SDP Research Report

Assessing and Managing Milk-borne Health Risks for the Benefit of Consumers in Kenya

A. Omore, S. Arimi, E. Kangethe, J. McDermott, S. Staal
E. Ouma, J. Odhiambo, A. Mwangi, G. Aboge, E. Koroti and R. Koech

Smallholder Dairy (R&D) Project
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ilri-ma@AfricaOnline.co.ke

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TABLE OF CONTENTS

PREFACE

This research report presents an analysis of the public health hazards associated with milk marketing in Kenya. The study underlying the report was a response to concerns among some key players and stakeholders in the dairy industry regarding the public health risks from hazards that may be transmitted through milk. The concerns arose, in part, due a perceived increase in the sale of raw milk since market liberalisation in 1992. The DFID funded Smallholder Dairy (Research & Development) Project (SDP) that is jointly implemented by the Ministry of Agriculture and Rural Development (MoARD), Kenya Agricultural Research institute (KARI) and the International Livestock Research Institute sponsored the study. It was conducted in collaboration with the Department of Veterinary Public Health, Pharmacology & Toxicology, University of Nairobi and the Kenya Medical Research Institute.

A wide spectrum of key players and stakeholders including those in the public and private sector were consulted before and during the study, to determine the research questions and activities that were required to address the concerns expressed. Those who participated in the consultative process included the three institutions that implement SDP (MoARD, KARI, ILRI), the Kenya Dairy Board, the Kenya Dairy Processors Association, the Kenya Bureau of Standards, public health officials of the Ministry of Health and the MoARD's Department of Veterinary Services. Recommendations from the study were presented to the same stakeholders on 14th February 2000 (see Annex 2).

The report presents a summary of the main findings including recommendations (Executive Summary) and the Main Report that carries details of data collection methodology, brief description of laboratory methods, data analyses and the identification of critical control points (CCPs) for the improvement of milk quality and reduction of heath risks. Details of laboratory analyses and questionnaire are in Annex 1.

It is hoped that the findings and recommendations contained herein will significantly contribute to the creation of a more favourable milk market environment for all stakeholders.

EXECUTIVE SUMMARY

It is estimated that nearly 90% of marketed milk in Kenya is sold to consumers without passing through a pasteurization process (Omore *et al.,* 1999). Key players in this "informal" milk marketing system include many small-scale market traders (often referred to as "hawkers"). These traders generally each sell less than 120 litres of raw milk per day; these sales enable them to earn a daily income equal to approximately twice the national average. While these small-scale milk traders link dairy producers (mainly smallholders) to their consumers in a cost effective way, there is public concern, but without quantified information, that this "informal" milk marketing may pose public health risks. The research problems addressed were therefore a) lack of accurate information on milk-borne health risks, b) need for practical steps to optimize milk quality; and, c) need for a basis to begin to define the trade-offs that the dairy industry in Kenya should go for in terms of quality assurance on the one hand and cost and restrictions on traders on the other.

To address these problems, this document reports research carried out in 1999/2000 through the MoARD/KARI/ILRI Smallholder Dairy (R&D) Project which assessed the quality (bacterial counts and levels of butterfat and contaminants) of milk marketed raw, and which quantified potential zoonotic health hazards (*Br. abortus* and *E. coli* 0157:H7) and the levels of anti-microbials (antibiotics and antibacterials) present in milk marketed formally (i.e. after pasteurization) and "informally" (i.e. without pasteurization). Using the Hazard Analysis Critical Control Points (HACCP) principles as a tool and guideline, the health risks associated with each milk-borne hazard are estimated and, based upon the results, recommendations are given on how to minimize the identified risks.

The research was carried out with randomly selected households, market agents and retail outlets in Nakuru and Narok districts (representing areas of low human and cattle population densities with extensive dairy production systems) and in Nairobi and Kiambu districts (representing areas of high human and cattle population densities with intensive dairy production systems).

The structure of the dairy industry

The dairy industry in Kenya, which we define to include both formal and informal market pathways, is to a very large extent dependent on the annual marketed surplus from smallholder dairy producers, which in 1997 was an estimated 1,093 m. litres (Omore *et al,* 1999). Of this amount, only 133 m. litres (12%) passed through pasteurization and "formal" marketing by Kenya Cooperative Creameries (KCC) and some 45 private milk processors[1]. The remainder (an estimated 88%) was sold raw through: (i) direct sales to consumers, either individual or institutional, which accounted for 633 m. litres (58%); and (ii) co-operatives, self-help groups and small traders (milk bars, kiosks and mobile traders) who sold some 327 m. litres to consumers (30%). While these quantities and their proportions will vary somewhat year to year, it is widely accepted that most milk will continue to be marketed and consumed without having first been pasteurized.

The research being reported in this study found that small-scale market agents (mobile/itinerant traders, shops/kiosks and milk bars) on average sold 50-120 litres/day, traveled double the distance traveled by coop milk collectors (av. 26 km), and in a number of cases collected milk from intermediaries. Complementary surveys by SDP found that the small market agents incurred minimal variable costs, obtained high returns (20-25%) to their labour, paid farmers

[1] The 133 m. litres is equivalent to 70% of the estimated total of 190 m. litres from small and large scale farms that was processed in 1997 (44 m. litres by KCC and 146 m. litres by other private processors). KCC alone processed slightly over 200 m. litres in 1992/93 when milk marketing was liberalised, indicating that the total volume of processed milk has remained about the same over the period. Though consumption per capita of processed milk may have decreased slightly (due to increase in population), the common belief that a significant market share for pasteurised milk has been displaced by raw milk sales is not backed by these figures.

prices that were 7% - 65% higher than those paid by processors and charged consumers 20-50% less per litre for raw milk than the price consumers paid for pasteurized, packaged milk. In addition more than 10 times the number of jobs were created per unit of milk compared to those in the processing and marketing of pasteurized milk.

Consumer surveys by SDP have shown that shops and mobile traders without chilling facilities were the main purchase points for most consumers of raw milk in urban areas. In Nairobi, these purchase points served 63% and 32% of raw milk consumers, respectively; while in Nakuru town, shops and mobile traders comprised 52% and 31% of purchase points for raw milk, respectively. In rural Nakuru most consumers (82%) purchased their milk at the producer-gate. Retail prices for raw milk were highest in Nairobi (KSh 32/litre) and lowest in Nakuru rural (KSh 18/litre), and both were cheaper than pasteurized milk (KSh 40/litre). In addition, consumers' stated preference was for raw milk, mainly because of its lower cost.

This consumer preference for raw fresh milk was reflected even in Nairobi, where pasteurized milk is readily available; 29% of Nairobi households purchased on average 6 litres/hh/month of raw milk in comparison to 93% of households in both Nakuru urban (av. 23 l/hh/m) and rural (av. 24 l/hh/m). Pasteurized milk was purchased in Nairobi, Nakuru urban and Nakuru rural by 78%, 34% and 5% of sample households, respectively. As income class increased more pasteurized and raw milk was consumed, showing that besides price, taste preferences are important determinants of raw milk consumption.

In relation to public health risks from milk-borne pathogens, it is important to note that all sample households in urban areas and virtually all sampled households (96%) in Nakuru rural reported that milk (whether pasteurized or raw) was boiled prior to its consumption [2]. However, a small proportion of rural households (6%) consumed home-made naturally fermented milk.

Assessment of milk-borne health risks

Adulteration

Adulteration by addition of water to milk may introduce chemical and microbial health hazards as well as reducing the nutritional and processing quality, palatability, and market value of the milk. Overall, 4.7% and 10.4% of samples from consumer households and market agents, respectively, had specific gravity below 1.026kg/litre and therefore suspicious of adulteration by added water. The results showed that the number of samples to which water had been added varied widely by season and by area of sampling and that there was no obvious effect of the type of market agent. Milk market agents in Nakuru and Narok had more samples with added water (up to 27%) than Nairobi and Kiambu (4-15%). There were also indications that there was addition of solids particularly in Nakuru where up to 9% of samples in the wet season had a specific gravity >1.032. Adulteration as determined by the Solids-Not-Fat (SNF) index classified more than double the proportions above as adulterated, after correcting the specific gravity for the fat content of the milk, but there was still no obvious effect of the cadre of market agent.

The minimum butterfat content in whole milk as set by the Kenya Bureau of Standards (KEBS), is 3.25%. Urban households in Nairobi and Nakuru purchased more milk (33-53%) with butterfat content below this level than households in Nakuru rural (18%). The stated preference for raw milk in Nakuru rural may be linked to the more wholesome nature of the milk traded in rural areas, as well as to its ready availability. Most pasteurized and packaged milk has a lower butterfat, often standardized at 2-3%, than the milk that is marketed without processing.

[2] The common pasteurisation of milk is at 72 ^{o}C for 15 seconds. The pasteurisation curve gives the highest temperature required to kill all pathogens as 89^{o}C for one second. Boiling attains a higher temperature and duration thereby destroying all pathogens, though this compromises milk flavour (because serum proteins are denatured) and the nutritive value of the milk mainly due to loss of soluble vitamins (mainly B1 and C) (Kon 1975). Crucial vitamins that can only be obtained from animal food sources such as Vitamin A and B12 are not much affected by heat.

Milk bacteriological quality

Bacterial counts in milk reflect the temperature of the milk; time elapsed since milking and level of hygiene. Whereas total bacterial counts will mainly reflect time elapsed since milking and ambient temperature (if milk is not chilled), coliform counts are especially associated with level of hygiene since they are mainly of fecal origin. High bacterial counts in milk increases the risk of bacterial infection directly or through the toxins produced by the bacteria. In this study, the geometric means of total and coliform bacterial colony forming units (cfu) per ml in raw milk from farmer groups (mostly short market chains) were 8,000,000 and 15,000 respectively, 3-5 times lower than the mean of all samples and market chains. Average bacterial counts in milk from households in Nakuru rural were the lowest (1,300,000 total cfu/ml and 800 coliform cfu/ml), while the averages of samples from urban households were over 25 times higher than those from rural households. These results show that while the majority of milk from short market chains and rural households met the KEBS minimum standards for raw milk of 2,000,000 cfu/ml and 50,000 coliform cfu/ml, respectively, the samples from long market chains and from urban households did not.

In the same way, a high proportion of the samples of processed (pasteurized and packaged) milk did not meet their respective standards. In the processed milk samples from Nairobi and Nakuru, 82%-89% exceeded the KEBS defined threshold of 30,000 cfu/ml, while 59%-70% of samples exceeded the threshold of 10 coliform cfu/ml. Therefore, in this carefully designed study, it was shown that the majority of samples of milk, whether marketed raw or processed (pasteurized and packaged), did not attain their respective KEBS standards.

The study considered critical control points (CCPs) and the milk market pathways associated with significantly high bacterial counts. For total bacterial counts, these were the wet (compared to the dry) season and the market pathways from farms to shops/kiosks and milk bars. There was no significant difference in milk quality as measured by coliform counts between the various market pathways. The previous training of milk handling personnel on practices to ensure good milk quality was shown to have a positive effect on the level of hygiene. However, the lack of a cold chain was the overriding factor contributing to high levels of bacterial growth.

Zoonoses

The prevalences of *Br. abortus* and *E. coli* 0157:H7 (a newly recognized fecal coliform that causes bloody diarrhoea and kidney damage) were quantified. *Br. abortus* antibodies (as determined by ELISA and by Milk Ring Test (MRT)) were detected in 5% and 4%, respectively, of the samples from consumer households, and in 2% and 3%, respectively, of samples from market agents. Almost all positive samples at the market-level were from large bulk quantities of milk from dairy co-operatives and milk bars in Nakuru and Narok districts where extensively grazed cattle (and hence ease of infectious disease spread) predominate. Two consumer households (out of 420) in Nakuru reported having had a member diagnosed with brucellosis in the previous one year. The ELISA test classified nine (8%) of pasteurized milk samples as positive, six of which were from one milk processor in Nakuru. The higher prevalence of *Br. abortus* antibodies[3] in bulked milk reflects a potential higher health risk if such milk is not heat-treated before consumption.

Milk can get contaminated by *E. coli* 0157:H7 from cow or human fecal material resulting from unhygienic milk handling. The prevalence of the hazard was low as only two out of 264 samples from consumer households tested positive (one was from Nairobi and the other from Nakuru). This prevalence translates to a potential risk of exposure to the pathogen of about three times each year, for a daily consumer of non-heat treated milk.

[3] Antibodies are natural protective proteins produced by mammals following infection and are often used as surrogate measures for infection, in which case a positive test result equals potential disease risk. However this risk is eliminated after heat treatment even though an antibody test would still read positive.

Cultural, biochemical and molecular speciation of tubercle bacilli from TB patients did not result in isolation of *Mycobacterium bovis*. Though these findings vindicate the long held official position indicating absence of bovine tuberculosis in Kenya, they need to be verified and periodically monitored in other areas, given the risk posed by frequent movement of pastoral livestock across borders from neighbouring countries. The sampling strategy used implies that if no *M. bovis* is detected, one can be 95% confident that the maximum prevalence of bovine TB in the district is not greater than 2%. If *M. bovis* were present in Kenya, those at the greatest risk of acquiring bovine TB would be those pastoralists who consume raw milk and/or other raw animal products.

Anti-microbials

Antibiotics and other anti-bacterials (collectively called antimicrobials) residues in foods may contribute to the development of bacterial resistance. Five families of anti-microbials were screened: β-lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides. Their observed levels were compared to the maximum residue limits (MRLs) recommended by the European Union. Residues of these anti-microbials in milk most often originate from poor milk handling and hygiene at the farm-level. However, unconfirmed reports suggest that some unscrupulous milk market agents may add antibiotics, among other chemicals, to lengthen the shelf life of milk.

In this study, 9% and 6% of consumer- and market-level samples, respectively, were positive for one or more of the five families of anti-microbials, indicating that a consumer who takes milk daily is at risk of consuming milk with drug residues about twice every month. The HACCP analyses showed that the residues were moderately associated with the farmer-to-mobile milk trader pathway and highly associated with milk collected from rural consumer households, which had about three times (15%) the prevalence of those from urban areas (4%). The proportion of samples testing positive for residues moderately decreased with increasing levels of bulking, perhaps indicating dilution to below threshold levels; co-operatives who sell large amounts of milk had a lower proportion of samples with anti-microbials than samples from milk bars and small mobile traders who sell smaller volumes of milk. The residues were detected in 8% of pasteurized milk samples. These findings would imply that the residues are more likely to originate at the farm-level but do not rule out market-level practices which introduce anti-microbials to milk. Though most reports of the negative effects of anti-microbial abuse have in the past mainly been attributed to mis-use of human drugs, these findings suggest that potential risks emanating through milk are high and should be given urgent attention.

Market risk factors

An analysis to identify homogenous groups of market agents in relation to milk quality indicators grouped together most small-scale traders (milk bars, shops/kiosks and mobile traders), irrespective of whether or not they had been licensed. Three homogenous groups were of small traders that together comprised 85% of the market agents who were sampled. Virtually all of these goups of small traders were associated with milk of relatively neutral bacterial quality compared to other groups, use of plastic containers, traded low volumes of milk (average = 107 l/day, bulked from 3-4 sources), had little training (less than 10% were trained in milk quality control) and had relatively short working experience as milk traders (av. 3yrs). The other homogenous groups that comprised 15% of sampled market agents sold larger qualities of milk. One group of traders was of medium scale market agents who bulked and sold 402 litres/day on average. The other group was of large-scale agents such as dairy cooperatives. They were associated with milk of relatively better quality milk, sold over 5,000 l/day on average, had trained personnel in milk handling practices (69% of respondents were trained) and had been in business for a relatively long period (over 15 yrs). This group was also associated with the use of metal containers and non-piped (mainly borehole or well) water. The homogenous groups identified indicated that profit margin per litre of traded milk increases quickly to about KSh 10/litre with increasing scale of business up to about 108 litres of milk sold/day and thereafter the margins decline gradually.

In cases where no intermediary was involved (i.e., sales from farms to directly to a retail outlet), the small-scale trader group travelled the longest distance averaging 30 km from point of milk collection to point of retail, mainly in urban areas. Their milk was sampled at the retail points within, on average, 1.5 hours of milk collection. If one or more intermediary was involved (43% of milk samples that did not directly originate from farms), the average additional distance travelled and time taken were 5km and 1.5 hours, respectively. Nevertheless, the bacterial quality of this milk was only marginally worse than that from pathways without intermediaries (except for milk travelling very short pathways mainly to coops that was significantly of better bacterial quality). In most cases, more than half of the milk samples exceeded the KEBS standard for total plate counts (2,000,000 cfu/ml) and nearly half had exceeded the standard for coliform counts (50,000 cfu/ml). This may reflect bacterial counts at the stationary phase of growth and suggests that most milk had degraded (and therefore a critical control point) before reception by most market agents.

Small market agents sold milk of worse quality than large-scale market agents such as dairy coops. This was partly attributed to the use of non-food quality plastic containers that was significantly associated with higher coliform counts. Non-food quality plastics were used most frequently by mobile traders (89%); by contrast only 10% of the large-scale market agents (such as dairy co-operatives) used plastic containers. The reasons given by the small-scale traders for using the non-food quality plastic containers was their low cost and the risk of confiscation of their containers by regulators.

Post harvest losses incurred by the market, as determined by the fate of leftover milk from previous day's sales, indicated that on average, one in every four traders of all cadres recorded leftovers of about 7% of the volume of previous day's milk sales. However, only 2% of traders recorded leftover milk (from previous day's milk collection) that was thrown away. The rest of leftover milk was consumed by the family or sold at a lower price. Notably, there were very few samples that had evidence of any chemical preservatives (used to lengthen shelf life and reduce spoilage). Though not tested, only 2% of traders indicated that they used hydrogen peroxide (one milk-bar and one large-scale mobile trader)[4]. None of the agents sampled in this study said that they used lactoperoxidase or anti-microbials, though 3% said they used other unspecified preservation methods. The vast majority indicated that they used hot water and soap/disinfectant to clean containers (overall mean proportion= 89%), indicating a conscious effort by the majority to improve hygiene and reduce spoilage.

Management of milk-borne health risks: conclusions and recommendations

General

1. The liberalisation of milk marketing in 1992 led to considerable changes, including increased private sector participation through a large number of market agents who collect, transport, process and distribute milk. **Most of these agents are small-scale and they play an important role in the marketing of milk by linking the majority of producers and consumers in a cost effective way**.

Milk Disposal and Consumption Issues

2. Though potential public health hazards resulting from bacterial pathogens were found in the milk sampled in this study, the common consumer practice of boiling milk prior to consumption eliminates all such health risks. **The practice of boiling of raw milk by consumers should therefore be reinforced through appropriate media campaigns**.

[4] Hydrogen peroxide is easily converted to water upon heating and therefore undetectable in boiled milk. The resultant oxidation of milk proteins may however lead to undesirable off-flavours.

3. A potential source of milk-borne health risks is home-made naturally fermented milk consumed by a small proportion (6%) of rural households. This is because natural fermentation may only reduce, but not eliminate milk-borne health risks. **Consumers of home-made naturally fermented milk should therefore be advised to boil the milk and to use commercially available methods of souring milk before consuming the fermented product**.

Milk Collection and Bulking Issues

4. Bulked milk from many sources increase the risk of contracting milk borne zoonoses in those households that do not boil milk prior to consumption. **Bulked milk such as those traded by dairy co-operatives, should therefore be sent for processing or screened before sale to minimize these health risks**.
5. Many mobile traders (hawkers) were not licensed to sell milk. They traded small quantities and generally used low cost plastic containers, which were associated with poor milk hygiene. These containers were preferred by the traders partly because of the risks of confiscation of containers used for unlicensed marketing of milk. It is probable therefore that the lack of licensing of this group to sell milk contributes to the relatively poor quality of the milk. **A systematic programme should therefore be mounted to incorporate these traders in the licensed milk market through certification following training to ensure the use of easily sterilisable containers and the sale of milk meeting standards defined to meet the requirements of their consumers.**
6. This carefully designed study showed that the majority of samples of milk, whether marketed raw or processed (pasteurized and packaged), did not attain their respective KEBS standards. These standards are based on those currently imposed in countries where all milk flows through cold-chain market pathways and only sold after pasteurisation. In light of the finding that time taken to the first sale transaction point and high local ambient temperatures were the major factors contributing to non-compliance, **it is recommended that the KEBS standards for marketed milk should be reviewed. The review should take account of the predominant milk handling practices in Kenya and throughout Eastern Africa, the collection and sale of raw milk and its boiling in the home of the purchaser before consumption**. The review should consider defining standards appropriate to these milk-handling practices.
7. The study also showed that training in hygienic milk handling practices and in measures ensuring quality control was beneficial in reducing the bacterial load in marketed milk. **There is therefore the urgent need to transfer practical milk hygiene technologies and institute simple and practical training courses in hygienic milk handling for those involved in the raw milk trade as recommended in 5) above**. Pilot testing of appropriate mechanisms for such training should be the first step.

Milk Production Issues

8. The study showed that anti-microbial residues in marketed milk were more likely to originate at the farm level, although the findings did not rule out their addition by market agents. **More research is required to define risk factors for antibiotic and anti-bacterial residues in milk, particularly at the farm-level, as a basis for the development of training and extension materials for their safe use.**
9. Since most milk had degraded before reception by the market agents, **technologies to reduce bacterial growth before or at the first milk sale transaction point are needed.** This calls for specific locally suitable and practical technologies (e.g., cooling or the Lactoperoxidase Milk Preservation System) that can be validated and disseminated to reduce bacterial growth and improve milk quality.

MAIN REPORT

1. INTRODUCTION

The basis for the existence of specific standards and regulations for hygiene and handling of marketed milk is primarily to protect consumers from milk-borne public health hazards. The standards and regulations, which have largely been borrowed from western models, restrict milk handling to cold chain pathways and pasteurization. Whereas nearly all marketed milk in western countries abide by the standards and regulations, they have largely failed in most of the developing world where raw milk sales predominate. Kenya is one of those countries where current regulations and some officials continue to stress pasteurisation despite the fact that only about 12% of marketed milk is pasteurized and the rest sold raw through informal market channels that handle small quantities, about 100lts/day per unit (Figure 1)[5].

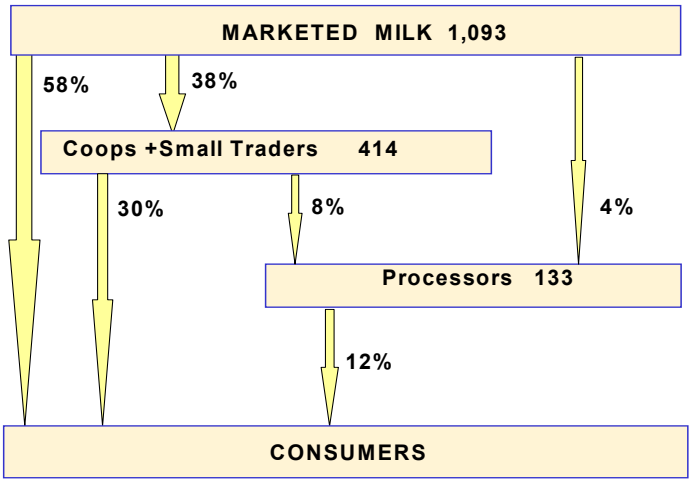


Figure 1. Marketed National Milk Flow from Smallholders in Kenya ('000 MT)
(Adapted from Omore et al., 1999)

The continued dominance of the market by un-pasteurised milk in Kenya indicates that current restrictions against raw milk sales restrict the majority of traders from scaling up. Indications are that improved market participation by more market agents selling consumer preferred milk products would result in increased benefits to farmers, market agents and consumers. These benefits include improvement in levels of income, creation of employment and competitive prices. However there has been public concern and debate, but without quantified information, that encouraging the sale of raw milk by small traders may pose public health risks. This study was designed to produce quantitative information to inform this debate and propose needed interventions. The research problems addressed by this report therefore were:

a) lack of accurate information on milk-borne health risks, and
b) need to define practical steps to optimize milk quality.

Answers to these problems were considered fundamental in addressing the need to define the trade-offs that the dairy industry in Kenya should go for in terms of quality assurance on the one hand and cost and restrictions on traders on the other. They were addressed under specific topics of **risk assessment** and **management**:

[5] Total smallholder dairy production in 1997 was estimated at about 1,700 MT, 64% of which was marketed. This production was conservatively estimated to account for at least 70% of total dairy production in Kenya (Peeler and Omore, 1997).

Questions in risk assessment

- Are milk-borne hazards present in informally marketed milk and at what prevalence?
- Do the hazards pose significant health risks?
- What are the risk factors involved?

Questions in risk management

- Can public health be safeguarded without sacrificing efficiency in liberalised dairy markets?
- Are there practical technical (e.g., handling) and policy options to safeguard public health in informal dairy markets?

To **assess risk**, the study quantified the major milk-borne public health hazards associated with raw and/or informal milk marketing pathways by

- Determining the extent of and evaluating the public health hazards of bovine brucellosis, tuberculosis and other bacteria (including fecal coliforms and entero-pathogenic *E. coli* 0157:H7 in particular) transmitted through milk in target study sites and to extrapolate the impact to other areas;
- Determining the extent of and evaluating the public health hazards of anti-microbials in marketed milk;
- Evaluating the milk handling and hygiene practices of farmers, market agents and consumers; and,
- Estimating the risk for each of anti-microbials, zoonotic organisms (*Brucella abortus* and *Mycobacterium bovis)* and other bacteria in the main unpasteurized milk market pathways.

In addition, pasteurised and packaged milk samples from various retail outlets were assessed for anti-microbials, total and coliform bacteria and antibodies to *Brucella abortus*[6].

To **manage risk,** the study

- Assesses the health risks that the milk-borne hazards pose to consumers; and;
- Makes recommendations on how to safe-guard the risks and to protect public health without discouraging informal milk markets. The recommendations are given to form a basis for communicating the risk information[7] to stakeholders and consumers.

The principles of Hazard Analysis Critical Control Points (HACCP)[8] system were used as a tool and guideline to assess and manage the risks noted above. Five main steps are involved in HACCP: 1) assessment of risks in the food chain, 2) determination of the critical control points (CCPs) and 3) critical limits (CL) for ensuring food safety, 4) development of monitoring systems, and 5) implementation of procedures for verification. The first two steps are reported here, based on risk analyses at consumer- and market-levels, and recommendations made on the latter three.

