Occurrence of *Brucella* and *Mycobacteria* Species in Raw and Fermented Camel Milk along the Value Chain

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ABSTRACT---- ***The study sought to determine the presence of Brucella and Mycobacteria species in raw and fermented camel milk (suusa) along the value chain in Kenya. Fresh milk, fermented milk and blood samples from camels were collected at production, bulking (Isiolo) and at market centres (Nairobi, Eastleigh). Serological tests were used for screening and confirmation of Brucella species while molecular techniques using Line Probe Assay was used to analyse for presence of Mycobacteria species of major clinical relevance. At production, incidence of Brucella species was 17% using confirmatory Antigen Rapid Brucella Antibody Test (ARBAT). Line probe assay identifiedatypical and typical Mycobacteria species which includedM. avium, M. kansasii, M. intracellulare, M. malmoense and Mycobacteria tuberculosis complex (MTBC). Fresh camel milk at production had higher incidence of Mycobacteria species than fermented milk (suusa) made from it. Mycobacteria tuberculosis complex (MTBC) an important human strain associated with infection of tuberculosis in humans was identified in raw and fermented camel milk. Occurrence of Brucella species and Mycobacteria tuberculosis complex in camel milk greatly poses a public health concern to consumers as well as in the commercialization of indigenously produced fermented camel milk product, suusa, in Kenya.***

Keywords---- *Brucella*, *Mycobacteria*, zoonotic, camel milk, *suusa*

1. INTRODUCTION

Camels play important socioeconomic roles within the pastoral and agricultural systems in the arid and semiarid zones of Asia and Africa. In Northern and Eastern Kenya pastoralists depend on camel milk and milk products to provide meat and milk to the population in the arid and semi-arid lands (ASAL). Raw and fermented camel milk known as *suusa* have found way to varied markets including local urban centres within areas of production and urban towns in the entire Northern Kenya and big cities like Nairobi. Raw camel milk is left to ferment in smoke-treated gourds by pastoralists to become *suusa* at ambient temperature ranging from 26–29^{o}C, for 1–2 days (Tezira *et al*., 2005). Camel milk accounts for 15% of the total national milk production approximately 4000 litres daily production (FAOSTAT, 2012), indicating that most people have taken to the consumption of fresh camel milk and *suusa*. However, consumption of unpasteurized milk and milk products can serve as a vehicle for transmission of infection to humans with *Brucella* and *Mycobacteria* organisms. The practise of drinking raw camel milk and milk products by the pastoral community and other non-pastoral consumers may pose a risk to these infections (Akweya *et al*., 2012).

Brucella species are host specific intracellular pathogens that are able to cause severe disease in both mammals and humans. In humans, chronic *Brucella* infection can lead to reproductive defects as well as undulant fever. *Brucella* is a gram negative, non-spore forming, coccobacillus which lives in a microaerophilic environment inside its host (Weimer, 2001). Camels are susceptible to *Brucella* species especially *B. arbutus* and *B. melitensis* (Mayada *et al*., 2012). Camels are infected through contaminated feeds and water, through the respiratory tract by inhaling dust or droplets. The bulls that are infected may pass the organisms through semen to females (Mayada *et al*., 2012). The *Brucella* organisms, once in lactating camels localize themselves in mammary tissues and are excreted in milk when the camel is milked. The pastoralists consume camel milk in raw form with a belief that in that state, the milk is therapeutic to other health complications in their community such as asthma and diabetes (Akweya *et al*., 2012).With such beliefs and practices, the pastoral community is potentially exposed to brucellosis, a zoonotic infection. Milk ring test (MRT) and blood serological tests are used principally for diagnosis of the disease. Tests for detection of *Brucella* antibodies in milk are considered to be principal methods for detection of infected herds and for diagnosis of brucellosis in individual animals. Solid phase chromatographic immunoassay can also be used for the qualitative detection of *Brucella arbutus* antibody in

