher risk of feeding AFB1contaminated animal feeds.

Aflatoxin M1 (AFM1) is the principal hydroxylated AFB1 metabolite present in milk of cows fed with a diet contaminated with AFB1[11,12] and excreted within 12 hours of administration of contaminated feeds[11]. AFM1 in milk has been shown to decline as contaminated feed is withdrawn, with no traces of aflatoxin in milk being detected after 3-4 days of withdrawal[11,12]. AFM1 has been shown to be hepatocarcinogenic at 50ppb in Fischer male rats

***Corresponding author:**
Erastus K. Kang'ethe
Department of Public Health, Pharmacology and Toxicology
University of Nairobi, Kenya
P. O. Box 29053-00625
Tel: +254 722 363 873
E mail: aekiambi@yahoo.com.

with the potency of 2-10% of that of AFB1 and also induces low incidences of intestinal adenocarcinomas[13,14]. AFM1 found in milk is usually about 3% of the dietary intake of AFB1[15].

Muriuki and Siboe[16] reported contamination of maize meal in Nairobi with AFB1 and B2 (0.4-20ugperkg). This showed a high population exposure to aflatoxins considering the consumption rate of 0.4kg/person /day. Lewis *et al*[17] reported contamination of maize during the aflatoxicosis outbreak in Eastern Kenya in 2004 with many samples exceeding 100ppb. Kadera et *al*[18], reported locational differences in Fumonisin contamination of maize in Western Kenya. Considering that young children are weaned on to cow's milk and grain based porridges and they are not immune competent at this early age, consumption of milk contaminated with AFM1 may further suppress their immunity and make them susceptible to other diseases. Gong *et al*[19] reported a correlation between stunting and aflatoxin exposure in Benin, West Africa, and while in Kenya, Okoth and Ohingo[20] reported a significant correlation between wasting and aflatoxin exposure in children under 3 years of age in Kisumu, Kenya. Kwashiorkor, a severe protein-energy–deficient disease in children has been linked to AFB1 exposure[21].

This study was initiated to assess the knowledge and practices of urban dairy farmers and feed millers about aflatoxin in feeds and milk, determine the prevalence and quantify the levels of AFB1 and AFM1 in animal feeds and milk respectively from urban environs in Kenya.

Methods

This work was carried out in the Department of Public Health Pharmacology and Toxicology, University of Nairobi, Kenya, between February 2006 and March 2007. Community appraisals and sampling were done between June and July 2006 and sample extraction and analysis was done in August and September 2006.

Selection of study sites and sample size calculation

Five urban centers (Nairobi, Machakos, Nyeri, Nakuru and Eldoret) were purposively selected for this study based on urban agriculture and livestock keeping activity. Machakos is situated south east of Nairobi and bordered by districts where there had been frequent outbreaks of human aflatoxicosis in 2004. Maize was incriminated as the source of the poisoning[17]. The municipality has dairy herd of improved and local breeds and large farms of beef animals. Nakuru and Eldoret are in the Kenya highlands, the grain basket of Kenya and have high livestock keeping activities[22]. Nyeri is to the North of Nairobi, in the central highlands with high dairy production activity but a grain deficit region with chances of animal feeds from other parts finding their way into this lucrative market. Nairobi forms the biggest market for dairy products because of its high population estimated at 3.7 million[23] and per capita consumption of 156 litres for 1996[24].

Livestock extension officers in each urban centre listed the urban dairy farmers in their areas. The list formed our sampling frame. Sample sizes from each study site were calculated using the method of[25] to ensure the samples contain sufficient dairy farmers' representatives. In- Nyeri 180; Machakos 140; Eldoret 150; Nakuru 160 dairy households were selected for sampling.

Participatory appraisals and household survey

One day participatory meetings were held with urban livestock keepers who were in the livestock extension list, feed manufacturers and agrochemical shop owners in each urban centre. They were informed of the goals of the survey and their willingness to participate was sought. The survey instrument took care of the bias in the knowledge about aflatoxins emanating from these pre-sampling meetings. Where the respondent answered in the affirmative that he/she had knowledge on aflatoxins in milk, he/she was requested to inform the source of such knowledge. This was taken into account when assessing the awareness level about aflatoxins. The questionnaire administration and sample collection were done within 5 days following the participatory meeting in each centre. The exception was in Nakuru where the household questionnaire was not administered because a similar study had just concluded a participatory urban appraisal targeting a number of health risks associated with urban livestock keeping, where aflatoxins in milk were discussed. Nakuru was therefore skipped in fear of biasing data on knowledge and practices related to aflatoxins.