[6] Antibodies to *Brucella abortus* were assessed in pasteurised milk to assess the potential risk if bulked milk is not pasteurised

[7] Risk communication is the final step in risk analysis. It involves productive interactions between policymakers and stake holders

[8] HACCP is a risk analysis tool and system of process control aimed at ensuring food safety. Originally designed in the early 1960's to control post harvest processing to ensure safe foods for astronauts, the system is now widely applied along the food chain from farm to table to identify and prevent microbial, chemical and physical hazards in food before they occur by a) correcting deviations as soon as they are detected and b) prevent their occurrence. A useful and detailed description can be found in a guidebook by USDA (1997)

2. SAMPLING METHODOLOGY

2.1. Study area and population

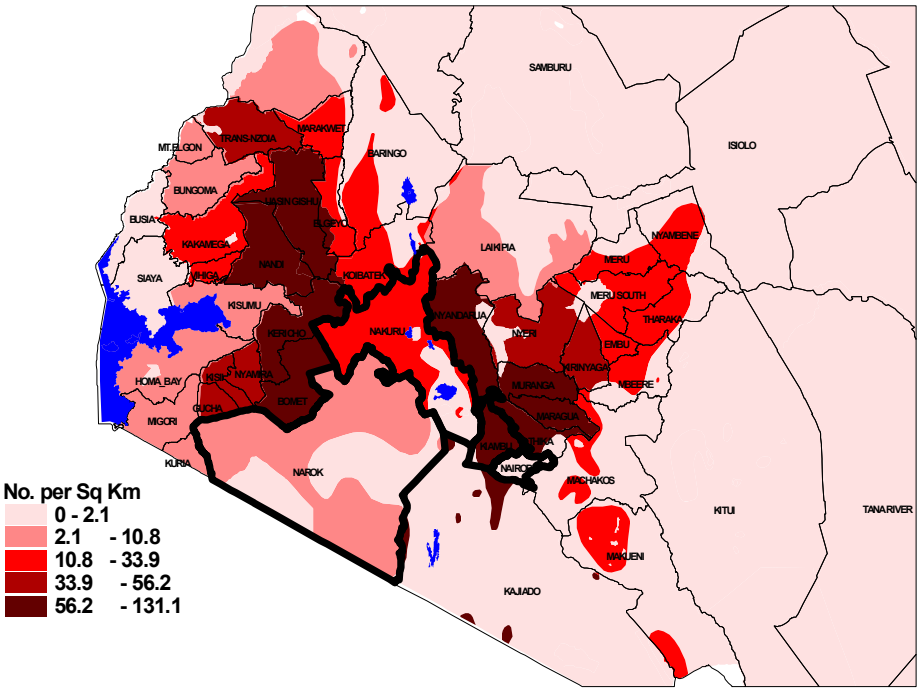
The foci of the study were pathways that do not involve industrial pasteurization. Survey data were collected within the context of household characterisation, consumer/dairy demand and market/transactions costs studies conducted by SDP. At the consumer-level the study was carried out in Nairobi and Nakuru districts representing both urban and rural populations. At the market-level the study was carried out in two sites representing a range from intensive peri-urban and high market access (IHMA) represented by Nairobi and Kiambu districts to more extensive production systems with medium market access (EMMA) represented by Nakuru and Narok districts. Estimates of consumer concentration, number of market agents and main production system at each site are given in Table 1.1.

Table 1.1 Study population: estimates of consumer concentration, market agents and cattle density (dairy and zebu) at each site[a] and stratification criterion

Acronym	District	Consumers			Market agents		Cattle Production system			
		Market access	Human density/ km^2	No. Small traders	No. Coops/ SHGs[b]	No. Processors	AEZ[c] Potential	Density /km^2	Main breed	Main feeding system
SITE1 (IHMA)	Kiambu +Nairobi	High	>500	>1000	16	8	High	100	Exotic	Intensive
SITE2 (EMMA)	Nakuru	Med.	150	>300	6	>20	High	48	Exotic	Extensive
	Narok	Low	28	<100	0	0	Med	52	Zebu	Extensive

[a]Estimates derived from Peeler and Omore (1997) and SDP systems and sub-systems studies.
[b]SHG=Self help groups; [c]AEZ=Agro-ecological zone potential.

Map 1. Study areas and dairy cattle density



2.2. Data collection and analyses

Data collection

For the consumer-level survey, thirty representative clusters per district were selected out of the available 120 Central Bureau of Statistics determined clusters in Nairobi and Nakuru. Seven households were then randomly selected from each of the 30 clusters, making a total of 210 households per district. Out of these, 212 and 222 raw milk samples (fresh or boiled) were collected during the first (dry) and second (wet) season, respectively, for laboratory analyses from every household that consumed unpasteurized milk.

Respondents at the market-level were randomly selected within IHMA (Site 1) and EMMA (Site 2). At each site, sub-locational areas and divisions in which they are located were randomly selected based on a household characterisation survey conducted earlier. Additional divisions were randomly selected in Nairobi to account for the higher number of market agents in the city. A total ten divisions were selected this way from Site 1 (Nairobi Central, Makadara, Westands, Kasarani, Kibera, Kikuyu, Kiambaa, Limuru, Lari and Githunguri) and six divisions from Site 2 (Nakuru Municipality, Bahati, Molo, Njoro, Rongai and Mau).

A total of 262 and 270 informal market agents in both sites responded during the first (wet) and second (dry) seasons, respectively (Table 1.2). All informal market agents that bulk large quantities of milk (dairy co-operatives, self-help groups) in the two distinct sites were included. Dairy cooperative collection points and smaller market agents (milk-bars, shops, kiosks and small mobile traders) were randomly sampled at their retail outlets in selected sub-locations. In addition, 145 pasteurised milk samples were collected during the first season and tested for quality to compare adherence to set standards.

Table 1.2. Households and market agents surveyed & sampled in each site and season[a]

Category	Nairobi		Kiambu		Nakuru		Narok	
	S1	**S2**	**S1**	**S2**	**S1**	**S2**	**S1**	**S2**
Consumers / month	1/99	10/99	-	-	2/99	9/99	-	-
Nairobi urban households	49	53	-	-	-	-	-	-
Nakuru urban households	-	-	-	-	57	56	-	-
Nakuru rural households	-	-	-	-	105	113	-	-
Total households	**49**	**53**	**-**	**-**	**162**	**169**	**-**	**-**
Market agents / month	5/99	2/00	5/99	2/00	6/99	11/99	6/99	1/00
Cooperative Societies	0	0	16	16	6	3	0	0
Coop. Collection centres	0	0	23	11	0	2	0	0
Self help groups (SHGs)	0	0	0	0	2	5	0	0
Milk-bars/Snack-bar	49	53	10	13	23	18	10	9
Milk-shops/Kiosks	29	31	14	23	19	20	3	4
Mobile trader (Hawker)	15	15	14	15	29	32	0	0
Total informal market agents	**93**	**99**	**77**	**78**	**79**	**80**	**13**	**13**
Pasteurised milk samples	**82**	**-**	**-**	**-**	**63**	**-**	**-**	**-**

[a] Dashes (-) imply 'not applicable' while zeros (0) imply 'no observation'.
Relative seasonal contrasts in precipitation during the surveys were as follows.
Consumer surveys: season one (S1) was relatively dry and season two (S2) was relatively wet.
Market surveys:- season one (S1) was relatively wet and season two (S2) was relatively dry.
There was no striking seasonal difference in precipitation during both studies.

Attempts were made to survey and collect milk samples from the same households or informal traders during the two seasons. Where it was not possible to interview the same respondent during the second season, a replacement was selected within the same locality. Major reasons for not interviewing the same respondent were because either the family had moved or the same market agent could not be traced. The numbers of non-repeat respondents were 69 (16%) consumer households and 142 (27%) market agents (106 in Site 1 and 36 in Site 2). Most non-repeat market respondents were small mobile traders.

Laboratory analyses

From each respondent, milk samples were collected in sterile 50ml (aseptically) and non-sterile 250ml plastic tubes for laboratory analyses. The milk samples were kept in a cold box and transported within 5 hours of collection to the laboratory at either the Department of Dairy Technology, Egerton University or the Department of Public Health, University of Nairobi - depending on proximity of area of sampling. The aseptically collected milk samples were analysed for the hazards of total bacteria, coliforms, brucellosis, anti-microbial agents while the non-aseptically collected samples were tested for adulteration (added water and solids) and butterfat (BF) content.

Tests requiring fresh milk samples (bacterial counting, specific gravity (SG) for adulteration and Milk Ring Test (MRT) for brucellosis) were done immediately after sampling. BF content was also determined from fresh samples. Milk samples were thereafter frozen for later testing for brucellosis, antimicrobial residues and heavy metals. Coliform bacteria were inoculated into a nutrient medium for later sub-culture in selective media and biochemical tests. Details of laboratory analyses are presented in Annex 1.

Data analyses

Consumer and informal market-level survey and laboratory data were analysed to:

- identify sources of consumed milk;
- quantify the prevalence of brucellosis;
- assess milk bacteriological quality;
- assess the presence of inhibitors (antimicrobials) in milk;
- assess milk handling practices by consumers;
- assess consumer perception on quality of milk;
- assess milk handling practices by market agents;
- assess the association of milk quality and public health hazards with market concentration (size and number) and behaviour including profit margins; and
- quantify the impact of milk-borne public health hazards on humans.

Two strategies were used to identify CCPs for each hazard along the market chain. The first was description of laboratory assessments for each type of trader, area and season (Sections 3 & 4). Market agents were re-classified into four broad groups roughly according to hierarchy in the milk market chain: farmer groups (coops, collection centres and SHGs), milk bars, shops/kiosks and small mobile traders.

The second was to conduct multiple regression and multivariate analyses in SAS to evaluate risk factors for each health hazard and identify associations among principal components and clusters (Section 5). These analyses were used to identify CCPs along various market pathways including consumer outlets (Section 6). Details of each analytical step are presented under each section.

3. LABORATORY HAZARD ANALYSES: DESCRITIVE RESULTS AND DISCUSSION

3.1. Assessment of informally traded (raw) milk quality

3.1.1. Adulteration

Adulteration of milk implies addition or subtraction of any of its components including addition of water (reduces SG) or addition of solids such as flour or sugar and removal of butterfat (BF (increases SG). Such interference may introduce chemical and microbial health hazards besides reducing its nutritional and processing quality, palatability, and market value. The SG of milk measured at about 20^{o}C is normally 1.026 – 1.032 kg/litre. The SG depends on the protein and fat content: the SG of fat is 0.93, solids-non-fat (SNF) is 1.6% and water is 1.0 kg/litre. If the milk is mixed with air, for example by bumping during transport, the SG will be 1.020 kg/litre or lower (FAO, 2000). See details of method in Annex 1.

Results and discussion

Added water and solids

Overall, 4.7% and 10.4% of samples from consumer households and market agents, respectively, had SG below the KEBS standard of 1.026kg/litre and therefore suspicious of adulteration by added water (Table 3.1). Adulteration was highly variable by area and season but without significant differences among cadres of market agents. Among samples from consumer households, the highest proportion of this adulteration was found in the first (dry) seasonal consumer survey in Nairobi where 22% of milk samples had specific gravity < 1.026 kg/litre[9]. This proportion was substantially higher than that of Nakuru urban (0%) and rural (1%). The reverse was true in the second (wet) season with more samples in Nakuru having added water compared to Nairobi. The relatively high proportion of added water in Nairobi in the dry season may indicate a tendency to add water to traded milk by market agents during periods of low milk supply and high milk prices.

Table 3.1: Adulteration of milk with water (SG<1.026) and solids (SG>1.032)

District	Season 1				Season 2			
	Added water		Added solids		Added water		Added solids	
	n	%	n	%	n	%	n	%
Consumer households								
Nairobi	10	22	0	0	0	0	0	0
Nakuru urban	0	0	2	4	3	5	8	15
Nakuru rural	1	1	9	9	6	5	7	6
Market Surveys IHMA								
Coops/Coll. Centres/SHGs	5	13	0	0	1	4	0	0
Milk-bars/Snack-bar	4	7	0	0	8	15	2	4
Milk-shops/Kiosks	5	12	2	5	4	9	0	0
Small mobile traders	3	10	1	3	4	14	0	0
Market Surveys in EMMA								
Coops/Coll. Centres/SHGs	0	0	0	0	1	10	0	0
Milk-bars/Snack-bar	0	0	0	0	5	19	0	0
Milk-shops/Kiosks	0	0	0	0	4	17	0	0
Small mobile traders	0	0	0	0	8	27	1	3

[9] FA0 suggests specific gravity lower than 1.01 kg/litre for adulteration of milk collected by tanks and likely to me mixed with air due to shaking (FAO, 2000)

Added water was detected in 7-13% of milk samples from a range of market agents along the market chain in Kiambu and Nairobi in the wet season (S1). Similar proportions (4-15%) were recorded in the dry season (S2) but there was no noticeable trend between types of market agents. In contrast, market agents in Nakuru and Narok had milk with marked seasonal variation in added water from none in the wet season to 10-27% in the dry season. Addition of water therefore seems to be a practice that generally occurs across seasons in IHMA but only associated with the dry season in EMMA. These proportions indicate a large variation in added water by season and area and may be attributed to relative changes in milk supply and prices.

Overall, 5.9% and 1.0% of samples from consumer households and market agents, respectively, had SG above KEBS standard of 1.032kg/litre and therefore suspicious of adulteration by added solids (Table 3.1). These figures were also highly variable ranging from 0% to 15% and with no obvious pattern.

Butterfat

Urban areas had substantially more milk with below KEBS minimum standard for whole milk BF content of ≥3.25% (Table 3.2). At the market level, the proportion of samples with low BF% was more uniform among market agents in Kiambu and Nairobi (IHMA) but was higher among smaller traders than at cooperatives and collection points in Nakuru and Narok (EMMA). BF content above 6% was recorded in up to 10% of consumer samples from Nakuru rural. Most pasteurized and packaged milk has a lower butterfat, often standardized at 2-3%, than the milk that is marketed without processing

Table 3.2: Milk with butterfat content below KEBS minimum limit of 3.25%

District	Season 1		Season 2	
	n	% BF<3.25	n	% BF<3.25
Consumer households				
Nairobi	9	50	23	43
Nakuru urban	19	33	30	54
Nakuru rural	19	18	23	20
Market Surveys in IHMA				
Coops/Coll. Centres/SHGs	9	24	9	33
Milk-bars/Snack-bar	12	21	20	32
Milk-shops/Kiosks	16	37	13	25
Small mobile traders	8	28	8	29
Market Surveys in EMMA				
Coops/Coll. Centres/SHGs	1	13	1	10
Milk-bars/Snack-bar	5	15	7	39
Milk-shops/Kiosks	6	27	7	35
Small mobile traders	9	31	7	23

Low BF% in traded milk could be an intentional practice by traders who may remove cream to sell separately from the skimmed milk to make more profit. The separation of cream from milk may also be well intended to enable boiling of unsold milk in readiness for mixing with following day's milk, thereby lowering the overall BF% of the mixture. Separate layers of milk in the same container often yield different BF% if the milk is not mixed before dispensing because BF tends to congregate at the top.

Solids-Not-Fat and Total Solids

Solids-not-fat content (SNF) and total solids (TS) were calculated using Richmond formulae[10] as follows: SNF= (0.22 x BF + (0.25 x SG)+ 0.72) and TS = SNF+BF. These parameters

[10] The SG reading used in the Richmond Formulae is the last two digits (e.g., 30 is used instead of 1.030)

standardize the solids content as milk with high BF and SNF could have the same SG as milk with low BF and low SNF contents. Overall means for SG, SNF and TS are given in Table 3.3.

Table 3.3. Overall means for adulteration indices

Parameter	N	Mean	SD	Range
Specific gravity (SG)	472 (427)[a]	1.028 (1.029)	0.002 (0.003)	1.020-1.034 (1.022-1.060)
Solids-not-fat (SNF)	455 (400)	8.6 (8.9)	0.5 (0.7)	6.6-10.2 (6.8-16.6)
Butter Fat (BF) %	476 (403)	3.7 (3.8)	0.9 (1.1)	0.5-8.0 (1.0-8.0)
Total solids (TS) %	455 (400)	12.3 (12.8)	1.2 (1.6)	8.4-18.2 (8.8-20.8)

[a] Figures for consumer milk samples are in brackets.

The mean SNF and TS for bovine milk is given as 8.6% and 12.7%, respectively (O'Conner, 1995). The average TS for milk samples obtained at the market-level was lower at 12.3% but this figure was not significantly different at 5% from the given mean. There was also no significant difference in the mean TS and SNF between milk handled by various cadres of traders. Though the proportions of samples with SNF less than the normal standard of 8.5% were also not significantly different between milk handled by various cadres of traders, they were significantly higher than the specific gravity index that classifies milk as adulterated with added water at the threshold of SG<1.026 kg/litre. Whereas the overall proportion of market agents with milk with SNF<8.5% ranged from 29.3% - 35.1%, the proportion of those agents with milk with SG<1.026kg/litre ranged from 9.7% - 12.5%. This suggests that actual levels of adulteration were much higher.

3.1.2. Milk bacteriological quality

Bacterial counts in milk reflect the temperature of the milk; time elapsed since milking and level of hygiene. Whereas total bacterial counts will mainly reflect time elapsed since milking and ambient temperature (if milk is not chilled), coliform counts are especially associated with level of hygiene since they are mainly of fecal origin. Common bacteria in milk are given in Table 3.4.

Table 3.4. Bacterial types commonly associated with milk.

Bacteria	Effect on milk / consumers
***Lactococci**: L. lactis-diacetylactis, L. lactis, L. cremoris*	Flavour production and fermentation
***Lactobacillus:** L. lactis L. bulgaris, L. acidophilus, Leuconostoc lactis, Propionibacterium*	Acid production/fermentation
Pseudomonas, Bacillus cereus	Spoilage
Enterobacteriaceae	Pathogenic and spoilage
***Staphylococci**: Staph. aureus*	Pathogenic
***Streptococcus**: Strep. agalactiae,*	Pathogenic
Brucella	Pathogenic
Mycobacteria	Pathogenic
Coliforms (mostly introduced through poor hygiene)	Some are pathogenic (e.g., *E. coli* 0157:H7)

Source: Adapted from O'Connor (1995)

The major factor in total bacterial growth is time elapsed since milking (Illustrated in Figure 3.1). Under ambient temperatures that prevail in the tropics, a bacterial cell in milk with a typical generation time of 20 minutes will multiply to 2,000,000 cells, the threshold set by the KEBS for total bacterial plate counts (TPC) in raw milk, within 7 hours. However, if the generation time is reduced to two hours by lowering the temperature of the milk to below 10^{o}C, the same bacterial cell would only multiply to only 32 cells within the same period (FAO, 1979). With higher initial

load of bacterial cells due to unhygienic milking, the time taken to these thresholds reduce considerably.

Poor hygiene often arises from poor handling at the farm, collection centres, during transportation and at retail points. Common sources of bacterial contamination, especially coliforms, are faeces (of animal or human origin), personnel, water and containers. A high bacterial count reduces the shelf life of milk and enhances the risk of milk-borne bacterial infections and intoxication if the milk is not properly heated or if thermally injured pathogens recover under suitable temperatures (Andrew and Russel, 1984; Kayihura *et al*., 1987). See details of method in Annex 1.

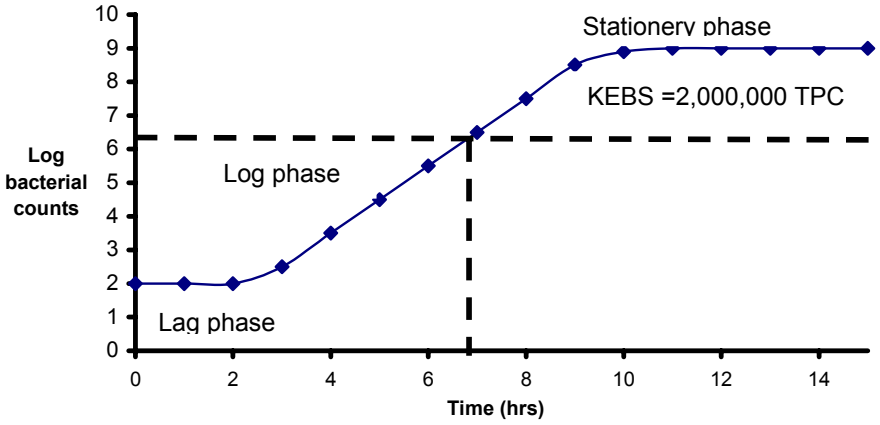


Figure 3.1. Typical multiplication of a bacterial cell over time at ambient temperatures
(Adapted from FAO, 1979)

Results and discussion

Milk quality

The average total plate counts (TPC)/ml in milk from farmer groups were much lower at $7.9x10^6$ (Log_{10} 7.2) compared to the overall average of $39.8x10^6$ (Log_{10} 7.6) (Table 3.5). Similarly, coliform plate counts (CPC)/ml in milk from farmer groups were also much lower at $15x10^3$ (Log_{10} 4.2) than the average for the whole sample ($50x10^3$ or Log_{10} 4.7). TS and SNF were uniform across market-level samples.

Table 3.5: Market-level: Means of bacterial counts by market agents

Parameter	Milk market agents									
	Farmer grp		Mobile trader		Shop/kiosk		Milk bar		Overall	
	n	Mean	n	Mean	n	Mean	n	Mean	n	Mean
Total Plate Counts /ml '000[a]	68	7,900	106	39,800	128	39,800	158	79,400	471	39,800
Coliform Plate Counts /ml '000 [a]	70	16	111	63	135	63	158	100.0	485	50
Solids-not-fat (SNF) [b]	68	8.6	111	8.5	123	8.5	143	8.6	455	8.6

[a] Standard deviations (SD) for geometric means of total and coliform bacterial counts were between 10-20% and 20-30%, respectively. [b] SD for all SNF means was 0.5.

At the consumer level, milk samples from Nakuru rural (short market chain) had markedly lower bacterial counts than milk collected from consumers in urban areas (long market chain)(Table 3.6). Milk sold to households in Nairobi had the highest total and coliform bacterial counts.

Table 3.6: Consumer-level: Means of bacterial counts by location of households

Parameter	Nairobi		Nakuru urban		Nakuru Rural		Overall	
	n	Mean	n	Mean	n	Mean	n	Mean
Total Plate Counts /ml '000 [a]	102	316,200	113	20,000	217	1,300	433	10,000
Coliform Plate Counts /ml '000 [a]	98	50	113	20	215	1	427	5

Standard deviations (SD) for geometric means of total and coliform bacterial counts were between 10-30% and 30-50%, respectively.

Classification of bacterial counts according to KEBS

Milk from urban areas had strikingly higher proportions of milk with unacceptable TPC and CPC of 61-84% and 39-69%, respectively; compared to milk from Nakuru rural (Table 3.7). However it is notable and striking that as high as 35% of milk samples from Nakuru rural (very short and direct market chain) did not meet KEBS standards for total bacterial counts. This shows the unsuitability of these standards under local circumstances, as the majority cannot meet them. The proportion of households in Nakuru with milk with unacceptable total counts was less in the wet season (27-61%) than in the dry season (35-82%).

Table 3.7. Consumer-level: Milk samples with unacceptably high bacterial counts according to KEBS (total counts above 2 million c.f.u/ml and coliform counts above 50,000 c.f.u/ml)

District	Total counts >2 million c.f.u/ml		Coliform counts >50,000 c.f.u/ml	
	n	%	n	%
Dry season				
Nairobi	41	84	22	48
Nakuru urban	47	82	22	39
Nakuru rural	36	35	11	11
Wet season				
Nairobi	44	83	36	69
Nakuru urban	34	61	29	52
Nakuru rural	31	27	17	15

It was noted that many urban households purchase milk from stationary or mobile milk traders while some are supplied directly by farmers or market agents. In sales through traders, the milk goes through multiple intermediaries and containers that may not be hygienic. Most households and informal milk sale points did not have milk-cooling facilities to slow down bacterial growth. Distances travelled (and time spent) by producers or market agents to consumers in Nakuru rural were shorter than in Nakuru urban while some respondents, mainly in rural areas, were also milk producers themselves. These variations contributed to the large range in bacterial counts ranging from very low counts in milk from Nakuru rural to the high counts in urban centres. These factors will be further investigated in Section 5.

Among market agents at both sites, bacterial counts seemed to increase as the milk moved up the market chain. Milk bars, shops/kiosks and small mobile traders had markedly higher proportion of their milk with bacterial counts above KEBS standards as compared to cooperatives and collection centres (Table 3.8). This may reflect bacterial counts at the stationary phase of growth and suggests that most milk had degraded, and therefore a critical control point, before reception by most market agents. Seasonal differences were not clear-cut. Statistical differences for these and other market risk factors are assessed in Section 5.

The overall picture at both the consumer and market levels is that bacterial counts increase (and quality decreases) as milk passes through increasing numbers of intermediaries. The generally high proportions of raw milk samples with total counts that did not achieve KEBS standards suggests that long duration and lack of a cold chain between milking and sale may be major factors contributing to rapid bacterial multiplication (see analyses in Section 5 where theses effects are quantified). The lack of a cold chain and resultant rapid bacterial growth also applies to most outlets for processed milk without chilling facilities (see Section 3.2 below).

Table 3.8. Market-level: Milk samples with unacceptably high bacterial counts according to KEBS (total counts above 2 M cfu/ml and coliform counts above 50,000 cfu/ml)

Season/District	Total counts >2 million cfu/ml		Coliform counts >50,000 cfu/ml	
	n	%	n	%
Wet Season				
Surveys in Nairobi and Kiambu (IHMA)				
Coops/Coll. Centres/SHGs	24	71	13	37
Milk-bars/Snack-bar	47	89	33	61
Milk-shops/Kiosks	29	74	28	65
Small mobile traders	26	90	20	69
Surveys in Nakuru & Narok (EMMA)				
Coops/Coll. Centres/SHGs	5	63	4	50
Milk-bars/Snack-bar	24	75	18	56
Milk-shops/Kiosks	15	83	9	47
Small mobile traders	22	79	18	64
Dry Season				
Surveys in Nairobi & Kiambu (IHMA)				
Coops/Coll. Centres/SHGs	6	38	5	29
Milk-bars/Snack-bar	38	76	23	47
Milk-shops/Kiosks	32	68	25	51
Small mobile traders	14	64	11	41
Surveys in Nakuru and Narok (EMMA)				
Coops/Coll. Centres/SHGs	6	60	4	40
Milk-bars/Snack-bar	21	91	16	70
Milk-shops/Kiosks	19	79	12	50
Small mobile traders	23	85	16	59

3.2 Assessment of formally traded (pasteurised) milk quality

Assessment of quality of pasteurised and packaged milk was conducted alongside that of informally marketed milk to 1) compare the performance of each milk pathway in accordance with respective standards, and 2) in response to complaints about high rates of spoilage of pasteurised milk by a section of dairy industry stakeholders.