whole blood, plasma, serum and milk (Bionote, 2005).Animal brucellosis can be transmitted by both vertical and horizontal transmission. Horizontal transmission occurs through ingestion of contaminated feed, skin penetration, via conjunctiva, inhalation and udder contamination during milking (Radostits *et al.,* 2007). Animal health workers, butchers, farmers, and those who are habitually consume raw milk and come in contact with animals are at high risk for brucellosis (Chukwu, 1987). In man, transmission occurs as a result of ingestion of milk, contact via skin abrasion, mucous membranes and inhalation (Seifert, 1996; Radostits *et al*., 2007).

Tuberculosis (TB) is the major contributor to the global burden of disease especially in developing countries (Kiine *et al*., 2006). Tuberculosis is an important zoonotic disease caused by *Mycobacterium* species. It is transmitted form animals to humans through consumption of unpasteurized milk and its by-products, sputum, urine, visceral organs, nasal discharges or aerosols (Mayada *et al*., 2012). *M. tuberculosis* complex, a group that also includes *M. tuberculosis, M. africanum and M. microti*. *M. Bovis* the causative agent of bovine tuberculosis and *M. tuberculosis,* the causative agent of tuberculosis in humans, are genetically and antigenically very similar and cause identical clinical disease in humans. GenoType *Mycobacteria* direct (GTMD) is a qualitative *in vitro* test for diagnostic purposes based on nucleic acid sequence based amplification (NASBA) and DNA strip technologies. It permits the genetic detection direct from decontaminated samples of five *Mycobacteria* species: *Mycobacteria avium, Mycobacteria kansasii, Mycobacteria intracellulare, Mycobacteria malmoense* and *Mycobacteria tuberculosis* complex. Nucleic acid sequence-based amplification (NASBA), also known as "self-sustained sequence replication" (3SR) **(**Guatelli *et al*., 1990**)** and Transcription mediated amplification (TMA) (Gill and Ghaemi, 2008). It is a sensitive transcription-based amplification system (TAS) for the specific replication of nucleic acids *in vitro*. The complete amplification reaction is performed at the predefined temperature of 41^{o}C. Three enzymes are involved in this homogeneous isothermal reaction: avian myeloblastosis virus (AMV), reverse transcriptase (RT), RNase H and T7 DNA dependent RNA polymerase (DdRp). Due to the integration of RT into the amplification process, the method is especially suited for RNA analytes like mRNA, rRNA or genomic RNA (Deiman *et al*., 2002). Line-probe assays also referred to as reverse hybridization, are a family of novel DNA strip-based tests that use PCR and reverse hybridization methods for the rapid detection of mutations associated with drug resistance using an automated system (Angkana, 2009).Therefore this study sought to determine the presence of *Brucella* and *Mycobacteria* species in the critical control points in the camel milk value chain.

2. METHODOLOGY

Study site and sampling procedure

The study was conducted along the camel milk value chain from Isiolo camel producing areas to Nairobi urban consumption centre in Kenya. The study site was purposively selected due to the high number of camels as well as high production and consumption of camel milk and *suusa*. Production, bulking and marketing were identified as critical control points hence sampled. A stratified sampling procedure was therefore applied with control points being the strata. At production, due to the high mobility and scattered nature of the pastoral camel herds as well as necessity to obtain consent from herd owners, sampling was purposive and limited to accessible herds whose owners agreed to participate in the study. A total of 36 blood and 96 milk samples were collected. Pooled milk samples from the herds were collected for analysis of *Mycobacteria* and *Brucella* Milk samples were collected from each container at the herd level and later pooled to make a representative a total of 42 milk samples. At bulking centres, 7 cooling hubs identified at the time of the study (June 2013) were sampled. A total of 35 milk samples were randomly collected from the cooling hubs. At the market centres, 19 women were identified selling camel milk from Isiolo herds at Eastleigh, Nairobi. A pooled milk sample from each container was collected from each woman to obtain a representative of 19 samples of fresh and fermented camel milk (*suusa*). The samples were then transported to the Isiolo Veterinary laboratory and Central Veterinary Laboratory (Kabete, Nairobi) within 6 hours of sample collection in a cool box containing cooling cumulators at 8^{o}C.