Milk sample collection

Milk samples from urban smallholder dairy farming households were collected in sterile 15 millilitres tubes. Milk samples were also obtained from farmers bringing milk to milk processing plants or to large dairy cooperatives outlets. Only those who brought over 20 litres were sampled. This was aimed at capturing milk from medium and large scale dairy

farmers outside the urban areas although they were not the targeted group in this study.

Marketed milk was purchased from various supermarkets in the urban centers. Majority of the milk was either pasteurized whole or ultra heat treated or pasteurized whole skim or ultra heat treated skim milk. All the samples were transported in cool boxes to the laboratory, frozen until analyzed within 3 months of collection.

Sampling of animal feeds

Since majority of the farmers either buy one bag of animal feed (70Kg), or in small convenient amounts for a few days use, a sample of about 500g was taken from each bag/ household for analysis after mixing the contents in the bag.

From feed manufacturers' bags of animal feed were randomly selected depending on the layers of bags and height and a sample from each bag (100g) collected. A sample consisted of composite feeds from a minimum of twenty bags for each feed type. A questionnaire to capture knowledge of aflatoxins and practices was administered during sampling.

Agro-vets are private enterprises that specialize in stocking agricultural inputs, veterinary drugs and animal feeds. Five agro-vets were recruited from each urban centre from which livestock feeds were sampled, following the system used for feed manufacturers. However, few had large stores of feed. Majority kept few bags of each feed type depending on throughput.

Enzyme immunoassay for aflatoxin M1 (AFM1) in milk

Milk samples were thawed and centrifuged before they were analysed using an ELISA kit for M1 [Ridascreen® Aflatoxin M1] purchased from r-biopharm, Germany. Manufacturer's instructions were followed. Samples were run in duplicates. Where the sample had an OD reading above the reading of 40 parts per trillion (ppt) standard, the sample was diluted and re-tested. The kit had a sensitivity of 5ppt.

Analysis of Aflatoxin B1 (AFB1)

Sample preparation

The manufacturers recommendations for sample preparation were followed. Twenty grams of each sample after thoroughly mixing were ground and added into a screw cap bottle. One hundred millilitres of methanol/distilled water (70:30) were added and mixed for 10 minutes at room temperature. The extract was filtered through whatman® filter paper number 1. The filtrate was used in the analysis after diluting100µ l of the sample filtrate with 600µ l of the sample dilution buffer.

Enzyme immunoassay for total AFB1

Competitive enzyme immunoassay for AFB1 was done using Aflatoxin kit [Ridascreen® Aflatoxin] obtained from r-biopharm, Germany. The manufacturer's recommendations were followed except 20g of the sample and 100 mls of methanol/ water (70:30v/v) instead of two grammes of the sample and 10mls of methanol/ water were used to extract the AFB1. Any sample having more than 13.5ppb was diluted further and re-tested. This concentration was the sensitive upper limit of the standard curve. The kit had a sensitivity limit of 1.8ppb.

Data analysis

Data were entered in a Microsoft® Access Database. Tables and quantitative analyzes were produced using GenStat Eighth Edition ver 8.1, **R** ver. 2.4.

Results

Knowledge and Practices related to AFB1 and AFM1

Urban smallholder dairy farmers

The study targeted about 470 households, 140 in Machakos, 150 in Eldoret and 180 in Nyeri. Although some targeted households were not reached for one reason or another, a high proportion of the households 80% in Eldoret, 83% in Machakos and 81% in Nyeri were sampled. Table 1 shows the summary of knowledge and practices about aflatoxins among the respondents in the three urban centres.

Men were more knowledgeable than women on issues regarding aflatoxins in general (60% and 40% men and women) or in milk (70 and 30% men and women). The participatory meetings helped to inform 70.% (84/126) of the respondents about aflatoxin in milk, while, neighbours (7%, 9/126) and radio programmes (19.0%) were the other channels through which people got the information.

Most of the respondents 68% (259/383) did not know how animals get aflatoxins; that animals could pass aflatoxin to humans through milk and how to protect themselves from being exposed to aflatoxins through cow's milk. Of these, 49% were women and 51% men and this was significant ($p<$

0.05). Seventy eight percent of the respondents did not know how to protect themselves from exposure to aflatoxin MI.

Most of the urban smallholder dairy farmers (81%; 310/383) reported that they used commercial feeds to supplement their animals. Sixty eight percent (262/383) used dairy meal as the favored feed supplement (Table 1). A higher percentage of male (82%; 139/169) to female headed households (80%; 169/211) supplemented their animals with commercial feeds.