Sampling of pasteurized milk and quality tests performed

Pasteurised and packaged milk samples were collected from retail outlets in Nairobi and Nakuru town. In Nairobi, 82 samples (packets) of pasteurised milk from six processors were purchased during the month of May/June, 1999 from supermarkets, shops and kiosks. Similarly, 63 samples from seven processors were obtained from Nakuru giving a total of 145 samples. All the samples were purchased and analysed on the day of expiry or "sell by date" -- normally three days after pasteurisation, during which bacterial counts should not exceed specified standards. These standards assume cold-chain specifications of below 4^{o}C. Standard methods (KEBS 1996) for total counts and coliform counts for pasteurized milk were followed during preparation, incubation and enumeration of the counts. The KEBS standards on

processed milk were used to interpret the results. These are 30,000 total counts/ml and 10 coliforms/ml. The method applied for bacterial counting was similar to that used for informally marketed milk samples (Annex 1).

Results and discussion

In Nairobi, 67 out of 82 samples (82%) had total counts above 30,000/ml while 48 out of 63 samples (59%) had coliforms above 10/ml. In Nakuru 56 out of 63 samples (89%) had total counts above 30,000/ml and 44 (70%) had coliforms above 10/ml (Table 3.9). A substantial number of samples had total counts and coliform counts above 1,000,000/ml. Therefore, only a small proportion (18% in Nairobi and 11% in Nakuru) met the KEBS (1996) standards.

Table 3.9. Bacterial counts in pasteurised milk above KEBS

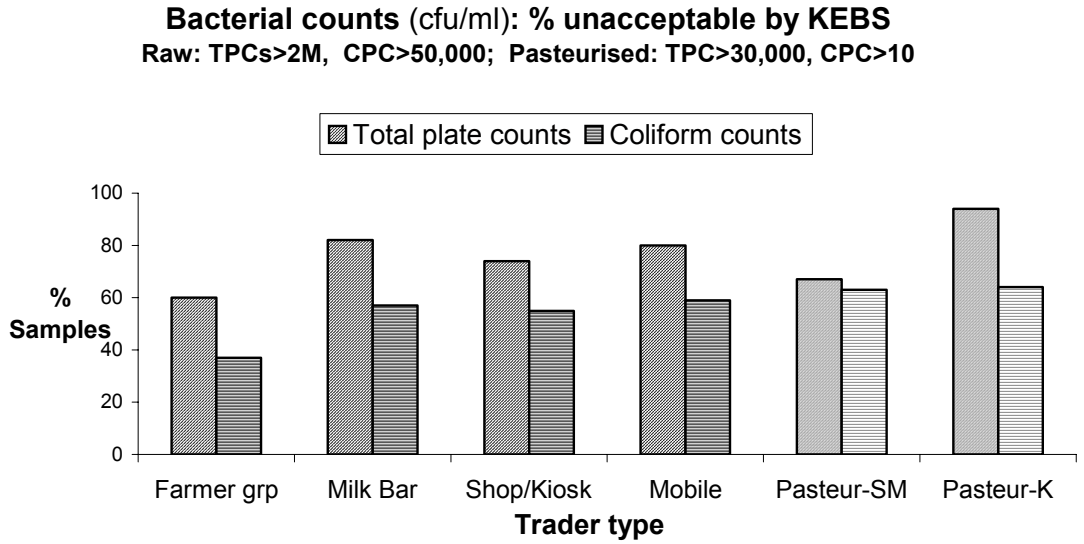
Site	Retail point	Total counts > 30,000 c.f.u./ml		Coliform counts >10 c.f.u./ml	
		n	%	n	%
Nairobi	Supermarkets	14	52	17	63
	Shops & kiosks	53	96	31	56
Nakuru	Supermarkets	13	81	14	63
	Shops & kiosks	43	91	34	72

A higher proportion of samples from shops and kiosks (over 90%) in both Nairobi and Nakuru, had total counts of above 30,000/ml as compared to 52% of samples from supermarkets in Nairobi and 81% in Nakuru (Table 3.9). These proportions were significantly different ($p<0.05$).

The lower proportion of samples from supermarkets with above 30,000/ml total counts in Nairobi is an indication of the benefits of keeping milk chilled after pasteurization. About 60% of all samples from outlets in Nairobi and Nakuru had coliform counts above 10/ml but samples from supermarkets did not consistently have lower coliform counts compared to retail outlets without chilling facilities.

These results show that by the time pasteurised and packaged milk reaches the expiry date, bacterial population in the milk will be quite high. A number of factors may contribute to this. Firstly, it could reflect the original heavy load of bacteria in raw milk before pasteurization. Pasteurization (not sterilization) usually reduces the percentage of bacteria to about 1% and this number can be substantial if the original bacterial load in raw milk is high. Secondly, it should also be borne in mind that bacterial cells can recover after thermal injury under the favorable tropical temperatures that prevail during transportation or at retail outlets that do not have chilling facilities such as kiosks (Andrew and Russel, 1984; Kayihura *et al*., 1987). Lastly, there is the possibility of re-contamination after pasteurization. KEBS standards only assure quality immediately after pasteurization and assume that the milk is chilled at all retail points.

The comparison of pasteurised milk with informally traded raw milk suggests that the majority of milk in both pathways do not adhere to respective KEBS standards (Figure 3.2). The standards should therefore be reviewed in the context of the absence of a cold chain common in both pathways. It is noteworthy that chilling of pasteurised milk at supermarket retail points does not markedly reduce average bacterial counts compared to pasteurised milk purchased from kiosks. This indicates that a CCP for pasteurised milk may exist during transportation from processing plants to retail points, or that temperatures are not kept low enough at retail outlets, even those with chilling facilities.



Key: Pasteur-SM & Pasteur-K = Pasteurised milk from Supermarkets and Kiosks, respectively.
Note different standards used for pasteurised and raw milk.

Figure 3.2. Comparison of bacterial counts in milk among cadres of milk traders

3.3. Zoonotic Health Hazards

3.3.1. Brucellosis

Brucellosis is a milk-borne zoonosis that is a major reason for advocacy of milk pasteurization or boiling before consumption. The major species of concern in marketed milk, *Brucella abortus*, causes a febrile flu-like illness in humans. *Br. abortus* was tested using the traditional brucella Milk Ring Test (MRT) and a recently validated indirect Milk ELISA (Nielsen et al., 1996). Both are surrogate tests for brucella antibodies[11]. See details of method in Annex 1.

Results

Overall prevalence of brucellosis at consumer-level as determined by both ELISA and MRT were 4.9% and 3.9%, respectively (Table 3.10). At the informal market level, ELISA and MRT classified 2.4% and 3.4%, respectively, as positive. Informally traded bulked raw milk from dairy co-operatives and milk bars had the highest proportion of ELISA and MRT positive samples. Nearly all these samples were from Narok District where extensively grazed pastoralist zebu herds predominate. The ELISA test classified nine (8.2%) of pasteurised milk samples as positive. Six of the nine positive samples were from one milk processor in Nakuru. Agreement between the test results were poor (*Kappa* = 0.32, 95%, confidence interval = 0.07-0.56) to moderate (*Kappa* = 0.40, 95% confidence interval = 0.19-0.60) for the market- and consumer-level samples, respectively, with the ELISA test classifying more samples as positive. Two consumer households in Nakuru reported having had a member diagnosed with brucellosis in the previous one year.

[11] Antibodies are natural protective proteins produced by mammals following infection and are often used in immunological tests as surrogate measures for infection, in which case a positive test result equals potential disease risk. However after effective heat treatment that kills the pathogen, a positive antibody test result will not equal potential risk of infection.

Table 3.10. Numbers and proportions of milk samples from consumer households and various market agents (two seasons) testing positive for *Br. abortus* antibodies

Source of milk samples	Antibody Prevalence			
	MRT		ELISA	
	n	%	n	%
Consumer households				
Urban consumers (Nairobi and Nakuru)	10	4.7	11	5.1
Rural consumers (Nakuru)	7	3.2	10	4.6
Informal market agents in Nairobi & Kiambu (IHMA)				
Coops/collection centers/Self help groups	3	4.8	2	3.1
Milk Bars	1	0.8	2	1.6
Milk Shops/kiosks	2	2.1	1	1.0
Small mobile traders	1	1.7	0	0
Informal market agents in Nakuru &Narok (EMMA)				
Coops/collection centers/Self help groups	0	0	0	0
Milk Bars	9	15.0	5	12.2
Milk Shops/Kiosks	4	4.4	0	0
Small mobile traders	0	0	2	3.4
Pasteurised milk in Nairobi & Nakuru urban	-	-	9	8.2

The test results generally reflect previous findings from serological studies in cattle (e.g., Kagumba and Nandokha, 1978; Kadohira *et al*., 1997) indicating higher farm-level prevalence of brucellosis in extensive and/or communal grazing areas than in smaller stall-fed herds. Kagumba and Nandokha (1978) reported a prevalence of 10% bovine brucellosis in extensive production systems in Nakuru, and Kadohira *et al*., (1997) reported a 2% apparent prevalence of bovine brucellosis in the smallholder farms in Kiambu. Human brucellosis is also more common where extensive cattle production systems predominate. Muriuki *et al*., (1997) found that as high as 21% of human flu-like cases reported in health facilities in Narok were diagnosed as brucellosis.

3.3.2. *E. coli* 0157:H7

E. coli 0157:H7 is a newly recognized bacterial zoonosis that causes haemorrhagic colitis (HC) and haemolytic ureamic syndrome (HUS) in humans. The strain is found in gut and fecal material of affected cows and humans. Milk can get contaminated through contamination with cow faeces or unhygienic handling. Dairy cattle in many countries have been shown to harbour *E.coli* 0157:H7 and to act as a source of contamination of milk (and other foods), water and the environment (Youko Miyao *et al*., 1998; Aloysio *et al.,* 1999; Cobbold and Desmarchelier, 2000). This report covers results from 264 samples. See details of method in Annex 1.

Results and discussion

The 264 samples examined for E. coli 0157:H7 were from Nairobi season one and two surveys, and Nakuru urban and Nakuru rural season one survey (Table 3.11). Three highly suspect isolates on BCM ^{TM} 0157:H7(+) medium were recovered from three different milk samples out of the 91 samples with faecal *E.coli.* Two of the isolates, one from Nairobi, and one from Nakuru urban, were serologically confirmed to be *E. coli* serotype 0157:H7. The Nakuru isolate produced verocytotoxin 1. The third suspect isolate that could not be serotyped (only 0157:H7 specific antiserum was used) and did not produce verocytotoxin was from Nakuru urban. No *E. coli* 0157:H7 was detected in the 33 *E. coli* positive samples from Nakuru rural.

Table 3.11. Numbers of unpasteurised consumer milk samples screened for *E. coli* and isolation and identification of strain 0157:H7

Milk sample and test details	Number			
	Nairobi urban	Nakuru urban	Nakuru rural	Total
Examined for coliforms	99	58	104	264
Positive for *E. coli*	37	21	33	91
Suspect *E. coli* 0157:H7 on BCM^{TM} medium	1	2	0	3
Serologically confirmed *E. coli* 0157:H 7	1	1	0	2
Verocytotoxin1 producing *E. coli* 0157:H7	0	1	0	1

E.coli 0157:H7 is a rare strain among *E.coli* organisms. It therefore requires screening of a large number of samples in order to detect it. The larger the sample and the higher the proportion of faecal *E.coli* in any set of milk samples, the greater the chance of isolating the strain. The culture results showed many samples had high total counts and coliform counts, and also faecal *E. coli*, thus providing a fair chance of recovering strain 0157:H7. The pathogen was recovered from two out of 264 samples. This translates to a recovery rate of only 0.8%, which is low but significant for a number of reasons. Currently the strain is highly acknowledged as an important food-borne zoonosis. HUS may lead to permanent kidney damage caused by potent verocytotoxins produced by one of the isolates. This damage has previously necessitated a transplant (Riley *et al*. 1983, Flowers *et al*. 1992; Jay 1992). The role of *E.coli* 0157:H7 in causing these diseases here in Kenya is not clear. What the finding in this study show, however, is that those who consume unpasteurised or unboiled milk are at risk of getting infected. The prevalence of 0.8% would imply that a consumer taking marketed milk on a daily basis (as most Kenyans do) is at risk of exposure to *E. coli* 0157:H7 bacteria at least three times each year. Fortunately this exposure would rarely translate into an infection given that all households in urban areas and over 95% of the households in rural areas (excluding pastoral areas) reported boiling milk (and thereby destroying the organism) before consumption (Ouma et al., 2000).

As far as we know, this is the first time *E.coli* 0157:H7 has been recovered from marketed raw bovine milk in Kenya. Its origin could be from dairy herds or milk handlers. The fact that isolations were made from two towns, which are far apart (150km), may indicate that its occurrence is widespread. If its origin is dairy cows, which contribute milk that is sold in the urban centres, then the spread would be even bigger considering the spatial distribution of the dairy herds. Farm level studies would help ascertain the actual origin of the pathogen.

3.3.3. Bovine Tuberculosis

Though zoonotic bovine tuberculosis has never been officially reported in Kenya[12], the situation has not been widely studied to rule in or rule out the disease since the comprehensive reviews in the 1960's by FAO/WHO/GoK experts (Myers and Steele, 1969). The only indication that the situation could still be the same has been the lack of any reports of TB from passive reporting systems such as post mortems in abattoirs and speciation of *Mycobacteria* from TB patients at KEMRI. Available hospital records showed that approximately 180,000 patients were suspected to have TB nationally between 1990 and 1999[13]. The national overall ratio of human *pulmonary* TB to *extra-pulmonary* TB (often more associated with M*. bovis infection*) for the decade was 4:1. There was a general increase in suspected cases of both forms of TB over the period, the general rate of which cannot be explained by population growth or increase in number of hospital visits alone: extra-pulmonary TB (EXPTB) cases increased by 26% annually,

[12] The zoonosis is endemic in all neighbouring countries
[13] Suspect cases were determined through clinical diagnosis and acid-fast staining method

significantly higher than the rate of increase of pulmonary TB cases (17%). Most of the increase in cases has been attributed to the high prevalence of the human immunodeficiency virus (HIV) infection. Suspected EXPTB occurrence nationally was uncorrelated with production system or population densities of either cattle or humans.

Narok District was chosen for this study because it is perceived as a relatively high-risk area for zoonotic *M. bovis* infection with predisposing factors being extensive/pastoral livestock grazing and raw milk consumption habits by the Maasai. The hospital records in the district indicated about 120,000 outpatient-visits and 2,603 clinical diagnoses of TB made in 16 health centres in 1999. Of these suspect TB cases, 272 were confirmed through acid-fast staining. Most (89%) of the acid-fast positive samples were from patients with pulmonary symptoms and the rest (11%) were from patients with extra-pulmonary symptoms with or without pulmonary symptoms. The district contributed about 8% of all TB morbidity cases reported nationally in 1999 (MoH, 1998).

Sampling and laboratory analyses

Sampling of patients was opportunistic and covered geographical and livestock production system variation in the district (lowland/extensive zebu cattle grazing system to highland/semi-intensive dairy cattle system). All patients reporting to the 16 health facilities in the district and suspected to be suffering from tuberculosis by health clinicians were sampled early in the mornings on three consecutive days (to enhance chances of isolation of *Mycobateriacae*) for a period of nine months. The samples were thereafter taken to the Narok District Hospital where acid-fast staining was done to identify suspected cases.

A total of 149 suspect (acid-fast positive) sputum and three sub-mandibular biopsy aspirates from 134 patients were cultured for speciation of *M. bovis* from other mycobacteria at the Respiratory Diseases Centre at Kenya Medical Research Institute (KEMRI) using standard methods. Culture positive samples with were further subjected to DNA typing by PCR at the Microbiology Laboratory at Sokoine University of Agriculture in Morogoro, Tanzania. See details of method in Annex 1.

Results and discussion

Of the 37 samples that were culture positive for *Mycobacteria*, none resulted in the isolation of *M. bovis* using both biochemical and PCR detection methods. The sampling strategy used implies that we can be 95% confident that the maximum prevalence of bovine TB in the district is not greater than 2% (Cannon and Roe, 1982). It is useful to mention here that *M. bovis* was also not isolated using molecular diagnostic methods in another recent extensive survey by researchers at KEMRI and UK collaborators among human populations in North Eastern Province in Kenya (Githui et al., 2000) also did not identify any TB case associated with *M. bovis*.

Though these findings vindicate the long held official position indicating absence of bovine tuberculosis in Kenya, they need to be verified and periodically monitored in other areas, given the risk posed by frequent movement of pastoral livestock across borders from neighbouring countries. If *M. bovis* were present in Kenya, those at the greatest risk of acquiring BTB would be those pastoralists who consume raw milk and/or other raw animal products.

3.4. Anti-microbial Residues

Antimicrobial (antobiotic and synthetic antibacterials) agents in milk are undesirable because of their negative health effects on individuals and communities continually exposed to such risks. These include hypersensitivity, drug resistance and specific tissue damage (Schultz *et al*, 1963; Moffit *et al*, 1974; Oslon and Sanders, 1975; Nijsten *et al*, 1996). Antimicrobial abuse promotes drug resistance by killing all but the most potent bacteria strains. This puts selective pressure on microbial evolution and helps create 'superbugs' that are immune to attack by common, less expensive antibiotics. They also inhibit organisms required in the processing of cultured milk products. Antimicrobial residues in milk most often originate from farm-level practices with

regard to non-observance of withdrawal periods after anti-microbial therapy. In Kenya, unconfirmed reports indicated that unscrupulous milk market agents might be using bacterial growth inhibitors, including hydrogen peroxide and antibiotics, to lengthen the shelf-life of marketed milk. Penicillin residues have been demonstrated in 1.2% of milk deliveries at Kenya Co-operative Creameries (Chewulukei, 1978) and general veterinary drugs have been found in slaughter-house meat (Mdachi and Murilla, 1991 and Muriuki, 1992).

This study screened five common families of anti-microbials using the Charm AIM anti-microbial inhibition screening kit (Charm Sciences Inc., USA) that detects any of a range of commonly used antimicrobials. A more specific test, Charm ROSA, was used to assess the presence of β-lactams and tetracyclines specifically. To measure agreement, both tests were experimentally compared by testing milk samples from eight lactating dairy cows injected with therapeutic doses of intra-mammary and intra-muscular preparations of Penicillin G and 10% oxytetracycline. One pre-treatment and five post-treatment milk samples were collected daily from the eight cows and tested.

The use of hydrogen peroxide was not evaluated in the laboratory because the practice was found to be non-existent among small market agents during PRAs.

Results and discussion

Overall, 37 (9.4%) and 27 (5.7%) of consumer- and market-level samples, respectively, were positive on the Charm AIM test (Table 3.12), indicating that a consumer who takes milk daily is at risk of consuming milk with drug residues at least twice every month.

Table 3.12. Numbers and proportions of consumer- and market-level samples testing positive for anti-microbials on Charm AIM test in both seasons.

Source of sample	n	%
Consumer households		
Urban consumers (Nairobi and Nakuru)	8	4.0
Rural consumers (Nakuru)	29	15.0
Informal market agents in Nairobi/Kiambu (IHMA)		
Coops/collection centres centres/Self-help groups	1	1.5
Milk Bars	10	9.4
Milk Shops/kiosks	5	5.5
Small mobile traders	4	7.1
Informal market agents in Nakuru/Narok (EMMA)		
Coops/collection centres centres/Self-help groups	0	0
Milk Bars	2	3.8
Milk Shops/Kiosks	0	0
Small mobile traders	5	10.0
Pasteurised milk in Nairobi & Nakuru	9	8.2

The proportion of consumer-level samples from rural areas with antibiotic residues were three times those from urban areas. Among informal market level samples, the proportion testing positive for residues decreased with increasing levels of bulking with milk bars and small mobile traders having a much higher proportion of samples with anti-microbials compared to samples from dairy co-operatives. This perhaps indicates dilution of the residues to below threshold levels for detection by the test. Nine out of 110 (8.2%) pasteurised milk samples had residues.

Whereas agreement between the two tests was inconclusive, these results indicate that the problem of anti-microbial residues in milk needs to be tackled at both the farm and market levels. To begin with, there is the need to define farm-level causal relationships to complement these data. Such information would be necessary to devise appropriate measures to reduce residues at both these levels.

The higher proportion of consumer-level milk samples with anti-microbial residues as detected by the Charm-AIM test kit seems to indicate that the residues are more likely to originate at the farm-level than due to bad market-level practices. On the other hand, the increased residues as milk moves up the market chain and bulking occurs (as indicated by pasteurised milk) seems to suggest that anti-microbial agents may be added after the first milk sale transaction. Any dilution effects of bulking on anti-microbial residue levels need further investigation.
These apparent high levels of antibiotic residues in marketed milk, as detected by the Charm-AIM test, need to be evaluated against the background of the results of the Charm-ROSA test and the experiment conducted to compare the two tests. None of the consumer- and market-level milk samples collected from the field that were positive on the Charm-AIM test was positive on the Charm-ROSA test as well. And in the experiment, the Charm-AIM test classified as many as seven out of eight samples as having Penicillin G or oxy-tetracycline residues up to the fifth day post-drug administration, compared to the Charm-ROSA test that classified only one by the same day.

Given that all the bacterial health risks can be eliminated by the common consumer practice of boiling (see section 6.2.2), anti-microbial residues stands out as the major health risk identified in this study that cannot be eliminated by heat treatment. Though most reports of the negative effects anti-microbial abuse have usually been attributed to mis-use of human drugs, these findings suggest that potential risks emanating through milk (and probably other animal product foods as well) are high and should be given urgent attention.

4. MARKET RISK FACTORS: DESCRIPTIVE RESULTS AND DISCUSSION

4.1. Major milk marketing pathways

Most milk samples (57.4%) were directly sourced from farms and did not pass through intermediaries. Table 4.1 shows the source for milk samples by type of trader/respondent. All pathways with more than nine observations were included in subsequent descriptive and statistical analyses. Own and another's farm sources were considered the same source.

Table 4.1. Milk marketing channels and respondents (both seasons)

Trader/Respondent	Source of milk				
	Own Farm	Another's Farm	Coops/ SHGs	Small Traders	Overall (%)
Coops/SHGs	0	**69**	2	0	71 (13.7%)
Milk bar	**19**	**79**	**39**	**27**	164 (34.3%)
Shop/Kiosk	**6**	**68**	**23**	**36**	133 (27.1%)
Mobile trader	**3**	**97**	9	5	114 (24.9%)
Total (%)	**28** (6.3%)	**313** (51.1%)	**73** (13.2%)	**68 (13.4%)**	481 (100%)

Descriptive statistics by the various milk-marketing pathways are in Table 4.2. A distinction is made between pathways without intermediaries (i.e., milk originating directly from farms) and those with one or more intermediaries (i.e., from another market agent).

Table 4.2. Descriptions of continuous variables by various pathways

Variable	***Pathways without intermediaries***			***Pathways with ≥ 1 intermediary***
	Farm-Farmer group (n=86)	Farm-small traders (all) (n=231)	Overall (N=439)	Farm-intermediary (ies) -small milk traders (n=129)
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Time since collection (hrs)	1.6 (2.0)	1.5 (1.7)	1.5 (1.8)	1.5 (1.7)
Distance from collection point (km)	19.2 (18.2)	29.5 (37.8)	29.6 (34.0)	36.5 (35.4)
No. of sources bulked (lts)	75.8 (412.2)	3.5 (7.2)	24.1(220)	3.5 (9.0)
Amount traded/day (lts)	2326 (6319)	114.0 141.1)	466.1 (2617)	139 (330.2)
Period in business (yrs)	15.6 (15.6)	2.5 (2.9)	5.0 (8.9)	2.6 (3.8)
Margin (Ksh/Lt)	1.0 (7.0)	5.4 (6.8)	4.2 (8.1)	4.3 (10.0)
Total Plate Counts /ml '000 [a]	19,900	50,100	39,800	50,100
Coliform Plate Counts /ml '000 [a]	20	79	50	50
Solids-not-fat (SNF) %[b]	8.6	8.5	8.6	8.6

[a] Standard deviations (SD) for geometric means bacterial counts were between 20-28% about the mean.
[b]SD for all SNF means was 0.5.

Among those without intermediaries, a distinction is made between those supplying large scale traders (farmer-groups and those supplying small traders (milk-bars, shops/kiosks and mobile traders). Though milk from those with one or more intermediaries travelled longer distance (and taken longer time) relative to milk from pathways without intermediaries, the average bacterial counts were generally similar. This may reflect bacterial counts whose growth had reached the stationery phase. The same descriptive statistics are given by trader type in Table 4.3.

Table 4.3: Descriptions of continuous variables by market agents

Parameter	Milk market agents				
	Farmer grp (n=55)	Mobile trader (n=113)	Shop/kiosk (n=121)	Milk bar (n=150)	Overall (N=439)
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Time since collection (hrs)	1.8 (2.2)	1.4 (1.6)	1.4 (2.0)	1.5 (1.3)	1.5 (1.7)
Distance from collection point (km)	17.7 (11.5)	24.4 (28.6)	32.5 (39.9)	35.2 (37.9)	29.6 (34.0)
No. of sources bulked (lts)	103.1 (480)	4.0 (8.9)	2.6 (5.9)	3.5 (7.8)	17.4 (183.2)
Amount traded/day (lts)	3743 (7697)	94.6 (113.5)	63.5 (71.1)	162.6 (183.5)	466.1 (2617)
Period in business (yrs)	21.4 (5.3)	3.1 (3.4)	1.8 (1.8)	2.4 (2.9)	5.0 (8.9)
Margin (Ksh/Lt)	1.7 (3.9)	6.9 (4.6)	4.6 (11.4)	2.8 (6.4)	4.2 (8.1)

4.2. Scale of business

The average amount of milk traded by small traders (small mobile, milk bars and shops/kiosks) ranged from 64-163 litres/day and these were bulked from an average of 3.3 (SD=7.6) sources, while farmer groups traded much higher quantities (mean=3,743 litres; SD=7,697) bulked from hundreds of farmers.

4.3. Time and distance to retail outlets

On average, milk traders travelled about 30 km (SD=34.0) and were sampled at their retail points within 1.5 hours (SD=1.7) of milk collection (Table 4.3). Those that sourced milk from other market intermediaries were therefore sampled after about 3 hrs since milk collection from farmers. Milk sold in milk-bars travelled the farthest (mean=35.2km; SD=37.9).

Figure 4.1 indicates that by the time of sampling (8-10am), more than half of milk samples did not meet the KEBS standard for total plate counts of 2,000,000 cfu/ml ($\log_{10}$6.3) and nearly half of the samples did not meet the KEBS standard for coliform counts of 50,000cfu/ml ($\log_{10}$4.7). Since most milk samples were collected by 10.00am each morning, the high bacterial counts suggest more than 5 hours had elapsed between milking and sampling, enough generation time for a bacterial cell to reach the exponential or stationery phase of growth.