2.1 Detection of *Brucella* species in milk samples

2.1.1 Milk Ring Test (MRT)

The test was performed according to the manufacturer's instruction. MRT was performed on individual milk samples. Antigen and milk samples were brought to the room temperature prior to performing the test.

2.1.2 Rose Bengal Precipitation Test

Approximately 20µl (one drop) of the antigen was placed on each square of the plate. Approximately 20µl (one drop) of the test serum was placed alongside the antigen. With an applicator stick, the antigen and the sera were mixed thoroughly. The plate was then shaken gently for 5 minutes. The results were read immediately after 5 minutes by examining for agglutination. Any degree of agglutination was taken as a positive result.

2.1.3 Anigen Rapid *Brucella* Antibody Test kit (Bionote).

Approximately 20µl (one drop) of the test serum was slowly added using a capillary tube to the sample well and then 4 drops of the assay diluent was added. The test results were interpreted after 20 minutes. The presence of only one purple

colour band within the result window indicated a negative result while the presence of two colour bands (Test band and Control band) within the result window, no matter which band appeared first, indicated a positive result.

2.1.4 Line Probe Assay for detection of *Mycobacteria* species

Fresh milk and *suusa*samples (5 ml) were decontaminated using 5% oxalic acid and concentrated by centrifugation (10 min, 1200 x g, 4°C). The test kit was adapted from Hainlifescience. The test is based on nucleic acid sequence based amplification and DNA strip technology permits the genetic detection direct from decontaminated samples of five *Mycobacteria* species: *Mycobacteria avium, Mycobacteria kansasii, Mycobacteria intracellulare, Mycobacteria malmoense* and *Mycobacteria tuberculosis* complex. RNA was extracted using magnetic beads capture technique and amplification was followed using the nucleic acid sequence based amplication technique. Reverse hybridization then followed with detection using DNA strips.

2.2 Statistical analysis

Logistic regression was used to quantify risk of occurrence of zoonotic pathogens *Brucella* and *Mycobacteria* species along the value chain. Chi square was also used to determine significant difference along the value chain.

3. RESULTS

3.1 Occurrence of *Brucella* species along the *suusa* value chain

Table 1 shows that there was no significant ($p>0.05$) difference in the number of positive samples for *Brucella* along the value chain. The prevalence risk increased from 14%, 23% and 26% at production, bulking and marketing respectively. The likelihood of obtaining samples positive with the *Brucella* organism at processing is 1.8 times higher than obtaining the same at production and 2.1 times higher at the market than at production. However, incidence of 20% occurrence of *Brucella* organisms was observed along the value chain. The results indicate that the seroprevalence of brucellosis in raw camel milk chain and subsequent products like *suusa* is on the increase as shown in table 1.

Table 1: Detection of *Brucella* species along the *suusa* value chain

		Screening tests		Confirmatory test		
Node	**N**	**RBPT (+ve)**	**MRT (+ve)**	**ARBAT (+ve)**	**Prevalence risk (%)**	**Likelihood ratio (C.I.)**
Production	36	6	-	6		
	42	-	11	-	14	Reference
Bulking	35	-	15	8	23	1.778 (0.5 – 5.7)
Market	19	-	11	3	26	2.143 (0.5 – 8.2)
Total (inc. %)	**96**	**6 (17)**	**37 (39)**	**6 (17)**		

Key: RBPT- Rose Bengal precipitation test; MRT-Milk Ring Test; ARBAT-Anigen Rapid *Brucella* Antibody Test
(inc%)-incidence, C.I. – confidence interval