The methods of tending the animals varied across the households in the four centers as shown in table 1. Of the eight respondents who practiced tethering, 73% (6/8) were women.

Storage facilities at the farmer households were not ideal for keeping animal feeds. Of those who stored on a raised place, under humid conditions 86% (144/168) had samples positive for AFB1, while 76% (60/70) of the farmers who stored in a dry place, the samples were positive for AFB1. Of those farmers who stored on the floor, under dry humid conditions, 80% (44/55) and 60% (9/15) who stored under humid conditions, the samples were positive for AFB1.

Table 1: Diary households' knowledge and practices about aflatoxins

Attributes	Municipalities			Over all % (N=383)
	Eldoret %	Machakos %	Nyeri %	
Knowledge				
Heard of Aflatoxin (n=245	19.2	42.0	38.8	64.0
Know animals get aflatoxicosis (n=252)	39.8	29.8	31.3	65.6
Heard of aflatoxin in milk (n=126)	12.7	31.7	55.6	33
Practices				
Use dairy meal to supplement (n=273)	31.5	31.5	41.7	71
Zero graze (n=163)	19	39.3	41.7	42.6
Graze indoor at night (n=143)	49	17.3	33.6	37.3
Open field grazing (n=16)	25	56.3	18.8	4.2
Tethering (n=8)	25	25	20	2.1
Storage of feed				
Raised and dry (n=82)	22	12	64.4	21.4
Raised and humid (n= 25)	84	4	12	6.5
On floor and dry (n= 43)	30.2	30.2	39.5	11.2
On floor and humid (n= 26)	57.7	15.4	26.9	6.8

Aflatoxin B1 in animal feeds

Knowledge and practices of feed manufacturers

Twenty six feed manufacturers were interviewed and majority made feeds mostly for cattle (89%) and for chicken(85%). Sixty five percent of the manufacturers reported importing raw materials for feed manufacture. However, 54% purchased raw materials directly from farmers. Eighty percent carried out with 70% Aflatoxin B1 in feeds obtained from urban diary farmers, quality control tests, with 70.1% carrying out proximate analysis. Aflatoxin B1 testing was done by 50% of the manufacturers, but not on regular basis. Sixty nine percent of the manufacturers reported using mycotoxin adsorbents (Mycotoxin binders) in chicken and dog feeds. Of these, 39% came from Nakuru, 15% Nyeria and 8% from Nairobi.

Aflatoxin B1 in feeds obtained from urban dairy farmers

Four hundred and twelve feed samples from urban dairy farmers were analyzed and 85% [352/412] were positive for aflatoxin B1. Majority of the positive samples (26%) came from Eldoret as shown in table 1. Seventy percent (248/352) of the samples had aflatoxin levels that exceeded 5ppb the WHO/FAO[26,27] limit for feeds destined for dairy animals. Nyeri had the lowest (69%) testing positive while Machakos had the highest number of samples with aflatoxin B1 exceeding 5ppb. The average aflatoxin level for the feed samples with aflatoxin B1 exceeding 5ppb was 21.1±43.1ppb.

Table 2: Summary of mean Aflatoxin levels (ppb) of animal feeds from various sources by municipality

Municipality (n= samples)	% positive	% exceeding 5ppb	mean ppb ±SD	Range ppb
Urban Smallholder Dairy Farmers				
Nyeri (n=118)	68.6	49.2	13.6 ±10.0	4.0 to 63
Eldoret (n=108)	98.1	61.1	23.2±23.2	4.2 to178.2
Machakos (n=99)	94.9	73.3	27.7±74.9	3.6 to 595
Nakuru (n=87)	80.5	58.6	17.4 ±11.1	1.8 to 58
Feed Manufacturers				
Nyeri (n= 14)	100.0	42.9	6.4±4.9	1.9 to 15.8
Eldoret (n-18)	88.9	66.7	13.9±12.8	1.9 to 49
Machakos (n=1)	100.0	100	43.8±0.0	43.8
Nakuru (n=171)	77.8	43.3	26.0±44.5	0.9 to 280
Nairobi (n=390)	84.6	56.4	13.0±15.9	0.9 to 280
Agrochemical Shops				
Nyeri (n=19)	89.5	31.6	8.9±8.5	1.9 to 28.7
Eldoret (n=58)	93.1	72.4	17.0±34.6	1.8 to 238
Machakos (n=29)	79.3	43.3	17.6±19.6	2.0 to 64.4
Nakuru (n=69)	84.1	43.5	46.0±8.4	2.0 to 46 2