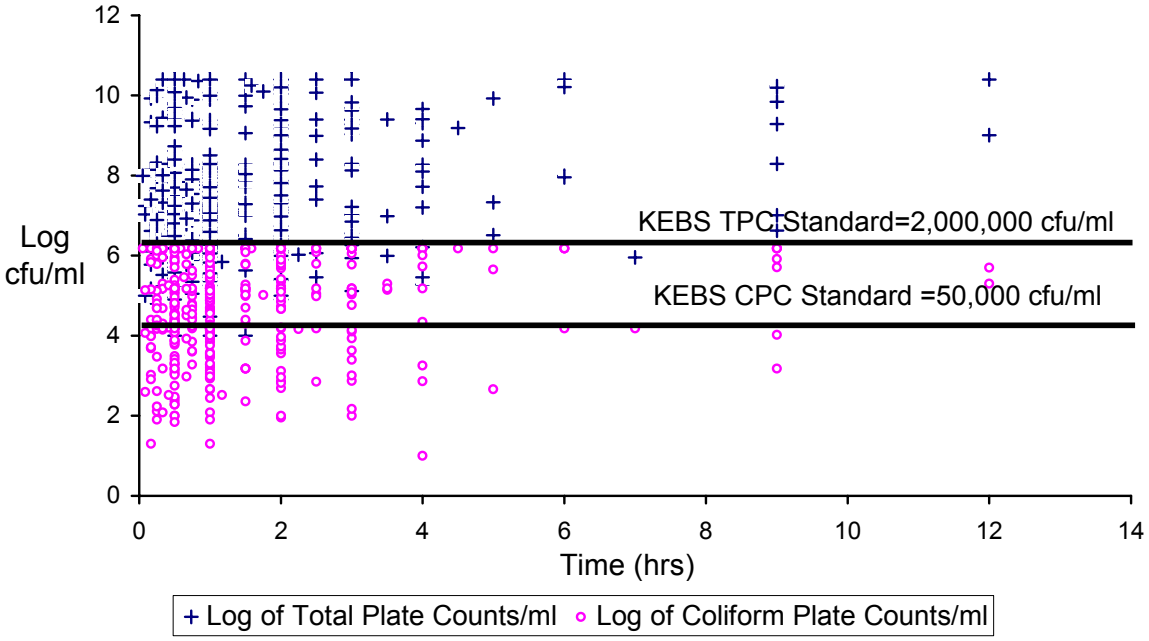


Figure 4.1. Bacterial counts vs time since milk collection by market agents

4.4. Profit margin

The overall profit margin per litre of milk ($\prod$)[14] was KSh. 4.20/litre (SD=8.10) (Table 4.3). Smaller business selling lower quantities of milk generally enjoyed higher profits. Small mobile traders enjoyed the highest profits (KSh 6.90/litre) while dairy coops had the lowest profits (KSh. 1.70/litre).

4.5. Milk handling

Methods of milk handling were markedly different between scales of business (Table 4.4). The use of plastic containers was recorded because many are not food-grade quality besides being more difficult to clean compared to metal containers. Smaller market agents used more plastic containers (up to 89% for mobile agents) than larger scale market agents such as dairy cooperatives that used plastic containers in only 10% of cases, the rest being mainly aluminium metal churns. Three quarters of all market agents dispense milk by pouring versus scooping. The method used to dispense milk was considered to have implications on milk quality because of the possibility of recontamination if milk is scooped repeatedly.

[14] Profit margin ($\prod$) per litre of milk for each market agent was estimated by the following formula from annualised figures: $\prod$ = total revenue – total cost (cost of procuring milk + fixed cost + intermediary costs + statutory costs + labour costs + rent + contingency fees). The cost of procuring milk was the product of quantity purchased x procurement price + fare. The annual equivalent value of fixed assets (capital recovery cost) was calculated using the formula: $A = Z[\frac{(1+i)^{ni}}{(1+i)^{n}-1}]$

Where: A = capital recovery cost of item; z (replacement cost of the item) = initial cost – salvage value (includes zeros); *i* = real rate of return on capital invested elsewhere and n = useful life of the item (excludes zeros).

Table 4.4: Descriptions of categorical variables by market agents

Variable	Market agents									
	Farmer group		Milk bar		Shop/kiosk		Small mobile trader		Overall	
	n	%	n	%	n	%	n	%	N	%
Container type										
Plastic only	6	10	59	37	90	69	101	89	258	55
Metal only	56	86	70	43	21	16	7	6	154	32
Plastic and metal	1	2	33	20	17	13	4	4	55	12
Other combination	1	2	0	0	2	2	2	2	5	1
Method of dispensing milk										
Scooping	20	28	59	36	25	18	17	15	121	25
Pouring	51	72	107	64	112	82	98	85	369	75
Quality test before receiving										
None	6	9	19	12	30	23	31	27	86	18
One or more tests done[15]	59	91	143	88	100	77	83	73	386	82
Quality test prior to sale										
None	27	62	27	16	34	25	36	31	124	25
One or more tests done	44	38	139	84	103	75	79	69	366	75
Fate of left-over milk										
Thrown away	1	20	1	1	1	3	0	0	3	2
Used by family or sold	4	80	68	99	37	97	20	100	209	98
Method of preservation										
Not treated	39	55	15	9	27	20	55	47	127	28
Boiling	3	4	38	23	48	35	6	5	95	19
Refrigeration/Chilling	25	34	109	66	56	41	52	45	242	47
Antibiotics added	0	0	0	0	0	0	0	0	0	0
Hydrogen peroxide added	0	0	1	1	0	0	1	1	2	2
Lactoperoxidase added	0	0	0	0	0	0	0	0	0	0
Other additives	4	7	3	2	6	4	1	1	14	3
Water source										
Piped/tap	29	41	154	93	111	82	102	89	397	81
Other (e.g. river, roof, well)	42	59	12	7	25	18	13	11	92	19
Mode of cleaning containers										
Cold water alone	2	3	0	0	2	1	2	2	6	1
Hot water alone	8	12	2	1	4	3	1	1	15	3
Cold water with soap/disinf.	16	20	8	5	5	4	6	5	36	7
Hot water with soap/disinf.	45	45	150	90	222	89	102	89	426	89
Other	0	0	5	4	4	3	4	3	13	11
Training										
None	38	57	147	91	129	95	107	96	421	88
One or more months	29	43	14	9	7	5	5	4	55	12

[15] Tests included adulteration (lactometer test) organoleptic (smell, taste), alcohol, boiling. Match stick and temperature

On average, 28% of milk from all traders was not treated for preservation in any way, 17% was refrigerated/chilled and 19% was boiled. Though the boiling was mainly done to lengthen shelf life, all bacterial pathogens are killed in the process (Table 4.4). Notably, hardly any chemical preservatives were recorded as being used to lengthen shelf-life. Only 2% of traders indicated that they used hydrogen peroxide (one milk-bar and one large scale mobile trader) and none indicated addition of lactoperoxidase or anti-microbials though 3% said they use other unspecified preservation methods. Piped water source was reported more often (80%) than water from other sources, especially by mobile traders (89%). Alternative water sources were wells, roof catchments and rivers. Many traders indicated that they used hot water and soap/disinfectant to clean containers (overall mean proportion= 89%), indicating a conscious effort by the majority to reduce spoilage and improve hygiene. This practice needs to be reinforced besides other improved handling practices.

4.6. Training and experience

Overall, only 12% of milk handlers had received any form of training in milk handling and quality control but this had a wide range from only 4% of mobile traders to 43% of dairy cooperative staff (Table 4.4). Small traders had been in business for about 2.5 yrs (SD=2.9), substantially less than farmer groups (mean=21.4yrs; SD=5.3) (Table 4.3). This may indicate a high turnover in the milk market business, or an expanding market with several recent entrants. These factors need to be considered in any milk hygiene improvement efforts.

4.7. Post-harvest losses

Post harvest losses incurred in the market may be roughly assessed through the fate of leftover milk from previous day's sales. On average, one in every four traders of all cadres recorded leftovers of about 7% of the volume of previous day's milk sales. However, only 2% of traders recorded leftover milk that was thrown away from previous day's milk collection (Table 4.4). The rest of leftover milk was consumed by the family or sold. This is likely to be forced consumption or sale at a lower price. Table 4.5 and 4.6 gives the amounts left over by major milk market agents and pathways, respectively.

Table 4.5: Descriptions of amount of left over milk by market agents

Parameter	Milk market agents				
	Farmer grp (n=7)	Mobile trader (n=21)	Shop/kiosk (n=37)	Milk bar (n=70)	Overall N=137)
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Amount left-over yesterday (lts)	454 (755)	2.9 (2.4)	11.2 (27.6)	21.1 (34.6)	38.0 (189.2)

Table 4.6. Descriptions of amount of left over by various pathways

Variable	*Pathways without intermediaries*			*Pathways with ≥ 1 intermediary*
	Farm-Farmer group (n=15)	Farm-small traders (n=75)	Overall (n=90)	Farm-intermediary (ies) -small milk traders (n=45)
	Mean (sd)	Mean (sd)	Mean (sd)	Mean (sd)
Amount left-over previous day (lts)	156.6 (513.0)	15.0 (33.3)	38.6 (212.4)	36.7 (133.1)

4.8. Major constraints

The five most commonly cited constraints by traders are given in Table 4.7. Competition was the most commonly cited constraint followed by spoilage, harassment, seasonality and trasportation problems. Trasportation problems were mainly due to bad roads. Mobile milk traders mainly cited harassment.

Table 4.7. Ranking of the five most frequently cited constraints

Constraint	% traders citing constraint under each rank				Most affected trader type and proportion citing constraint (%)	
	1	2	3	4		
Competition %	53	30	12	6	Milk-bars	(40%)
Spoilage %	39	25	25	11	Milk bars	(42%)
Harassment %	38	35	18	9	Mobile traders	(46%)
Seasonality %	41	32	18	8	Coops	(41%)
Transportation %	29	35	22	14	Milk-bars	(30%)

Table 4.8 shows subjective scores (see key below the table) of market agents and retail premises. Over 74% of milk handlers and over 65% of premises were judged as good or very good, respectively.

Table 4.8. Personal hygiene and premises cleanliness scores

Trader	Personal hygiene score (%)			Premises cleanliness score (%)		
	1-2	3	4	1-2	3	4
Coops/Collection Centres/SHGs	78	18	4	80	18	2
Milk-bars/Snack-bar	86	13	1	79	19	1
Milk-shops/Kiosks	75	25	0	65	29	6
Small mobile traders	74	26	0	-	-	-

Key

Score	Personal Hygiene	Cleanliness of premises
1 = Very good	Clean protective clothing, wearing hat/head dress, boots, good health	Wall tiles/white wash walls, ceiling board, formeca counter, clean storage, running water
2 = Good	Clean non-protective clothing, wearing hat/head dress, normal shoes, no signs of ill health	Concrete floor, normal walls, no ceiling board, clean surfaces, ordinary wooden counter, clean storage, water available
3 = Fair	No hat, no shoes, not ill	Concrete floor, normal walls, no ceiling board, clean surfaces, ordinary wooden counter, clean storage, no water
4 = Poor	Dirty, no hat, no boots, no shoes, signs of ill health	Non-concrete floor, mud walls, no roof, dirty surfaces and equipment, no water

5. REGRESSION AND MULTIVARIATE ANALYSES – RESULTS AND DISCUSSION

This section first assesses variation between levels of stratification, correlations among key variables (to identify and deal with potential problems of collinearity), and investigates risk factors in the informal milk marketing environment for each health hazard (total and coliform bacterial counts, adulteration, brucella antibodies and antimicrobial residues). These were done in SAS using relevant statistical procedures (PROC VARCOMP, PROC CORR and PROC REG).

Second, multivariate analyses were conducted for market-level variables and milk quality indicators by factor and cluster analysis (Gockowski and Baker, 1996) using PROC FACTOR and PROC FASTCLUS in SAS. The two procedures were used to calculate associations among principal components[16] and clusters, respectively. Variables included were those with significant association to milk quality indicators of bacterial counts and adulteration. These were market access strata, market pathways, milk handling practices, experience, time, and profit margin.

Table 5.1. Variance components of each hazard attributable to each site, area and season (Restricted Maximum Log-likelihood Estimation)

Level	Hazard							
	Log total counts/ml	%	Log coliform counts/ml	%	SNF	%	MRT/ ELISA +ve	%
Study Site[a]	0.03	1	0	0	0	0	0	0
Area (Division)	0.43	16	0.16	11	<0.01	7	<0.01	0
Season	0.33	12	0.03	2	<0.01	2	<0.01	1
Error	1.89	70	1.28	87	0.04	90	0.01	99

[a]Kiambu and Nairobi districts (were classified into one category representing high production intensity and market access) vs Nakuru and Narok districts (classified as extensive production and medium market access)

Differences within sites (division-to-division) for bacterial counts and solids in milk accounted for more variation than between sites. Seasonal variation was minimal except for total bacterial counts (12%).

5.1 Correlation and Regression analysis

Correlation and regression analyses are presented in Tables 5.2 and 5.3, respectively. Prior to regression analyses, variables that were highly correlated in the correlation table and assessed to measure the same effects as other variables were dropped. Total and coliform bacterial counts were normalised through logarithmic (base 10) transformation before analysis.

[16] A principal component is a linear combination of variables with coefficients equal to the eigenvectors (customarily taken with unit length) of the correlation or covariance matrix. Eigenvectors correspond to each of the eigenvalues and associated principal components and are used to form linear combinations of the Y variables.

Table 5.2. The results of correlation analysis of the full dataset showing correlations at *p*<0.01 *p*<0.05 and *p*<0.10

	Log of Total PC	**Log of Coliform PC**	Wet Season	Intensive / high mkt access	Farm-Coop	Farm-Milk bar	Farm-Shop/ kiosk	Farm-Mobile trader	Coop-Milk bar	Coop-Shop/ kiosk	Coop-Mobile trader	Mobile trader-Milk bar	Mobile trader – Shop/ kiosk	Hrs since collect-ion	Distance (Km)	**SNF**
Log of Total PC																
Log of Coliform PC	**+++**															
Wet Season	**+++**	**++**														
Intensive/high mkt access	**+++**															
Farm-Coop	**---**	**---**		++												
Farm-Milk bar	**+**			---	++											
Farm-Shopkiosk			++		+++	++										
Farm-Mobile trader				---												
Coop-Milk bar				+++	---	---	---	---								
Coop-Shop/kiosk				+++	---	---	--	---	++							
Coop-Mobile trader	**++**			++	---	-	-	--	+++	+++						
Mobile trader-Milk bar			+++	++	---	---	---	---								
Mobile trader_Shopkiosk				++	---	---	---	---	-							
Time since collection (hrs)	**+++**		+													
Distance (Km)	**+++**	**+++**		+++	---			++	+			+++		+++		
Solids-not-fat (**SNF)**	**+++**		**+++**				**--**									
Warm weather				+++												
Milk separated			---		---		---		+++				++		++	
Quality test before receiving			-	+++			---	---			++					
Quality check before sale	**+++**		+	+++	+++	++					+					
Milk preservation			+	+++	+++	---	--	++	---		+++	--	--			
Plastic container		**+++**		---	---	---		---	---		-		---			
Piped water	**++**	**+++**	-			++		+++	+++			++			+++	
Scoop vs pouring				+++					+++			+++				
Training		**++**	++	+++	+++	--		---		-			-	+++		
Drugs								**++**								
Milk sale volume (lts)					++									+++		
Period in business	**-**	**---**			+++		---	---					-		---	
Number bulked			+		+++									+++		**+++**
Brucella ab +ve			**+**			**+**										
Margin/Lt (KSh)	**-**		**--**	**---**	**---**	**---**	**-**	**+**					**+**			**-**

Key: Positive correlation at p<0.01 (+++), p<0.05 (++) and p<0.10 (+); Negative correlation at p<0.01 (---), p<0.05 (--) and p<0.10 (-)

Table 5.2. cont'd

	Hot weather	Milk separated	Quality check before receiving	Quality check before sale	Milk preservation	Plastic container	Piped water	Scoop vs pouring	Training	**Drugs**	Milk sale volume (lts)	Period in business	Number bulked	**Brucella ab +ve**
Milk separated	-													
Quality test before receiving	+++	++												
Quality check before sale	+++	--	+++											
Milk preservation	+	---	+++	+++										
Plastic container		+++	+++											
Piped water		+++		---	+++									
Scoop vs pouring	+++			+++		+++	++							
Training		--	+			+++	---	+						
Drugs	**+++**	**++**	**++**				**+**		**-**					
Milk sale volume (lts)		-				+++	---		+++					
Period in business		---	-				---		+++		+++			
Number bulked	+					++	--		+++		+++	+++		
Brucella ab +ve														
Margin (Ksh/lt)							**++**	**--**	**--**			**--**		

Key: Positive correlation at p<0.01 (+++), p<0.05 (++) and p<0.10 (+) ; Negative correlation at p<0.01 (---, p<0.05 (--) and p<0.10 (-)

Table 5.3. OLS model explaining associations between risk factors and milk quality

Parameter	TPC R^2 = 0.61; F(32,299)=14			CPC R^2 = 0.56; F(32,299)=11			SNF R^2 = 0.15 F(32,299)=5.9		
	Est.	S.E.	*p*-value	Est.	S.E.	*p*-value	Est.	S.E.	*p*-value
Intercept	2.92			1.36			8.5		
Study strata									
IHMA	**1.01**	**0.30**	**<0.01**	-	-	>0.10	0.15	0.05	0.01
Wet Season	**0.69**	**0.13**	**<0.01**	**-0.25**	**0.11**	**0.02**	**0.13**	**0.06**	0.05
Quality measures									
Log of TPC	N/A	N/A	N/A	**0.51**	**0.04**	**<0.01**	-	-	>0.10
Log of CPC	**0.88**	**0.06**	**<0.01**	N/A	N/A	N/A	-	-	>0.10
Solids-not-fat (SNF)	**0.74**	**0.35**	**0.04**	-	-	>0.10	N/A	N/A	N/A
Antimicrobials present	-	-	>0.10	-	-	>0.10	-	-	>0.10
Market pathways									
Farm-Milk bar[a c]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Farm-Shopkiosk[a]	**0.44**	**0.25**	**0.08**	**-0.43**	**0.19**	**0.03**	-	-	>0.10
Farm-Mobile trader[a ,d]	-	-	>0.10	-	-	>0.10	-	-	>0.10
IHMA*Farm-Coop[b]	-	-	>0.10	**-0.44**	**0.23**	**0.06**	-	-	>0.10
IHMA*Farm-Milk bar[b]	**0.66**	**0.34**	**0.05**	-	-	>0.10	-	-	>0.10
IHMA*Farm-Shop/kiosk[b]	-	-	>0.10	**0.49**	**0.26**	**0.06**	-	-	>0.10
IHMA*Farm-Mobile trader[b]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Coop-Milk bar[a]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Coop-Shop/kiosk[a]	-	-	>0.10	-	-	>0.10	**0.26**	**0.15**	**0.09**
Coop-Mobile trader[a]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Mobile trader-Milk bar[a]	-	-	>0.10	**-0.55**	**0.23**	**0.02**	-	-	>0.10
Mobile trader-Shop/kiosk[a]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Milk handling									
Milk separated[d]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Quality test done	-	-	>0.10	-	-	>0.10	-	-	>0.10
Milk preservation	-	-	>0.10	**-0.23**	**0.14**	**0.09**	-	-	>0.10
Plastic container	-	-	>0.10	-	-	>0.10[e]	-	-	>0.10
IHMA*Plastic container[b]	**-0.65**	**0.31**	**0.04**	-	-	>0.10	-	-	>0.10
Piped water	-	-	>0.10	**0.31**	**0.15**	**0.03**	-	-	>0.10
Scoop vs pouring	-	-	>0.10	-	-	>0.10	-	-	>0.10
IHMA*scoop[b]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Experience									
Training	-	-	>0.10	-	-	>0.10	-	-	>0.10
Period in business	-	-	>0.10	-	-	>0.10	-	-	>0.10
Other covariates									
Warm weather[d]	-	-	>0.10	-	-	>0.10	-	-	>0.10
Time since collection (hrs)	**0.09**	**0.04**	**0.01**	-	-	>0.10	-	-	>0.10
Milk sale volume (lts)	-	-	>0.10	-	-	>0.10	-	-	>0.10
Number bulked (units)	-	-	>0.10	-	-	>0.10	-	-	>0.10
Margin (Ksh/Lt)	**-0.02**	**0.01**	**0.09**	-	-	>0.10	-	-	>0.10

[a] Compared to Farm-Coop pathway; [b] Compared to interaction between EMMA and factor
[c] Variable associated (p<0.10) with brucella antibodies (OR=8.0) and
[d] Positively associated with antimicrobials at p<0.10): ORs for Farm-Mobile milk trader pathway, milk separated and warm weather were 2.7, 2.4 and 5.4, respectively. [e]Significant association using the complete dataset (p = 0.04).

It is evident from the simple correlation analysis in Table 5.2 that both measures of spoilage (total bacterial counts) and hygiene (coliform counts) had strong negative correlation with farmer-to-coop market channels. There were significant strong positive correlations between

total bacterial counts and season, site, distance and time factors. Coliform counts were mainly correlated with handling factors such as use of plastic containers and piped source of water. The regression analysis in Table 5.3 shows that pathways involving mobile milk traders (who currently do not qualify for licences) or milk originating from them were not associated with worse milk quality (high total and coliform counts) than other small traders with fixed premises such as milk bars or kiosks/shops. Increased solids in milk, as measured by SNF, had strong positive correlation with season, IHMA and milk sold at milk bars (collected from farms) and kiosks (collected from coops), and strong negative correlation with profit margin. The strong association between higher SNF and lower profits may indicate the non-effectiveness of milk quality checks currently practiced. The various pathways had significant positive correlations with milk quality testing (mostly by lactometer) and whether some method was used to preserve milk.

Factors associated with indicators of milk quality (TPC, CPC and SNF) in Table 5.3 were included in principal component analysis. The means of variables included in the analysis are in Table 5.4.

Table 5.4. Means of 353 milk quality and market handling variables available for analysis to identify principal components and clusters

Variable	Mean (n=353)	SD
Study stratum		
IHMA	0.58	0.49
Quality measures		
Log of Total Plate Counts	7.65	1.59
Log of Coliform Plate counts	4.80	1.16
Solids-not-fat (SNF)	8.56	0.49
Market pathways		
Farm-Coop	0.18	0.38
Farm-Milk bar	0.26	0.41
Farm-Shopkiosk	0.21	0.41
Farm-Mobile trader	0.29	0.45
Coop-Milk bar	0.08	0.26
Coop-Shop/kiosk	0.05	0.21
Coop-Mobile trader	0.03	0.16
Mobile trader-Milk bar	0.06	0.24
Mobile trader-Shop/kiosk	0.07	0.25
Milk handling		
Milk separated	0.44	0.50
Quality check before sale	0.27	0.44
Milk preservation	0.28	0.45
Plastic container	0.58	0.49
Piped water	0.82	0.38
Experience		
Training	0.14	0.34
Period in business	5.31	9.63
Other covariates		
Time since collection (hrs)	1.48	1.82
Margin (Ksh/Lt)	4.21	7.67

5.2. Principal component analysis of milk quality

The variables in Table 5.4 were included in principal component analysis following the method described by Gockowski and Doyle, (1996), Staal et al., (1998) and Vidal et al., (2000) by selecting those eigenvalues with principal components greater than one (Table 5.5). The eigenvalues correspond to each of the principal components and represent a partitioning of the total variation in the sample of market agents. Since the associated eigenvectors are orthogonal, the principal components represent jointly perpendicular directions through the space of the original variables and are uncorrelated with each other. The first nine principal components were rotated through varimax rotation option to improve interpretability.[17]

Table 5.5. Principal components associated with milk quality

Principal Component	Eigenvalue	% of total variation	Cumulative % of total variation
1.00	2.83	0.13	0.13
2.00	2.03	0.10	0.23
3.00	1.70	0.08	0.31
4.00	1.60	0.08	0.39
5.00	1.39	0.07	0.45
6.00	1.19	0.06	0.51
7.00	1.18	0.06	0.57
8.00	1.06	0.05	0.62
9.00	1.00	0.05	0.67

The nine principal components with eigenvalues greater than one together explain 67% of total variation, with the strongest principal component explaining a relatively modest proportion of total variation of 13%. The low proportional values illustrate the lack of close association between the individual variables or sets of variables.

The orthogonal rotated correlation coefficients of the original variables are shown in Table 5.6. The coefficients with weights >0.5 were used to define the axes extracted.

[17] The rotated principal components are also uncorrelated after an orthogonal transformation.

Table 5.6. Results of the principal component analysis showing weights of first nine axes extrated following varimax rotation[a]

Factor	F1	F2	F3	F4	F5	F6	F7	F8	F9
Variable	**LSEXP**	**LMQ**	**COOPINT**	**NOINT1**	**HMGN**	**MOBINT1**	**MOBINT2**	**NOINT2**	**LTIME**
Study stratum									
Intensive/high mkt access	-	-	-	-	-	-	-	-	-
Quality measures									
Log of Total PC	-	0.89	-	-	-	-	-	-	-
Log of Coliform PC	-	0.85	-	-	-	-	-	-	-
Solids-not-fat (SNF)	-	-	-	-	-	-	-	-	0.74
Market pathways									
Farm-Coop	0.70	-	-	-	-	-	-	-	-
Farm-Milk bar	-	-	-	-	-0.54	-	-	-	-
Farm-Shopkiosk	-	-	-	-	-	-	-	0.52	-
Farm-Mobile trader	-	-	-	0.60	-	-	-	-	-
Coop-Milk bar	-	-	-	-0.51	-	-	-	-	-
Coop-Shop/kiosk	-	-	0.68	-	-	-	-	-	-
Coop-Mobile trader	-	-	0.65	-	-	-	-	-	-
Mobile trader-Milk bar	-	-	-	-	-	0.89	-	-	-
Mobile trader-Shop/kiosk	-	-	-	-	-	-	0.89	-	-
Milk handling									
Milk separated	-	-	-	-	-	-	-	0.74	-
Milk preservation	-	-	-	0.65	-	-	-	-	-
Plastic container	-	-	-	0.61	-	-	-	-	-
Piped water	-0.58	-	-	-	-	-	-	-	-
Experience									
Training	0.69	-	-	-	-	-	-	-	-
Period in business	0.84	-	-	-	-	-	-	-	-
Other covariates									
Time since collection (hrs)	-	-	-	-	-	-	-	-	0.66
Margin (KSh/Lt)	-	-	-	-	0.70	-	-	-	-

[a] Only Weights >0.5 or <0.5 are presented and corresponding variables used to define respective axes

Key:

LSEXP: Large-scale & experience
LMQ: Low Milk Quality
COOPINT: Coop Intermediary
NOINT1: No Intermediary1
HMGN: High Margin
MOBINT1: Mobile Intermediary1
MOBINT2: Mobile Intermediary2
NOINT2: No Intermediary2
LTIME: Long time since collection

The first axis extracted (LSEXP) is highly correlated with milk sales by experienced market agents (training and long period in business), non-use of piped water and dairy cooperatives. The second axis (LMQ) is highly correlated with high bacterial counts and hence poor milk quality. The third axis (COOPINT) separates out sales through dairy cooperative intermediaries. Sales without intermediaries are divided into two axes, NOINT1 (fourth axis) and NOINT2 (eighth axis), depending on specific milk handling practices, mainly use of plastic containers or milk separation, respectively. Sales through mobile trader intermediaries are also divided into two axes, MOBINT1 (sixth axis) and MOBINT2 (seventh axis), depending on whether the milk is retailed at milk-bars or shops/kiosks, respectively. The fifth axis (HMGN) is highly correlated with high profits and non milk-bar sales. The last (9^{th}) axis extracted (LTIME) is highly correlated with long duration since milk collection and high solids in milk.