3.2 Occurence of *Mycobacteria* species along the *suusa* value chain

Two types of *Mycobacterial* groups were identified; the atypical *Mycobacteria* group that included *M. avium, M. intracellulare, M. kansasii* and *M. malmoense* at production level forming an incidence of 42% in raw camel milk. The typical *Mycobacteria* group identifiedwas *Mycobacteria tuberculosis* complex (MTBC) with an incidence of 26% in raw camel milk at production. The MTBC complex consists of *M. tuberculosis, M. bovis, M. africanum, M. canetti and M. microti*. Any of these can cause human tuberculosis. At the market level, the incidence of MTBC reduced to 21% despite the acid development during fermentation into *suusa* (Table 2).

Table 2: Occurrence of *Mycobacteria* species along the *suusa* value chain in Kenya.

Chain Node	**N**	***M. Avium***	***M. Intracellulare***	***M. Kansasii***	***M. Malmoense***	**MTBC**
Production	19	7 **(37)**	6 **(32)**	8 **(42)**	6 **(32)**	5 **(26)**
Market(*suusa*)	19	3 **(16)**	3 **(16)**	4 **(21)**	3 **(16)**	4 **(21)**

Key: MTBC is *Mycobacteria tuberculosis* complex; Figures in parenthesis represent the percentage incidence of occurrence

Table 3 shows the prevalent risk of *Mycobacteria* occurrence in the *suusa* value chain. The risk decreased along the value chain. There was a 58% and 42% risk of obtaining *Mycobacteria* at production and marketing respectively. The likelihood of obtaining samples positive with the *Mycobacteria* organism at production and marketing is equal (1).

Table 3: Prevalent risk of *Mycobacteria* along the *suusa* value chain

Chain Node	Total samples	Positive samples	Negative samples	Prevalence risk (%)	Likelihood ratio (C.I.)
Production	19	11	8	58	Reference
Marketing	19	8	11	42	1.0 (0.651-1.536)
Total	38	19	19		

Key: C.I. refers to confidence interval

It was found that some milk and *suusa* samples had all the 5 strains of *Mycobacteria* species as shown by the stained DNA strip in figure 1.

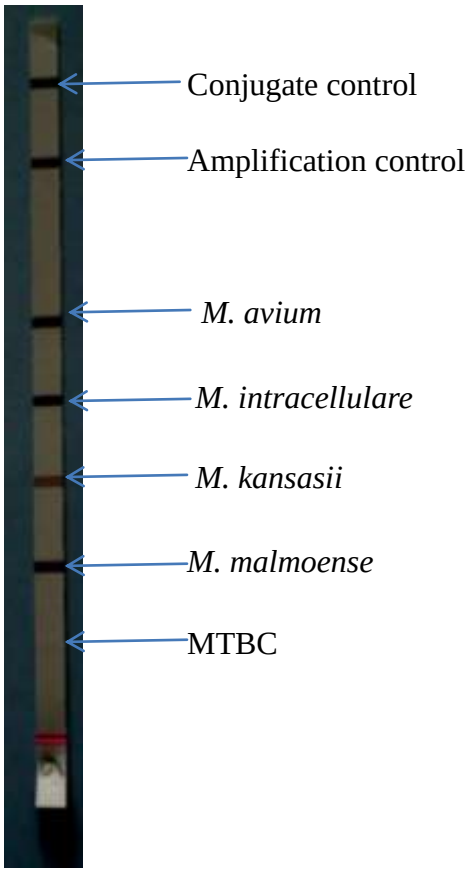

Figure 1: DNA strip stained with different *Mycobacterium* species.