Key: pbb = parts per billion

AFB1 in feeds from feed manufacturers

Two hundred forty three feed samples were taken for analysis and 197 (81%) were positive. Majority of the feeds 17 and 12% were made up of dairy meals and maize related feeds (maize germ, maize gluten and maize bran etc) respectively. Sunflower and cotton seed cakes contributed 7.0% each of the positive samples. Fifty eight percent (115/197) of the positive feed samples had aflatoxin B1 levels exceeding FAO/WHO limits while the feed samples with the highest amount of aflatoxin were from Nakuru and Nairobi with 280ppb each. Average aflatoxin B1 levels for the feed samples with levels e" 5ppb was 21.4±37.5ppb.

AFB1 in feeds from Agrochemical shops

A total of 175 samples from 28 different manufacturers were sampled from agro-vets and analyzed. Eighty seven percent (153/175) were positive for total aflatoxin B1. Sixty percent (92/153) of the positive feed samples had aflatoxin B1 levels above FAO/WHO limits. The feed sample with the highest recorded amount of aflatoxin B1 (238ppb) was obtained from Eldoret. Eldoret had the highest feed samples testing positive (93.1% (54/58). Nyeri had the highest proportion of samples [68%] with aflatoxin B1 levels below 5 ppb. The average aflatoxin level for the samples exceeding 5ppb was 13.0± 24.0 ppb.

Aflatoxin M1 in milk

Urban smallholder farmers

A total of 439 milk samples were collected (107 from Eldoret, 99 from Machakos, 112 from Nakuru and 121 from Nyeri) and analyzed for AFM1. Of these, 72% (315/439) had traces of AFM1 (Table 3). Nakuru municipality contributed the highest number of positive samples (27% 85/315) while Eldoret and Nyeri had the lowest number, 23% each. Thirty five percent of the positive samples had aflatoxin levels exceeding 0.05µ g/Kg (50 ppt), the FAO/WHO and EU permissible level of AFM1 and M2 for milk (26, 27). Two percent of the positive samples with aflatoxin M1 levels exceeding 50ppt had Aflatoxin M1 levels above 500ppt the USFDA limits. The highest amount of aflatoxin detected in a sample was 680ppt from a sample obtained from Machakos.

Medium and large scale dairy farmers

Eighty five samples were collected and 83% (71/85) were positive as indicated in table 3. About thirty five percent (25/71) of the positive samples had aflatoxin levels that exceeded 0.05µ g/Kg. The sample with the highest level of aflatoxin had 0.56µ g/Kg and was obtained from Eldoret.

Table 3: Summary of Aflatoxin M1 levels in milk samples obtained various sources by municipality

Municipality (n=samples)	% positive	% 50ppt exceeding	mean ppt ±SD	Range ppt
Urban Smallholder Dairy Farmers				
Nyeri (n=120)	60.8	3.3	33.8±68.7	5 to 460
Eldoret (n=107)	68.2	10.3	39.9±39.7	5.4 to 228
Machakos (n=99)	82.8	24.2	99.7±168.9	5.1-780
Nakuru (n=110)	77.3	20.9	83.3±129.3	5.2 to 550
Medium and large scale farmers				
Nyeri (n= 25)	76	0.0	20.2±29.0	5.2 to 50
Eldoret (n-16)	68.8	12.5	115.6±202.7	5.5 to 560
Machakos (n=7)	100	50.0	52.2±34.7	10.9 to 102.5
Nakuru (n=27)	89.9	55.6	65.1± 36.7	5.3 to 165
Nairobi (n=10)	100	50.0	99.8±97.3	10 to 245
Marketed milk				
Nyeri (n=10)	100	30	129.3±198.8	16.5 to 600
Eldoret (n=18)	100	22.2	36.4±24.5	5.8 to 74
Machakos (n=18)	94.4	16.7	33.1±17.0	11 to 67
Nakuru (n=19)	100	36.8	36.1±22.9	8.0 to 71
Nairobi (n= 24)	100	41.7	64.9±76.4	7.9 to 300

ppt = parts per trillion

Marketed milk

Eighty nine milk samples were purchased from different supermarkets in Eldoret (18), Machakos (18), Nairobi (24), Nakuru (19) and Nyeri (10]) representing 14 different milk processors. The milk was either whole or skim pasteurized and ultra heat treated milk. Of 89 samples, 99% (87/89) were positive for AFM1. Of the positive samples, 27 (31%) had AFM1 exceeding 50ppt. The sample with the highest aflatoxin M1 level had 600ppt and was purchased from Nyeri.