5.2. Clustering analysis

The FASTCLUS procedure in SAS was then used to assign a cluster to each observation to try to find homogenous groupings of market agents. The procedure employs a standard iterative

algorithm for minimising the sum of squared distances from the cluster means. Each observation is assigned to only one cluster. Several clusters were investigated and 6 clusters finally chosen to differentiate the observations along the 9 axes selected (Table 5.7). The variables have mean 0 and variance 1. Positive means indicate levels higher than the overall sample means and vice versa for negative means.

Table 5.7. Clustering of 353 market agents using new variables

		Means									**Relative Scale of Business**
Cluster	Freq	**LSEXP**	**LMQ**	**COOPINT**	**NOINT1**	**HMGN**	**MOBINT1**	**MOBINT2**	**NOINT2**	**LTIME**	(Litres sold/day)
1	**22**	**-0.31**	**0.29**	-0.19	0.04	**-1.47**	**0.29**	0.15	0.23	**0.48**	**Small** (44)
2	**158**	**-0.25**	0.06	0.16	-0.15	-0.19	0.06	0.03	0.21	0.03	**Small** (126)
3	**120**	**-0.37**	-0.01	-0.19	0.18	**0.58**	-0.18	-0.07	-0.17	0.08	**Small** (108)
4	**25**	**2.74**	**-0.29**	-0.22	-0.24	0.11	-0.10	-0.06	**-0.64**	0.07	**Large** (5,536)
5	2	-0.75	-1.37	-0.63	-0.24	3.62	-0.64	0.86	1.21	0.91	
6	3	0.42	1.22	0.19	0.92	-3.87	0.17	0.57	0.43	-0.11	
7	**22**	**0.89**	**-0.36**	**0.39**	0.27	-0.21	**0.35**	-0.18	-0.16	-0.03	**Medium** (367)

NB. Significant clusters and mean values in respective axes are bolded

Key:

LSEXP: Large-scale & experience
LMQ: Low Milk Quality
COOPINT: Coop Intermediary
NOINT1: No Intermediary1
HMGN: High Margin
MOBINT1: Mobile Intermediary1
MOBINT2: Mobile Intermediary2
NOINT2: No Intermediary2
LTIME: Long time since collection

Of the 8 clusters, five (bolded) have sizeable groupings of market agents with three clusters comprising small-scale businesses (Clusters 1-3) selling less than 126 litres/day on average (all negatively associated with LSEXP) and the other comprising larger-scale businesses (Clusters 4 and 7) selling over 360 litres/day (both positively associated with LSEXP). The five clusters are mainly separated on the basis of factors associated with scale of business, milk quality, type of intermediary and profit margins. Of the three clusters comprising small traders, a small group (Cluster 1) that sells very small quantities of milk is also associated with low milk quality, low profit margins and long duration between milk collection and re-sale. This group is abbreviated here as **small-scale poor quality low margin (SSPQLM).** However, the majority of small traders (Clusters 2 and 3) are largely neutral with regard to milk quality. Cluster 2 is abbreviated here as **small-scale neutral quality (SSNQ)**, and Cluster 3, which is further distinguished by high profit margins, is abbreviated here as **small-scale neutral quality high margin (SSNQHM)**. The small-scale clusters comprise 85% of market agents (Table 5.8).

Of the two clusters that sell higher volumes of milk, there is a medium-scale Cluster 7 with 22 traders that sells an average of 367 litres/day and a large-scale Cluster 4 with 25 market agents that sells an average of 5,536 litres/day (Table 5.8). Cluster 7 is negatively associated with LMQ and positively associated with COOPINT and MOBINT1 indicating low milk quality in pathways involving dairy cooperative and mobile trader intermediaries, respectively. It is here abbreviated **medium-scale poor quality / intermediary (MSPQI)**. Cluster 4 is highly associated with LSEXP indicating experience and trained staff and is here abbreviated **large-scale good quality (LSGQ)**. The cluster is negatively associated with LMQ and NOINT2 indicating sale of relatively good bacterial quality in pathways without intermediaries.

Table 5.8 gives means and proportions of five major milk quality and market variables obtained in the informal milk market survey.

Table 5.8. Means and proportions of milk quality and market handling variables for the major five clusters of market agents sorted by ascending scale of business

Cluster No.	1	3	2	7	4
Scale of business	**Small**	**Small**	**Small**	**Medium**	**Large**
Abbreviation	**SSPQLM**	**SSNQHM**	**SSNQ**	**MSPQI**	**LSGQ**
Number of market agents	22	120	158	25	22
Study strata					
IHMA (Nairobi/Kiambu) (%)	75.8	44.0	70.2	68.8	74.2
EMMA (Nakuru/Narok) (%)	24.2	56.0	29.8	31.2	25.8
Quality measures					
TPC (geometric mean)	158,500 x 10^3	39,800 x 10^3	50,100 x 10^3	12,600 x 10^3	10,000 x 10^3
% 'Bad milk' (>2,000x10^3 cfu)	83.9	85.3	75.2	62.5	51.6
CPC (geometric mean)	79 x 10^3	100 x 10^3	50 x 10^3	10 x 10^3	12 x 10^3
% 'Bad milk' (>50x10^3 cfu)	62.5	62.1	54.0	31.3	32.3
Solids-not-fat (SNF) %	8.7	8.4	8.6	8.7	8.5
% Low solids (SNF < 8.5)	28.6	38.7	29.6	12.5	41.4
Cadre of Market Agent					
Farmer groups (%)	0.0	2.7	2.8	56.3	96.8
Milk bar (%)	48.5	24.8	47.3	12.5	0.0
Shop/kiosk (%)	42.4	28.2	32.6	6.3	0.0
Mobile trader (%)	9.1	44.3	17.4	25.0	3.2
Milk handling					
Milk separated (%)	54.5	45.1	50.9	43.7	9.7
Milk preservation (%)	12.1	22.0	24.7	68.8	32.3
Plastic container (%)	67.7	66.2	59.4	18.7	6.5
Piped water (%)	81.8	88.7	88.5	37.5	38.7
Intermediary involved (%)	33.3	24.8	38.1	12.5	3.2
Experience					
Training vs no training (%)	0.0	3.4	9.0	31.2	71.0
Period in business (yrs)	2.0	8.4	1.8	11.5	34.3
Other covariates					
Time since collection (hrs)	1.9	1.5	1.3	1.1	2.0
Distance from collection (Km)	28.8	28.9	33.7	23.4	15.7
Purchase price (Ksh/Lt)	22.2	17.9	20.2	17.6	16.0
Sale price (Ksh/Lt)	26.6	29.1	26.2	22.4	20.6
Milk sale volume (lts/day)	44.1	108.4	126.1	367.2	5536.0
Profit Margin (Ksh/Lt)	-7.8	9.7	2.7	1.9	1.2

Key:
SSPQLM = Small-Scale Poor Quality Low margin
SSNQ: = Small Scale Neutral Quality
SSNQHM: = Small Scale Neutral Quality High Margin
MSPQI: = Medium-Scale poor Quality / Intermediary
LSGQ: = Large Scale Good Quality

The differences and similarities in the descriptive statistics in the Table are evident. The two largest groups of small traders (SSNQHM and SSNQ) include all categories of small traders (both mobile and those with fixed premises) and enjoy the highest profits that basically reflect returns to labour given the low capital investment. However there is a small homogenous group of small traders (SSPQLM) comprising some 6% of all traders with very poor quality milk, little experience and apparently operate at a loss. None in the group has received any training in milk quality. A higher proportion of the groups of small traders operate in areas of relatively lower market access (Nakuru and Narok) compared to the high market access areas of Nairobi and Kiambu. The large-scale group (LSGQ) has markedly better milk quality indicators and

nearly all are dairy farmer cooperatives with over three decades of experience on average and a high proportion of trained staff (71%). Though shorter market chains without intermediaries and shorter duration from milk collection may explain part of the improved milk quality, the association between the group and a high proportion of trained staff is also a key factor. The use of non-plastic containers also distinguishes this group. All these factors can be considered to be important in contributing to the relatively good milk quality.

The homogenous groups identified indicate some trade-off between profit and scale of business: profit margin increases quickly with increasing scale of business from negative profit at 44litres sold per day to KSh 9.7/litre at 108 litres of milk sold/day and thereafter the margins decline gradually. It is also noteworthy that the group with the highest profit margins has the lowest total bacterial counts among small traders and the group with lowest profit margins has the highest overall bacterial counts. This emphasises the benefit of keeping bacterial counts low and overall good hygiene. The big difference between the purchase and sale prices in Cluster 3 seems to be the main reason for the higher profits. These are likely to be mainly traders who know the market well and are able to move milk relatively quickly from high supply areas where prices are low to deficit areas where prices are high.

6. IDENTIFICATION OF CRITICAL CONTROL POINTS (CCPs)

6.1. Risk factors in market channels and consumer outlets

Risk factors in market channels

Results of the OLS regression (Table 5.3) and multivariate analyses (Tables 5.4 to 5.8) were used to identify critical control points (CCPs) along market channels. All market points with coefficients that were significant at $p<0.10$, and risk factors with weights of 0.5 or more were considered to be potential CCPs.

6.1.1. CCPs for Total Plate Counts

CCPs for relatively high TPC in milk (compared to the farm-coop pathway) were pathways from farms to shops/kiosks. There were also some significant differences in TPC between contrasting areas of market access. Whereas the pathway from farms to milk bars in IHMA had milk of significantly worse quality than the same pathway in the extensive and low market access area (EMMA). The use of plastic containers in EMMA was significantly associated with worse bacterial quality than the same practice in IHMA. Time elapsed since collection had a modest association with TPC. Every hour that elapsed increased TPC by 3%. Given the average TPC of 39, 800 x 10^3 (Log_{10} 7.6), every hour that elapses would add another 1,200,000 bacteria to the milk, though this would however depend on the phase of bacterial growth at the time (lag, log, stationary or death phase – see Figure 3.1). The clustering analysis in Table 5.7 shows that those selling milk of the worst quality were the small group of small traders (SSPQLM with the highest total bacterial counts and over 80% “bad milk” according to KEBS. Boiling of milk will eliminate any bacterial health risks.

6.1.2. CCPs for Coliform Counts

The farm to coop pathway had significantly worse milk quality as measured by coliform bacteria than pathways from farm to shops/kiosks and from mobile traders to milk-bars. There were also some significant differences in coliform counts between pathways in different market access areas: The pathway from farm to coop in EMMA sold worse quality milk than the same pathway in IHMA and the pathway from farm to shop/kiosk in IHMA sold worse quality milk than the same pathway in EMMA. Though not significant in the dataset modelled above, high coliform counts were associated with the use of plastic versus metal containers ($p=0.04$) among market agents using the complete dataset and scooping of milk versus pouring was also associated with higher coliform counts. Both handling practices should therefore be discouraged. Non-preservation of milk by cooling was associated with higher coliform counts, reflecting the benefit of cooling. Interestingly, in contrast to the case for TPC, wet season was associated with better quality milk. The use of piped water was associated with higher coliform counts, perhaps because such water is usually not flowing in many areas. The small-scale clusters (SSNQHM) had relatively high coliform counts compared to total counts. This indicates that hygiene is a particular problem for this cluster. Boiling of milk will eliminate any risks from coliform bacteria.

6.1.3. CCPs for Adulteration

Adulteration as determined by SNF was relatively uniform across various cadres of market agents and market pathways. Only the coop to shops/kiosks pathway had significantly higher SNF (indicating non-adulteration). Higher SNF was also positively associated with wet season and bulking of milk but negatively associated margin (KSh) per litre.

6.1.4. CCPs for Brucellosis and *M. Bovis*

Brucella antibodies were significantly associated with farm to milk-bar pathway (OR=8.0) with all samples being sourced entirely from extensive grazing systems (EMMA). This has health implications for the small fraction of consumers who may not boil milk before consumption, especially if it is bulked raw milk. The study did not identify any *M. bovis* and risks from the

pathogen are considered non-existent or very low. Boiling of milk will eliminate any risks from all zoonotic organisms.

6.1.5. CCPs for Antimicrobials

Antimicrobial residues were significantly associated with farmer to mobile milk trader pathway, milk separation and warm weather (ORs = 2.7, 2.4 and 5.4, respectively). It is more likely that the antimicrobial residues originated at the farm since no agent admitted adding antimicrobials to preserve milk and milk directly sourced from producers in the rural areas had relatively high anti-microbial levels in the consumer survey. More information on causal relationships at the farm-level are needed to devise appropriate farmer education materials that would include advice on withdrawal periods following therapy. Antimicrobial residues in milk were thus considered the most important health risk identified, given that it cannot be dealt with by heat treatment.

6.2 Risk factors and CCPs in consumer milk purchase points

Background information and details of consumption patterns of dairy products, including preference, are contained in a separate report (Ouma et al., 2000). Consumer perceptions and practices are briefly referred to in Section 6.2.2 below.

6.2.1 CCPs in consumer milk purchase points

Risk factors identified in analyses of consumer-level data largely reflected those from market-level analyses (Tables 5.9 and 5.10).

Table 5.9. Descriptors of quality measures in milk collected from consumer outlets, Comparison to market-agents (separate datasets)

Source of milk	Geometric mean TPC x 10^3		Geometric mean CPC x 10^3		SNF	
	Consumer	Market	Consumer	Market	Consumer	Market
Farm/own production	1,590	-	1.0	-	9.1	-
Home delivery	15,850	-	10.0	-	8.9	-
Farmer group/Coop	-	7,940	-	15.8	-	8.6
Shop/kiosk	79,430	39,810	25.1	63.1	8.8	8.6
Milk bar	-	79,430	-	100.0	-	8.6
Mobile trader	39,810	39,810	20.0	63.1	8.7	8.6
Local market	251	-	0.6	-	8.8	-

NB. Dash (-) = Not applicable

The influence of season and production potential/market access on both total and bacterial counts was similar to what was found with market-level data. The consumer milk retail points with the highest average total bacterial count/ml were shops and kiosks. Pathways serving these outlets were also identified in the regression analysis of market-level data as CCPs for TPC. Shops and Kiosks also had the highest mean coliform counts. Differences in factors influencing SNF were weak as shown by a very low R^2.

Table 5.10. OLS model explaining associations between risk factors and quality of milk collected from consumer households.

Parameter	TPC R^2 = 0.57; F(10, 265)=34			CPC R^2 = 0.56' F(10, 264)=34			SNF R^2 = 0.13; F(9,285)=4.6		
	Est.	S.E.	*p*-value	Est.	S.E.	*p*-value	Est.	S.E.	*p*-value
Intercept	4.3			1.14			9.3		
Study strata									
IHMA	1.16	0.25	<0.01	0.41	0.2	0.05	0.56	0.14	<0.01
Urban vs rural milk			>0.10	0.57	0.19	<0.01			>0.10
Wet Season	0.90	0.17	<0.01	-0.62	0.14	<0.01			>0.10
Quality measures									
Log of TPC	N/A	N/A	N/A	0.53	0.04	<0.01			>0.10
Log of CPC	0.75	0.06	<0.01	N/A	N/A	N/A	-0.09	0.04	0.02
SNF			>0.10	-0.19	0.08	0.02	N/A	N/A	N/A
Drugs			>0.10	0.57	0.26	0.03			>0.10
Retail points[a]									
Home delivery			>0.10			>0.10			>0.10
Shop/kiosk			>0.10	0.39	0.19	0.04			>0.10
Mobile trader			>0.10			>0.10			>0.10
Local market			>0.10			>0.10	-0.43	0.24	0.07

[a]Compared to own production

6.2.2 Consumer perceptions and practices to reduce milk-borne health risks

Reports of brucellosis and TB by household respondents were generally low (these exclude undiagnosed cases or those that respondents were not informed about by clinicians who treated household members) (Table 5.11).

Table 5.11. Reported and positively diagnosed brucellosis and general TB cases among consumers of raw milk.

Disease	Nairobi		Nakuru Rural		Nakuru Urban	
	n	%	n	%	n	%
Brucellosis	1	0.2	8	3	0	0
Tuberculosis	6	1.5	5	1.9	2	1.3

More consumers in Nairobi (65 %) were aware of the public health risks associated with raw milk consumption compared to Nakuru rural (23 %) and Nakuru urban (44 %). All urban households and 96% of household in Nakuru rural boiled raw milk (alone or in tea) before consumption (Figure 5.1). About 6% of households, mostly from Nakuru rural, consumed home-made fermented milk (often unboiled before fermentation) in the previous one month before each seasonal survey. Detailed reporting of consumer perceptions is contained in Ouma et al., (2000).

Boiling of raw milk (alone or in tea) attains a higher temperature than pasteurisation and therefore destroys all pathogens[18] (Figure 5.2). Given the very high proportion of households

[18] The common pasteurisation process keeps milk at 72 ^{o}C for 15 seconds. The pasteurisation curve (Figure 7) gives the highest temperature required to kill all pathogens as 89^{o}C for one second. Boiling attains a higher temperature and duration and therefore destroys all milk-borne pathogens.

that boil milk, the health risks from bacterial pathogens were determined to be very low. This practice should be encouraged, especially in rural / pastoral areas.

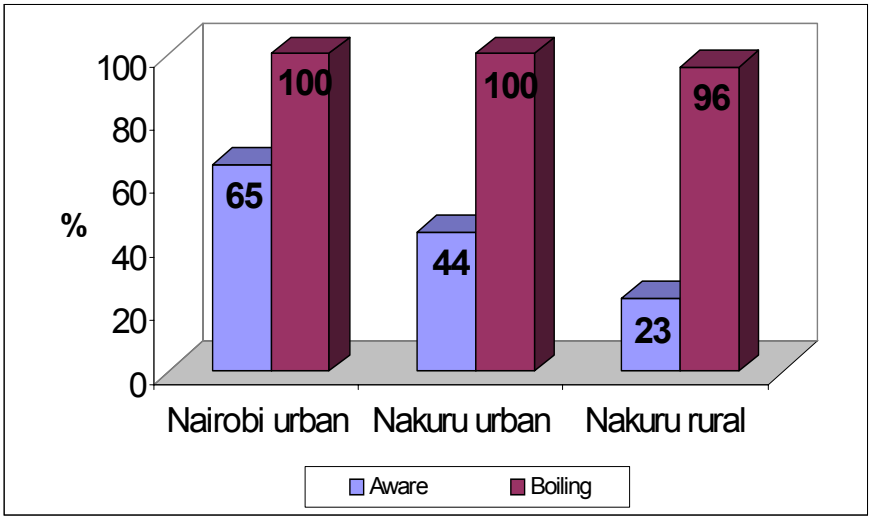


Figure 5.1. Proportion households that are aware of milk-borne risks and that boil milk in urban and rural areas

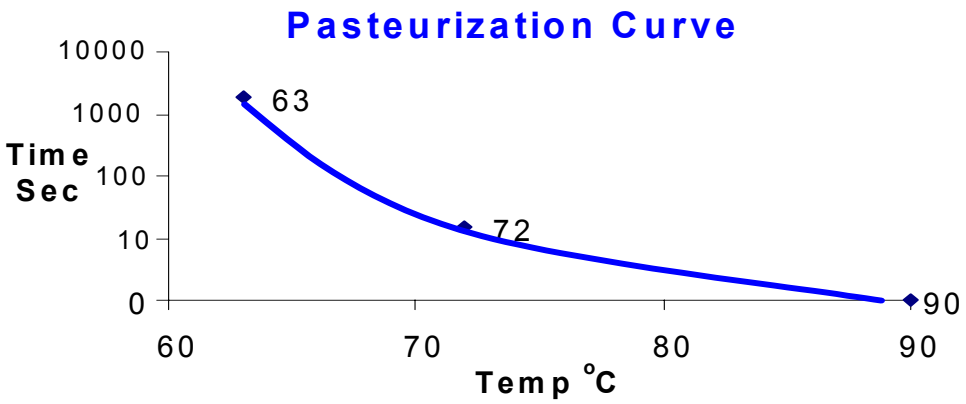


Figure: 5.2. The time-temperature requirements for pasteurisation

One area that requires attention is the consumption of traditionally fermented milk (*maziwa lala*) (consumed by 6% of households in rural areas in this survey). This milk is often not boiled before fermentation, which lowers the pH of milk from about 6.8 to about 4.5. Some pathogens

may not be affected by fermentation. For example, *Br. abortus* organisms are only mildly affected by acidity at this level (Farrel, 1996). In a related investigation, Minja *et al* (1998) found that the low pH level in sour milk only destroyed *M. bovis* after 66 hours. This would imply that home-made fermented milk could be a possible source of milk-borne infection to humans. The survival of these and other pathogens such as pathogenic *E. coli* in fermented milk also needs further investigation.

It is note-worthy all those who reported a member having a household member having been affected by brucellosis were from the Nakuru rural area where some unboiled and/or home-made fermented milk is consumed. It is also apparent that bulking of raw milk by large-scale raw milk market agents or failures in large-scale pasteurization can increase risks of infection with brucellosis, *E. coli* 0157:H7 or any zoonotic agent.

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ANNEX 1. DETAILS OF LABORATORY METHODS

Determination of specific gravity (density) and butterfat content

Addition of water or solids was tested by specific gravity (SG) using a lactometer equipped with a thermometer at a standardised milk temperature of 20^{o}C and readings taken below the meniscus. Butterfat content was determined using the Gerber method. Briefly, 10 ml of concentrated sulphuric acid (BDH, specific gravity 1.820 kg/litre), was first pipetted into a Gerber butryometer followed by addition of 11ml milk and 1ml amyl alcohol. The mixture in the butyrometer was tightly closed with a stopper, contents thoroughly mixed, centrifuged at 1200rpm for 5 minutes after which the butyrometer was placed in a water bath at 60-63^{o}C before reading the butterfat percentage. Standards from the KEBS were used to classify proportions above and below acceptable limits.

Determination of total and coliform plate counts

Samples were assessed for total viable bacterial counts (TPC) and coliform plate counts (CPC) using direct culture methods described by Marshall (1992), Speck (1984) and as adopted by KEBS (1976) Standard Specification for Unprocessed Whole Milk. Ten-fold serial dilutions of ach sample from 10^{-1} to 10^{-8} were prepared in phosphated sterile water diluent of 0.0425g of potassium dihydrogen phosphate (KH_2PO_4) per litre and standard pH 7.2. The wide range of dilutions was selected due to expected wide variation in bacterial counts.

Dilutions for culture for total plate counts ranged from 10^{-4} to 10^{-8}. From each dilution, 1 ml was transferred using a sterile pipette into a 90-mm diameter disposable petri dish. This was mixed thoroughly with 15 - 20 ml of sterilized (autoclaved at 121^{o}C for 15 minutes) and cooled to 45 – 47^{o}C molten standard plate count agar (APHA-Oxoid). After cooling and solidification of the medium, plates were incubated in inverted positions at 32^{o}C for 48 hrs. Following this period, plates with colonies ranging from 25 – 250 colony forming units (cfu) were selected, counted using a colony counter and computed following guidelines by Speck, (1984) and KEBS (1996).

For coliform plate counts, sample dilutions ranging from 10^{-1} to 10^{-4} were cultured in molten violet red bile (VRB) agar using the same volumes and procedure as above. After cooling and solidification of the medium, all the plates were over-layed with a thin layer of the same VRB medium and incubated at 37^{o}C for 24hrs. Plates showing typical red coliform colonies in the countable range of 15 – 150 cfu per plate were chosen, counted and computed following guidelines by Speck, (1984) and KEBS (1996). The lower range of countable colonies for coliforms (compared to total counts) is advised by KEBS (1976).

Brucella Milk Ring Test (MRT)

Testing for antibodies to *Br. abortus* in fresh milk is currently mostly done using MRT. The test works on the principle that antibodies to *Br. abortus* present in milk agglutinate with haematoxylin stained *Br. abortus* antigen and rise to the op layer with the fat globules to form a deep blue ring in the cream top layer. If no antibodies are present, the cream that separates out is white and the skim milk below is blue. The test often detects a high proportion of false positives (low sensitivity) due to positive reactions from samples taken shortly after parturition, near the end of lactation period, or from mastitic quarters (MacMillan, 1990). MRT was conducted by pipetting 1 ml of milk into a 1.2ml Skatron tubes (Skatronas, Lier, Norway), adding and mixing one drop of stained *Br. abortus* antigen. The tubes were thereafter incubated at 37^{0}C for 1hr. A positive control was included with each set of tests.

Indirect Milk ELISA Test

The method described by Neilsen *et al*., (1996) (sensitivity = 95% and specificity = 99%) was adopted with slight modification. Briefly, polystyrene 96-well flat bottomed plates were coated with 100μl of 0.5mg/well of *Br. abortus* smooth lipopolysacccharide antigen in coating buffer

(0.06 M carbonate buffer pH 9.6) and kept overnight in a humid box. The plates were thereafter washed five times with phosphate buffer (0.01M phosphate buffer of pH 7.2 containing 0.05% Tween-20 and 0.15M NaCl), dried and blocked using 200ul/well of 0.1% gelatin and incubated at 25^0C for 30 mins. The plates were washed again, dried and milk samples added at 100 μl/well diluted 1:2 in milk diluent (0.01M phosphate buffer, pH 6.3, containing 0.15M NaCl, 0.05% Tween-20, 15 mM EDTA and 15 mM EGTA). The plates were shaken for 2 minutes in an orbital shaker and incubated for 28 mins at 25^0C. The plates were then washed and 100μl/well of monoclonal antibody conjugated (dilution 1:1600) to horse radish peroxidase added and incubated for 1hr at 25^0C. The plates were washed again, dried and the substrate (0.05M Citrate buffer pH 4.5 containing 1mM hydrogen peroxide and 4mM ABTS) added at 100 μl/well. The plates were incubated for a maximum of 15 mins and the absorbance read at 414 nm. Brucella positive and negative serum and milk controls were included. The control serum samples were diluted 1:50, while milk samples were diluted 1:2 in the milk diluent. Each milk sample was tested in duplicate. The modification in this procedure was that the cut-off value was determined by using twice the mean of the negative control samples (Savingy and Voller,1980) and not by the targeted reading described by Wright *et al*., (1985). This test was applied to both raw and pasteurised milk samples.