4. DISCUSSION

4.1 Occurrence of *Brucella* species along the *suusa* value chain

The increase in incidence may be associated with the varying husbandry and management practices that are emerging in livestock systems as urbanization sets in pastoral areas (Abuo -Eisha, 2000). Peri-urban dairy camel keeping has led to intensification of camel keeping (Noor *et al*. 2012). Under these conditions, *Brucella* organisms may form a web of circulation in re-infections from the fields, water and the handlers. In Northern Kenya, most pastoralists are keeping dairy animals around towns to supply milk to residents and beyond. The dairy animals mostly kept are lactating camels and dairy goats. In this mixed system, the *Brucella* organisms may be shed in the environment by smaller ruminant stock that are known to be carriers and cause infection to lactating camels (Abbas and Agab 2002, Musa *et al*., 2008).

Spontaneous fermentation of raw camel milk into fermented milk (*suusa*) is a predisposing factor for the occurrence of *Brucella* in the final marketed product (*suusa).* Brucella organism is able to survive in fermented milk. After 10 days of storage at 4°C, *B. abortus* was recovered in fermented milk at a level of 10^5 CFU/ml, despite the low pH below 4.0 (Zuniga *et. al*., 2005; Falenski *et al*., 2011). The pastoral community has the culture of consuming raw camel milk due to their perception that it has good taste and flavor (Akweya *et al*., 2012).Animals are reservoirs of human brucellosis. The infection is mainly transmitted to humans through the ingestion of raw milk or unpasteurized dairy products contaminated with one of the *Brucella* species pathogenic to humans.

Brucella organism has been known to survive dessication and starvation outside the host cell for years due to presence of lipopolysaccharide in their cell membrane (Rittig *et al*., 2003). *Brucella* is known to survive in soil in an inactive form and will only be reactive when in host cells and dusty milking environment. Another predisposing factor is lack of vaccination of the camels against brucellosis in the study area. This is because camels are kept in a pastoral system where the herders move from one place to another making access and monitoring of the camel herds difficult for the veterinary officers. Consumption of raw camel milk and *suusa* made from the same at production and market level potentially exposes the population to risk of infection with *Brucella* organism.

Spread of the *Brucella* organsism in the study area could probably be due to movement of infected animals to disease free herds. Proximity of infected herds to non-infected herds is another probable risk factor which occurs at watering points where camels come together. Other probable risk factors in the study area are: poor management, abortion, milking more animals by a single person, herding with other ruminants and survival of the organisms in the environment (mostly soil), may also play a role in epidemiology of the organism **(**Abbas *et al*., 1987; Radwan *et al.*, 1992; Abuo -Eisha, 2000**)**.

4.2 Occurrence of *Mycobacteria* species along the *suusa value* chain

Mycobacteria normally survive acid conditions due to their lipid cell walls that are hydrophobic. The reduction in occurrence from production to marketing may be attributed to the slow growing nature and fastidiousness of *Mycobacteria*, especially in competing for nutrients with other more aggressive microorganisms like lactic acid bacteria (Simone *et al*., 2008). The high lipid content in the cell wall of *Mycobacteria* species prevents its destruction by acidic, alkaline and attack by antimicrobial compounds like lysozyme in both the intracellular and extracellular environment. This explains the presence of *Mycobacteria* species in fermented camel milk (*suusa*) despite its acidic nature. The most significant risk factor for the occurrence of *Mycobacteria* in the *suusa* value chain is the lack of pasteurization of camel milk before processing into *suusa*as well as consumption of unpasteurized product exposes the population to infection with tuberculosis. *Mycobacteria* species are intracellular organisms where they colonize and multiply within the white blood cells of the host. Since milk contains white blood cells, *Mycobacteria* may be shed in milk from an infected camel. *Mycobacterium avium paratuberculosis* may be present in milk in free suspension.