Association between cattle rearing system, supplementation and aflatoxin in milk

Table 4 shows the relationship between cattle under zero grazing system, supplemented with commercial feeds or not and the likelihood of getting the samples positive for AFM1

Table 4: Association of supplementation, zero grazing and aflatoxin in milk

Grazing system		Aflatoxin		OR*
Zero grazing	**Supplementation**	**Positive**	**Negative**	
Yes	Yes	119	33	
Yes	No	4	6	5.4
No	Yes	77	41	
No	No	29	19	1.3
Yes		123	39	
No		104	60	1.8
	Yes	196	74	
	No	31	25	

* OR= Odds Ratio

Discussion

Knowledge about aflatoxin M1 in milk varied among men and women in the three urban centers. Machakos had the highest level of awareness about aflatoxin (42%) compared to the other urban centres (Nyeri 39%, Eldoret 19%). This was not surprising since Machakos and its neighboring districts have had three outbreaks of human aflatoxicosis prior to this study and Ministry of public health and sanitation had mounted health education campaigns in bid to control the epidemics. In urban dairy production, women in Kenya are engaged in more dairy production activities than men[28]. Women engage more in unpaid family labor while men engage in both paid labor and in cash sales of urban agriculture products[29]. It is imperative that women be empowered with the knowledge about the occurrence of aflatoxin B1 in animal feeds and aflatoxin M1 milk because they are responsible for family nutrition and are better placed to mitigate the risks posed by aflatoxin M1 in milk than their men counterparts.

The benefits accrued from supplementing animals in terms of more milk yields and consequently higher income returns were well appreciated across the gender with no significant difference between male and female headed households. Despite the above, women tended to choose less labor intensive methods of rearing cattle. The possible explanation why more women were engaged in tethering and open field grazing than men is that the two modes of tending are not labor intensive and therefore the systems allowed women more time for other household chores than would be the case in labor intensive systems of tending animals.

Contamination of animal feeds with AFB1 was prevalent throughout the farmer, producer and retailer chain in the four surveyed urban centers. The positive feed samples that exceeded the FAO/WHO limit were 70, 58 and 60% from urban farmer, manufacturer and market respectively. Animal feeds are manufactured from grains; the level of contamination reported in this study suggests that contaminated grains may have found their way into animal feeds. Lewis *et al*[17] reported that 35% of maize samples collected during the 2004 human aflatoxicosis outbreak in Kenya were contaminated with aflatoxin exceeding 100ppb and 7% above 1000ppb. Okoth and Ohingo[20] reported that 29% of children weaning flour in Kisumu, Kenya contained aflatoxin with levels ranging from 2-82 g/kg. Farmers in Tongaren and Kapsabet in Kenya indicated that the main uses of rotten maize were as livestock feed and in the preparation of local beer[30]. In Tanzania, AFB1 has been reported[31] in brewed beverages arising from use of contaminated grain or fruit during their preparation.

Most of the feed manufacturers (65%) reported importing raw materials for manufacturing animal feeds. A small proportion of the manufacturers (11%) sourced their raw materials from the National Cereals and Produce Board which would treat and test such grains for aflatoxin content. Only 50% of the manufacturers' reported rarely testing the raw materials for aflatoxin content citing the prohibitive costs of analysis and failure by Kenya Bureau of standards to remit results regularly. If control of aflatoxin laden raw materials is to be reduced, border entry points need to have well equipped laboratories to test for aflatoxins.

Levels of AFM1 contamination of fresh milk reported in this study are comparable to those reported elsewhere[32,33,34,35]. Studies in Brazil[36] in Greece[32] and in Colombia[37] have reported contamination of marketed milk with AFM1. However, in this study a higher proportion of samples exceeding the FAO/WHO limit of 0.05<g/Kg-1 is reported. Higher proportions have been reported in India[38] where 99% of the contaminated raw milk, milk based cereal weaning formula and infant formula exceeded the 0.05<g/Kg-1.

Conclusion

The levels of contaminated animal feeds and milk reported in this study with AFB1 and AFM1 should be a wake up call for stringent monitoring of raw materials and feed samples to prevent cattle exposure to aflatoxins contaminated feeds which would lead to excretion of AFM1 in milk and eventually causing human exposure through consumption of contaminated milk.

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