Isolation of E. coli 0157:H7

For each milk sample cultured on VRB agar for coliform counting, emerging coliform colonies were, after counting, examined further for *E. coli* and subsequently 0157:H7 strain. Up to six coliform colonies per plate were purified on MacConkey agar and tryptose agar (Oxoid), and differentiated for *E. coli* by culturing on eosin methylene blue agar (Oxoid) and testing for indole, methyl red, vogues proskaeuer and citrate (IMViC) reactions. Confirmed *E. coli* isolates (IMViC++--) and suspicious weak positives were further cultured on selective indicator Biosynth medium (BCM^{TM}0157:H7(+); Biosynth Biochemica, Biosynth International Inc., USA) to observe any development of blue black colonies of *E. coli* 0157:H7. The blue black colonies were cultured on non-selective tryptose soy agar and sero-grouped using latex slide agglutination test (Oxoid). The latex beads were coated with specific rabbit antibody reactive with the 0157 somatic antigen. The strains of *E. coli* 0157:H7 isolated in this study were tested for their potential to produce verocytotoxins I (VTI) and II (VT2). The organisms were cultured on brain-heart infusion agar (Oxoid) at 37^oC for 24 hours and toxins extracted from the growth using polymyxin B solution. The polymyxin B extracts were tested for VTI and VT2 in V-bottom microtitre plates using reverse passive latex agglutination test (Oxoid).

Laboratory isolation of Mycobacteria

Sputum samples were liquefied with N-acetyl-L-cysteine and decontaminated with sodium hydroxide. The alkali was neutralized with a buffer or distilled water, centrifuged at 3,000 rpm for 30 minutes and the concentrated sediment sample was inoculated onto Loewenstein Jensen medium with pyruvate and without glycerol (Baron and Finegold., 1990) and incubated at 37^oC for 4-6 weeks. Growth on this medium is indicative of *M. bovis*. The colonies were subjected to biochemical tests to differentiate and identify *M. bovis*. In addition, molecular/DNA typing by polymerase chain reaction (PCR) was done (courtesy of Sokoine University of Agriculture, Morogoro, Tanzania).

Charm AIM Test

All samples were screened using the Charm AIM-96 anti-microbial inhibition assay screening kit (Charm Sciences Inc., USA) according to manufacturer's recommendations. The test kit detects β-lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides at levels above maximum residue limits (MRLs) recommended by the EU (EU MRLs for the two antibiotics commonly used in Kenya, penicillin G and oxytetracycline, are 4ppb and 100ppb, respectively).

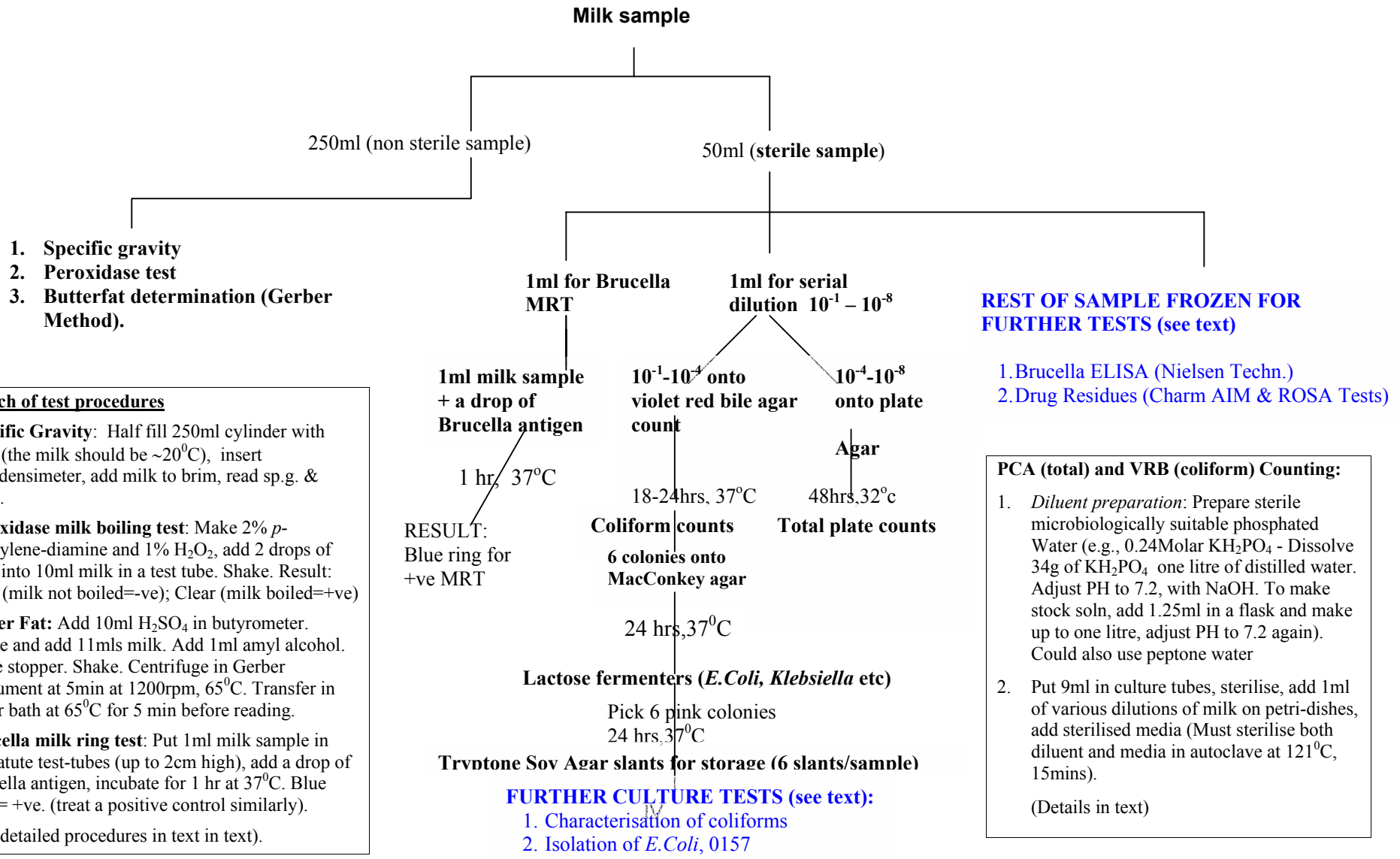
Briefly, 50μl of each sample was added in duplicate to the supplied microtitre plate followed by 200μl of a mixture of *Bacullis* st*earothemophilus* spore tablet and lyophilised medium dissolved

in 22ml of deionized water. The plate was then sealed and tightly secured by screws and incubated for 3-4 hours. Positive and negative controls were also included in the assay. The positive milk control consisted of antibiotic free milk determined using *Micrococcus lutea* inhibition assay mixed with penicillin G or sulfamethazine standard. 200μl of bacterial spores suspension and lyophilised medium in de-ionized water were added to 50μl of the positive control milk. The negative control consisted of 50μl of negative control tablet dissolved in distilled water and 200ul of the test bacteria and media dissolved in deionized water. Test results were read using colour contrasts and scored from 1-5 (negative = 1-3 and positive = 4-5).

Charm ROSA test

All samples tested positive by the Charm-AIM kit were subsequently analysed using the new (United States Food and Drug Administration approved) Charm-ROSA test (Charm Sciences Inc., USA) to identify specifically those containing β-lactams and tetracyclines. Lower detection limits for the Charm-ROSA kit are 2ppb and 125ppb for penicillin G and oxy-tetracycline, respectively.

FLOW DIAGRAM SUMMARISING LABORATORY ANALYSIS OF MILK SAMPLES



Sketch of test procedures

Specific Gravity: Half fill 250ml cylinder with milk (the milk should be ~20^{0}C), insert lactodensimeter, add milk to brim, read sp.g. & temp.

Peroxidase milk boiling test: Make 2% *p*-Phenylene-diamine and 1% H_2O_2, add 2 drops of each into 10ml milk in a test tube. Shake. Result: Blue (milk not boiled=-ve); Clear (milk boiled=+ve)

Butter Fat: Add 10ml H_2SO_4 in butyrometer. Shake and add 11mls milk. Add 1ml amyl alcohol. Place stopper. Shake. Centrifuge in Gerber instrument at 5min at 1200rpm, 65^{0}C. Transfer in water bath at 65^{0}C for 5 min before reading.

Brucella milk ring test: Put 1ml milk sample in miniatute test-tubes (up to 2cm high), add a drop of Brucella antigen, incubate for 1 hr at 37^{0}C. Blue ring = +ve. (treat a positive control similarly).

(See detailed procedures in text in text).

PCA (total) and VRB (coliform) Counting:

1. *Diluent preparation*: Prepare sterile microbiologically suitable phosphated Water (e.g., 0.24Molar KH_2PO_4 - Dissolve 34g of KH_2PO_4 one litre of distilled water. Adjust PH to 7.2, with NaOH. To make stock soln, add 1.25ml in a flask and make up to one litre, adjust PH to 7.2 again). Could also use peptone water
2. Put 9ml in culture tubes, sterilise, add 1ml of various dilutions of milk on petri-dishes, add sterilised media (Must sterilise both diluent and media in autoclave at 121^{0}C, 15mins).

   (Details in text)

ANNEX 2. PROCEEDINGS OF A STAKEHOLDERS WORKSHOP

Kenya Agricultural Research Institute Headquarters

14th February 2001

List of participants

NAME	INSTITUTION	ADDRESS	TELEPHONE NO.
1. M. Waithaka	Brookside	Box 236 Ruiru	54623
2. J. E. Mwangi	Brookside	Box 236 Ruiru	54623
3. Paul B. Capstick	Consultant - Analabs	Box 24780 Nairobi	632033
4. T. T. Dolan	Consultant - Analabs	Box 24780 Nairobi	732870/72
5. Nigel Bremmer	Consultant – Trident	Box 24344 Nairobi	884473
6. Mark Rotich	DFID – East Africa	Box 30465 Nairobi	717609
7. Emily Ouma	Egerton University	Box 536 Njoro	
8. P. L. Shalo	Egerton University	Box 536 Njoro	037-61276
9. Robert Ouma	Egerton University	Box 536 Njoro	630743
10. Brian Dugdill	FAO	FAO – Rome - Italy	+39 06 57053937
11. David W. Taylor	ILRI	Box 30709 Nairobi	630743
12. John McDermott	ILRI	Box 30709 Nairobi	630741
13. L. Njoroge	ILRI	Box 30709 Nairobi	630743
14. Steve Staal	ILRI	Box 30709 Nairobi	630743
15. James M. Lomax	ILRI	Box 30709 Nairobi	630743
16. W. Thorpe	ILRI	Box 30709 Nairobi	630743
17. E. A. Mukisia	KARI HQs	Box 51811 Nairobi	583149
18. Festus Murithi	KARI HQs	Box 5784 Nairobi	583301
19. George Orinda	KARI HQs	Box 5784 Nairobi	583301
20. Amos Omore	KARI/ILRI	Box 30709 Nairobi	630743
21. Michael Waithaka	KARI/ILRI	Box 30709 Nairobi	630743
22. D. M. Mwangi	KARI-Muguga	Box 30148 Nairobi	
23. Margaret Syomiti	KARI-Muguga	Box 30148 Nairobi	32404
24. S. W. Mwendia	KARI-Muguga	Box 30148 Nairobi	32404
25. Cecilia G. Chege	KDB	Box 30406 Nairobi	
26. Julius Makapila	KDB	Box 30406 Nairobi	244064
27. Mbuo Wanganagwa	KDB	Box 28 Runyenjes	62040 (Embu)
28. V. K. Ngurare	KDB	Box 30406 Nairobi	244064
29. Muhoho Kenyatta	KDPA/KDB/Brookside	Box 67498 Nairobi	711090
30. A. Irungu	KEBS	Box 54974 Nairobi	502210/19
31. E. M. Maindi	KEBS	Box 54974 Nairobi	502210/19
32. T. W. Wanjohi	KEBS	Box 54974 Nairobi	502210/19

List of Participants (cont'd)

NAME	INSTITUTION	ADDRESS	TELEPHONE NO.
33. Joseph Kibunja	Kikuyu Dairy Coop. Soc.	Box 50 Kikuyu	32/76
34. Paul Kiamburi	Kikuyu Dairy Coop. Soc.	Box 50 Kikuyu	0154-32176
35. Peter Itunga	Kikuyu Dairy Coop. Soc.	Box 50 Kikuyu	0154-32176
36. C. Wilson	Kilifi Plantations Ltd	Private Bag, Kilifi	
37. Patrick Mahulo	Kisumu Municipality	Box 105 Kisumu	23416
38. Joe Carvalho	Land O'Lakes	Box 45006 Nairobi	748526
39. Josephine Kirui	Land O'Lakes	Box 45006 Nairobi	748526
40. Michael Kibinge	Land O'Lakes	Box 45006 Nairobi	748526
41. Duncan Muya	Limuru Milk Processors	Box 563 Limuru	0154-71369
42. Phyllis Nganga	Limuru Milk processors	Box 563 Limuru	0154-71369
43. P. M. Low	Maradju Ltd	Box 362 Naivasha	0311-21148
44. G. K. Mbogori	Meru Dairy Coop. Union	Box 6 Meru	0164-30081/82
45. B. K. M. Tumbo	Ministry of Health	Box 30016 Nairobi	717077
46. C. K. Langat	Ministry of Health	Box 5 Kapsabet	0326-2380
47. Sarah Toroitich	Ministry of Health	Box 11 Kericho	21346/31191
48. H. Muriuki	MoARD	Box 30028 Nairobi	718528
49. A. W. Wokabi	MoARD	Box 30028 Nairobi	718528
50. F. K. Njoroge	MoARD	Box 599 Muranga	22449
51. G. N. Gichungu	MoARD	Box 30028 Nairobi	718528
52. J. K. Mutunga	MoARD	Box 44 Nakuru	213260
53. J. Kiptarus	MoARD	Box 34188 Nairobi	722637
54. J. P. Cheruiyot	MoARD	Box 34188 Nairobi	722601
55. L. N. Chege	MoARD	Box 728 Kiambu	0154-22607
56. P. K. Langat	MoARD	Box 60 Kapsabet	0326-2349
57. S. I. Waithaka	MoARD	Box 68 Muranga	22449
58. S. M. Gichuki	MoARD	Box 68 Muranga	22449
59. W. C. M. Theuri	MoARD	Box 68 Muranga	22449
60. A. M. Mugambi	MoARD - PDALE-Nairobi	Box 34188 Nairobi	335577
61. S. C. Koech	MoARD - PLPO-RUP	Box 530 Nakuru	214287
62. J. P. Nthuli	MoARD - Vetlabs Kabete	P.O. Kabete	631289
63. H. G. Mwangi	MoARD- DLPO-Narok	Box 5 Narok	0305-22215
64. Stephen N. Kimani	Nakuru Municipality	Box 124 Nkuru	037-40361

List of Participants (cont'd)

NAME	INSTITUTION	ADDRESS	TELEPHONE NO.
65. D. N. Ravindra	Premier Dairy Ltd / KDB	Box 1522 Kericho	0361 72235/7/8
66. David K. Kurgat	Small milk trader	Box 1015 Kapsabet	0326-2164
67. J. K. Kamau	Small milk trader	Box 11415 Nairobi	577669/576709
68. Joseph Kiratu	Small milk trader	Box 176 Njoro	
69. Paul Njenga	Small milk trader	Box 1061 Limuru	
70. Peter Kiarie	Small milk trader	Box 123 Naishi	
71. Joseph Githigi	Spin Knit Dairies Ltd	Box 78377 Nairobi	351123/24
72. Mahul Shah	Spin Knit Dairies Ltd	Box 78377 Nairobi	532412/3
73. Joseph Mwangangi	Technoserve	Box 14821 Nairobi	445556
74. C. Schwab	Tetra Pak	Box 78340 Nairobi	534300
75. Anne W. Mwangi	University of Nairobi	Box 30709 Nairobi	882902/630743
76. E. K. Kangethe	University of Nairobi	Box 29053 Nairobi	631325
77. Elizabeth Koroti	University of Nairobi	Box 450 Eldoret	62105
78. G.O. Aboge	University of Nairobi	Box 29053 Nairobi	630743
79. Hellen Ommeh	University of Nairobi	Box 29053 Nairobi	631277 Ext. 27002
80. Reuben Koech	University of Nairobi	Box 29053 Nairobi	630743
81. S. M. Arimi	University of Nairobi	Box 29053 Nairobi	631298

Introduction

These proceedings summarise the presentations and discussions at a workshop, the theme of which was *"Ensuring good quality milk in a liberalized market*", held at KARI Headquarters on 14th February 2001. The study that was the subject of the workshop was conducted in 1999 and 2000 by the MoARD/KARI/ILRI Smallholder Dairy Project (SDP) in collaboration with the Department of Public Health, Pharmacology and Toxicology of the University of Nairobi and the Respiratory Diseases Research Unit of the Kenya Medical Research Institute. The objectives of the Workshop were to:

1) Present to stakeholders and key-players in the dairy sub-sector the findings of the SDP study on health risks in marketed milk.
2) Review recommendations for reducing health risks and improving milk quality.
3) Develop and agree on a plan of action.
4) Agree the roles of different institutions in the action plan.

Dr. E. Mukisira (AD - Animal Production, KARI), on behalf of Dr. J. Wafula (DD – Research and Technology Transfer, KARI), gave introductory remarks that focused on the importance of the smallholder dairy sub-sector and the nutritional value of good quality milk. This was followed by a short presentation on the background of the SDP by Mr. H. Muriuki (AD – Animal Production, MoARD and Manager of SDP). Dr. A. Omore (Veterinary Epidemiologist attached to SDP) then presented a summary of the study results. Dr. S. Staal (Agricultural Economist, ILRI) summarised the policy issues related to milk marketing and public health. Mr V. Ngurare (MD, KDB) ended the presentations with a summary of the role of the KDB, its concerns about milk quality, the constraints it faces and the process of change that it was undergoing.

A key message from the presentations was that the liberalisation of milk marketing in 1992 led to considerable changes, including increased private sector participation through a large number of market agents who collect, transport, process and distribute milk. Most of these agents are small-scale who, besides generating income for themselves, play an important role in the marketing of milk by linking the majority of producers and consumers in a cost effective way. However, there is concern that the current regulatory environment is not supportive of their milk marketing activities and that this impacts negatively on producers and consumers. The study and workshop proposed ways through which the milk-borne public health risks identified could be reduced without impeding the efficient marketing of milk.

Following the presentations, the workshop participants formed three working groups to review the recommendations for reducing health risks and improving milk quality. The recommendations were reviewed under these areas: 1) Milk disposal & consumption issues; 2) Milk collection and bulking issues; and, 3) Milk production issues. Each group identified recommendations/interventions, plan of action, institutional roles for implementation and financing. These were reported and discussed in a plenary. One major recommendation was for training of all milk market and processing agents on ensuring good milk quality before certification that would allow each trainee to engage in milk marketing.

Finally, the plenary authorised the MoARD to take the lead in appointing a representative committee to follow up the issues and report at a follow-up stakeholders' meeting to be held in about a year's time.

A wide spectrum of stakeholders and key-players from all over the country attended the workshop (over 80 participants altogether). They represented the public sector organisations (MoARD, KARI, ILRI, Ministry of Health, Municipal Councils, Kenya Dairy Board, Kenya Bureau of Standards, Universities), private sector players (various members of the Kenya Dairy Processors' Association, Tetra Pak, small-scale milk traders) and NGOs.

Plenary Discussions/Comments

Question Is milk hawking a short-term phenomenon or will it be with us for a long time to come?

Answers / Comments We think the informal milk markets will be with us for a long time. The reason being that even in more industrialised countries such as in Brazil, informal milk marketing is growing. The markets will be around for a long time because they serve a large section of farmers and consumers who would otherwise not be served. In addition consumer preference for raw milk, mainly due to it's lower cost, is a major driving factor.

The policy should not be to discourage informal milk marketing but rather to encourage the growth of formal channels while at the same time formulate ways to make informally marketed milk safe.

The question should NOT be "either/or". Both channels exist because there is a demand for them. The consumer has played a big role in this. The survey results revealed what is currently happening in milk marketing. The question should be: "where do we go from here?"

Informal milk marketing arose after liberalisation and before the emergence of the current processors. The policy will be to discourage sale of raw milk over time while at the same time promote processing. Processors insist that if a market is assured, they will absorb all milk produced.

According to MoARD and SDP, the dairy industry does not just mean formal processing; it refers to the whole picture. Milk hawking was not a result of liberalisation. That particular way of selling milk has been in existence for a long time but has only come to light because the whole picture of the dairy industry is now being given attention (beyond the 12% of marketed milk being handled by KCC and other processors).

As shown in the survey, processors have not necessarily met their quality standards any better than informal raw milk traders.

KDB should not only concern itself with milk going to urban areas. Most milk is consumed in the rural areas where the largest numbers of consumers are in direct contact with farmers.

Though its activities are currently centred in urban areas, the KDB is concerned with the whole milk marketing chain from production to consumption.

Question What is being done about antibiotic and antibacterial residues?

Answer On the issue of antibiotics residues in milk, KDB is working with many stakeholders including the Departments of Veterinary Services and Public Health whose role is to address such issues.

Question Clarify the recommendation on boiling of milk. Does it refer to raw or pasteurised milk as well?

Answer The recommendation to boil milk refers to *raw milk only* as pasteurisation achieves the same effect of destroying pathogens in milk. Most health risks are likely to be from consumption of home made fermented milk. If raw milk is fermented without boiling, pathogens may survive. The problem that consumers of such milk face is that if milk is boiled before hand, natural fermentation is often not successful. It is advisable to use commercially available fermentation products after boiling to obtain good flavour.

Question Does bovine TB exist in Kenya? Your survey shows it does not. How accurate are your results?

Answer Bovine TB was not found in the area where the survey was conducted, though confirmation through diagnosis is still pending. The sampling strategy used implies that one can be 95% confident that the maximum prevalence of bovine TB in Narok District is not greater than 2%. This is in agreement with the official Government of Kenya stand, based on surveys in the 1960's that did not find bovine TB in cattle populations.

Question What is the impact of boiling on the nutritional value of milk?

Answer Firstly, the informal trade channels have met the needs of poor families in Kenya because of the lower milk prices. Secondly, most consumers boil milk whether pasteurised or not. The nutrients that are lost in the heating process are mainly the water-soluble Vitamins B and C (most of which are commonly available from cereals, fruits and vegetables). The fat-soluble Vitamins A and D are not destroyed by heating and are left intact. Vitamin A, which is not destroyed by heat, is of more concern since it is the one that is most deficient in diets of poor families. The nutritional losses through boiling or any form of heat-treatment is therefore not of great concern.

Question What are the long-term employment and other policy implications?

Answer Though the informal channel currently employs more people per litre of milk delivered to consumers, we should not forget that formalising milk marketing might eventually have the same effect through a 'multiplier effect'.

Comment Fingers should not point at us (informal milk traders) alone because the research has shown that both informal and formal milk traders have not been able to meet their set standards. What we require is training.

Outcomes of the Review of Recommendations

All workshop participants agreed with the general conclusion that **"most milk market agents are small scale-scale and play an important role in the marketing of milk by linking the majority of producers and consumers in a cost-effective way**". The Workshop participants through formation of three working groups deliberated upon the other recommendations contained in the report. The recommendations were reviewed under these areas: 1) Milk disposal & consumption issues; 2) Milk collection and bulking issues; and, 3) Milk production issues. Each group identified recommendations/interventions, plan of action, institutional roles for implementation and financing. Groups were asked to consider adding/revising recommendations and identify gaps for further work.

Group 1: Milk disposal and consumption issues

Chairman: D.M. Mwangi *Rapporteur: J. Kiptarus*

Issue	**Recommendations/ Interventions**	**Plan of action**	**Implementation & financing**
1. Reinforcement of the practice of boiling raw milk before consumption to eliminate all health risks.	Boil or pasteurise raw milk. The practice of boiling of raw milk should be reinforced through appropriate media campaigns targeting those consumers who currently do not boil milk before consumption.	Define, harmonise and enforce standards. Investigate more avenues for private sector financing.	KEBS KDB
2. Consumption of home made naturally fermented raw milk as a potential source of zoonoses.	Consumers of naturally fermented raw milk to be advised to boil the milk and use commercially available methods of souring. Train small milk traders to improve milk quality. Implement intensive programme of training and public awareness on clean milk handling from production-to-consumption.		

Group 2: Milk collection and bulking issues

Chairman: J.P. Cheruiyot *Rapporteur: E. Ouma*

Issue	**Recommendations/ Intervention**	**Plan of action**	**Implementation & financing**
3. Bulks of raw milk increase the risk of contracting zoonosis	It should be mandatory to send bulks of raw milk for processing to minimise health risks. Mount programmes to improve animal health.	Mount practical training programmes. Conduct seminars. Pass messages through barazas. Use mass media. Produce and disseminate farmer bulletins.	KEBS KDB MoARD
4. Incorporate small mobile milk traders into the licensed milk market.	Incorporate small mobile milk traders into the licensed milk market following training and certification.		
5. Review national standards to conform to local realities.	The KEBS standard for marketed milk should be reviewed, to take into account the predominant milk handling practices in Kenya and eastern Africa, and collection and sale of raw milk and its boiling in the home of the purchaser before consumption.		
6. Mount programmes to improve milk quality	Institute simple and practical training courses and public awareness campaigns on hygienic milk handling for all milk market agents. Good hygiene to be observed from farm to table – aim to improve personal hygiene, cleanliness of the milk-shed, use of clean water and utensils. Encourage quality control. Encourage use of appropriate containers and transportation.		

Group 3: Milk production issues

Chairman: E. K. Kang'ethe *Rapporteur: R. Ouma*

Issue	**Recommendations/ Intervention**	**Plan of action**	**Implementation & financing**
7. More farm-level information required tounderstand how antibiotic residues in milk come about.	Conduct follow-up study to generate more farm-level information on risk factors and causal relationships of antibiotic residues. Used generated farm-level information to complement current knowledge at the market level to design appropriate training and extension materials, and to mount public awareness campaigns on the safe use of antibiotics for milk producers, market agents and processors.	Need a collaborative study on antibiotic residues similar to that conducted by SDP, UoN, Egerton University, and KEMRI Seek some private sector financing (esp. milk processors)	DTI KDB MoARD KARI ILRI Universities of Egerton and Nairobi
8. Rapid bacterial degradation of milk.	Promote charcoal cooling. Train farmers on the need to cool milk. Promote use technologies such as the FAO/WHO approved Lactoperoxidase Milk Preservation System – to be used at collection centres (not recommended for use by individual farmers).	Test the technologies first	MoARD FAO KDB

Public Health Committee: Appointment, Terms of Reference and Representation

At the end of the Workshop, the Kenya Dairy Board and Ministry of Agriculture and Rural Development were requested by the workshop participants to appoint a representative committee to coordinate the follow-up of issues raised.