Of significant importance was the occurrence of MTBC in both fresh and fermented camel milk with an incidence of 10.2% and 8.2%. *Mycobacterium tuberculosis* complex (MTBC) which is a group of pathogenic*Mycobacteria* species that includes *M. tuberculosis, M. bovis, M. africanum, M. canetti*and *M. microti* responsible for causing tuberculosis infection in humans. *Mycobacterium bovis* causes tuberculosis in a broad range of mammalian hosts including cattle and other ruminants, felids, canids, lagomorphs, porcids, camelids, cervids and primates including humans. Although fermentation results in formation of lactic acid and hence lowers the pH which is detrimental to many pathogenic microbes, *Mycobacteria* species are known to survive in soured milk for up to 14 days (Kazwala, 1998). *Mycobacteria* species have a cell wall that is composed of over 60% of lipids which comprises of three major components i.e., mycolic acid, cord factor and wax-D (Alderwick *et al*., 2007; Brennan, 2003). Mycolic acids are unique alphabranchedlipids which determine the permeability of *Mycobacteria* cell wall due to its strong hydrophobic nature. It forms a lipid shell around the organism and thus affects permeability properties at the cell surface. Mycolic acid is an important factor responsible for virulence in MTBC, because they defend *Mycobacteria* from the attack of cationic proteins, lysozyme and oxygen radicals in the phagocytic granule. They are also known to protect extracellular *Mycobacteria* from complement deposition in serum.

An important finding was that the milk samples showed mixed infection where 2 or more bands specific for a certain type of *Mycobacteria* species were observed as shown in figure 1. This is an indication of contamination from both the environment as well as from the animal. Environmental opportunistic/atypical *Mycobacteria* are normal inhabitants of natural waters, drinking waters, and soils (Falkinham, 2002). *M. avium* is an environmental organism that has been isolated from natural water, soil and dust, drinking water, plant and plant products (Falkinham, 2002). *M. intracellulare* has also been isolated from soil and water. *M. kansasii* is frequently isolated from tap water aswell river and lake water (Taillard *et al*., 2003). Environmental reservoir for *M. malmoense* is soil and water (Falkinham, 2002). Production site was characterised by milking using unsuitable containers, dust and soil. Marketing of fermented camel milk (*suusa*) was characterised by sale in the open air markets along the road sides. These act as risk factors for contamination with the *Mycobacteria* species from dust. Animals with pulmonic lesions from MTBC will excrete the organism in exhaled air, in the sputum and in the faeces and milk (Kinne *et al*., 2006).

Unpasteurized contaminated milk and other secretions or tissues from any of the isolated species can serve as the source of infection for humans. In cows, one cow can secrete enough viable bacilli to contaminate milk of up to 100

cows, when their milk is pooled (Kazwala, 1998).Mixing of milk from different suppliers as is practiced in the cooling hubs in the study area increases the chances of gross contamination of camel milk with *Mycobacteria*. Tuberculosis in the study area has been reported to have a prevalence of 338 and incidence of 137 people per 100,000 people (Ministry of health, 2012).The habit of drinking raw camel milk and preparing spontaneously fermented milk from unpasteurized milkamong the pastoral community greatly increases the chances of acquiring tuberculosis disease.

The contamination of raw camel milk by *Mycobacteria* species is apparently inevitable, even under sanitary conditions, due to the ubiquitous nature of these microorganisms. Only the heat treatment of raw milk using commercial pasteurization protocols can ensure the adequate destruction of *Mycobacteria* contaminants. In particular, unpasteurized, *Mycobacteria* contaminated milk poses a serious risk to HIV patients (Falkinham, 2002).

5. CONCLUSION AND RECOMMENDATIONS

The occurrence of *Brucella* and *Mycobacteria* species especially MTBC in camel milk value chain poses a risk of infection with brucellosis and tuberculosis respectively to consumers. Value addition in camel milk handling from production to market, especially through heat treatment and use of proper hygienic practices will improve safety of the camel raw milk and products like '*suusa*' made from it. Pasteurization of camel milk is recommended before processing into *suusa* which is sufficient to destroy *Brucella* and *Mycobacteria* species. There is need to carry out more studies at molecular level to identify the prevalent *Brucella* strain and other *Mycobacteria* strains in Kenya's informal camel milk value chain to fill the gap of information.

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