The proposed terms of reference for this committee are as follows:

- Finalise recommendations (interventions, plan of action, institutional roles for implementation and financing) agreed at the workshop
- Ensure institutional ownership and consensus on issues raised with regard to ensuring good milk quality in a liberalised market
- Oversee the proposed testing of interventions to improve milk quality
- Set up appropriate reporting mechanism to stakeholder/key-player institutions
- Convene a follow-up key-player/stakeholders' meeting to report progress

After consultations, it is proposed that the committee should consist of representatives of the MoARD (Dairy and Beef Branch (DBB)), the Kenya Dairy Board (KDB), the Kenya Dairy Processors Association (KDPA), Dairy Industry Stakeholders' Association (DISAK), small-scale milk traders, the Kenya Bureau of Standards, Ministry of Health (Department of Public Health) and the Smallholder Dairy Project. It is proposed that the MoARD (Head of DBB) will chair the meetings of the committee.

ANNEX 3. PUBLICATIONS ARISING FROM THE STUDY

1. Aboge, G.O., Kangethe, E.K., Arimi S.M., Kanja L.W., Omore, A.O. and McDermott, J.J. 2000. Antimicrobial agents detected in marketed milk in Kenya. *Paper presented at the 3rd All Africa Conference on Animal Agriculture (AACAA), 6-9 November, Alexandria, Egypt, 2000*

2. Arimi, S.M., Koroti, E., Kangethe, E.K., Omore, A.O., McDermott, J.J., Macharia, J.K. and Nduhiu; J.K. 2000. E*. coli* 0157:H7 risk from informally marketed milk in Kenya. *Paper presented at the 3rd All Africa Conference on Animal Agriculture (AACAA), 6-9 November, Alexandria, Egypt, 2000.*

3. Kang'ethe E.K., Arimi S.M., Nduhiu J.G., Macharia J.K., Omore, A.O., and McDermott J.J. 2000. The prevalence of brucellosis antibodies in marketed milk in Kenya and its public health implications. *Paper presented at the 3rd All Africa Conference on Animal Agriculture (AACAA), 6-9 November, Alexandria, Egypt, 2000*

4. Mwangi, A., Arimi, S.M., Mbugua, S., Kang'ethe, E.K., Omore, A.O., McDermott, J.J. and Staal, S. 2000. Application of HACCP to improve the safety of informally marketed raw milk in Kenya. In: *Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics (ISVEE), 6-11 August 2000, Beckenridge, Colorado, USA. pp544-546*

5. Omore, A.O., McDermott, J.J., Staal, S. Arimi, S.M. and Kang'ethe, E.K. 2000. Analysis of public health risks from consumption of informally marketed milk in sub-Saharan African countries. In: *Proceedings of the 9th International Symposium on Veterinary Epidemiology and Economics (ISVEE), 6-11 August 2000, Beckenridge, Colorado, USA. pp547-549*

ANTIMICROBIAL AGENTS DETECTED IN MARKETED MILK IN KENYA

Aboge, G.O.[1], Kang'ethe E.K.[1], Arimi S.M[1]; Omore, A.O.[2,3], McDermott, J.J.[3], Kanja L.W[1], Macharia J.K[1], Nduhiu J.G.[1] and Githua, A.[1]

[1]Department of Public Health, Pharmacology and Toxicology, University of Nairobi. P.O. Box 29053, Nairobi, Kenya. [2]Kenya Agricultural Research Institute (KARI), P.O. Box 57811, Nairobi Kenya. [3]International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya.

Summary

Drug residues in foods are a major public health concern in many countries, especially where most food sales bypass official quality assurance channels. In common with many tropical countries, sales of unpasteurized milk in Kenya account for over 85% of marketed milk. This milk is either sold directly from producers to consumers or via various cadres of informal market agents. Besides residues that may arise from lack of adherence to withdrawal times following cow therapy, there have been concerns that some antimicrobial agents may be added to informally marketed milk to extend its shelf life.

As part of a large study to assess public health hazards associated with marketed milk, samples were collected seasonally between January 1999 and January 2000 from raw (unpasteurized) milk consuming households and informal market agents of various cadres. Pasteurised milk samples were also collected from retail points and tested for comparison. All samples were screened for antimicrobial residues using charm AIM-96 and Charm-ROSA (Charm Sciences Inc, USA) tests. The former detects a wide range of anti-microbials, and the latter detects β-lactams and tetracyclines specifically, at levels above maximum residue limits (MRLS) recommended by the European Union (EU). The Charm-AIM screening test showed that 9.4% and 5.7% of samples from consumer households and market agents had antimicrobial residues above EU MRLS, respectively. It was concluded that antimicrobial residues were more likely to have originated at farm-level than because of poor market handling practices.

Key words: *Anti-microbial residues, marketed milk, Kenya.*

Introduction

Antimicrobial agents in milk are undesirable because they cause hypersensitivity (Oslon and Sanders, 1975), drug resistance (Nijsten *et al*, 1996) and specific tissue damage (Schultz *et al*, 1963; Moffit *et al*, 1974) in humans. They also inhibit organisms required in the processing of cultured milk products. In Kenya anti-microbial agents of aminoglycosides, β-lactams, sulfonamides and tetracyclines are used extensively for treatment of livestock diseases. Anti-microbial residues have been reported in milk following all routes of administration (Suliman *et al.,* 1990; Roudant *et al.,* 1990) and ingestion of contaminated feed (McEvoy e*t al.,* 2000). Penicillin residues have been demonstrated in 1.2% of milk deliveries at Kenya Co-operative Creameries (Chewulukei (1978) and general veterinary drugs have been found in slaughter-house meat (Mdachi and Murilla, 1991 and Muriuki, 1992).

Since market liberalisation in 1992, the proportion of raw milk sold in urban centres has markedly increased, thereby raising public health concerns (Omore *et al*., 1999). Besides residues that may result from lack of adherence to withdrawal times following therapy, there have been concerns that some antimicrobial agents may be added to informally marketed milk to extend its shelf life. This paper describes the use of Charm AIM-96 and Charm-ROSA (Charm Sciences Inc, USA) kits to test for anti-microbial agents in milk informally and formally marketed by various market agents in Kenya

Materials and Methods

As part of a large study to assess public health hazards associated with marketed milk, samples were collected between January 1999 and January 2000 from 212 and 222 raw (unpasteurized) milk consuming households in the dry and wet season, respectively. At the market-level, 262 and 246 informal market agents were interviewed and milk samples collected from them during the two respective seasons. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. Nakuru and Narok districts represented extensive production systems and low population density (also medium market access). Nairobi and Kiambu Districts represented intensive production systems and high population density (also high market access). The informal market agents that were sampled included dairy co-operatives, milk bars, milk shops and mobile traders on foot, bicycle or motorised transport. Attempts were made during the wet season to

sample the same agent sampled in the dry season. Where this was not possible, substitution was done within the same locality. A total of 110 formally (pasteurized) marketed milk samples from Nairobi and Nakuru were also tested.

All samples were screened using the Charm AIM-96 anti-microbial inhibition assay screening kit (Charm Sciences Inc., USA) according to manufacturer's recommendations. The test kit detects β-lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides at levels above maximum residue limits (MRLs) recommended by the EU (detection levels and EU MRLs for the two antibiotics commonly used in Kenya, penicillin G and oxytetracycline, are 4ppb and 100ppb, respectively). Briefly, 50µl of each sample was added in duplicate to the supplied microtitre plate followed by 200µl of a mixture of *Bacullis* st*earothemophilus* spore tablet and lyophilised medium dissolved in 22mls of deionized water. The plate was then sealed and tightly secured by screws and incubated for 3-4 hours. Positive and negative controls were also included in the assay. The positive milk control consisted of antibiotic free milk determined using *Micrococcus lutea* inhibition assay mixed with penicillin G standard or sulfamethazine standard. To 50µl of the positive control milk, 200µl of bacterial spore and lyophilised media was added. The negative control consisted of 50ul of negative control tablet dissolved in distilled water and 200ul of the test bacteria and media dissolved in deionized water. Test results were read using colour contrasts and scored from 1-5 (negative = 1-3 and positive = 4-5).

All samples tested positive by the Charm-AIM kit were subsequently analysed using the new United States Food and Drug Administration approved Charm-ROSA (Charm Sciences Inc., USA) test to identify specifically those containing β-lactams and tetracyclines. Lower detection limits for the Charm-ROSA kit are 2ppb and 125ppb for penicillin G and oxy-tetracycline, respectively. In addition, results from the two tests were experimentally compared by testing milk samples from eight lactating dairy cows injected with therapeutic doses of intra-mammary and intra-muscular preparations of Penicillin G and 10% oxytetracycline. One pre-treatment and five post-treatment milk samples were collected daily from the eight cows and tested.

Results and Discussion

Overall, 37 (9.4%) and 27 (5.7%) of consumer- and market-level samples, respectively, were positive on the Charm AIM test (Table 1). The proportion of consumer-level samples from rural areas with antibiotic residues were three times those from urban areas. Among informal market level samples, the number with residues decreased with increasing levels of bulking with milk bars and small milk traders having much higher proportion of samples with anti-microbials compared to samples from dairy co-operatives. Nine out of 110 (8.2%) pasteurised milk samples had residues.

Table 1. Numbers and proportions of consumer- and market-level samples testing positive for anti-microbials on Charm AIM test in both seasons.

Source of sample	Number	%
Consumer households		
Urban consumers (Nairobi and Nakuru)	8	4.0
Rural consumers (Nakuru)	29	15.0
Informal market agents in high market access and intensive production area (Nairobi/Kiambu)		
Coops/collection centres centres/Self-help groups	1	1.5
Milk Bars	10	9.4
Milk Shops/kiosks	5	5.5
Small mobile traders	4	7.1
Informal market agents in medium market access and extensive production area (Nakuru/Narok)		
Coops/collection centres centres/Self-help groups	0	0
Milk Bars	2	3.8
Milk Shops/Kiosks	0	0
Small mobile traders	5	10.0
Pasteurised milk in Nairobi & Nakuru	9	8.2

The higher proportion of consumer-level milk samples with anti-microbial residues as detected by Charm-AIM test kit would imply that the residues are more likely to originate at the farm-level than because of bad market-level practices. On the other hand, the increased residues as milk moves up the market chain and bulking occurs (including of pasteurised milk) seems to suggest that anti-microbial agents may be added after the first milk sale

transaction. Further investigation, including any dilution effects on anti-microbial residue levels, need further investigation.

These apparent high levels of antibiotic residues in marketed milk as detected by the Charm-AIM test need to be evaluated against the background of the results of the Charm-ROSA test and the experiment conducted to compare the two tests. None of the consumer- and market-level milk samples that were positive on the Charm-AIM test was positive on the Charm-ROSA test as well. And the Charm-AIM test classified as many as seven out of eight experimental samples as having Penicillin G or oxy-tetracycline residues up to the fifth day post-treatment, compared to the Charm-ROSA test that classified only one by the same day.

Whereas agreement between the two tests is inconclusive, these results indicate that the problem of anti-microbial residues in milk needs to be tackled at both the farm and market levels. To begin with, there is the need to define farm-level causal relationships to complement these data. Such information would be necessary to devise appropriate measures to reduce residues at both these levels.

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RISK OF INFECTION FROM *E. coli* 0157:H7 THROUGH INFORMALLY MARKETED RAW MILK IN KENYA.

Arimi, S.M.[1], Koroti, E.[1], Kang'ethe, E.K.[1], Omore, A.O.[2,3*], McDermott, J.J.[3], Macharia, J.K.[1], Nduhiu, J.K.[1] and Githua, A.M.[1].

[1]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya; [2]Kenya Agricultural Research Institute (KARI), P.O. Box 57811, Nairobi, Kenya; [3]International Livestock Research Institute (ILRI), P.O. Box 30709, Nairobi, Kenya.

Summary

E. coli 0157:H7 is a newly recognised bacterial zoonosis that originates from the gut of infected cattle. It causes potentially fatal haemorrhagic enteritis, haemolytic uraemic syndrome and kidney damage in humans. Epidemiological data on *E. coli* 0157:H7 infection and transmission in developing countries remain scarce but it is suspected that consumption of unpasteurised milk is an important vehicle for its transmission to humans, as milk can easily be contaminated with cattle faeces during milking. Given the high proportion of informal sales of unpasteurized milk in many tropical countries, *E. coli* 0157:H7 has been one of several zoonoses of concern.

Between January 1999 and January 2000, survey data and raw milk samples were collected seasonally from households consuming unpasteurised milk in rural and urban locations in central Kenya. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. Laboratory samples were assessed for bacteriological quality by total and coliform counts. Selective media were used sequentially to screen for faecal coliforms and *E. coli* 0157:H7. Suspect *E. coli* 0157:H7 colonies were also serotyped and tested for production of verocytotoxins.

E. coli was recovered from 91 out of 264 samples (34%) and *E. coli* 0157:H7 serotype identified in two samples (<1%). One of the two isolates produced verocytotoxins. As in many studies, the recovery rate of this serotype was low, but the finding is significant from a public health perspective. Our consumer studies have shown that over 95% of consumers of unpasteurised milk boil the milk before consumption and potential health risks from this zoonosis are therefore quite low. As informal milk markets without pasteurisation technology are likely to remain dominant for the foreseeable future, there is the need to further emphasise the importance of boiling raw milk before consumption, especially among pastoral communities where this practice is not common.

Key words: *E. coli O157:H7, unpasteurised milk marketing, Kenya*

Introduction

Since the first reported foodborne illness associated with *Escherichia coli* 0157:H7 (*E. coli* 0157:H7) in 1982 in Michigan and Oregon, USA (Riley *et al*.,1983), the organism has been isolated from a variety of foods and from cattle faeces in many countries (Jay, J.M., 1992; Abdul Tapif *et al*., 1996; Youko Miyao *et al*., 1998; Aloysio *et al*., 1999). Accumulated research data have led to the recognition of this organism as an important foodborne pathogen and a zoonosis. Otherwise known as enterohaemorrhagic *E. coli* (EHEC), *E. coli* 0157:H7 causes haemorrhagic colitis (HC) leading to bloody diarrhoea and haemolytic ureamic syndrome (HUS) in humans due to the production of potent verocytotoxins; HUS is associated with serious kidney damage and renal failure (Jay, 1992; Besser *et al*., 19993).

Human infection is associated with the consumption of a number of contaminated foods among them meat, especially undercooked ground beef, raw milk, yoghurt, salamis, cheese and unpasteurised apple cider (Riley *et al*., 1983; Doyle and Shoen, 1984; Doyle, 1992; Tildenet *et al*., 1996). Human beings and cattle carry the pathogen in their intestines and faeces are therefore a source of contamination of foods, water and the environment. The faeces and bacteria may contaminate udders and milking equipment and get into the milk during milking and handling if adequate hygiene practices are not observed.

Most of what is known about *E. coli* 0157:H7 has emanated from developed countries. In Kenya, there is little information on the organism and the role milk and other foods play in its transmission. Milk is widely consumed in Kenya, mostly in its natural liquid form, or fermented, or in tea. Sales of raw (unpasteurised) milk captures over 85% of the marketed milk (Omore *et al*., 1999). Since the liberalisation of milk marketing in the country in 1992 and subsequent increased sales of unpasteurised milk to urban consumers, concerns have been raised regarding transmission of foodborne diseases to consumers. As part of a larger consumer and milk market study, the

microbiological quality of unpasteurised milk purchased by consumer households was assessed and coliform isolates screened for *E. coli* 0157:H7 to establish its occurrence and evaluate potential human health risks. Consumer practises that may reduce the risks of infection were also studied.

Materials and Methods

Between January 1999 and January 2000, 212 and 222 raw (unpasteurized) milk consuming households were surveyed in the dry and wet season, respectively. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. The geographical units selected from each site also covered a variation from low to high income classes. Nakuru district represented extensive production systems and low population density (also medium market access). Nairobi represented intensive production systems and high population density (also high market access). Attempts were made during the second (wet) season to interview and sample the same respondent as in the first (dry) season. Where this was not possible, substitution was made within the same locality. *E. coli* 0157:H7 was isolated after screening for coliforms by plating and counting of colony forming units (c.f.u). Milk samples were collected in sterile 50ml plastic tubes in the mornings and transported to the laboratory in ice-cooled boxes. Analysis commenced within six hours of sample collection. This report covers results from 264 samples that were processed and that had plates with coliform colony forming units.

Sample preparation and culture for bacteriological quality assessment.
For each sample, tenfold serial dilutions (10^{-1} to 10^{-7}) were prepared in sterile phosphate buffered water diluent (0.0425g of potassium dihydrogen phosphate per litre (final concentration) of distilled water), pH 7.2. Dilutions to culture for total counts and coliform counts were based on the expected microbial load in the samples.

Total plate count and coliform count
One millilitre of 10^{-4} to 10^{-7} dilutions of milk was pipetted into 90mm diameter disposable petri dish and mixed well with 20 ml of sterile standard plate count (SPC) agar (APHA; Oxoid). The SPC agar was prepared by dissolving 23.5g of powder in one litre of distilled water, sterilised by autoclaving at 121^{o}C for 15 minutes and cooled to 45^{o} – 47^{o} in a waterbath.The sample cultured for total counts was also cultured for coliform counts. Sample dilutions from 10^{-1} to 10^{-4} were cultured; 10^{o} and 10^{-5} dilutions were sometimes included. One millilitre of each dilution was pipetted into 90 mm diameter disposable petri dish and mixed with about 20 ml of violet red bile (VRB) agar (Oxoid). After cooling and solidification of the medium, all the plates were covered with a thin layer of the same VRB agar medium. The medium was prepared according to the recommendations of the manufacturer by suspending 52g of powder in one litre of distilled water, bringing it to boil to dissolve completely and cooling to 45^{o} – 47^{o}C in a waterbath.

After cooling and solidification of the poured media, SPC and VRB agar plates were incubated inverted, at 32^{o}C for 48 hours for total counts and at 37^{o}C for 24 hours for coliform counts. SPC agar plates with countable colonies between 25 and 250 c.f.u./plate and VRB agar plates with countable colonies between 15 to 150 c.f.u./plate were chosen for counting with the aid of colony counter (Gerber).

Screening for E. coli 0157:H7
After counting the number of coliforms for a sample, the coliform c.f.u were examined for the presence of *E. coli*. In order to increase chances of detecting *E.coli* and strain 0157:H7 in particular, up to six colonies per plate were purified on MacKonkey agar (Oxoid) and differentiated for *E. coli* by plating on eosine methylene blue agar (Oxoid) and testing suspect colonies for indole, methyl red, vogues proskaeuer and citrate (IMViC) reactions. Identified *E. coli* isolates were further cultured by streaking onto selective indicator Biosynth culture medium (BCM^{TM} 0157:H7(+) (Biosynth Biochemica, Biosynth International Inc., USA) and incubated at 35^{o}C for 24h for identification of blue black colonies of *E. coli* 0157:H7. The BCM^{TM} 0157:H7(+) medium was prepared according to the instructions of the manufacturer. Briefly, 80g of the powder was dissolved completely in 1 litre of distilled water containing 5ml N, N-dimethylformammide (Sigma). After cooling to 50^{o}C in a water bath, 5ml of 0.2% (w/v) sodium novobiocin (Sigma) and 0.2ml of 0.1% (w/v) potassium tellurite (Sigma), both filter sterilised, were added to the medium, mixed and the medium poured into petri dishes. It was then allowed to solidify and dry at room temperature.

Serogrouping of isolates
Blue black colonies on BCM^{TM} 0157:H7(+) medium were cultured onto non-selective tryptose soy agar (Oxoid) and serogrouped using latex slide agglutindon test (oxoid) to confirm that they were *E. coli* 0157:H7 and hence potential producers of verocytotoxin (VT). Latex beads coated with specific rabbit antibody reacts with the 0157 somatic antigen causing agglutination.

Test for production of verocytotoxins
The organisms were cultured onto brain heart infusion agar (Oxoid) at 37°C for 24h and toxins extracted from the growth using polymyxins B sulphate (sigma) solution. The polymyxin B extracts were tested for VT1 and VT2 in V-bottom microtitre plates using reverse passive latex agglutination (RPLA) test kit (Oxoid).

Results and Discussion

Total counts and coliform counts
The bacteriological quality of the milk (total viable counts and coliform counts) was interpreted according to the Kenya Bureau of Standards (KEBS) guideline specifications for whole unpasteurized milk (1976). According to the standard, milk containing a total bacterial count of up to 1 million per millilitre is classified as very good; 1 million to 2 million as good; 2 million as bad and >5 million as very bad. Similarly, milk containing coliform counts up to 1000 per millilitre is classified as very good; 1000 to 50,000 as good; 50,000 to 500,000 as bad and >500,000 as very bad. Milk classified as bad is not acceptable within the regulations for marketing.

Eighty six percent of the milk samples in Nairobi and 88% in Nakuru urban had total counts of >2 million/ml with no significant difference between the two towns (Table 1). A repeat sampling in Nairobi showed the same high proportion (85%) of unacceptable bad quality milk. In Nakuru rural a fairly high proportion of milk, but relatively less than that from Nairobi and Nakuru, had total counts >2 million/ml. The results of the coliform counts showed a picture similar to total counts in both urban and rural areas. In Nairobi, 46% of the milk had coliform counts >50,000/ml and in Nairobi urban 45%. Repeat sampling in Nairobi showed an increase in count from 46% to 71%. These high counts show that milk bought by households for consumption in the two urban centres is of poor bacteriological quality. By contrast, only 12% of the milk from Nakuru rural had coliform counts >50,000/ml showing that most of the milk was of good quality.

Table 1: Milk samples from consumer households containing unacceptably high total and coliform bacterial counts

District/area of Study	Samples with unacceptable high counts			
	Total Counts >2,000,000 c.f.u[a]/ml		Coliform counts >50000 c.f.u./ml	
	n	%	n	%
Nairobi urban (dry season)	49	86	46	46
Nairobi urban (wet season)	53	85	52	71
Nakuru urban (dry season)	58	88	58	45
Nakuru rural (dry season)	104	41	104	12

[a]c.f.u. = colony forming units

The high number of bacteria in raw milk is a reflection of poor production and handling hygiene during milking, transportation to the market, storage at selling points and even at home. Initial loads at the production stage may be high. Unsanitary handling during transportation from source to sale points may add to the contamination. Coupled with these, long holding times in warm tropical weather by vendors and even by households before pasteurisation or boiling encourages rapid microbial multiplication. In the areas studied and particularly in the urban centres, milk goes through a number of handling stages without adequate control of hygiene or cooling and this favours contamination and multiplication of bacteria in the milk before the household buys it. Many households in the urban centres, and especially those with low incomes, buy small quantities of raw milk from traders or from nearby milk shops, milk bars, kiosks, and street vendors (stationary or mobile on bicycles or motorised vehicles). Most households lack cooling facilities and use plastic containers, which are difficult to clean. The distances travelled and/or the time spent on the way from producer to consumer is sometimes long. All these factors contribute to the poor bacteriological quality of the milk.

In Nakuru rural, milk was relatively of better bacteriological quality (Table 1). Although cooling facilities were not readily available, time spent from producer to consumer was generally shorter than in the urban centres especially in Nakuru. Some of the respondent households were also milk producers themselves, consuming some and selling the remainder to neighbours.

E. coli 0157:H7
A total of 264 milk samples that were cultured for coliform counts yielded 845 coliform colonies that were screened for *E. coli* and subsequently *E. coli* O157:H7. Three *E. coli* isolates from three different samples, one from Nairobi and two from Nakuru urban, produced blue black colonies on BCM™ 0157:H7 medium and were regarded highly

suspect for strain 0157:H7 (Table 2). Two of these three isolates reacted positively with 0157:H7 specific antibodies. Thus two isolates (one from Nairobi and one from Nakuru urban) out of 264 milk samples were serologically confirmed to be *E. coli* 0157:H7. This translates to a recovery rate of 0.8%. The organism is a rare strain among a huge population of *E. coli* organisms (Jay, 1992) which therefore requires examination of a large number of isolates in order to detect it. The finding is, however, significant considering the importance of the pathogen in causing haemorrhagic colitis with bloody diarrhoea and haemolytic ureamic syndrome which often leads to kidney failure (Jay, 1992; Besser *et. al*., 1993). One of the isolates from Nakuru urban produced verocytotoxin one (VT1). The finding is also significant considering the low infective dose of 700 organisms or less (Turtle and Gomez, 1999) and, in this case, the large number of people who buy unpasteurised milk for consumption. They, if consuming unboiled or unpasteurized contaminated milk, stand a high risk of getting infected. Fortunately, over 95% of the households boil milk before consumption, which destroys this and other pathogens. Consumers should therefore protect the milk from recontamination after the heat treatment.

Table 2: Numbers of unpasteurised consumer milk samples screened and isolation and identification of *E. coli* 0157:H7

Milk sample and test details	Number			
	Nairobi urban	Nakuru urban	Nakuru rural	Total
Examined for coliforms	102	58	104	264
Positive for *E. coli*	37	21	33	91
Suspect *E. coli* 0157:H7 on BCM™ medium	1	2	0	3
Serologically confirmed *E. coli* 0157:H 7	1	1	0	2
Verocytotoxin1 producing *E. coli* 0157:H7	0	1	0	1

It is clear from the total bacteria and coliform counts that milk sampled from consumer households, particularly in the urban centres, had heavy loads of coliforms. Consequently, faecal *E. coli* is expected to be in high numbers, which increases the chances of some milk being infected with strain 0157:H7. Since the milk were from consumers, it is difficult at this point to indicate the main sources and entry points of 0157:H7 into the milk. However, contamination with cattle or human waste and contaminated water (Jay, 1992; Cobbold and Desmarchelier, 2000) at the different stages of handling (farm level, market level and consumer level) are the broad possible sources. In many areas, there have been difficulties with obtaining water, especially in the dry seasons. At the farm level, besides cows faeces, *E. coli* mastitis could contribute to the presence of 0157:H7 in milk. Unhygienic handling and infected handlers may also contaminate marketed milk. Since the results under discussion are only a part of an ongoing study, a clearer picture of the occurrence of *E. coli* O157:H7 in the milk will emerge after completion of the market and farm level studies.

In Kenya, diarrhoea is one of the commonest diseases of children caused by a variety of pathogens including *E. coli* (Sang *et al*., 1992). Cases of kidney failure in humans are fairly common. The role of *E. coli* 0157:H7 in the causation of enteritis and renal failure in Kenya is yet to be established and needs to be given attention. One of the 0157:H7 isolates from the milk produced verocytoxin and these toxins are associated with kidney damage and kidney failure. As far as we know, this is the first time *E. coli* 0157:H7 has been isolated from milk in Kenya. Its origin could have been from cows or from human beings.

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THE PREVALENCE OF ANTIBODIES TO *BRUCELLA ABORTUS* IN MARKETED MILK IN KENYA AND ITS PUBLIC HEALTH IMPLICATIONS.

Kang'ethe, E.K.[1]. Arimi, S.M.[1], Omore, A.O[2,3], McDermott, J.J.[3], Nduhiu, J.G.[1], Macharia J.K.[1] and Githua, A[1].

[1]Department of Public Health, Pharmacology and Toxicology, University of Nairobi, P.O. Box 29053, Nairobi, Kenya; [2]Kenya Agricultural Research Institute (KARI), P.O. Box 57811, Nairobi, Kenya; [3]International Livestock research Institute (KARI), P.O. Box 30709, Nairobi, Kenya.

Summary

The risk of infection by milk-borne brucellosis is one reason for public health regulations which discourage informal milk markets that sell unpasteurized milk. However, these regulations are not generally implemented in many developing countries. Kenya is a typical example, with over 85% of milk sales passing through informal channels. Consumer practices to reduce or eliminate potential infection by milk-borne health hazards under these circumstances have rarely been studied.

Seasonal survey data were collected between January 1999 and January 2000 from informal milk market agents of various cadres and from households consuming unpasteurized milk in rural and urban locations in central Kenya. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. In addition, pasteurized and packaged milk samples from five processors were collected. Samples were screened for antibodies to *Brucella abortus* using the milk ring test (MRT) (unpasteurized milk) and indirect antibody ELISA (both unpasteurized and pasteurized milk).

Milk samples originating from farms in the extensive production system and those containing milk from many sources were associated with higher antibody detection proportions. Five percent of all raw milk samples collected from consumer households and 4% of samples collected from various levels of bulking of market samples were positive to the ELISA. There was poor to no agreement between the two antibody detection tests. All urban consumers and 96% of rural consumers of unpasteurized milk indicated that they boil the milk (in tea or otherwise) before consumption. The implications of these results on milk marketing in Kenya are discussed.

Key words: *Brucella abortus, unpasteurised milk, milk marketing, Kenya.*

Introduction

Bovine brucellosis is a zoonosis commonly caused by *Brucella abortus*. The disease in cattle causes abortions and is mainly spread by material contaminated by body fluids. In humans, brucellosis presents as a febrile flu-like illness and is common among pastoralists in Africa (Berman, 1981; Chukwu, 1987; Nicoletti, 1984; Seifert, 1996) and Kenya (Muriuki *et al*., 1994). It is less prevalent in intensive smallholder production systems (Kadohira *et al.,* 1997). The prevention of brucellosis infection in humans is a major reason for the advocacy of milk pasteurization worldwide. Despite the existence of regulations that require milk pasteuriuzation, over 75% of milk marketed in many developing regions is sold raw through informal channels (Staal, 2000). The informal milk markets thrive because they provide social and economic benefits to smallholder producers, small market agents and consumers in terms of higher farm-gate prices, creation of employment and competitive consumer prices. In Kenya, over 85% of marketed milk is not pasteurized and is sold through informal market pathways (Omore *et al*., 1999). Concerns about human health risks from these market pathways need to be addressed in the context of consumer practices, such as boiling, to reduce or eliminate potential infection by milk-borne health hazards, without discouraging the markets through which the majority of smallholders sell their milk.

Application of serological diagnostic tests for bovine brucellosis has been achieved in diverse areas using the Rose Bengal Plate Test (RBPT) (Kagumba and Nandokha, 1978; Turkson and Boau, 1992) and complement fixation test (CFT) (Cargill *et al.,* 1982 and Sutherland *et al*., 1986). Until recently, only the Milk Ring Test (MRT), with a sensitivity of about 89% (Nicoletti, 1969; Hunter and Allen, 1972) was available for detection of brucella antibodies in fresh milk. A more accurate indirect ELISA (sensitivity = 95% and specificity = 99%) for testing brucella antibodies in milk has also been recently improved and validated (Kerkhofs *et al*., 1990; Nielsen *et al*., 1996). The milk ELISA is more sensitive than MRT, CFT and RBPT (Sutherland *et al*., 1986; Kerkholfs *et al*., 1990 Neilsen *et al*., 1996; Kerby *et al* 1997) and reportedly is able to detect antibodies in dilutions of up to 1:100 (Forschner and Buegner,1986). This paper presents results of a study on the occurrence of *Br. abortus* antibodies in informally and formally marketed milk in Kenya using the MRT and milk ELISA tests and it evaluates consumer practices to reduce potential milk-borne health risks from consumption of raw milk.

Materials and methods

As part of a large study to assess public health hazards associated with marketed milk, samples were collected between January 1999 and January 2000 from 212 and 222 raw (unpasteurized) milk consuming households in the dry and wet season, respectively. At the marker-level, 262 and 246 informal market agents were interviewed and sampled during two seasons. Informal market agents sampled included dairy co-operatives, milk bars, milk shops and kiosks and mobile traders on foot, bicycle or motorised transport. A total of 110 formally (pasteurized) marketed milk samples from retail outlets with and without refrigeration facilities in Nairobi and Nakuru were also tested.

Respondents were randomly selected within production system (extensive and intensive) and human population density (urban, peri-urban and rural) strata. Nakuru and Narok districts represented extensive production systems and low population density (also medium market access). Nairobi and Kiambu Districts represented intensive production systems and high population density (also high market access). Informal market agents sampled included dairy co-operatives, milk bars, milk shops and mobile traders on foot, bicycle or motorised transport. Attempts were made during the wet season to interview and sample the same respondent as in the dry season. Where this was not possible, substitution was made within the same locality. Samples were screened using the milk ring test (MRT) (unpasteurized milk) and indirect antibody ELISA (both unpasteurized and pasteurized milk).

Brucella Milk Ring Test (MRT)

The MRT works on the principle that antibodies to *Br. abortus* attach themselves to fat globule agglutinins in milk which rise to the surface of the milk and cluster in the cream layer. When haematoxylin stained *Br. abortus* antigen combines with brucella antibody (if present), a complex which adheres to the fat globules in the cream layer of milk is formed. The test often detects a high proportion of false positives (low sensitivity) due to positive reactions from samples taken shortly after parturition, near the end of lactation period, or from mastitic quarters (MacMillan, 1990). MRT was conducted by pipetting 1 ml of milk into a 1.2ml Skatron tubes (Skatronas, Lier, Norway), adding and mixing one drop of stained *Br. abortus* antigen. The tubes were thereafter incubated at 37^0C for 1hr and results read. A positive control was included with each set of tests.

Indirect milk ELISA

The method described by Neilsen *et al*., (1996) was adopted with slight modification. Briefly, polystyrene 96-well flat bottomed plates were coated with 100µl of 0.5mg/well of *Br. abortus* smooth lipopolysacccharide antigen in coating buffer (0.06 M carbonate buffer pH 9.6) and kept overnight in a humid box. The plates were thereafter washed five times with phosphate buffer (0.01M phosphate buffer of pH 7.2 containing 0.05% Tween-20 and 0.15M NaCl), dried and blocked using 200ul/well of 0.1% gelatin and incubated at 25^0C for 30 mins. The plates were washed again, dried and milk samples added at 100 µl/well diluted 1:2 in milk diluent (0.01M phosphate buffer, pH 6.3, containing 0.15M NaCl, 0.05% Tween-20, 15 mM EDTA and 15 mM EGTA). The plates were shaken for 2 minutes in an orbital shaker and incubated for 28 mins at 25^0C. The plates were then washed and 100µl/well of monoclonal antibody conjugated (dilution 1:1600) to horse radish peroxidase added and incubated for 1hr at 25^0C. The plates were washed again, dried and the substrate (0.05M Citrate buffer pH 4.5 containing 1mM hydrogen peroxide and 4mM ABTS) added at 100 µl/well. The plates were incubated for a maximum of 15 mins and the absorbance read at 414 nm. Brucella positive and negative serum and milk controls were included. The control serum samples were diluted 1:50, while milk samples were diluted 1:2 in the milk diluent. Each milk sample was tested in duplicate. The modification in this procedure was that the cut-off value was determined by using twice the mean of the negative control samples (Savingy and Voller,1980) and not by the targeted reading described by Wright *et al*., (1985).

Results and Discussion

Indirect ELISA classified more consumer- and market-level samples as *Br. abortus* positive than MRT (Tables 1). Overall prevalence of brucellosis at consumer-level as determined by both ELISA and MRT were 4.9% and 3.9%, respectively. At the informal market level, ELISA and MRT classified 2.4% and 3.4%, respectively, as positive. Informally traded bulked raw milk from dairy co-operatives and milk bars had the highest proportion of ELISA and MRT positive samples. Nearly all these samples were from Narok District where extensively grazed pastoralist zebu herds predominate. The ELISA test classified nine (8.2%) of pasteurised milk samples as positive. Six of the nine positive samples were from one milk processor in Nakuru. Agreement between the test results were poor (*Kappa* = 0.32, 95%, confidence interval = 0.07-0.56) to moderate (*Kappa* = 0.40, 95% confidence interval = 0.19-0.60) for the market- and consumer-level samples, respectively, with the ELISA test classifying more samples as positive. Two consumer households in Nakuru reported having had a member diagnosed with brucellosis in the previous one year.

Table 1. Numbers and proportions of milk samples from consumer households and various market agents (two seasons) in rural and urban areas in Kenya testing positive for *Br. abortus* using MRT and ELISA antibody tests

Source of milk samples	Antibody Prevalence			
	MRT		ELISA	
	n	%	n	%
Consumer households				
Urban consumers (Nairobi and Nakuru)	10	4.7	11	5.1
Rural consumers (Nakuru)	7	3.2	10	4.6
Informal market agents in high market access and intensive production area (Nairobi/Kiambu)				
Coops/collection centers/Self help groups	3	4.8	2	3.1
Milk Bars	1	0.8	2	1.6
Milk Shops/kiosks	2	2.1	1	1.0
Small mobile traders	1	1.7	0	0
Informal market agents in medium market access and extensive production area (Nakuru/Narok)				
Coops/collection centers/Self help groups	0	0	0	0
Milk Bars	9	15.0	5	12.2
Milk Shops/Kiosks	4	4.4	0	0
Small mobile traders	0	0	2	3.4
Pasteurised milk in Nairobi & Nakuru	-	-	9	8.2

The test results generally reflect previous findings from serological studies (e.g., Kagumba and Nandokha, 1978; Kadohira *et al*., 1997) indicating higher farm-level prevalence of brucellosis in extensive and/or communal grazing areas than in smaller stall-fed herds. Kagumba and Nandokha (1978) reported a prevalence of 10% bovine brucellosis in extensive production systems in Nakuru, and Kadohira *et al*., (1997) reported a 2% apparent prevalence of bovine brucellosis in the smallholder system in Kiambu. Human brucellosis is also more common where extensive cattle production systems predominate. Muriuki *et al*., (1997) found that as high as 21% of human flu-like cases reported in health facilities in Narok were diagnosed as brucellosis (tests were done using Rose Bengal Plate test).

Boiling of raw milk (alone or in tea) achieves higher temperatures and duration than those attained during pasteurisation. These conditions, like pasteurisation, destroy all zoonotic health hazards. Given the very high proportion of households that boil milk, the health risks from bacterial pathogens were determined to be very low. One area that requires attention is the consumption of traditionally fermented milk (*maziwa lala*), by 6% of households (mainly in rural areas) in this survey. This milk is often not boiled before fermentation, which lowers the pH of milk from about 6.8 to about 4.5. *Br. abortus* are only mildly affected by acidity at this level (Farrel, 1996). In a related investigation, Minja et al., (1998) found that the low pH level in sour milk only destroyed *Mycobacterium bovis* after 66 hours. This would imply that home-made fermented milk could be a possible source of milk-borne infection to humans. It is note-worthy that the number of consumer households reporting a member having been affected by brucellosis was generally low. These households were in Nakuru rural sample area where more unboiled and/or home-made fermented milk is consumed. It is apparent that bulking of raw milk by large-scale raw milk market agents or failures in large-scale pasteurisation can increase risks of infection with brucellosis.

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APPLICATION OF HACCP TO IMPROVE THE SAFETY OF INFORMALLY MARKETED RAW MILK IN KENYA

Mwangi, A.[1], Arimi, S.M.[2], Mbugua, S.[1], Kangethe, E.K.[2], Ouma, E.A.[3], Omore, A.O.[3,4], McDermott, J.J.[3] and Staal, S.J.[3]

[1]Dept. of Food Technology & Nutrition and [2]Dept. of Veterinary Public Health, University of Nairobi, P.O. Box 29053, Nairobi, Kenya; [3]International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya; [4]Kenya Agricultural Research Institute, P.O. Box 57811, Nairobi, Kenya.

Food safety standards require monitoring from production-to-consumption. The Hazard Analysis Critical Control Points (HACCP) process, recommended by FAO/WHO[1], is now a widely accepted methodology in risk analysis for industrially processed foods. HACCP identifies the points in a process that are hazardous, their risk factors and potential level of risk so that "critical control points" for remedial action can be implemented. Controls are specific actions taken to prevent hazards. The application of HACCP is a major challenge in developing countries where food markets are mostly informal. Market channels for milk range from direct sales of liquid milk or processed dairy products from producers to consumers, to a long chain involving combinations of private traders on bicycle, public or private transport, milk bars and kiosks, dairy farmer groups, small-scale and industrial processors. About 88% of marketed milk in Kenya is sold unprocessed, outside regulated channels. This study attempts to adapt a HACCP methodology to assess health risks at different points in the informal dairy marketing network.

Materials and Methods

Between March and May 1999, 162 raw milk traders of various cadres were identified and their milk handling practises studied. Traders were selected in a random sample, stratified on proximity to consumers (Nairobi) and producers (Kiambu). Milk handling practices for each trader were both observed and recorded on a questionnaire. Questions included milk procurement (source, time of collection, distance travelled, quality control procedures, type of handling vessels, bulking (mixing of milk from different sources), mode of transport and prices paid); milk handling (time to re-sale, storage, method of cleaning, water source); milk sale (type of buyers, quantities sold, packaging, prices received); and hygiene of premises and personnel. In addition, variable and fixed costs were estimated. One or more milk samples were collected at retail points in sterile tubes from each market agent and total and coliform bacteria in the milk counted using the Standard Plate Count method. Boiling and adulteration of sampled milk were also investigated. Bacterial counts were estimated for 80 pasteurised milk samples, purchased from retail shops and tested on the last day of "sell by date"

Two strategies were used to identify critical points (CPs) that were associated with high total and coliform counts in raw milk. The first was descriptive, to define dummy variables for all potential CPs (combinations of sources of milk and agent) and estimate statistics for each CP or group of CPs. These included the calculation of proportions with counts above national standards and the plotting of bacterial counts versus time since collection for each CP to visually assess trends. The second strategy was to include all potential CPs and milk procurement, handling and sale variables in stepwise regression models of the logarithm of total and coliform bacterial counts as dependent variables in the Proc REG procedure ($p<0.05$ for entry and retention) in SAS. Time since collection of milk was forced into all final models.

Results and Discussion

About 75% of milk samples were collected within two hours of their receipt by traders. Market points with one or more intermediate steps comprised 41% of samples collected. Direct sales occurred between producers and dairy co-ops (20%), hawkers (15%), milk-/snack-bar (13%) and kiosks/shops (12%). Bacterial counts were high (Table 1). At this early point in the retail chain, 58% and 82% of raw milk samples did not meet national standards for coliform and total bacterial counts, respectively. Approximately 13% of samples were adulterated with water. Interestingly, 70% of pasteurised samples did not meet national standards for bacterial counts.

Table 1. Descriptive statistics of milk bacterial counts and some continuous variables during the first seasonal survey of market agents in Kiambu and Nairobi

Variable	Number of obs.	Range	Median	% with counts above national standards [a]
Total bacterial counts (x 10^6/ml)	179	0.25 - 25,100	1,490	82 (70)[b]
Coliform counts (x 10^3/ml)	178	0.10 - 1,540	149	58 (73)[b]
Time since collection of milk (hrs)	159	0.03 - 7	1	-
Milk temperature (^{o}C)	171	11 - 31	21	-
Distance travelled (Km)	140	0 - 200	15	-

[a] Kenyan national standards (maximum bacterial counts/ml) for 'good' milk are: 2,000,000 and 50,000 for total and coliform counts, respectively, for raw milk; and, 50,000 and 10 for total and coliform counts, respectively, for pasteurised milk . [b] Figures in parentheses are proportions of pasteurised milk samples with counts above acceptable limits for 'good' milk.

Complete data for the regression analysis were obtained for 103 samples. Two market channel types (retail agents other than dairy co-ops and multiple selling steps) and three risk factors: scooping of milk, higher milk temperature and piped water were associated with higher coliform counts (the three risk factors were also associated with higher total bacterial counts) (Table 2). Using both complete and incomplete data records (154 samples), high coliform counts were also associated with the use of plastic versus metal containers ($p=0.03$). Time in the market chain and distance to retail points showed no significant association with total or coliform counts ($p>0.05$).

The generally high bacterial counts and lack of association with time show that most bacterial growth occurred before milk entered the market. Given a previous finding that milk sampled from farms had low bacterial counts[2], we hypothesize the existence

Table 2. Regression models for $\log_{10}$ of total and coliform bacterial counts in milk collected from market agents during the first season in Kiambu and Nairobi

Parameter	Estimate	s.e.	*p*-value
a) Regression model for $\log_{10}$ total counts (unit)			
Intercept	2.74		
Time since milk collection (hrs)	0.14	0.14	0.33
Milk temperature (^{O}C)	0.20	0.05	<0.01
Method of dispensing milk (scooping vs. pouring)	0.90	0.38	0.02
Water source (Piped vs. river, well or roof catchment)	1.43	0.39	<0.01
b) Regression model for $\log_{10}$ coliform counts (unit)			
Intercept	-0.25		
Time since milk collection (hrs)	0.09	0.11	0.38
Milk temperature (^{O}C)	0.13	0.04	<0.01
Method of dispensing milk (scooping vs. pouring)	1.02	0.29	<0.01
Water source (Piped vs. river, well or roof catchment)	1.18	0.34	<0.01
CPs without intermediaries selling milk to bars/shops/kiosks/hawkers *vs*. points selling milk to dairy co-ops	1.13	0.38	<0.01
CPs with >1 intermediary *vs*. points selling milk to dairy co-ops	0.79	0.37	0.04

of one (or more) CP(s) between farm and milk market agent. There are numerous possibilities (e.g. time held on farm, bulking) which deserve further investigation. Association of piped water source with higher counts was unexpected and may reflect a relative shortage of water from piped sources. Better milk quality from dairy co-ops is likely due to higher hygiene standards (mainly testing for adulteration, use of aluminium containers and chilling equipment). Otherwise, most milk samples were not chilled and the high bacterial counts (both raw and pasteurised) can be partly attributed to the general lack of a cold chain. One option is the adoption of the lacto-peroxidase system (LPS) for milk preservation[3]. However, the widespread adoption of LPS will require its widespread acceptance by national policy makers. The majority of milk that currently reaches consumers, both from informal and formal agents, is below Kenyan national standards. Thus, the boiling of milk, now done by the majority of consumers, should continue to be encouraged. This study shows that some practices of informal market agents, such as scooping of milk and use of plastic containers, could be improved by extension and training. Since bacterial counts were already high on reaching the informal market agents, we will focus on studies to investigate potential CPs on-farm and between farm and market agent. At present, the public health risks from informally marketed milk appear low, particularly when compared to the substantial socio-economic benefits obtained from this system.

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ANALYSIS OF PUBLIC HEALTH RISKS FROM CONSUMPTION OF INFORMALLY MARKETED MILK IN SUB-SAHARAN AFRICAN COUNTRIES

Omore, A.O.[1,3], McDermott, J.J. [1], Staal, S.[1], Arimi, S.M.[2] Kang'ethe, E.K.[2] and Ouma, E.A.[1]

[1]International Livestock Research Institute, P.O. Box 30709, Nairobi, Kenya;
[2]Dept. of Vet. Public Health, University of Nairobi, P.O Box 29053, Nairobi, Kenya;
[3]Kenya Agricultural Research Institute, P.O. Box 57811, Nairobi, Kenya.

Despite policies to discourage them, informal milk markets account for over 80% of milk sales in most sub-Saharan African (SSA) countries. Informal milk market agents include farmer dairy co-operatives, small traders using bicycles and public or private transport and small retail outlets, such as dairy kiosks, and shops. Studies conducted by the International Livestock Research Institute (ILRI) and national collaborators (e.g., in Kenya[1]) show that convenient delivery and lower prices (reflecting lower handling and processing costs) are the principal benefits for consumers. Current milk handling and safety regulations in most SSA countries are derived from models in industrialised countries. These may not be appropriate for local market conditions where such regulations may unnecessarily inhibit efficient milk marketing. An important step in developing targeted policies more supportive of market participation of the majority is to collect quantitative and qualitative information about milk-borne health risks under different production and marketing situations. This paper gives an over-view of on-going activities in central Kenya aimed at assessing public health risks from informally marketed milk and presents preliminary results of milk quality and handling practices of informal milk market agents and consumers.

Materials and Methods

Seasonal survey data were collected from 250 informal milk market agents and 230 households (hh) consuming raw (unpasteurised) milk in rural (Kiambu and Nakuru Districts) and urban (Nairobi City and Nakuru Town) locations in Kenya between January 1999 and January 2000. These sites also represented contrasting levels of market access and types of dairy production systems. Respondents were randomly selected within production system (extensive and intensive) and human population density (urban and rural) strata. Data on milk handling practises by consumers and market agents, dairy product consumption and preferences were collected using a questionnaire. Raw milk samples were collected from each milk market agent at retail points and from each consuming household for laboratory assessments. In addition, 110 pasteurised milk samples were collected from retail outlets with and without chilling facilities and subjected to the same tests for comparison.

Total and coliform bacteria in the milk samples were counted using the Standard Plate Count method; brucellosis status was investigated using the Milk Ring Test (MRT) and the indirect ELISA[2] (the latter is more sensitive (96.5%) and specific (>99.5%)); selective media and biochemical tests were used to isolate *E. coli* and *E. coli* 0157:H7; and, drug residues were screened using Charm AIM test kit (Charm Sciences Inc., USA) to detect β-lactams, tetracyclines, aminoglycosides, macrolides and sulphonamides at levels above maximum residue limits (MRLs) recommended by the European Union (EU). In addition, risks of zoonotic tuberculosis are being investigated through speciation of *Mycobacteriaceae* isolated from patients suspected to be suffering from tuberculosis.

Results

Consumption is mainly of liquid milk. Raw fresh milk was purchased by 29% of households in Nairobi (average = 5.5 litres/hh/month) in comparison to 93% of households in both Nakuru urban (average = 22.5 litres/hh/month) and rural (average = 24.3 litres/hh/month). The total liquid milk equivalent of pasteurised milk and processed dairy products consumed in Nairobi, Nakuru urban and Nakuru rural were 15.6, 3.8 and 0.2 litres/hh/month, respectively. Pasteurised milk was purchased in Nairobi, Nakuru urban and Nakuru rural by 78%, 34% and 5% of sample households, respectively. More raw and pasteurised milk was purchased as income class increased. All households in urban areas and 96% in Nakuru rural reported boiling milk prior to consumption, mainly as an ingredient in other foods, mostly tea. Most consumers expressed a preference for raw over pasteurised milk.

Milk quality as judged by total and bacterial counts was generally low. This is discussed further in a companion paper[3]. The main zoonotic health risks examined to date were for brucellosis and coliforms. Interestingly, brucellosis antibody detection by ELISA varied by milk source. *Br. abortus* antibodies were not detected in raw milk sold in urban areas but were found at low levels (2-5%) in milk sampled from consumers in rural areas and at higher levels (25%) in pasteurised milk (Table 1).

Table 1. Proportions of raw milk samples from consumer households and various market agents in rural and urban areas in Kenya testing positive for *Br. abortus* using MRT and ELISA antibody tests.

Source of milk	Antibody Prevalence - Season 1			Antibody Prevalence - Season 2		
	Number tested	MRT Positive %	ELISA Positive %	Number tested	MRT Positive %	ELISA Positive %
Urban consumers	105	9.5	0	107	0	0
Rural consumers	106	5.6	1.8	114	0.8	4.8
Informal market agents	239	3.3	5	239	1.2	4.2
Formal market agents	110	-	25	-	-	-

Of 258 milk samples tested for faecal coliforms, 22% and 1% contained *E. coli* and *E. coli* 0157:H7, respectively. This mirrored the high bacterial counts found in 162 milk samples collected from informal milk agents in Nairobi and Kiambu[3]. Another important health risk is from anti-microbial residues in milk. Residues exceeding EU MRLs were detected in 4-16% and 8% of informally traded and pasteurised milk samples, respectively.

Discussion

The variation in detection of brucellosis reflects past findings that show high variation of the disease by cattle production systems.[4,5] Over 70% of marketed milk in Kenya is from smallholder herds without brucellosis.[5] The results indicate that bulking of milk from many areas and production systems could pose significant health risks if the milk is not pasteurised or adequately boiled.

The high bacterial counts mainly reflect poor hygiene and a long time-lag between milking and sale of the milk[3]. Future efforts will focus on improving milk quality by informal market agents by training and extension on appropriate handling containers, milk temperature regulation and other factors. Of greatest risk in this regard is raw milk purchased from multiple-source markets, often at great distances. Market agents who currently bulk and retail raw milk could reduce health risks by processing or screening their milk prior to sale. Actual health risks from bacterial contamination are already judged to be low because of the common consumer practice of boiling milk before consumption, a practice that should be further encouraged. This practice may decrease the need for strict implementation of regulations preventing raw milk marketing. Of concern is the high proportion of samples with drugs above EU MRLs, This suggests that many farmers do not observe prescribed withholding times. Market agents may also use anti-bacterials to increase milk storage time. Further studies will determine which drugs are involved and when and how they are administered.

Many studies on zoonotic health risks in SSA have focussed at the farm-level without assessing actual risks to consumers. Similar studies by ILRI and its partners in Ghana and Tanzania and further analyses of the data from Kenya will provide additional risk information. When this information is combined with economic data on market efficiency, recommendations will be developed to support dairy markets serving resource-poor producers without impeding the efficient marketing of milk. These recommendations will not only inform policy decisions on raw milk marketing in SSA but also in the many regions of the world with similar circumstances.

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ANNEX 4. QUESTIONNAIRES FOR MARKET AND CONSUMER LEVEL SURVEYS